ISSN: 0976-3031

Available Online at http://www.recentscientific.com

CODEN: IJRSFP (USA)

International Journal of Recent Scientific Research
Vol. 9, Issue, 4(J), pp. 26202-26205, April, 2018

International Journal of Recent Scientific Research

DOI: 10.24327/IJRSR

Research Article

EVALUATION OF GROWTH AND ANTIOXIDATIVE POTENTIAL OF PEA (*PISUM SATIVUM* L.) SEEDLINGS IRRADIATED WITH DIFFERENT LED LIGHTS

Sanjita Abhijita[1]., Pallavi Sharma[1] and *Amarendra Narayan Misra[1,2]

[1]Centre for Life Science, Central University of Jharkhand, Brambe, Ranchi – 835205, Jharkhand, India

[2]Khallikote University, Berhampur-760001, Odisha, India

DOI: http://dx.doi.org/10.24327/ijrsr.2018.0904.2012

ARTICLE INFO

Article History:

Received 5th January, 2018
Received in revised form 20th February, 2018
Accepted 8th March, 2018
Published online 28th April, 2018

Key Words:

Pea (*Pisum sativum* L.), Light quality, Antioxidative, Catalase, Peroxidase, Superoxide dismutase

ABSTRACT

Plant growth, development and metabolism are strongly influenced by light characteristics such as spectral quality, intensity and duration. This study aimed to evaluate the growth and antioxidative activities of pea (*Pisum sativum*) seedlings growing under different light spectra (white, blue and red LED) on different days. Maximum growth was found in blue light followed by white light and red light. The activities of catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD) were also higher in roots and leaves of pea seedlings grown under blue light compared to white light and red light. These findings suggest that blue wavelength can reduce oxidative stress and thus benefits the growth of pea seedlings.

INTRODUCTION

Light, which is considered as a vital environmental factor regulates the growth and developmental processes of plant (Joshi *et al.,* 2014). The main feature of any light driven system is the capacity to capture light. In the photosynthetic phenomenon, chlorophylls and carotenoids pigments present in leaves absorb light (Kusaba *et al.,* 2009). A leaf in general absorbs ~85% of photosynthetic active radiation (PAR), indicating that leaves capture light more efficiently (Baker *et al.,* 2007). Plant species have diverse responses to light quality, but red and blue wavelengths mostly have the strongest impacts on the growth of plant. From the PAR, leaves robustly absorb ultraviolet, blue and red regions (400–700 nm), while far-red (700–800 nm) and green light of 550 nm are generally reflected or transmitted (Casal, 2013; Demotes-Mainard *et al.,* 2016). An optimum light condition regulating the plants activity is an ecofriendly approach to get better quality plants (Liu, 2012). Light quality, intensity and photoperiodic duration not only increase the yield but also accumulate essential phytochemicals, which are beneficial for plants. Blue light helps in development of chloroplast, chlorophyll synthesis and stomatal opening (Senger, 1982) whereas red light has role in seedling growth, photosynthetic complexes assembling, and enzyme induction (Hopkins and Huner,2009; Liu *et al.,* 2011). By controlling qualities of irradiation spectrum higher yield and faster growth at a specific radiation, and plants of optimum nutritional value can be attained. Earlier generally fluorescent and incandescent bulbs were in use as light sources for plant cultivation (Kim *et al.,* 2004a). Though these sources are used to increase photon flux they contain wavelengths that are outside the PAR spectrum, and hence are very poor in increasing the plant growth (Kim *et al.,* 2004a). Compared to those sources, light emitting diodes (LED), which provide exact wavelength and very closely illuminate the plants are currently in use throughout the world (Liu, 2012; Yang and Lee, 2009).

