ISSN: 2394-1391
Original Article

Indian Journal of Biology
Volume 4 Number 2, July - December 2017
DOI: http://dx.doi.org/10.21088/ijb.2394.1391.4217.10

Effect of Different Visible Light Wavelengths on Seed Germination and Photosynthetic Pigment Contents in Vigna unguiculata (L.) Walp

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Abstract

Among the various naturally occurring abiotic factors regulating plant development, light (radiations) plays an important role in photosynthesis, photoperiodism and photomorphogenesis. The present investigations aimed to study the effects of different colour of light on seed germination, hypocotyl growth, mobilization efficiency (ME), vigor index (VI), biomass production and content of photosynthetic pigments (Chl a, Chl b and Carotenoids) in *Vigna unguiculata* (L.) Walp. *cv.* Kanchan, an important annual herbaceous food legume crop. Germination rate was found comparatively high in natural light with 95% germination at 84 hours. Individually, red light showed maximum % germination (98) at 84 hours while, green light showed almost no germination even after 96 hours. Blue light and yellow light caused significant reduction in % germination to 71 and 56, respectively, at 84 hours. The seedlings grown under different lights showed variation in biomass production (fresh weight of root and shoot). Root and shoot growth were highest in red light and the order of biomass production was red > yellow > natural > blue > green. ME and VI of cowpea seedlings revealed maximum (610.41 and 4.41, respectively) in red and minimum in yellow light (163.38 and 1.49, respectively). Both, ME and VI could not be determined under green light due to absence of well-marked hypocotyl. The data on photosynthetic pigments indicates synthesis of photosynthetic pigments highly dependent on light quality. Highest content of chlorophyll a, chlorophyll b and carotenoids were observed in red, yellow and natural light, respectively. The findings indicate possibility for using light quality for manipulation of germination and seedling health of crop plants in general and *V. unguiculata* in particular.

Keywords: Visible Light Wavelengths; Germination; Photosynthetic Pigments; Seedling Vigour; *Vigna Unguiculata.*

Introduction

Among the various naturally occurring abiotic factors regulating plant development, light (radiations) plays an important role in photosynthesis, photoperiodism and photomorphogenesis. Solar light consists of electromagnetic radiation with wavelengths ranging 400 to 700 nm (violet, blue, green, yellow, orange and red). Nature has produced a number of light absorbing molecules that enable organisms to respond to changes in the natural light environment. The changes in the light signal/quality (wavelength) influences various physiological processes (i.e. intra- and inter-cellular differentiation, seed germination and seedling growth, photosynthesis, flowering etc.), depending on the species and developmental stage or studied organ (He *et al.*, 2017). Green light, in the process of seed germination of *Arabidopsis*, stimulates the early elongation of the stems, antagonizing the growth inhibition by light whereas the white and red light, in ferns can delay the chlorophyll loss due to senescence (Burescu *et al.*, 2015). It has been reported earlier that light is absolute factor regulating the seed germination in numerous plant species (Jala, 2011). Over a study period of 27 days, Jala (2011) found that

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Received on 08.08.2017, **Accepted on** 25.09.2017

the seeds of *Nepenthes mirabilis* under white and red light germinated first and those under green light were the last once to germinate and the highest average speed of emergence was also recorded highest for seedlings under red light. Pigments are molecules that absorb light usually in the range of 320 to 760 nm and their biosynthesis in germinating seeds is highly dependent on light quality they perceive. Burescu *et al.* (2015) studied the effect of different wavelengths LED lights on the growth of Spruce (*Picea abies* L.) plantlets and observed increased biosynthesis of chl a, chl b under blue and yellow light, respectively. Carotenoid synthesis was also significantly enhanced in yellow light treated plantlets. However, all pigments tested were found lower in plants grown under green light than in other treatments. Considering this, the present study was conducted to study the effect of different colours of light (natural, red, blue, yellow, and green) on seed germination, hypocotyl growth, biomass production, mobilization efficiency (ME), vigor index (VI) and photosynthetic pigments in Cowpea/ Lobia (*Vigna unguiculata* (L.) Walp., an important pulse crops cultivated in the semi-arid and sub-humid tropics for its grain and fodder purposes.

Materials and Methods

Cowpea/Lobia (*Vigna unguiculata* L.) Walp. *cv.* Kanchan was used as experimental material for germination using Knop's solution as nutrient medium. Knop's solution (10X) was prepared by mixing 0.8 mg of $Ca(NO_3)_2.4H_2O$, 0.2 mg of KNO_3, 0.2 mg of K_2HPO_4, 0.2 mg of $MgSO_4.7H_2O$ and traces of $FeSO_4$ in 50 ml of distilled water (DW). This stock solution was further diluted with DW for the preparation of 250 ml nutrient solution for germination of seeds.

Hundred surface sterilized seeds of *V. unguiculata* were grown in a series of 5 petri dishes, each containing equal amount of sand and moistened with 30 ml of nutrient solution. These petri dishes were exposed to light of different wavelength (i.e. natural, red, yellow, green and blue light) provided using polypropylene film light filters for the duration of 96 hours.

