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Production of Poinsettia (*Euphorbia Pulcherrima*) with Light Emitting Diodes Compared with The Traditional High Pressure Sodium Lamp

> Sabir Thapa Plant Science

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Sabir Thapa

Supervisor Associate Professor Sissel Torre

Faculty of Veterinary Medicine and Biosciences Department of Plant Sciences Norwegian University of Life and Sciences (NMBU)

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Ås, October 2016

Abstract

Use of chemical are commonly used as tools for the commercial pot plant producers to grow strong, dwarfed, and compact plants. Although, these growth regulators have adverse effects on human health and the environment. So, this issue has motivated researchers to search for alternative methods for growth regulation. The aim of this study was to test light emitting diodes (LEDs) with different light qualities alone or in combination with high pressure sodium lamps (HPSs), to investigate their effects on compactness, flowering time and transpiration of poinsettia (Euphorbia pulcherrima). Similarly, to understand more of the growth regulation, hormone analysis was performed on some of the plant material. Two experiment, one in a growth chamber experiment and one in a greenhouse compartment were performed at SKP (Senter for Klimaregulert Planteforskning), Norwegian University of Life Science (NMBU). The growth chamber experiment was conducted to compare growth and hormonal content (auxin, abscisic acid, cytokinin and gibberellin) of shoots developed with HPS and compared with LED (20% blue and 80% red). However, LED with 20% blue and 80% red light used in the growth chamber experiment did not induce differences in morphology or hormonal content of poinsettia cv Christmas Eve compared to the traditionally HPS. In the greenhouse compartment experiment to assess effect of LEDs (blue LED and Green LED) alone or in combination with HPS towards compactness in cv Christmas Day HPS + blue (150 +50 µmol $m^{-2} s^{-1}$) and HPS +green LED (150 + 50 μ mol $m^{-2} s^{-1}$) have a potential to reduce shoot length in poinsettia compared to HPS (200 μ mol m⁻² s⁻¹) alone but the results were dependent on the background irradiance from natural light. Green light reduced transpiration, chlorophyll content in leaves and anocyanin content in bracts compared to blue light and reduce the external quality. Flowering time in poinsettia is very robust and no differences in flowering time was observed in any of the experiments. Thus it is concluded that blue LED in combination with HPS light are efficient in reduction of plant height without changing the flowering time and will to improve the external quality compared to green LED.

Abbreviations

ABA	Abscisic acid		
ABA-GE	ABA gluoside-ester		
ADT	Average Daily Temperature		
ANOVA	Analysis of Variance		
ARF	Auxin Response Factors		
СК	Cytokinin		
CO ₂	Carbon Dioxide		
CRY	Cryptochorme		
cv	Cultivar		
DIF	Difference in Day and Night Temperature		
DPA	Dihydrophaseic Acid		
DT	Day Temperature		
DW	Dry Weight		
EOD	End of Day		
FR	Fra-Red Light		
FW	Fresh Weight		
GAs	Gibberellins		
HPS	High Pressure Sodium		
hr	Hours		
IAA	Indole-3-acetic acid		
IAA-Asp	IAA-Aspartate		
IAA-Glu	IAA-glutamate		

IR	Infrared Radiation
LED	Light Emitting Diodes
mRNA	Messenger Ribonucleic Acid
NCED	Nine-Cis- Expoycarotenoid Dioxygenase
NT	Night Temperature
PA	Phaseic Acid
PAR	Photosynthetically Active Radiation
PGR	Plant Growth Regulators
РНҮ	Phytochorme
R:FR	Ratio of Red and Fra-red light
RH	Relative Humidity
SDP	Short Day Plant
SE	Standard Error
UV	Ultra-Violate Ray

1. Introduction

An important quality trait of pot plants is compactness. Different chemical growth inhibitors are commonly used to make pot plants strong, dwarfed, and compact. However, adverse effects on human health and the environment have motivated the research towards alternative methods for growth regulation (Kuwar 2010; M Ashraful Islam et al. 2012). Research on alternative environmental strategies to grow compact plants like manipulation on temperature, relative humidity (RH), photoperiod and light qualities have increased. Some of the techniques like use of specific light sources or screening of daylight by selective plastic films or by manipulation the red /far-red ratio are commonly used by greenhouse producers today but more knowledge in this area is needed to optimize the production (Kuwar 2010).

Light is the most important environmental factor in greenhouse production and it acts as the information centre for photoperiodism, phototropism, and photomorphogenesis and is the source of energy for photosynthesis and well as controlling stomatal movements (Aphalo 2006). Visible light consists of different wavelengths which ranges from 400-700 nm and consists of blue light (400-500 nm), green light (500-600 nm), red light (600-700) and far red photons (>700 nm) (Runkle 2007).

High pressure sodium lamps (HPS) are the common light source in greenhouses today. However, use of light emitting diodes (LEDs) have increased interest by growers. The most unique aspect of LEDs is the availability of narrow-spectrum light at wavelengths of primary interest for plant growth and development. LED technology have developed rapidly and have higher energy conversion efficiency than traditional light sources, low directional heat emission, longer lifetime and smaller loss in efficiency with age. However, the best light spectrum for efficient plant production and quality depends on the production aims, plant species and production systems. There is a need for more knowledge on intelligent use of LEDs in crop production. Also, many of the experiments with LEDs as light source are performed in controlled growth chambers. However, in order to optimize these processes and to understand the responses in real production systems we also need to test the light qualities in greenhouses environment together with natural light. For ornamentals like poinsettia it is very important that the flowering time is fast and not delayed due to changes in light quality. Furthermore, the transpiration, which is usually affected by light quality, should be optimal and ensure efficient nutrient uptake. Thus, the aim of this study was to test LEDs with different light qualities alone or in combination with HPS to investigate their effects on compactness, flowering time and transpiration. In order to understand more of the growth regulation, hormone analysis was performed on some of the plant material.

2. Literature Review

2.1. Poinsettia Production

Poinsettia, (*Euphorbia pulcherrima*) are Short Day Plants (SDP) and belong to the family *Euphorbiaceae* with about 2000 species (Yang et al. 2012). These plants are native in Mexico and Guatemala and spread all over the world except the arctic region. In wild, they usually have small woody shoots and grow up to 3 meter (Huang, 2007) and consisting of single female flowers surrounded by individual male flowers making cup shaped structure called cyathium (Ecke 1976). Poinsettia also consist of modified red leaves called bract/bracts which give ornamental value to poinsettia plants. more than the flower which is only a conspicuous organ (Rowell & Coolong 2010) (Figure 1).



Figure 1. Poinsettia, a common landscape plant in the tropics (Huang 2007).

2.1.1. The flower of poinsettia

As mentioned earlier, poinsettia inflorescence consists of a cluster of cyathia. In the centre of the inflorescence primary cyathium is develop which is the first flower of the inflorescence, and secondary cyathia are the three flowers that subtend the primary cyathium. Each cyathium bears one nectary gland which is enveloped by a symmetrical, uniserate

involucre. A single pistillate flower in the centre of the cyathium is encircle by variable number of staminate flowers (Rao 1971).

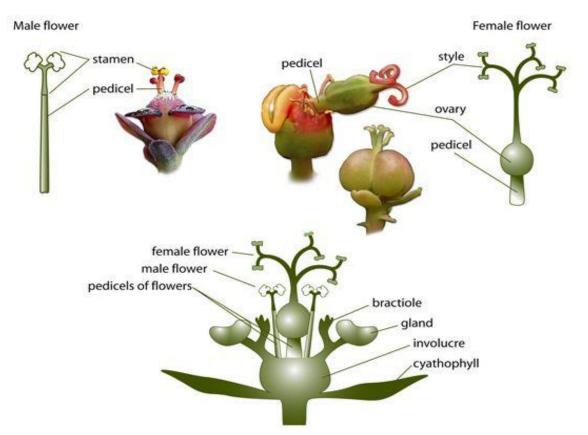


Figure 2. Inflorescence of *Euphorbia pulcherrima* (P. Berry, R. Riina n.d. retrived http://www.euphorbiaceae.org/pages/about_euphorbia.html)

Short days are required for the initiation and development of floral structures in *Euphorbia pulcherrima* since it is known as facultative short-day plant. The time of flower initiation to anthesis is influenced by temperature while the flowering process is triggered by photoperiod. Optimal temperature for flower development is supposed to be between 23-26 °C whereas when the average daily temperature (ADT) increases from 16 to 22 °C, the rate of flower development increases. Thus, in these temperatures the rate of flowering increases but the night temperature should not exceed 22 °C (Ecke III et al. 2004).

Poinsettia plants are indoor potted plants and regarded as a symbol of Christmas (Rowell & Coolong 2010). Since they are SDP they need long nights for initiation of flowering (Wang et al. 2003) with 12.5 hours of critical day length which naturally occurs during November – December (Kristoffersen 1969). However, in Norway most poinsettias are grown with automatic short day curtains and produced with 10 hours' photoperiod.

Different plant growth regulators (PGRs) such as Chlormequat, Daminozide or Paclobutrazol are commonly used (de Castro et al. 2004) by greenhouse grower to make pot plants strong, dwarfed, and compact. The growers are requested to meet some pre-set height specifications for successful production and to reduce shipping challenges and increasing the plant value (Sørensen et al. 2006; Clifford et al. 2004). Alternative non chemical environmental strategies like manipulation on temperature, RH, photoperiod and manipulation of light qualities are also commonly used by the commercial growers as tools to grow compact plants (Clifford et al. 2004).

One of the most practiced method to reduce plant height and stem elongation is by using temperature DIF and Temperature drop. Temperature DIF refers to the difference in Day temperature (DT) and night temperature (NT). A negative DIF (– DIF) is when the NT is warmer then the DT, and this temperature regime usually suppresses plant height (Berghage 1998). Lowering of temperature before sunrise by 5 to 15° F for about 30 minutes is another strategy known as temperature drop which have similar inhibitory effect on stem elongation as negative DIF (Berghage 1998; Runkle 2014). The effect of drop treatment differs between plant species. The shoot length of basil was longest at +24 DIF and shortest at 0 DIF but little effect was found when they were exposed to positive DIF. In contrast The effect of +24 DIF was opposite in lemon balm (Figure 3) (Gislerød 2016).

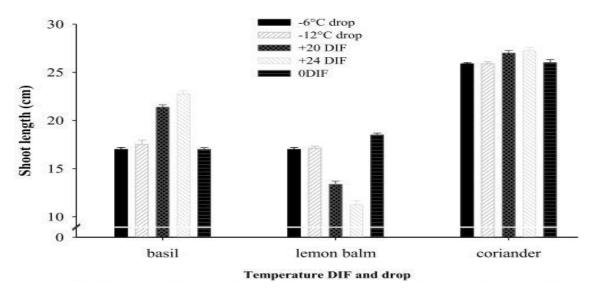


Figure 3. Effect of Temperature DIF and Temperature Drop on shoot length of Basil, Lemon balm and Coriander (Gislerød 2016)

Poinsettia respond strongly to negative DIF and becomes very compact compared to positive DIF (Myster and Moe, 1995). However, the flowering is delayed and an increase in

the post-harvest abscission of cyathia is common when poinsettia is grown under negative DIF. The energy consumption in the greenhouse is also much higher in a negative DIF regime compared to positive DIF due to a higher heating demand during night. Thus, a temperature drop is more common in commercial greenhouse poinsettia production. Moreover the temperature drop is also to achieve as supplementary lighting is required on the morning but it also results in a larger energy consumption but far from the energy demand required in the negative DIF regime (Moe et al. 1992).