Lighting conditions might arouse the photoxidative changes in plants that lead to altered antioxidant defense system and generation of reactive oxygen species (ROS). A minimal level of ROS is produced in all plants. Under optimal conditions, the balance between generation and consumption of ROS are controlled by antioxidant defense system (Foyer and Noctor, 2005). Plants detoxify ROS by up-regulating antioxidative enzymes such as peroxidase (POD), catalase (CAT) and

*Corresponding author: **Amarendra Narayan Misra**
Centre for Life Science, Central University of Jharkhand, Brambe, Ranchi – 835205, Jharkhand, India

superoxide dismutase (SOD) (Yuan *et al.,* 2012). SOD provides the first line of defense against the harmful effects of ROS. The SODs convert $O_2^{\cdot-}$ to H_2O_2. The H_2O_2 formed is then scavenged by CAT and POD. Catalase decomposes H_2O_2 into $H_2O$and O_2, while POD scavenges H_2O_2 using co-substrates such as phenolic compounds. CAT decomposes H_2O_2 generated during photorespiratory pathway (Scandalios *et al.*, 1997). As oxidative stress is a component of most environmental stress, crop plants can thrive better in these conditions if they maintain high level of antioxidants. In this paper, we investigated the growth and activity of major antioxidative enzymes in white, blue and red light grown pea seedlings.

MATERIALS AND METHODS

Plant material and growth conditions

Pea (*Pisum sativum* L.variety RKL) seeds were first surface sterilized (0.5% NaClO), soaked in distilled water at room temperature (25°C) overnight and then sown on 4 layers of moist germination papers in plastic trays and were allowed to germinate for 3-4 days. To keep the seedlings moist, they are daily watered with distilled water. Pea seedlings of uniform size were individually put in a foam cube, mounted into a thermocol plate with holes, and placed in a container (25cm×15cm×7cm) filled with Hoagland solution (0.5N) continuously aerated by air pump. A 12 hr (day/night) photoperiod was maintained. The Hoagland media was changed at an interval of a week.

Light treatments

The light source was LED. The peak emission of white, blue and red LED was 430 nm, 462 nm and 659 nm respectively. All treatments maintained a 12 hr photoperiodic condition and the equal light intensity of 135 $\mu molm^{-2}s^{-1}$ photosynthetic PFD, which was kept constant, by maintaining the distance between the LEDs and the plant canopy. Plants were harvested for experiments on 7^{th} and 14^{th} day of sowing.

Plant growth measurements

Length of stem and root were measured by scale. Fresh and dry weights were measured using weighing balance. For measuring dry weight, plant samples were kept in petriplates and placed inside hot air oven for 3 days at 70 °C before weighing.

Antioxidative activity

Fresh leaf and root tissues (0.5 g) were crushed in mortar and pestle with 5.0 ml extraction buffer, consisting 50 mM phosphate buffer of pH 7.5 and 1 mM EDTA. The homogenate was centrifuged at rpm of 15,000 for about 15 minutes. Supernatant was taken and used to measure the activities of CAT, POD and SOD. Protein determination was done according to Bradford (1976).

Catalase Activity

CAT activity was estimated by observing the H_2O_2 decomposition (Chance and Maehly, 1955). This was detected by noting the decrease in absorbance at 240 nm of reaction mixture consisting of 3 mL 250 mM sodium phosphate buffer (pH6.8), 0.3 mL 100 mM H_2O_2 and 0.3 mL enzyme extract. One unit of CAT meant the enzyme amount needed to decompose 1 mmol H_2O_2 min^{-1} under these specific assay conditions. Specific activity of CAT was denoted in U mg^{-1} protein min^{-1}.

Peroxidase Activity

The POD activity in leaves and roots was determined according to Amako *et al.* (1994) using pyrogallol substrate. The experiment was performed at 25°C and the reaction mixture comprised of 1500 µl 100 mM phosphate buffer, 1000 µl 60 mM pyrogallol and 480 µl 0.6 mM H_2O_2 solutions. To the assay mixture, 20 µl enzyme extract was mixed and increase in absorbance of the mixture was recorded continuously every 30 sec (for three minutes) at 430 nm. Specific activity of POD was denoted in Umg^{-1} protein min^{-1}.