2.2. Light

Light is form of radiant energy, narrow band of energy within continuous electromagnetic spec trum, which ranges from radio waves to gamma rays (Diffey 2002; Hopkins & Huner 2009). Light has characteristics of a particle and a wave which are required for complete description of its behavior. The light particles are known as photons whose energy level is determined by the waves or frequency or colours i.e. $E\lambda = hv = hc/\lambda_{vacuum}$ (where $E\lambda$ is a quantum, or the amount of energy that one photon has, h is Plank's constant, v is frequency, λ is wavelength, and c is speed of light in vacuum). Light of wavelengths between 400-700 nm act as the signal axis for photoperiodism, phototropism, photomorphogenesis, senescence and photosynthesis which is also known as visible light or Photosynthetically Active Radiation (PAR) (Aphalo 2006). In another word, photosynthetically active radiation, designates the spectral range of solar radiation from 400 to 700 nanometers that photosynthetic organisms are able to use in the process of photosynthesis. In addition, those regions of the light spectrum we notice as blue (400-500nm), green (500-600 nm), red (600-700 nm) and far red (700-740 nm) are called visible light. The ultraviolet (100-400nm) and infrared (more than 750 nm) regions of the spectrum, which our eyes cannot detect are referred to as ultraviolet or infrared radiation, respectively as described in figure 4. (Hopkins & Huner 2009).

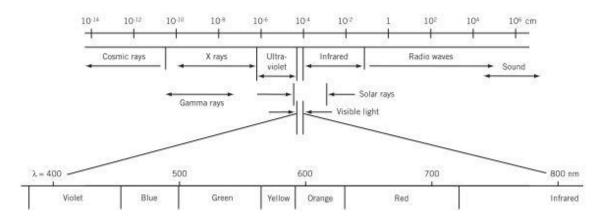


Figure 4. The electromagnetic spectrum. Visible radiation, or light, represents only a very small portion of the total electromagnetic spectrum (Hopkins & Huner2009).

Intensification of photosynthetic productivity of the plant relies on its ability to sense, measure, and react to light quality, quantity, and direction (Briggs & Olney 2001). Plants absorb light through pigments called photoreceptors which can be grouped into two groups of pigments, one group called mass pigments and another sensor pigments. The mass pigments like chlorophylls, absorbs large fraction of incident light as compared to sensor pigments due to high concentration in the plant tissues. Furthermore, sensor pigments sense the light environment and modify plants behavior and developmental plans as per the environmental condition. Red (R) and far red (FR) lights are sensed by the phytochromes, blue/UV-A lights are sensed by cryptochromes and phototropin (Aphalo 2006) whereas UV-B is sensed by plant through photoreceptor UVR8 which was recently described at the molecular level (Heijde & Ulm 2012). In general, far-red light reverse red light activation of phytochromes where phytochromes plays a central role for adapting light environment, sensing of shade, flowering and many other plant processes. The cryptochrome, blue light receptors control stomatal movements, plant stature, anthocyanin accumulation and flowering. Phototropin contributes to leaf expansion, phototropism, stomatal movements and chloroplast accumulation and avoidance (Kami et al. 2010).

2.2.1. Effect of Light Qualities in growth of Plants

The different light spectrums with different colours or wave lengths reaching to plant surface is referred to as light quality. Both quality, intensity, and duration of light influence on plant growth. red and blue light are important in photosynthesis and growth but green light is mostly reflected from the plant but is still believed to have a role in photosynthesis and growth (M. A. Islam 2013; Manohar 2011).

Different photoreceptors are present in plant which all are involved in sensing different wavelength of light (Smith 2000). Basically, blue lights stimulate phototropism, control of seed germination, stomatal opening, while R: FR light are responsible for changes in leaf area expansion and leaf morphology, stem elongation, leaf/stem dry weight ratio, shoot/root dry weight ratio, and photosynthetic capacity. (Aphalo 2006; Aphalo & Lehto 1977). Low ratio of R: FR stimulates stem elongation while high ratio of blue light inhibits stem elongation at high level of irradiance. Furthermore blue light also promote axillary shoot production by suppressing apical dominance in plants (Appelgren 1991).

The responses of plants to blue light spectrum differs from plants to plant. Studies shows increased specific leaf area and biomass yield in soybean, potato and lettuce by reduced level of blue light. The lettuce, spinach and mustard treated with low blue light had also increased leaf area with no change in dry mass (Dougher & Bugbee 2001a).

Plants grown under shaded habitats receive less amount of PAR at the canopy level e.g. tropical plants growing in the floor of tropical rainforest. In this condition huge amount of FR and green light are received by the plants compared to red and blue light which are absorbed by the canopy leaves of the taller trees. Thus, acclimation to natural shade conditions would appear to be a complex interaction of responses to both light intensity and light quality (Hopkins & Huner 2009).

Green light spectrum with wavebands (500–580 nm) have higher reflectance then Red and blue light and are sufficient in shaded environment (Wang & Folta 2013). Green light is able to enter into the canopy better than other wavelengths and enhance plant growth (Kim, Goins, et al. 2004a).Recently it was revealed that green light also has distinct effects on plant and affect plant processes via cryptochrome-dependent and cryptochrome-independent means (Folta & Maruhnich 2007).

Lettuce grown in monochromatic green light shows decreases in stomatal conductance (Kim, Goins, et al. 2004b) but in combination with blue and red light it improves plant biomass and chlorophyll content (Dougher & Bugbee 2001b). Frechilla et al. (2000) showed in studies with *Vicia faba* and *Arabidopsis thaliana* that green wavebands act as a modulator of stomatal aperture, reversing the blue light response. Furthermore, In sunflower, the opening of abaxial stomata was induced by monochromatic green light as well as light transmitted through its own canopy but adaxial stomata remained unresponsive (Wang et al. 2008). Klein et al. (1965) reported that additional green radiation wave bands (530 to 585 nm) caused growth repression

of *Targetes erecta* and *Sordaria fimicola* while Huh et al. (1997) says that plant height in *Hibiscus syriacus* with that high green light spectrum (500 to 600 nm) was increased and had higher plant height. Furthermore, *Lactuca sativa* treated with green fluorescent lamps had lower leaf area, high specific leaf area, lower shoot fresh and dry weight (Kim, Wheeler, et al. 2004).

2.2.2. Different Light Sources, HPS and LED used in Greenhouses

Year round production of greenhouse crops in Northern countries of Europe especially in Norway is only possible by using supplementary artificial lighting system in the winter (Moe et al. 2006; Singh et al. 2014). Moe et al. (2006) referring F Smith (1928, 1933) also stated that the previously used incandescent lamps in Norway were not sufficient to supplement enough spectral energy for artificial lightening in greenhouses. Further onwards, today High Pressure Sodium (HPS) lamps are commonly used by the producers as it can emit high amount of PAR and have high electric efficiency. HPS light discharges low amount of blue light (5%) (Figure 5) which is less compared to natural sunlight (18%) (in M Ashraful Islam et al. 2012).

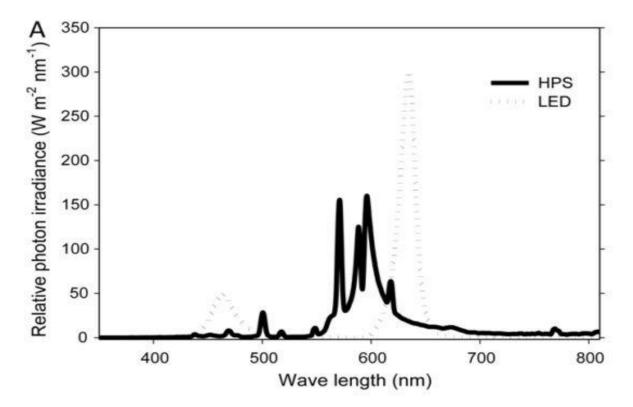


Figure 5. Light spectra of HPS (LU400/XO/T40) and LED lamps (SoLa-co round high power 162 W LED-light) (M. Ashraful Islam et al. 2012)

The new emerging lighting system in greenhouse production is light emitting diodes (LEDs) which are under research (Morrow 2008) and gives opportunities to select a specific light spectrum (Terfa et al. 2012). In addition, LED lights can provide more even light intensity with high energy efficiency, High Relative Quantum Efficiency, low heat stress to plants by stabling temperature in greenhouses, low maintenance cost and longevity although they needs high capital for lighting system (Massa et al. 2008; Singh et al. 2014). It is also found that LED light transmits less infrared (IR) radiation than HPS so more thermal energy is need to get desired results in greenhouse production (Dueck et al. 2012).

Euphorbia pulcherrima is one of the commercial potted plant in North America, Europe, Asia Australia and northern Europe (Ecke III et al. 2004) grown during winter season with the use of supplementary light due to deficiency of natural sunlight as mentioned earlier (M Ashraful Islam et al. 2012). The common lamp type in poinsettia production today is HPS but LEDs with different light qualities have been tested in different experiments to study growth and morphological changes. The experiment done by Islam et al. 2012 found the height of different cultivars of *Euphorbia pulcherrima* was reduced in LED light with 20% blue light and 80% Red light as compared to HPS with 5% blue light (Figure 6 and Figure 7). The strongest reduction was found in Christmas Spirit and the height was reduced by 34% in both greenhouse experiment and growth chambers compared to HPS. 'Christmas Eve' showed 27% and 21% height reduction in greenhouse and chamber experiments, respectively.

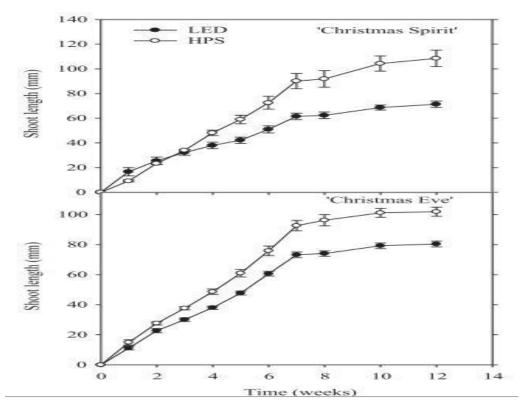


Figure 6. Effect of LED light and HPS on the shoot length of two cultivars of *Euphorbia pulcherrima* under short day condition at light irradiance of 100 μ mol m⁻² s⁻¹ in growth chamber(M Ashraful Islam et al. 2012)

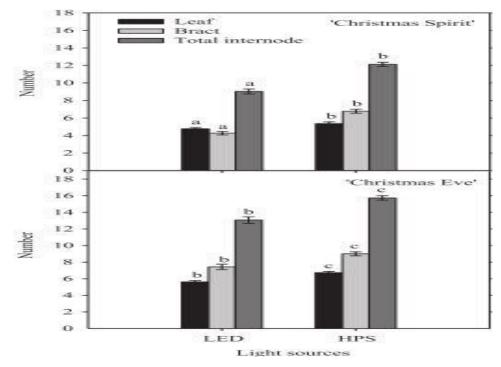


Figure 7. Number of leaves, bracts and total internodes of poinsettia plant grown in greenhouse compartment under LED and HPS light sourcesM Ashraful Islam et al. (2012).

Euphorbia pulcherrima grown under LED light also had shorter petioles, reduced leaf and bract area, shorter and fewer internode, decreased chlorophyll content as compare to HPS. Furthermore the specific leaf area and the specific bract area was also found to be reduced in LED light but there was no significance difference in dry matter content in leaves, bract, and shoots between these two light treatments (M Ashraful Islam et al. 2012) (Figure 5 and 6).

In another experiment which was performed to analyse internal and external quality parameters of pot roses (Rosa \times hybrida 'Toril') it was found that LED (80% red and 20% blue) grown plants had higher chlorophyll and anthocyanin content as compared to HPS. It was also supported that the stem length were shorter in the LED light as compare to HPS lighting system (Terfa et al. 2012).

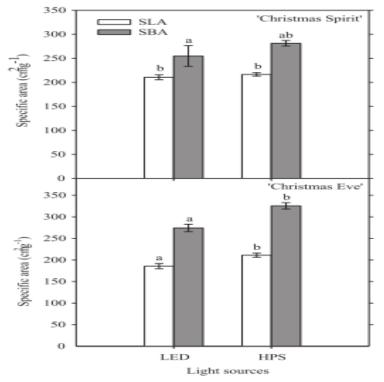


Figure 8. Difference in specific leaf area and specific bract area of different cultivar of Poinsettia grown greenhouse compartment under LED and HPS lighting condition (M Ashraful Islam et al. 2012)

2.2.3. Effect of Light Qualities in Photosynthesis and Stomatal Responses

Photosynthesis is a process where light energy transforms into chemical energy (Govindjee 1967) through absorption of a photons by chlorophyll. The photosynthetic responses fluctuates considerably between species while altering light qualities (Terfa et al. 2013) where blue light and red light spectrums are absorbed effectively as compare to the other spectrum like green light (M. A. Islam 2013). The photosynthetic quantum yield begins to

drops hastily at wave lengths shorter than 400nm and greater than 680nm and remains maximum nearly at 600nm (Evans 1987). It was found that red light is vital in developing photosynthetic apparatus (Sæbø et al. 1995) where formation of chlorophyll, stomata opening and photomorphogenesis is characterized by blue light (Dougher and Bugbee 1998). Culture in vitro of *Betula pendula* when subjected to blue light (max recorded photosynthesis, 82 µmol $CO_2 \text{ dm}^{-2} \text{ h}^{-1}$) have low Photosynthetic capacity while high Photosynthetic capacity exposed to red and/or far-red light spectrum (max recorded photosynthesis, 40 µmol $CO_2 \text{ dm}^{-2} \text{ h}^{-1}$). The chlorophyll content was also found higher in plantlets cultures irradiated with B light (Sæbø et al. 1995).

A number of environmental aspects like relative humidity, CO₂ concentration and light may affect the stomatal responses of plants (Merilo et al. 2014). The stomatal response towards the light depends upon two aspects, Photosynthetic independent and photosynthetic dependent opening (Lawson 2009). Zeiger et al. (2002) mention the photosynthesis dependent component as blue light specific response where stomatal opening is rapidly induced. The plasma membrane H+-ATPase is activated through a signal transduction cascade by the blue light photoreceptor, Phototropins is also believed to involved in photosynthesis dependent opening of stomata (Shimazaki et al. 2007). Moreover red light response is also photosynthetic dependent aspect (Sharkey & Raschke 1981) where vigorous photosynthesis is caused by lowered intercellular CO₂ concentration which induces stomatal opening (Roelfsema et al. 2002). The response of stomatal opening is mightily encouraged by blue light then red light whereas green light was almost unsuccessful. It was found that stomatal opening of Xanthiwm stnarium L was about 10 times higher in Red light (wavelengths between 630 and 680 nm) compared to blue light (wavelengths between 430 and 460 nm) resulting in a conductance of 15 centimoles per square meter per second. However, the stomatal response was marginal towards green light (Sharkey & Raschke 1981).

2.2.4. Effect of light qualities on Growth Hormones

Hormones are chemical signal molecules (Wolff & Landrigan 1994) produced in very low concentration by the plants to regulate growth and development (Davies 2010). Many hormones are involved in growth and development of plant individually or in cluster. The major classes of plant hormones are auxin, gibberellin (GA), cytokinin, abscisic acid (ABA), ethylene, brassinosteroid, salicylic acid (SA), jasmonate and strigolactone. (Taiz and Zeiger 2010). Furthermore, this review will focus on four of the five classical hormones: auxins, abscisic acid, cytokinins, and gibberellins but not ethylene.

Auxin

Auxin was the first to be discovered as a plant hormone (Hopkins & Huner 2009). It has a principal role in cell division, cell expansion, cell differentiation, lateral root formation, flowering and tropic responses (Davies 2010). Auxin is produced in meristematic regions and other actively growing organs such as coleoptile apices, root tips, germinating seeds, and the apical buds of growing stems. Auxin is also actively synthesised in young, rapidly growing leaves, developing inflorescences, and embryos following pollination and fertilization (Hopkins & Huner 2009). Indole-3-acetic acid, 4-chloroindole-3-acetic acid, phenylacetic acid, indole-3-butyric acid, and indole-3-propionic acid are five naturally occurring (endogenous) auxins in plants (Simon & Petrášek 2011).

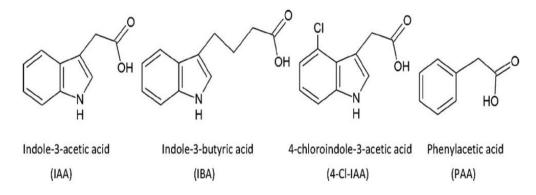


Figure 9. Chemical structure of four endogenous auxins. Indole-3-acetic acid (IAA), indole-3butyric acid (IBA), 4-chloroindole-3-acetic acid (4-Cl-IAA) and phenylacetic acid (PAA) (Simon & Petrášek 2011).

During the progress of research many auxin compounds are synthesised (Figure 9) and are involved in controlling the growth and development of crops (Woodward & Bartel 2005). Although large concentration of auxins is toxic to dicots and less to monocots and some of them are used as herbicides such as 2,4-Dichlorophenoxyacetic acid (2,4-D) and 2,4,5-Trichlorophenoxyacetic acid (2,4,5-T) to control weeds (Fischer & Neuhaus 1996). Moreover, some of these formulated auxins especially 1-naphthaleneacetic acid (NAA) and indole-3-butyric acid (IBA) are used to stimulate root growth or to prevent fruit drop in orchards (Blythe et al. 2007).

Indole-3-acetic acid (IAA) a major auxin that directly interacts with the F-box protein TIR1 (Transport Inhibitor Response 1) and promotes the degradation of the aux/IAA transcriptional repressor to trigger diverse auxin-responsive genes (Dharmasiri et al. 2005). IAA is synthesized in plant via multiple pathways. biosynthesis pathways of IAA from tryptophan (trp) are the YUCCA (YUC) pathway, the indole-3-pyruvic acid (IPA) pathway, the indole-3-acetamide (IAM) pathway, and the indole-3-acetaldoxime (IAOx) pathway (Mashiguchi et al. 2011; Sugawara et al. 2009; Woodward & Bartel 2005). IAA mainly have unidirectional energy demanding transport know as polar transport which moves from the apical to the basal end of the shoot (basipetally) and from the basal to the apical end of the roots (acropetally) (Hopkins & Huner 2009).

IAA is a positive regulator of photomorphogenesis where IAA within the epidermis of plants is diminished by light receptors (Phytochrome) to reduce the stem elongation. Under low PAR and low R:FR ratio the level of IAA used to increase resulting in hypocotyl elongation in A. thaliana through increased activity of IAA-mediated gene expression (Vandenbussche et al. 2003). Likewise, levels of IAA are also affected by DT and NT temperature differences in A. thaliana where under negative DIF compared to positive DIF reduced IAA levels was found resulting in reduction in stem elongation (Thingnaes et al. 2003). The recent research done by Pashkovskiy et al. (2016) found that blue light changes the gene expression of photoreceptors by reducing mRNA levels of PHYA, PHYD, and CRY1. This reduction in mRNA levels result in increase in auxin response factors (ARFs). So, such increases of ARFs declines the sensitivity of plant cell to auxin (ARF3 and ARF4). Thus, this may be the reason for the reduced plant growth under blue light. In some plants, both Bioactive GAs and auxin are actively participated in regulation of stem elongation where the level of the active GA is affected by IAA. In pea, removal of the apical bud (source of auxin) reduced the endogenous level of GA1 and this was completely reversed after the application of IAA to the decapitated plant (O'Neill & Ross 2002).

Gibberellins (GAs)

Among 136 naturally occurring GAs (MacMillan 2002) shares identical chemical structures (diterpenoids, formed by four isoprenoid units with five carbons) but only some of them have intrinsic biological activity while other GAs act as metabolic precursors or deactivation products. Among these GAs few are bioactive GAs which have influence in stem length. GAs plays vital role in different physiological phenomena like seed germination,

transition to flowering and pollen development and also identified as promoter of stem elongation. (Taiz and Zeiger 2010).

Among the different plant growth hormones, GAs plays vital role for accelerating shoot elongation (Kayal et al. 2011) where deficiency of GA usually retard elongation and promote apical dominance to a greater degree (Golovatskaya 2008). GAs are also involved in expression of skotomorphogenesis and repress photomorphogenesis in contrast with light signals (Lau & Deng 2010). For instance in cowpea (*Vigna sinensis*) and hybrid aspen (*Populus tremula* × *tremuloides*) increased levels of GA and IAA enhanced internode elongation in EOD-FR light (Olsen & Junttila 2002). Islam et al. 2014, perform hormone profiling where the amount of GA in shoot tips was found to be reduce by 30% in *Euphorbia pulcherrima* displayed to EOD-R against EOD-FR resulting in a reduction in shoot elongation (Table 1). This reduction in shoot elongation was correlated with reduction in active GA level (Hansen et al. 1999). Furthermore, the stem was shorter and leaves were smaller when *Arabidopsis thaliana* mutants lacks endogenous GAs (Kurepin et al. 2012).

A study investigate by OuYang et al.(2015), concluded that significantly higher concentration of GA was observed under red light compared with blue light so this might be the reason for the greater height increase of the plants grown under red light. Moreover, green light spectrum also retards stem elongation and branching, reduced leaf specific surface density and plant seed productivity, and retarded plant transition to reproduction to a greater degree in deficiency of GA 4 and GA 1(Golovatskaya 2008).