Superoxide Dismutase Activity

SOD activity was estimated by Beauchamp and Fridovich (1971) method by checking its inhibiting capacity on photochemical reduction of nitrobluetetrazolium (NBT). One unit of SOD meant the enzyme amount required to cause half the maximum inhibition of NBT reduction.

Statistical analyses

Data presented here are the mean values ± standard errors (SE) of three independent experiments. Statistical analysis of all the parameters was done in Graph pad software (GraphPad prism 5.01 version, La Jolla, CA, USA). All data were subjected to Tukey's multiple comparison test of one-way ANOVA and P-values less than 0.05 were considered significant (*<0.05, **<0.01, ***<0.0001).

RESULTS

Effects of white, blue and red LED light on seedling growth

When compared to the control, there was an increase in stem length of blue and red light illuminated plants. Maximum stem length was found in blue light irradiated seedlings (Fig. 1a). Same trend was also found in case of root length (Fig 1b).

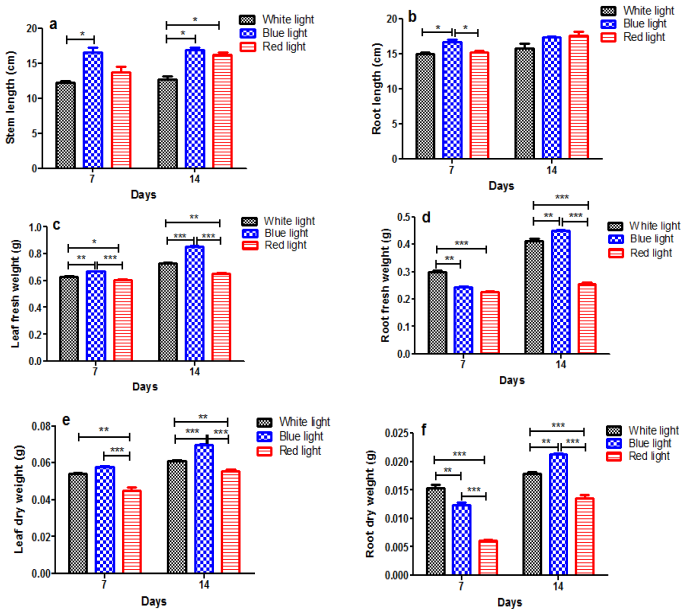

Figure 1 Stem length (a), root length (b), leaf fresh weight (c), root fresh weight (d) leaf dry weight (e) and root dry weight (f) of white, blue and red LED light grown pea seedlings. Values are means ± SE of triplicates and p-value denoted by * <0.05, **<0.01, ***<0.0001 were considered significant.

Leaf fresh weight was also maximum under blue LED light on both 7th and 14th day (Fig. 1c) while root fresh weight was maximum under white (control) LED light on 7th day (Fig. 1d). Lowest fresh weight of both leaves and roots were observed in red light conditions (Fig. 1c,d). The dry weights of leaves and roots also showed the similar pattern (Fig. 1e,f) .

Effects of white, blue and red LED light on catalase activity

Significant change was observed in the catalase activity in the leaves of pea seedlings grown under different LED light. It was maximum in blue light grown plants while minimum in red light grown plants on both days. On 7th day, plants under blue light showed 17.73% more catalase activity than white light and 33.95% more activity than red light (Fig. 2a). On 14th day there was 19.51% and 45.3% more activity in blue light than that of white light and red light respectively (Fig. 2a). In case of roots also, blue light exhibited highest activity under on all the days and red had least activity (Fig. 3a). Under red light activity decreased with increase of days.

Effects of white, blue and red LED light on peroxidase activity

In leaves, there was no significant change in peroxidase activity between control and blue light on both day. Significant difference between the blue and red light was observed on 14th day in the leaves of pea seedlings. It was maximum in blue light grown plants while minimum in red light grown plants on both days. Peroxidase activity was 18.18% and 57.3% more on 7th and 14th day respectively under blue light than red light (Fig. 2b). In roots of pea seedlings, no significant variation was found between any light conditions on 7th day while on 14th day highest activity was found under blue and minimum under red light with an increase of about 36.22% (Fig. 3b).