Table 1. Effects of end of day treatments with red and far red light in endogenous levels of gibberellins (ng g^{-1} dry weight) found in shoot tips of *Euphorbia pulcherrima* (Islam et al. 2014)

Gibberellins	EOD-R	EOD-FR
GA ₂₀	40.9 ± 5.5 a	$32.4 \pm 6.9 a$
GA29	279.8 ± 9.2 a	395.8 ± 39.8 b
GA1	$169.0 \pm 10.6 a$	$234.9 \pm 33.7 \text{ a}$
GA ₈	$281.2 \pm 6.1 a$	$521.6 \pm 59.9 \text{ b}$
GA ₅	35.5 ± 2.8 a	$31.6 \pm 7.5 a$
GA ₆	73.1 ± 3.3 a	77.8 ± 17.7 a
GA ₃	$200.0 \pm 5.4 a$	$246.2 \pm 30.6 a$
GA ₇	6.9 ± 1.7 a	$3.5 \pm 1.1 \text{ a}$
GA34	$13.7 \pm 1.4 a$	$11.6 \pm 3.3 a$
Total GA	1099.9 ± 38.6 a	1555.0 ± 159.0 b
GA ₈ /GA ₁	1.7 ± 0.1 a	2.3 ± 0.4 a
GA29/GA20	$7.1 \pm 1.0 a$	$13.3 \pm 2.9 a$
GA1/GA20	4.2 ± 0.4 a	$7.5 \pm 0.8 \text{ b}$
GA ₆ /GA ₅	$2.1 \pm 0.1 a$	$2.5 \pm 0.1 \text{ b}$
GA ₃ /GA ₅	$5.7 \pm 0.3 a$	$8.6 \pm 1.8 a$

Cytokinin (CK)

Cytokinins (CK) are plant hormones which are derivatives from thenitrogenous base adenine. The primary function of CK is to stimulate cell division in plant tissues. These hormones are also involved in shoot and root differentiation in tissue culture, growth of lateral buds and leaf expansion, chloroplast development, and delay of senescence (Hopkins & Huner 2009). Naturally occurring CKs are adenine derivatives having either an isoprenoid or aromatic side chain. At the N⁶ position. 2-isopentenyl adenine (2iP) and its hydroxylated forms zeatin (Z) and dihydrozeatin (DHZ) are representative of isoprenoid CKs. The two isomers of Z, *cisZ* (cZ) and *transZ* (tZ) differ in the position of their terminal hydroxyl group in the isoprenoid side chain. tZ and iP generally exhibit the highest activity whereas cZ has a weak biological impact only (Sakakibara 2006; Gajdošová et al. 2011). This occurs through either ethylene action or blocking the transportation of IAA. However, how endogenous CKs mediate photomorphogenesis is unclear (A. Islam 2013).

Abscisic acid (ABA)

Abscisic acid (ABA) is represented by a single 15-carbon compound formed by the methyl erythritol phosphate (MEP) pathway representing class of metabolites known as isoprenoids or terpenoids (Taylor et al. 2000; Nambara & Marion-Poll 2005). The name abscisic acid given because it was believed that this hormone is involved in the abscission of leaves and other organs (Hopkins & Huner 2009). The major functions of ABA in plant are control of cellular processes including seed development, dormancy, germination, vegetative growth and environmental stress responses (Xiong & Zhu 2003;Hopkins & Huner 2009). ABA is also responsible for encouraging stomatal closure to limit the water loss during transpiration (Xiong & Zhu 2003). Furthermore, ABA is involved in other developmental responses, including the induction of storage protein synthesis in seeds, heterophylly (leaves of different shape on the same plant), initiation of secondary roots, flowering, and senescence (Xiong & Zhu 2003; Hopkins & Huner 2009).

ABA biosynthesis occurs in roots, vascular tissue and in guard cells. ABA is synthesized by two pathways, one direct pathway in which ABA is synthesized from 15-carbon terpenoid precursor such as farnesyl diphosphate. In indirect pathway ABA is produced from the cleavage of a carotenoid such as β -carotene, based on structural resemblances between carotenoid pigments and ABA. The biosynthesis of ABA starts at chloroplast where carotenoid pigments are produced. Nine-cis- expoycarotenoid dioxygenase (NCED) is a critical enzyme which splits the 40-carbon carotenoid violaxanthin to produce a 15-carbon product, xanthoxin, and a 25-carbon by-product. An alcohol dehydrogenase convert Xanthoxin to abscisic aldehyde. which in turn oxidized to abscisic acid by abscisic aldehyde oxidase. The production site for enzyme NCED and xanthoxin may be in the chloroplast while the alcohol dehydrogenase and abscisic aldehyde oxidase are located in the cytosol. thus xanthoxin must be transported to the chloroplast into the cytosol, but mechanism of migration is not yet known (Taylor et al. 2000; Hopkins & Huner 2009). During the course of catabolism ABA is biologically inactivated through different steps. The principal metabolic is oxidation of ABA to phaseic acid (DPA) or into ABA gluoside-ester (ABA-GE) (Hopkins & Huner 2009).

As it is already mentioned that ABA regulate transpiration through its action on stomata function. High levels of ABA are produced by plant under drought condition resulting in stomatal closure. Nitsch et al. (2012) reported that in *Solanum lycopersicum* ABA levels in different ABA mutants showed strong correlation with plant height. In addition Nitsch et al. (2012) also mention that the ABA deficient double mutants *notabilis/flacca* (*not/flc*) in tomato had the lowest ABA levels and the lowest expression of ABA genes, resulting in smaller cell size and fruit size.

The endogenous level of ABA content increases when plants *of Lemna gibba* and *A. thaliana* were transferred to darkness but while treating *L. gibba* with red light the level of ABA declined which shows that Phytochrome may be involved in the changes of endogenous ABA levels (Weatherwax et al. 1996). Mostly the endogenous level of ABA has been tested under stressful conditions. For example, the shoot length of plants seems to be reduced during drought stress, when the turgor pressure is reduced. Meanwhile, the relation between light qualities and ABA levels has not yet been clear yet (Kraepiel & Miginiac 1997).

3. Materials and Methods

3.1. Experiment I. HPS and LED (20% blue and 80% red)

A chamber experiment was performed to compare hormonal content of shoots developed with HPS and LED. The cultivar Christmas Eve of poinsettia (*Euphorbia pulcherrima* Willd. ex Klotzsch) were used to experiment.

3.1.1. Plant propagation

Poinsettia's Cuttings with 6–7 leaves were rooted in Jiffy-7 (G3 Ljones Gartneri AS, Tørvikbygd, Norway) and potted in 13 cm plastic pots with Sphagnum peat (Vek-sttorv, Ullensaker Almenning, Nordkisa, Norway) The plants were kept for 6 weeks in a growth room having 20°C temperature, average relative air humidity (RH) of $70 \pm 5\%$, with an average of 0.7 k Pa water vapour pressure deficit (VPD), Light at a photon flux density of 80–90 µmol m⁻² s⁻¹ (Osram L 58 W/640 Cool White fluorescent tubes, Munich, Germany) was provided during an 18 hr photoperiod. Plants were pinched above 3–4 leaves and three side shoots per plant were allowed to grow.

3.1.2. Growth experiment

Flowering was induced by transferring the plants to the growth chamber with $21 \pm 2^{\circ}$ C temperature day and night. Light irradiance of 100 µmol m⁻² s⁻² for 10 hours of photoperiod was provided by high pressure sodium lamps (HPS, LU400/XO/T/40; General Electric Co., Fairfield, CT, USA). and LED with 20% blue and 80% red light. The CO₂ concentration was at ambient level where RH was adjusted to 70 ± 5%. Nutrient solution of an electrical conductivity (EC) of 1.5 mS cm⁻¹ (Red superba and Calcinit, Yara, Oslo, Norway) at pH 5.6–5.8 was provided daily.

After one month of growth the elongating part of the shoot tips (stem) (0.5–1 cm) from each of six plants of the cv Christmas Eve were harvested into liquid nitrogen. The samples were freeze dried using a freeze dryer machine (Heto Holten A/S, Gydevang 17-19, DK-3450 Allerød, Denmark). For each light treatment six samples, each consisting of three shoot tips from one plant, were used for hormone analysis. Of these, three samples were used for the analysis of auxin, abscisic acid (ABA), cytokinin (CK) and their metabolites, and the three other samples were used for gibberellin (GA) analysis.

3.1.3. Analyses of gibberellin, auxin, cytokinins, abscisic acid and their metabolites

Fifty mg dry weight (DW) of homogenised plant tissue was used for extracting auxin, gibberellin cytokinin and ABA as well as metabo-lites of these using 3 ml of isopropanol: water: glacial acetic acid (80:19:1, v/v). The samples were agitated in the dark for 24 h at 4°C. Deuterated forms of the hormones and their metabolites (Abrams et al., 2003; Chiwocha et al., 2003) were added as internal standards. These included [2H3]-dihydrophaseic acid (DPA), [2H5]-ABA-?-glucose ester (ABA-GE), [2H3]-phaseic acid (PA), [2H4]-7?-OH-ABA, [2H3]-neoPA, [2H4]-ABA, [2H4]-trans-ABA, [2H3]- indole-3-acteyl-leucine (IAA-Leu), [2H3]-IAA-alanine (IAA-Ala), [2H3]-IAA-aspartate (IAA-Asp), [2H3]-IAA-glutamate (IAA-Glu) and [13C4]indole-3-butyric acid (IBA) (all synthesised at Plant Biotechnology Institute-National Research Council, Canada, according to Abrams et al., 2003 and Chiwocha et al., 2003) as well as [2H5]indole-3-acetic acid (IAA) (Cambridge Isotope Labora- tories, Andover, MA, USA), [2H3]dihydrozeatin (DHZ), [2H3]- dihydrozeatin-riboside (DHZR), [2H5]-zeatin-O-glucoside (ZOG), [2H6]-isopentenyl adenosine (iPA) and [2H6]-isopentenyl adenine (iP) (OlChemIm Ltd., Olomouc, Czech Republic) and gibberellin; [2H2]GA1, [2H2]GA3, [2H2]GA4, [2H2]GA5, [2H2]GA6, [2H2]GA7. [2H2]GA8, [2H2]GA9, [2H2]GA12, [2H2]GA12-aldehyde, [2H2]GA15, [2H2]GA19, [2H2]GA20, [2H2]GA24, [2H2]GA29, [2H2]GA34, [2H2]GA44, [2H2]GA51 and [2H2]GA53 as internal standards. Dried elutes were re-solubilised in the mobile phase and analysed by an ultra-performance liquid chromatography (UPLC) MS/MS system.

3.1.4. Growth analysis

The growth analysis was performed after opening of cyatha. The shoot length was measured from the base of each shoot to the shoot apical meristem once in a week from beginning to the end of the experiment. Petiole length of four mature leaves on each shoot and the stem diameter at the middle of each shoot were measured. Similarly, the number of leaves and bract were counted and the average internode length were calculated by dividing final height by the number of leaves. transition leaves which had formed red color and were counted if the length exceeded 3 cm (petiole + bract) were demarcated as bract. Leaf area and bract area was measured by an area metre (Model 3100 area meter, LI- COR Biosciences). Fresh weight of leaves, bract and stem were measured and allowed to dry at 65°C until a constant mass was reached. Total chlorophyll content was measured by a chlorophyll content metre (Model CL-01, Hansatech Instruments, Norfolk, England) in the middle leaf of the three shoots on each plant.