Effects of white, blue and red LED light on superoxide dismutase activity

SOD activity in leaves was maximum in blue light grown plants while minimum in red light grown plants on both days. In leaves, it increased upto 33.29% on 14th day for white light compared to 7th day (Fig. 2c). On 14th day significant change was not observed between red and white light with maximum SOD in blue light having 29.75% more content than red light. In case of roots, blue light showed highest activity with about 45.45% and 39.4% more SOD than that under red light on 7th and 14th day respectively (Fig. 3c).

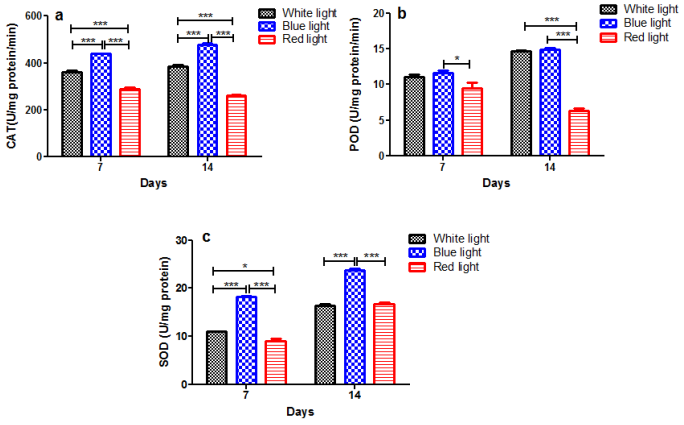

Figure 2 Activities of CAT (a) POD (b) and SOD (c) in leaves of white, blue and red LED light grown pea seedlings**.** Values are means ± SE of triplicates and p-value denoted by * <0.05, **<0.01, ***<0.0001 were considered significant.

DISCUSSION

Growth of seedling and its quality can be improved by LED irradiation (Johkan *et al.,* 2010; Liu, 2012; Wu *et al.,* 2007). In the experiment, influence of different LED light on pea seedling growth was studied. When the light radiation is very poor the seedlings become more elongated and have very compact roots that cannot uptake sufficient water and nutrients and thus the plants cannot have normal growth. Our results also showed compact roots in seedlings grown under red light compared to the seedlings grown under white and blue light (Fig. 1b) which might have caused poor development of leaves and reduced weights of both leaves and roots. The fresh weight of pea seedlings illuminated with blue LED light was greater than that of treated with white and red light. Leaves grow up healthy when roots energetically provide the plant fully with water and minerals.

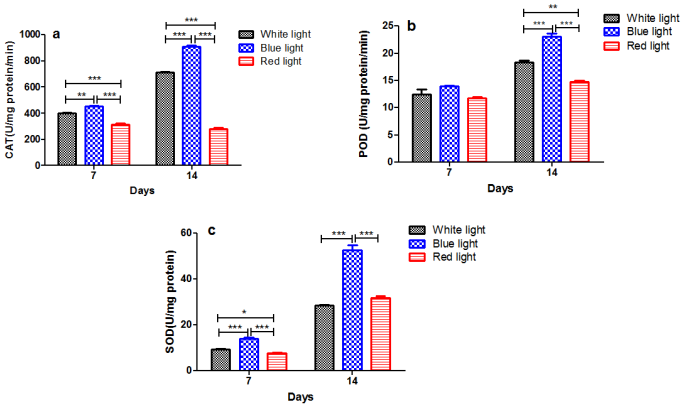

Figure 3 Activities of CAT (a) POD (b) and SOD (c) in roots of white, blue and red LED light grown pea seedlings. Values are means ± SE of triplicates and p-value denoted by * <0.05, **<0.01, ***<0.0001 were considered significant.