3.2. Experiment II. HPS and additional blue and green LED

3.2.1 Plant propagation

The experiments were conducted in a greenhouse compartment at SKP (Senter for Klimaregulert Planteforskning) Norwegian University of Life Science (NMBU). Shoot cuttings (3 cm to 5 cm) of the cultivar 'Christmas Day' of *Euphorbia pulcherrima* were selected from mother plants and planted in 3 white flat trays with 40 pots in each containing white moss peat "Sphagnum" - fine medium grade, 6% ash, pH 5.0-6.0 (Degernes Torvstrøfabrikk AS, Degernes, Norway) and fertilized with Kristalon Indigo NPK fertilizer containing Magnesium, and Yaraliva calcinit and covered with plastic films on 15th of September 2015.



Figure 10. Shoot cuttings with uniform height and good root transplanted in 12 cm black plastic pots containing white moss peat.

After four weeks on 26th October 2015, selected shoot cuttings with good root and uniform height were transplanted in 12 cm black plastic pots containing the same Sphagnum peat as described above. 40 plants were selected, pinched above 3 leaves and 3 lateral shoots were allowed to develop. The plants were moved to different light treatments when the new shoots were about 0.1-0.5 cm. During the propagation, the temperature was 20°C, and 16 hours with light was provided by high pressure sodium (HPS) lamps (Master Son-T Pia Plus 400w E E40 1sl) at 100 μ mol m⁻² s⁻¹ per day.

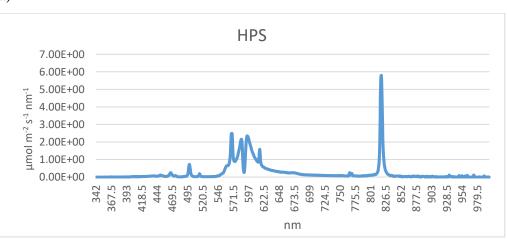
3.2.2. Experimental set-up

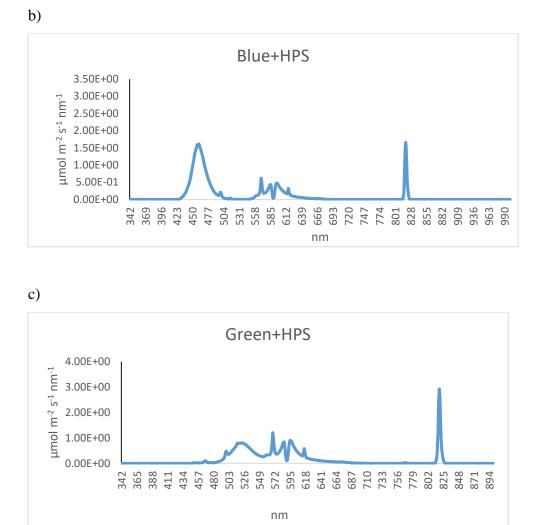
Three different lighting system (blue LED, green LED and HPS (HPS, LU400/XO/T/40; General Electric Co., Fairfield, CT, USA) were installed in a greenhouse with a photosynthetic active radiation (PAR) of 200 μ mol m⁻² s⁻¹ which was measured by LI-COR Model L1-250 Quantum sensor (Li-Cor Inc., Lincoln, NE, USA) light meter. Two of the light treatments were a combination of HPS (150 μ mol m⁻² s⁻¹) and LEDs (150 μ mol m⁻² s⁻¹) (Table 2). 40 plants were transfer to the light treatments, 10 plants in blue and green light treatments and 20 in HPS treatment. The plant density was the same for all three light treatments. The light spectrums of the different lamp types are described in figure 1.

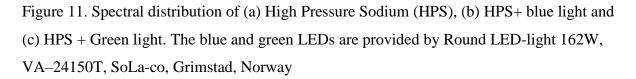
Table 2. Experimental set-up, PAR and the different lamp types used in the experiment

Lamp type	Lamp type			Total PAR
	Control (HPS)	HPS +Blue LED	HPS + Green LED	
HPS	200	150	150	200
Blue LED	-	50	-	200
Green LED	-	-	50	200

a)







The temperature during the experiment was 20°C and the relative air humidity (RH) was 70%. The plants were first treated with long days (16 hr) for 2 weeks and then 10 weeks with short days (10 hr). During the experiment 3 shoots were allowed to grow and the length of the shoots were measured once every week for 12 weeks. Then, the length from the base of the shoot to the shoot apical meristem area was measured with a ruler. In addition, sampling of water loss, GA analysis and fluorescence measurement were performed during the experiment. At the end of the experiment growth analysis, chlorophyll measurements Anthocyanin analysis of the bracts were done (see description below).

In the course of experiment water usage measurement was taken twice first on 16th December 2015 when the plants had still mainly green leaves and second on 19th January 2016. On 16th December each plant was watered, weighted and covered by plastic bag. These potted

plants were again weighted on 17 December to measure the water loss. The leaf area of each plants of treatments were measured by using leaf area meter and same for the second measurement.

3.2.3 GA Sampling

The sampling was done on 30th November, 2015 where 5 sample from each treatment were selected. The sampling was started at 13:25 and ended at 14:10. Apical tips of each plant were pinched, weighted and placed in tubes. These tubes are transferred to container containing liquid Nitrogen with temperature -200°c. The container was placed at -80°c and freeze dried before shipping to Chzech Republic for analysis. We have not got the results due to problems in the laboratory in Chezech Republic.

3.2.4 Florescence Measurement

Fluorescence was measured by using a fluorometer (Hansatech Instrument LTD, King's Lynn, Norfork, PE32 1JL, UK with HP sens type). The measurement was started on 7th December 2015 and measured once in a week till 18th January 2016. During the measurement the florometer's sensor closed clips were clipped to green leaves to dark-adapt the leaves and F0, Fm and Fv/Fm were measured from sensor after 15 minutes.

3.2.5 Chlorophyll analysis of leaves

The relative chlorophyll content was measured by using a Hansatech chlorophyll meter 19th January 2016. The measurement was taken 2 times on same leaf for each replication. Chlorophyll extractions were done from the same area. Then, three leaf discs each having 10-15 mm diameter were taken and placed in tubes with 5 ml N, N-dimetylformamid. These tubes from each plants from all treatment were stored in fridge for 4 days to extract chlorophyll completely from leaf discs. Spectrophotometer UV-1800 UV-VIS (Shimadzu, Kyoto, Japan) was calibrated by a tube with N, N-dimetylformamid (absorbance 0). Then the 2ml of each absorbance were put in a cuvette and placed in spectrophotometer. Each absorbance was measured at two wavelengths, 647 nm and 664 nm to quantity maximum amount of chlorophyll b and chlorophyll a respectively. The content of chlorophyll was determined in mg per dm3 by using following formulae

Chlorophyll $a = 12.64 A_{664} - 2.99 A_{647}$

Chlorophyll b = - 5.6 A_{664} + 23.26 A_{647}

The resultant from the calculation was multiply by 1.119 and 1.102 to get Chlorophyll a and Chlorophyll b in μ mol correspondingly.

Chlorophyll a (µmol. m^{-m}) = 1,119 × Chlorophyll a (mg. dm^{-m})

Chlorophyll b (μ mol. m⁻²) = 1,102 × Chlorophyll b (mg. dm⁻³)

3.2.6 Anthocyanin analysis of bracts

The amount of anthocyanin was analysed by taking discs from 3 bracts (10-15 mm diameter). The discs were placed in tubes with 5 ml methanol (CH₄O) and 1% Hydrochloric acid (HCl). These tubes from each plants from all treatment were stored in fridge for 4 days to extract Anthocyanin completely from leaf discs. Anthocyanin was measured by the use of a UV-1800 UV-VIS spectrophotometer (Shimadzu, Kyoto, Japan) with an absorbance peak at 530 nm.

3.2.7. Growth analysis

At the end of the experiment on 20th January 2015, again the water usage measurement was taken as described above. Growth analysis including width of plant, number of leaves and bracts, leaf area and bract area, plant height, shoot length, petiole length, and weight of the total plants without root were measured. An area meter (Model 3100 area meter, LI- COR Biosciences) was used to measure the leaf and bract area. At the end fresh weight of plant, leaves and bracts were taken and allowed to dry on drier at 32°C for 4 days and dry weight of bracts and leaves were taken. The shoots dry weight was measured after one week.

3.3. Data analysis

The growth data from was analysed using ANOVA one-way analysis of variance. Initially the data were noted in Microsoft excel 2013 and transferred to Minitab-16.2.1 for data analysis. Data for stem elongation was analysed in R software at 5% probability (p-value 0.05).

4. Results

Experiment I

4.1.1. Effect on Shoot length elongation under HPS and LED light

Euphorbia pulcherrima cv. Christmas Eve grown in controlled climate chambers did not show a significant difference in shoot elongation under LED and HPS light condition. The results show that the shoot length was shorter in HPS light condition then in LED light condition. However, the elongation pattern of shoot length along the growing period was not found significantly differ between treatments (Figure 12).



Figure 12. Effect on shoot length elongation of *Euphorbia pulcherrima* var. Christmas Eve under HPS and LED light Condition for 10 weeks of period. The shoots from 3 plants from each treatment were measured. The standard error mean shoot length of each plant was analyzed under p-value < 0.005. The shoot length elongation was superior in LED then HPS lighting condition along progressive time period of ten weeks.

4.1.2. Hormonal distribution

The effect of LED light and HPS light treatments on plant growth hormones IAA, GA, ABA, CK and their metabolites was analysed on elongating shoot tips of 'Christmas Eve'. The metabolites of auxin, IAA was found significantly different between the treatments while level of other metabolites IAA- Asp and IAA-Asp were found non-significant as shown in figure 13.

Among the different metabolites of Auxin IAA-glutamate (IAA-Glu) was found in a significantly lower concentration compared to IAA (Figure 15).

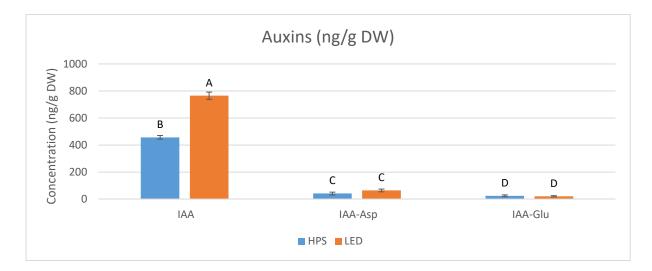


Figure 13. Endogenous level of IAA, IAA-Aspartate (IAA-Asp) and IAA-glutamate (IAA-Glu) in shoot tips of poinsettia (cv. Christmas Eve) exposed to LED and HPS light Treatments for 11 weeks. Mean values \pm SE are given. n = 3 with three pooled shoot tips in each. Different letters show the significant difference between the treatments based on ANOVA followed by Tukey's test at p \leq 0.05.

The level of ABA in the shoot tip was not significantly differenct between HPS and LED treated plants along with its metabolites DPA and PA while the level of metabolite ABA-GE was significantly different in the two treatments. A higher content of ABA-GE was detected in shoots from HPS plants compared to LED treated plants (Figure 14). High amount of PA was found in the shoot tips followed by DPA but the level of ABA-GE was found in lower concentration than PA and DPA.