Plants produce ROS in all conditions, even in normal and stress free environment. Oxidative damage, which may lead to apoptosis of cells, is caused by excessive amount of ROS production. Development of antioxidative defensive system prevents the plants against damage due to oxidative stress. In cells, ROS when produced are scavenged by metabolic activities of CAT, POD and SOD. CAT and SOD protect the plant cells from harmful ROS by removing the H_2O_2 and $O_2^{\cdot-}$, respectively. In this study, activities of CAT, POD and SOD were higher in roots and leaves of pea seedlings grown under blue light compared to white and red light. Similar to our results, CAT in leguminous plants was increased by irradiation with blue light and decreased by red light (Appleman and Pyfrom, 1955). Murakami (1940) studied the effect of monochromatic light on various enzymes, including catalase, in yeast and found an increase in catalase activity in the blue light than in red light. The activities of SOD, POD and CAT were the highest in *Morus alba* seedlings grown under blue light (Hu *et al.,* 2016). After 8-months treatment in alga *Anoectochilus roxburghii,* the SOD, POD, and CAT activities were significantly lower in the red than the other treatments. The enzyme activities were significantly higher in the blue treatment than control (Ye *et al.,* 2017). Under the red LED treatment, CAT activity in tomato leaves was markedlydecreased by about 18% compared to white LED light while the activity in blue LED-treated tomato was enhanced by 15% in leaves in comparison to that of white LED light treatment (Kim *et al.,*2013). Similar results were also reported

in *Rehmannia glutinosa* (Manivannan *et al*., 2015). Activities of CAT and POD were also higher in stevia plantlets grownunder blue light compared to red light (Simlat *et al*., 2016).

Highest antioxidant potential and growth was found in pea seedlings grown under blue light. Blue light photoreceptors cryptochrome and phototropin might be regulating the developmental process in *Pisum sativum.* The effect of blue light on CAT activity was suppressed when Ca^{2+} availability was decreased in *Triticum aestivum* seedlings (Causin *et al.,* 2015).The blue radiation signal transduction pathway may alter Ca^{2+} and H_2O_2 homeostasis and thus maintain the high CAT activity. The metabolism of auxin and its distribution in plants is affected by POD activity which thus controls the growth and developmental process of plants (Kim *et al.,* 2013). The exact detailed mechanism of blue light regulating the growth and antioxidant activity of plants remains to be elucidated.

CONCLUSION

The findings suggest that blue wavelength benefits the growth and quality of *P. sativum* seedlings by maintaining high CAT, POD and SOD activity.

Acknowledgments

The authors are thankful to Department of Biotechnology for the financial support of BUILDER project No.BT/PR-9028/INF/22/193/2013. SA acknowledges the award of JRF by CUJ and PS acknowledges the grant of UGC-FRP during the period of this work.

References

Amako K, Chen GX, Asada K (1994) Separate assays specific for ascorbate peroxidase and guaiacol peroxidase and for the chloroplastic and cytosolic isozymes of ascorbate peroxidase in plants. *Plant Cell Physiol* 35: 497-504

Appleman D and Pyfrom HT (1955) Changes in catalase activity and other responses induced in plants by red and blue light. *Plant Physiol* 30(6): 543-549

Baker NR, Harbinson J, Kramer DM (2007) Determining the limitations and regulation of photosynthetic energy transduction in leaves. Plant Cell Environ 30: 1107-1125

Beauchamp C, Fridovich I (1971) Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. *Analytical Biochem* 44: 276-287

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochem* 72: 248-254.

Casal JJ (2013) Photoreceptor signaling networks in plant responses to shade. *Annu Rev Plant Biol* 64: 403-427

Causin HF, Marchetti CF, Pena LB, Gallego SM, Barneix AJ(2015) Down-regulation of catalase activity contributes to senescence induction in wheat leaves exposed to shading stress. *Biologia plantarum* 59(1): 154-162

Chance B, Maehly AC (1955) Assay of catalase and peroxidase. Methods in enzymol 2:764-775

Demotes-Mainard S, Péron T, Corot A, *et al* (2016) Plant responses to red and far-red lights, applications in horticulture. *Env Exp Bot* 121: 4-21

Foyer CH and Noctor G (2005) Redox homeostasis and antioxidant signaling: a metabolic interface between stress perception and physiological responses. *Plant Cell* 17(7): 1866-1875