The Endogenous level of cytokinins metabolites were not significant with respect to the light treatments. The content of Isopentenyladenosine (iPA) was significantly higher in shoot tips than (cis) Zeatin-O-glucoside (Figure 15)

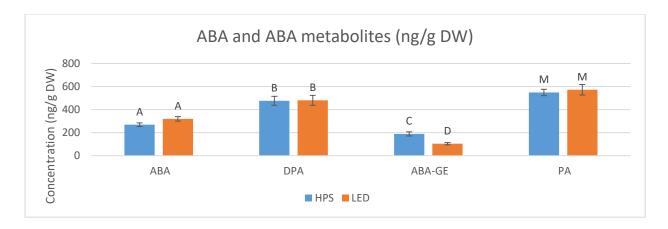


Figure 14. Endogenous levels of ABA (ABA + trans-ABA), dihydrophaseic acid (DPA), ABA glucose ester (ABA-GE) and Phaseic acid (PA) in shoot tips of poinsettia (cv. Christmas Eve) exposed to exposed to LED and HPS light Treatments for 11 weeks. Mean values \pm SE are given. n = 3 with three pooled shoot tips in each. Different letters show the significant difference between the treatments based on ANOVA followed by Tukey's test at p ≤ 0.05 .

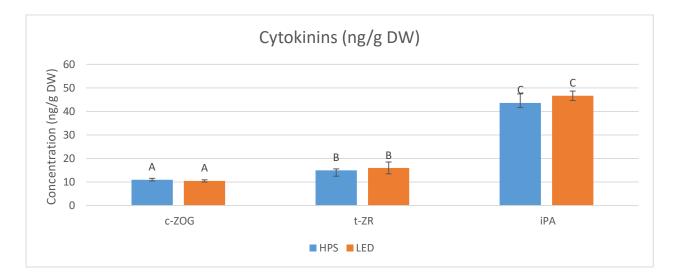
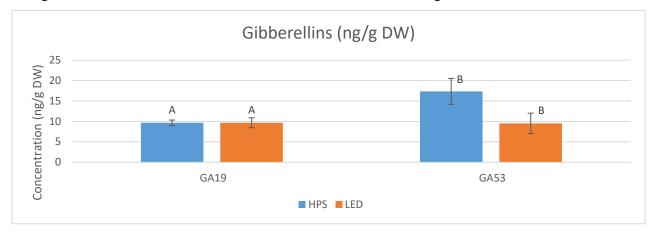


Figure 15. Endogenous levels of Cytokinins metabolites (cis) Zeatin-O-glucoside (c-ZOG), (trans) Zeatin-O-glucoside (t-ZR) and Isopentenyladenosine (iPA) in shoot tips of poinsettia (cv. Christmas Eve) exposed to exposed to LED and HPS light Treatments for 11 weeks. Mean values \pm SE are given. n = 3 with three pooled shoot tips in each. Different letters show the significant difference between the treatments based on ANOVA followed by Tukey's test at p ≤ 0.05

Very few GAs were detected in the poinsettia shoots. Further, the amount of GA 19 was almost same in the LED and HPS treatment and were not significantly different as shown



in figure 16. The data for GA 53 were unbalanced and non-significant to the treatments.

Figure 16. Endogenous levels of Gibberllins 19 and Gibberllins 53 in shoot tips of poinsettia (cv. Christmas Eve) exposed to LED and HPS light Treatments for 11 weeks. Mean values \pm SE are given. n = 3 with three pooled shoot tips in each. Different letters show the significant difference between the treatments based on ANOVA followed by Tukey's test at p ≤ 0.05

4.1.3. Bio metric analysis

The total chlorophyll content of cv. Christmas Eve was lower in plants grow under HPS then in LED but no significance difference was observed. Also, almost the same length of petioles was found in the plants grown in these light treatments. There was also no significant difference between the light treatments on number of leaves, leaf area, bract number and bract area. However, leaf area was slightly smaller while bract area was found slightly larger under HPS compared to LED.

Table 3. Effect of HPS and LED light treatments on different biometric parameters of *Euphorbia pulcherrima* var. Christmas Eve. The mean value of data was analysed using Tukey method (p-value< 0.05). The standard error of means is shown and similar letter in the same line indicates no significant differences.

Parameters	LED	HPS
Total chlorophyll content	$25.523 \text{ A} \pm 0.90$	23.067 A ±1.51
Petiole length (cm)	$3.6889~A \pm 0.22$	3.6111 A ±0.26
Leaf/internode no.	$5.8889 \; A \; \pm 0.48$	5.1111 A ±0.11
Leaf area	$177.63 \text{ A} \pm 26.90$	168.12 A ±17.98
Bract no.	$11.556 \text{ A} \pm 0.29$	11.555 A ±1.31
Bract area	$339.47 \text{ A} \pm 35.90$	370.00 A ±53.63

4.1.4 Fresh weight and Dry Weight Distribution

The fresh weight distribution shoot, leaf and bracts of poinsettia is shown in the figure 17. The fresh weight distribution of these different parameters were almost similar in both HPS and LED light treatments and are non-significance in both treatments. Furthermore, dry weight distribution of shoot, leaf and Bracts were also non-significance along the treatments and also percentage distribution of dry weight was also similar between treatments as presented in figure 18.

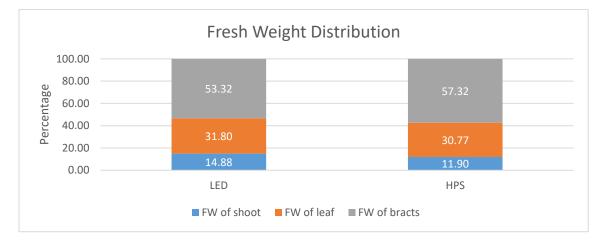


Figure 17. Percentage distribution of fresh weight of shoot, Leaf and bracts of poinsettia (cv. Christmas Eve) exposed to LED and HPS light in the controlled environment for 10 weeks in growth chamber.

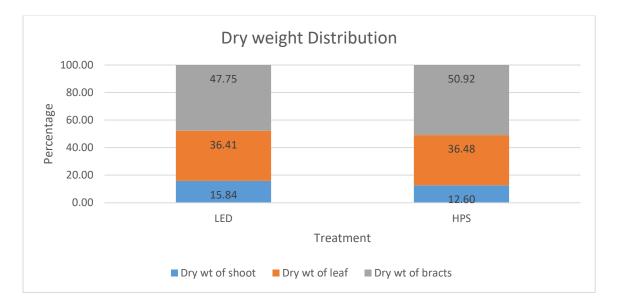


Figure 18. Percentage distribution of Dry weight of shoot, Leaf and bracts of poinsettia (cv. Christmas Eve) exposed to LED and HPS light in the controlled environment for 10 weeks in growth chamber.

Experiment II

4.2.1 Effect of blue and green LED

The shoot length of *Euphorbia pulcherrima* 'Christmas day'was significantly affected by light quality (P < 0.001). The shortest shoots were found on plants exposed to Blue + HPS and Green + HPS compared to HPS. However, no significant difference was found between Blue + HPS and Green + HPS.

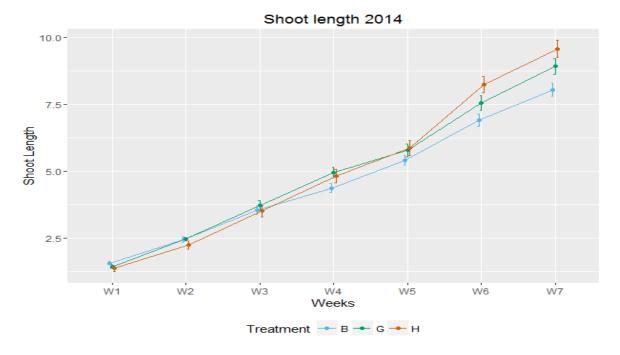


Figure 19. Shoot length of *Euphorbia pulcherrima* over time among different light treatments in year 2014. The length of the 5 plants shoots in three treatments (HPS, Blue + HPS, and Green + HPS) each with 3 shoots was measured. The standard error mean shoot length of each plant was analyzed under p-value < 0.001. The shoot length elongation was superior in HPS while suppressed in Blue + HPS+ HPS treatment along progressive time period of seven weeks.

In year 2014 and 2015 experiment, it was found significant changes in length of the shoots during the experimental period. In both years the length of shoots under HPS was longer as compared to Blue + HPS and Green + HPS LED lights as shown in figure 19 and 20. In 2014 no significant difference was found between HPS and Green + HPS. In addition, each week change in shoot length in each treatment showed significant difference. Whereas no significant difference was found between Green + HPS and Blue + HPS. However, while between HPS and Green + HPS as well as between HPS and Blue + HPS a significant

difference in shoot length was observed in 2015. Over the time period the change in shoot length was found to be significantly different but no significant difference was found between the weeks 11 and 10, 9 and 10, 12 and 11, 8 and 7 and 9 and 8 respectively (P < 0.001) as shown in the figure 20.



Figure 20. Shoot increase of *Euphorbia pulcherrima* over time among different light treatments in year 2015. The length of the 5 plants shoots in three treatments (HPS, Blue + HPS, and Green + HPS) each with 3 shoots was measured. The standard error of mean shoot length of each plant was analysed under p-value < 0.05. The shoot length elongation was superior in HPS while suppressed in Blue + HPS treatment along progressive time period of seven weeks.

4.2.2 Effect of light quality in Anthocyanin production

The content of anthocyanin in bracts was affected by light quality. The analysis showed that there was significance difference between the light treatments Green + HPS and Blue + HPS (P< 0.001) and HPS and Green + HPS (P< 0.001) in 2015. While, no significance difference was found between HPS and Blue + HPS (P< 0.001). The production of anthocyanin during year 2015 was found higher than in year 2014 In year 2014 there was no significant difference in production of anthocyanin among the treatments as in figure 21.

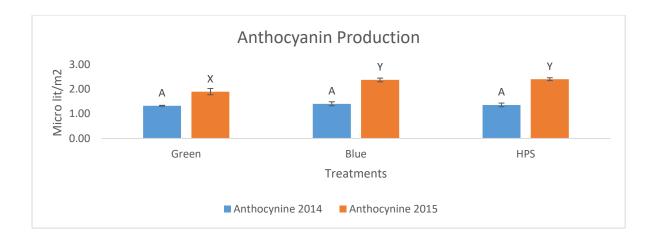


Figure 21. Comparison of Anthocyanin production in different light treatment in 2014 and 2015. The mean value of data was analysed and differentiate under p-value < 0.001. Error bars are the standard error of means while same letters are not significantly different among the treatments

4.2.3. Conductance and Water Loss per Leaf Area.

The transpiration of plants was measured right before the leaves start to change color to red and at the end of the experiment in the Greenhouse. Significant difference was found among the light treatments where HPS had higher conductance as compare to Blue + HPS and Green + HPS in the figure 22. In addition there was no significance difference between Green + HPS and Blue + HPS (P< 0.05) and Blue + HPS and HPS (P< 0.05). While significance difference was found between HPS and Green + HPS (P< 0.05).