Hopkins WG, Huner NPK (2009) Introduction to Plant Physiology, 4th ed., John Wiley& Sons, Inc., Hoboken, NJ, USA

HuJ, Dai X, Sun, G (2016) Morphological and physiological responses of *Morus alba* seedlings under different light qualities. *Not Bot Horti Agrobo* 44(2): 382-392

Johkan M, Sholi K, Goto F, Hashida S-N, Yoshihara T (2010) Blue light-emitting diode light irradiation of seedlings improves seedling quality and growth after transplanting in red leaf lettuce. *Hort. Sci*. 45: 1809-1814

Joshi P, Misra AN, Nayak L, Biswal B (2013) Response of mature, developing and senescing chloroplast to environmental stress. In: Plastid Development in Leaves during Growth and Senescence. (Eds. B Biswal, K Krupinska & UC Biswal), Advances in Photosynthesis and Respiration (Series Eds. Govindjee & TD Sharkey). Vol. 36, Chapter 28, pp. 641-668

Kim K, Kook HS, Jang YJ, Lee WH, Kamala-Kannan S, Chae JC, *et al* (2013). The effect of blue-light-emitting diodes on antioxidant properties and resistance to *Botrytis cinerea* in tomato. *J Plant Pathol Microbiol* 4: 49

Kim SJ, Hahn EJ, Heo JW, Paek KY (2004a) Effects of LEDs on net photosynthetic rate, growth and leaf stomata of chrysanthemum plantlets in vitro. Sci Hortic 101: 143-151

Kusaba M, Maoka T, Morita R, Takaichi S (2009) A novel carotenoid derivative, lutein 3-acetate, accumulates in senescent leaves of rice. *Plant Cell Physiol* 50: 1573–1577

Liu W (2012) Light environment management for artificial protected horticulture. *Agrotech* 1: 1-4

Liu XY, Guo SR, Xu Z, Jiao XL, Takafumi T (2011) Regulation of chloroplast ultrastructure, cross-section anatomy of leaves and morphology of stomata of cherry tomato by different light irradiations of LEDs. *Hort Sc.* 45:1-5

Manivannan A, Soundararajan P, Halimah N, Ko CH, Jeong BR (2015) Blue LED light enhances growth, phytochemical contents, and antioxidant enzyme activities of *Rehmannia glutinosa* cultured in vitro. H*ort Env Biotechnol* 56(1): 105-113

Murakami R (1940) Influence of monochromatic light on the action of enzymes. *J Agr ChemSoc* 16: 55-68

Scandalios JG, Guan LM, Polidoros A (1997) Oxidative stress and the molecular biology of antioxidant defenses. Cold spring Harbor Lab, Press Planvies NY, pp343-406

Senger H (1982) The effect of blue light on plants and microorganisms. *Photochem Photobiol* 35: 911-920

Simlat M, LêzakP, Mo´s M, Warcho M, Skrzypek E, Ptak A(2016) The effect of light quality on seed germination, seedling growth and selected biochemical properties of *Stevia rebaudiana* Bertoni. *Sci Hortic* 211: 295-304

Wu MC, Hou CY, Jiang CM, Wang YT, Wang CY, *et al*. (2007). A novel approach of LED light radiation improves the antioxidant activity of pea seedlings. *Food Chem*. 101: 1753-1758

Yang CM, Lee YJ (2009) Applicability of light-emitting diodes on agricultural production processes from viewpoint of photoperiod in plants. *Crop Env Bioinfo* 6: 192–200

Ye S, Shao Q, Xu M, Li S, Wu M, Tan X, Su L (2017) Effects of light quality on morphology, enzyme activities, and bioactive compound contents in *Anoectochilus roxburghii*. *Front Plant Sci* 8: 857

Yuan Y, Lingfei S, Shunqin C, Luqi H, Shuangshuang Q, Zhaochun Y (2012) Flavonoids and antioxidative enzymes in temperature-challenged roots of *Scutellaria baicalensis* Georgi. *Z Naturforsch* 67c:77-85