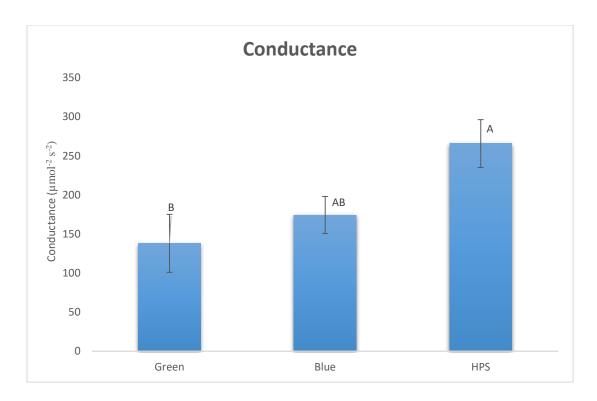


Figure 22. Amount of conductance in the different treatments. The mean value of data was analyzed and differentiate under p-value 0.05 Error bars shows the standard error of means while same letters are not significantly different. The conductance was found high in HPS as compared to other treatments.

The water loss and leaf area of the treatments was measure twice in middle and end of the experiment. The experiment result shows that rate of water loss per leaf area had no significant difference among the treatments. During the early stage no difference was found among treatments where Blue + HPS and HPS had slightly higher water loss per leaf area as compare to Green + HPS during late water loss measurement as in the figure 23.

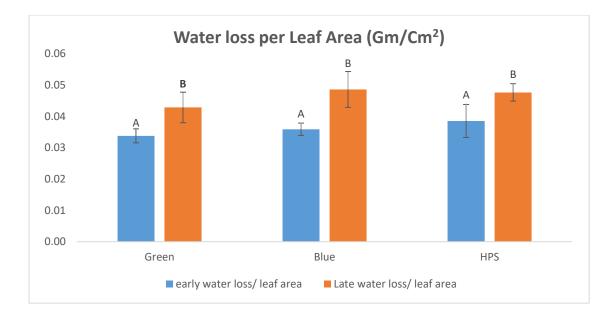


Figure 23. The amount of water loss per leaf area among the treatments with early and late water loss per leaf area. The mean value of data was analysed and differentiate under p-value 0.05 Error bars shows the standard error of means while same letters are not significantly different. no difference in water loss per leaf area was found among treatments where Blue + HPS and HPS had slightly higher water loss per leaf area.

4.2.4. Effect of light qualities on Chlorophyll content and Florescence content.

The total chlorophyll content on the leaves among the treatment was measured during the end period of the experiment. The total amount of chlorophyll a, chlorophyll b, and Total chlorophyll was calculated and found have significance difference among the treatments (P< 0.05). The content of chlorophyll a and chlorophyll b in the treatment with Blue + HPS led light has no significance difference with HPS and with Green + HPS led but Green + HPS led has significance difference with HPS (P< 0.05). In all Treatments the amount of chlorophyll a is higher than chlorophyll b while the ratio between chlorophyll a and chlorophyll b was found to have no significance difference among the treatments (P< 0.05) (figure 24)

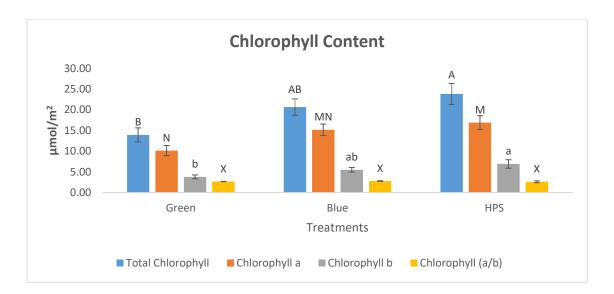


Figure 24. The comparison of amount of total chlorophyll, chlorophyll a, chlorophyll b and chlorophyll (a/b) content among the different light treatments. The mean value of data was analyzed under p-value < 0.05. Error bars shows the standard error of means while same letters are not significantly different. The amount of Total chlorophyll, Chlorophyll a and Chlorophyll b was higher in HPS among the treatments while no significance difference was found between ratio between chlorophyll a and chlorophyll b.

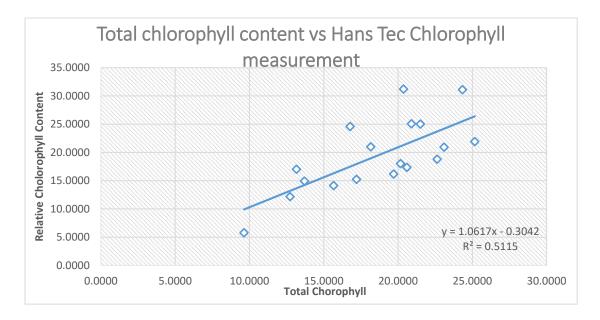


Figure 25. Relation between the Relative cholophyll content by Hans Tec instrument and total chlorophyll content measured by Spectrophotometer. The R²value was found to be very low (0.5115 with regression equation y = 1.0617x - 0.3042).

Beside the spectrophotometer, the relative chlorophyll content was also measured. The regression curve was constructed (figure 25) which shows a rather good reliability with R^2 value of 0.5115.

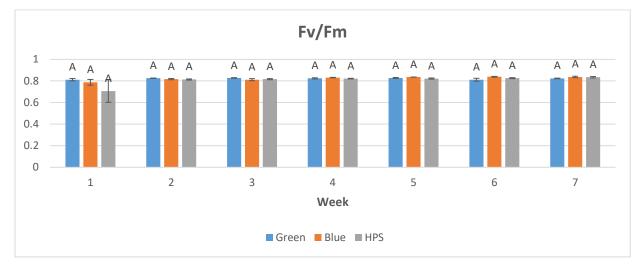


Figure 26. The effect of light stress on photosystem by measuring Fv/Fm in leaves of *Euphorbia pulcherrima* over the period of time. The mean value of data was analysed under p-value 0.05. The standard error of means is shown by Error bars while same letters are not significantly difference.

The health of the photosystem of *Euphorbia pulcherrima* was observed by using Fluorometers during the experiment. The effect of the stresses to the plant's photosystem was recorded by measuring Fv/Fm ratio. The ratio was found to be non-significant among the treatments over the duration of experiment (p-value<0.05)(figure 26).

4.2.5. Effect of light qualities on different Biometric Parameters

The number leaves in treatment Blue + HPS was significantly lower than other treatments while no significant difference was found in number of bract with higher number in treatment Blue + HPS. No significance difference was found in Petioles length, Plant width and leaf area where slightly longer petioles length was found in plants treated with Green + HPS and Blue + HPS then HPS. Plant width was found lower in plants treated with Blue + HPS but leaf area was lower in Green + HPS as compare to other treatments. In addition, the bract area treated with Blue + HPS has not significant difference with other treatments but Green + HPS has significantly different Bract area than HPS as shown in table 4.

Table 4. Effect of different light treatments on different biometric parameters of Euphorbia pulcherrima. The mean value of data was analyzed under p-value 0.05. The standard error of means was shown in tables while same letters are not significantly different.

Parameters/Treatments	Green + HPS	Blue + HPS	HPS
No. of leaves	$5.40 \text{ A} \pm 0.29$	3.86 B ± 0.12	5.53 A ± 0.33
No. of Bracts	$7.86 \text{ A} \pm 0.31$	$8.40 \text{ A} \pm 0.31$	7.86 A ± 0.44
Petiole length (cm)	$5.50 \text{ A} \pm 0.18$	5.50 A ± 0.22	5.42 A ± 0.12
Plant width (cm)	36.667 A ± 1.03	35.933 A ± 0.79	37.84 A ± 1.14
leaf Area (cm ²)	572.9 A ± 27.27	600.3 A ± 98.94	665.4 A ± 36.90
Bracts Area (cm ²)	$583.0 B \pm 94.50$	747.0 A B ± 87.24	942.2 A ± 80.47

4.2.6. Fresh weight and Dry Weight Distribution in different Light Treatments

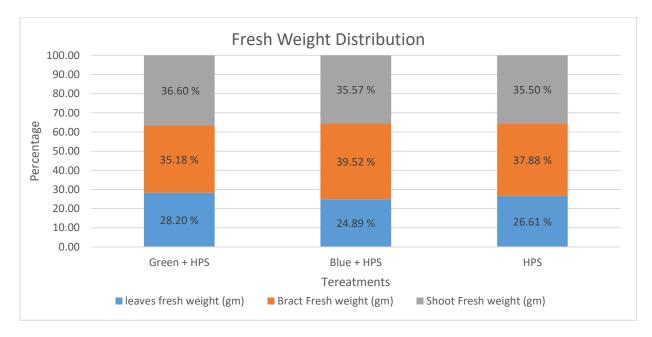


Figure 27. percentage distribution of fresh weight of Leaves, Bract and shoot in gram as effect of different light treatments Green+HPS, Blue+HPS and HPS light on *Euphorbia pulcherrima*. The mean value of data was analyzed under p-value 0.05

Small difference was observed for leaves fresh between Green + HPS and HPS and Blue + HPS and HPS while there was no significance difference between Green + HPS and Blue + HPS for total shoot fresh weight (figure 27) but the results analysis shows no significance difference for total shoot dry weight. Furthermore, both fresh weight of leaves and Dry weight of leaves was significantly high in *Euphorbia pulcherrima* grown under treatment HPS. In addition, there was no significance difference for bract fresh weight in treatments Green + HPS and HPS and Blue + HPS and HPS with high fresh weight of Bract was found in *Euphorbia pulcherrima* plants treated under HPS. The dry weight of Bract was found lower under Green + HPS + HPS which was significantly differ between *Euphorbia pulcherrima* plants Blue + HPS and HPS as shown in figure 28.

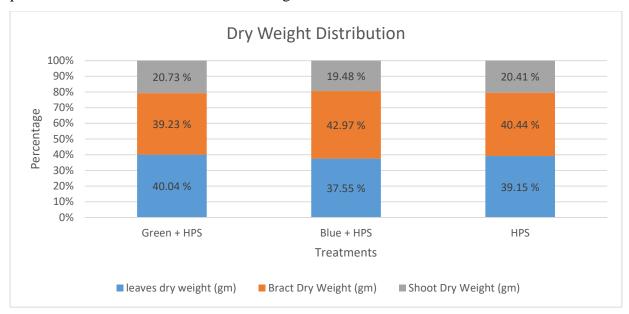


Figure 28. percentage distribution of dry weight of Leaves, Bract and shoot in gram as effect of different light treatments Green + HPS, Blue + HPS and HPS light on *Euphorbia pulcherrima*. The mean value of data was analyzed under p-value 0.05

5. Discussion

Many ornamental plants are produced year around in greenhouses at high latitudes although there is a limited availability of natural sunlight (Ieperen 2012). Maintenance of height and compactness of the ornamental plants in the greenhouse production system may be problematic due to low irradiance. Shoot elongation growth is usually enhanced in low light conditions and other climate factors must be manipulated in order to control the growth. Temperature is one important climate factor used as a tool by growers. This is possible due to development and widespread use of computer controlled environmental management especially in USA and Europe (Berghage 1998). Many researchers have attempted to investigate environmentally friendly and economically viable technology with motto to control height of the plants. Today, manipulation of light quality is a promising method due to the development of novel lighting technology like LEDs.

The ambition of this research was to test the hypothesis that LED lights with blue and red light affected morphology differently than HPS and how the different light affected the hormonal balance. Furthermore, it was tested if LEDs with green and blue light are useful to grow compact plants when provided together with the traditionally and commercially used lamp HPS in greenhouses. Different methods were used to examine whether these light qualities had specific effects on different physiological processes like shoot elongation, bract size and number, and transpiration. Two experiments, one on controlled growth chamber (LED with blue and red light versus HPS, experiment I) and a greenhouse compartment were performed with two different LEDS (blue and green) were tested in combination with HPS as control (Experiment II) the varieties; Christmas Eve and Christmas Day were used in the two experiments respectively.

Experiment I

This experiment was performed under controlled environment and poinsettia plants were subjected to two light treatments: HPS and LED (20% blue and 80% red). During the course of investigation, the height of *Euphorbia pulcherrima* var. Christmas Eve did not show a significant reduction in the shoot elongation under LED (80% red and 20% blue) compared to HPS light condition as we expected. The elongation pattern of shoot length along the growing period was only 1 cm different in shoot length after 10 weeks of growth. Whereas, M Ashraful Islam et al. (2012) reported height reduction by 20–34% when *Euphorbia pulcherrima* 'Christmas Spirit', 'Christmas Eve' and 'Advent Red' were grown under LED

with Blue light (20%) compared with traditional HPS lamps (5% Blue light), which is totally opposite to our results. The difference might be due to cultivar differences, as the effect of blue light on stem elongation is dependent on both species and cultivars (Terfa et al., 2013). Another important fact is that our experiment was performed without natural light. In the study of Islam et al. (2012), the experiment was done in a greenhouse with natural light in addition to supplementary light. In general, a higher irradiance induces more compact plants.

In another experiment, blue led light treated petunia showed increased length of the main stem, with 41 and 89% longer stems at the low and high irradiances correspondingly compared to plants grown under white LED light (Fukuda et al. 2015), which agrees to our findings..

There was also no significance difference between the light treatments (LED and HPS) on number of leaves, leaf area, bract number and bract area. While Islam et al. (2012) stated that LED light treated plants had shorter petioles, reduced leaf and bract area, shorter and fewer internode, decreased chlorophyll content as compare to HPS; which is different from our results. This might also be due to the difference in the experimental environment (aertificial vs natural light). Total fresh weight distribution and total dry weight distribution (shoot, leaf and bracts) were almost similar in both HPS and LED confirming that the two light qualities were very similar in this experiment, which as the findings of Islam et al. (2012).

Effect of light qualities varies among plant species where its effect on shoot elongation is facilitated by different phytohormones particularly gibberellin (GA) and auxin. For example in cowpea (Vigna sinensis) and hybrid aspen (Populus tremula \times tremuloides), enhanced internode elongation in EOD-FR light has been shown to correlate with increased levels of GA and IAA(Garcia et al. 2000; Olsen & Junttila 2002). Among the different phytohormones, IAA, GA, ABA, CK and their metabolites were identified in elongating shoot tips of 'Christmas Eve'. The results showed that the endogenous level of IAA, IAA-Aspartate (IAA-Asp) and IAA-glutamate (IAA-Glu) was found in the shoot tips. However, only IAA was significantly different between the treatments, while IAA-Aspartate (IAA-Asp) and IAA-glutamate (IAA-Glu) were non-significant and higher in LED light treated plants compared to HPS but this was not correlated with shoot length. The hormone sampling was done a few weeks after start of the short day and it is possible that the hormone content at this point is not representative for the shoot length at the end of the experiment. It could also be the IAA production in the shoot tip was delayed in the HPS treated plants compared to LED. The level of ABA in the shoot tip have not been found significant. ABA metabolites; the content of ABA-GE was found to be significantly different between the treatments with higher amount in HPS compared to LED.

High amount of PA was found in the shoot tips followed by DPA which is slightly lower in amount.

Isopentenyladenosine (iPA) was found higher in shoot tips while (cis) Zeatin-Oglucoside was found in lower amount among the different metabolites of cytokinins. GA metabolites were also non-significant. Thus, the hormone balance was not very different in the two light qualities like expected but the data fits well with the fact that the plant height was almost the same.

Experiment II

The growth and development of the plant relies on the different internal and external environmental factors. Among the different external factors light plays a vital role by controlling all physiological and morphological activities (Kraepiel & Miginiac 1997). In this experiment, two years, (2014 and 2015) growth data of Euphorbia pulcherrima cv. Christmas Day were analysed. A significant reduction in shoot length was observed in Euphorbia pulcherrima cv. Christmas Day in both years. Plants treated with HPS +Blue LED light and HPS+ Green LED light have reduced shoot elongation compared with HPS treated plants. Similar response and reduced shoot length due to blue light has been observed in Euphorbia pulcherrima (poinsettia) cultivars 'Christmas Spirit', 'Christmas Eve' and 'Advent Red,' grown under LED with blue light (20%) than traditional HPS lamps (5% Blue light) (M Ashraful Islam et al. 2012). For the first time, the results show that green LED can have similar suppressive effect on shoot length as blue light. Plants detect light qualities by different photoreceptor (Smith 2000). blue and green light is believed to be detected by the cryptochrome. The reason for shoot inhibition by blue light stated by Kigel & Cosgrove (1991) is may be due to blue light affecting cell expansion through changes in the cell wall properties while turgor increases as an indirect effect. The effect of green light may be similar but cell expansion was not measured in this experiment. From the growth data it was observed that plants developed in HPS + Green LED has significantly more leaves than HPS + Blue LED indicating that they probably have a higher number of internodes. Thus, it is possible to say green LED reduces internode length. The higher number of leaves also indicate that the floreal induction was later in green light comapares to blue. However, there wasn't differences in flowering time between the plants (results not shown).

The anthocyanin production was non-significant in the first year of experiment (2014) but the bracts developed in year 2015 had a higher content of antocyanins when developed with

blue LED. Further there was a significant difference between treatments Green + HPS and Blue + HPS (P < 0.001) and HPS and Green + HPS (P < 0.001) in 2015. The initiation of anthocyanin accumulation is a classic phytochrome dependent Low Fluence Responses (LFRs) (Hopkins & Huner 2009). Green light is not absorbed by the phytochrome system and therefore not very efficient in antocyanin production. Experiments with *Brasica olearacea* plants under lighting with 640 nm red LEDs shows the increase in anthocyanin content (Mizuno et al. 2011). The production of anthocyanin during year 2015 was found higher than in year 2014. The production was lower in Green + HPS as compare to Blue + HPS which is similar to finding of (Zhang & Folta 2012) where, green light is simultaneously delivered with blue light, then the level of anthocyanin is lower than blue light treatment alone But there was no significant difference in production of Anthocyanin in year 2014. This may be due to differences in natural light as well. A higher level of natural light was measured in 2015 compared to 2014 (Metrologic data, Ås).



Figure 29. Morphology of plant grown under HPS, HPS + Green LED light and HPS + Blue LED lights in the greenhouse compartments. Plants are grown under 200 μ mol m⁻² s⁻¹ with 20°C temperature and 70% relative air humidity.

The conductance was found to be significantly higher in plants grown with HPS as compared to HPS + LEDs but the rate of water loss per leaf area did not show significant differences among the treatments either in early or late period of development. The rate of water loss per leaf area was higher in the end of the experiment. According to the new analyses it was found that light qualities have a direct effect on stomatal opening and conductance while before it was thought that stomatal opening is light dependence of CO_2 assimilation (Sharkey & Raschke 1981). Kim, Goins, et al. (2004) found decreases in stomatal conductance when Lettuce was grown in monochromatic green light. It is also found blue light generally promotes stomatal opening more than other wavelengths (Zeiger1 & Zhu 1998; Zeiger et al. 2002). Thus, growing poinsettia in green light will reduce transpiration compared to blue light. It is not known if the difference is due to differences in stomatal opening or number of stomata. The reduced transpiration in the plants treated with green light had lower external quality than the plants treated with blue light. This was reflected with lower chlorophyll and antocyanin content.

The total amount of chlorophyll a, chlorophyll b, and total chlorophyll was calculated and found to be significantly different among the treatments. Christmas Spirit' and 'Christmas Eve' showed reduction in chlorophyll content while grown under led light (M. Ashraful Islam et al. 2012), which supports our finding that lower amount of chlorophyll a, chlorophyll b, and total chlorophyll is produced in green LED lights compared to HPS. It is possible that reduced uptake of elements like nitrogen is the reason for reduced chlorophyll due to reduced transpiration. The effect of light stress to the photosystem of *Euphorbia pulcherrima* cv. Christmas Day was measured where Fv/Fm ratio among the treatment was found to be nonsignificant. This implies that the different LED light sources do not have negative effect on the efficiency of photosystem II.

The number of leaves was slightly lower in Blue + HPS treatment, whereas the petioles length was slightly longer but not significant in LED lights + HPS treatments. Whereas, M. Ashraful Islam et al.(2012) reported different results with shorter petioles, reduced leaf and bract area, resulting in more compact plants, in LED lights as compared to HPS. The difference might be due to the effect of background HPS light in our experiment. Cucumber and tomato plants , treated with blue LED lamp reduced internode length on both plant species (Ménard et al. 2006), which is similar to our finding of compact plant under Blue + HPS treatment. The average width of the plants was slightly lower in Blue+ HPS treatment than Green + HPS and HPS, which is the reason for the plant being compact under Blue + HPS. The total fresh weight and total dry weight was high in HPS as compared to other treatments. Similar pattern was observed in the distribution of DM (%) in leaves, bracts and shoot between HPS and LED in cultivar 'Christmas Spirit' and 'Christmas Eve' (M. Ashraful Islam et al. 2012). This analysis shows that there are no differences in plants morphology, flowering and dry matter content grown in different light environments.

6. Conclusion

The study on effect of different light quality responses in poinsettia reveal that LED with 20% blue and 80% red light used in the growth chamber experiment did not induce differences in morphology or hormonal content of poinsettia cv Christmas Eve compared to the traditionally HPS. In the greenhouse compartment experiment conducted to assess effect of LEDs (blue LED and green LED) alone or in combination with HPS (HPS + blue (150 + 50 μ mol m⁻² s⁻¹) and HPS +green LED (150 + 50 μ mol m⁻² s⁻¹)) towards compactness in cv Christmas Day have a potential to reduce shoot length in poinsettia compared to HPS (200 μ mol m⁻² s⁻¹) alone but the results were dependent on the background irradiance from natural light. Green light reduced transpiration, chlorophyll content in leaves and anthocyanin content in bracts compared to blue light and reduce the external quality. Flowering time in poinsettia is very robust and no differences in flowering time was observed in any of the experiments. Thus, it is concluded that blue LED in combination with HPS light are efficient in reduction of plant height without changing the flowering time and will to improve the external quality compared to green LED.

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Norges miljø- og biovitenskapelig universitet Noregs miljø- og biovitskapelege universitet Norwegian University of Life Sciences Postboks 5003 NO-1432 Ås Norway