Mobilization efficiency (ME) in germinating seedlings of each light treatment was estimated by the method of Mohan *et al.* (1996) with the following formula:

ME = Dry weight of seedlings/ Dry weight of cotyledon X100

Vigor index (VI) of germinating seedlings in each light treatment was estimated by the method of Abdul-Baki and Anderson (1973) with the following formula:

VI = % germination X average hypocotyl length

The content of chlorophyll a, chlorophyll b, total chlorophyll and carotenoid were estimated in mg/ gm fresh weight according to method of Arnon (1949) with slight modification (Bansal *et al*., 1976). On 5th day 80% (v/V) acetone homogenate of plants was incubated at 4°C for 24 hours, and then the homogenate was centrifuged at 3000 revolution per minute for 15 minutes. The supernatant was used to determine OD of each sample/treatment at 480, 510, 630, 645, 652, 663 and 665 nm using Spectronic 20 Bousch and Lomb spectrophotometer. The pigment values were calculated as:

Total Chlorophyll = $O.D_{652}$ X 1000/ 34.5

Chlorophyll a = 15.6 X ($O.D_{665}$) – 2 X ($O.D_{645}$) – 0.8 X ($O.D_{630}$)

Chlorophyll b = Total Chl – Chl a

Carotenoids = 7.6 ($O.D_{480}$- 1.4 X $O.D_{510}$)

The actual pigment content (mg/g FW) was computed as pigment value X V/1000 X 1/W

Where V - volume of acetone extract (in ml) and W - weight of the leaf tissue used (in g).

Results and Discussion

The effects of different light treatments on germination of cowpea (*Vigna unguiculata* L.) seeds are summarized in figure 1. The extent/rate of germination has been found to depend on light quality (wavelength) and exposure period. The germination was recorded maximum (98%) in red light at 84 hours duration and its wavelength was found more suitable for germination than other lights tested. Similarly, in the study of Abdullateef and Osman (2011), red light (660 nm) had better influence on germination in *Stevia rebaudiana* Bertoni seeds than white light (400-700 nm). Thereafter, in natural light the rate of germination was much faster than other light treatments with maximum germination of 95% at 84 hours. Sharma and Sen (1975) also recorded highest percentage of germination in *Merremia* species with red light treatment. Blue and yellow light showed moderate germination (71 and 56%, respectively) at 84 hours. Almost no germination was seen under green light even after 96 hours. It has been reported earlier in case of *Nepenthes mirabilis* that seeds under white and red light germinated first and those under green light were the last ones to germinate (Jala, 2011). However, upon completion of germination, Jala (2011) recorded highest % germination in yellow light followed by red and natural light. The present results on germination show deviation from Jala (2011) but conform to observations of Colbach *et al.* (2002) on *Alopecurus myosuroides* and Ambika (2007) on *Chromolaena odorata* seeds.

The effect of different lights on biomass production in cowpea/ lobia is summarized in Table 1 in terms of root fresh weight (FW), shoot fresh weight (FW), seedling dry weight, cotyledon weight and hypocotyl length. Seedlings under red light gave highest biomass yield in terms of root, shoot and cotyledon fresh weight. The order of biomass production under different light qualities was red > yellow > natural > blue > green. Irradiation with blue was not suitable for biomass growth (particularly root) in *V. unguiculata*. It resulted in smaller cotyledons and leaves with relatively low FW and Seedling dry weight and caused significant reduction in hypocotyls length. Horizontal and vertical expansion of shoot, particularly leaves is genetically controlled developmental process (Tsukaya, 1998) and irradiation with blue light seems to cause imbalance in expression of concerned genes leading to inhibition of leaf expansion. For the lettuce crop, the fresh and dry weight accumulations were higher under the RB (red-blue) treatment (Mickens, 2012). Snowden (2015) also observed significant reduction in dry biomass in radish in the presence of green light at the high level among the comparable treatments. In contrast to present findings, green light stimulates the spruce (*Picea abies* L.) seed germination and plant growth whereas the blue light inhibits hypocotyl elongation (Burescu *et al.*, 2015).

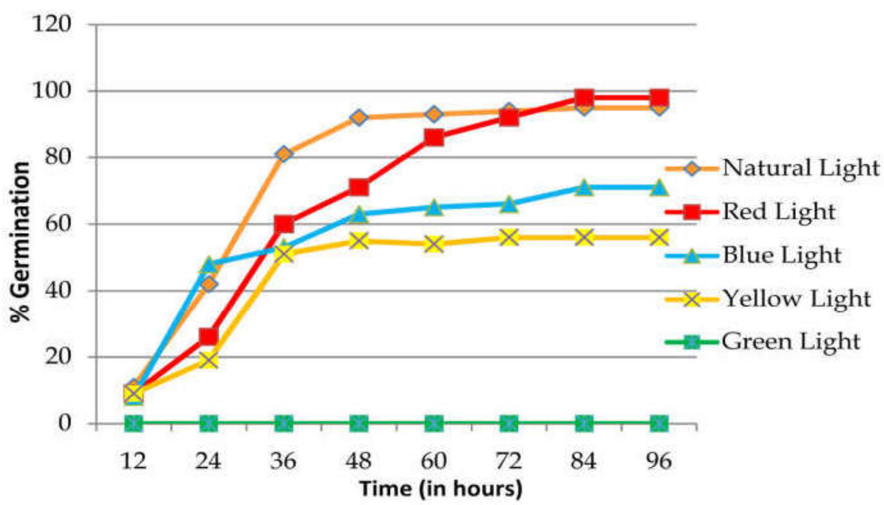

Fig. 1: Germination (%) in *V. unguiculata* in the presence of different light treatments

Table 1: Biomass production of root, shoot and hypocotyls of germinating seeds in *V. unguiculata* under different light treatments

S. N.	Treatments	Root FW (g)	Shoot FW (g)	Seedling DW (g)	Cotyledon DW (g)	Hypocotyl length (cm)
1	Natural Light	0.012±0.005	0.155±0.019	0.015±0.008	0.016±0.007	1.580±0.245
2	Red Light	0.014±0.008	0.186±0.017	0.018±0.007	0.020±0.009	1.900±0.236
3	Blue Light	0.005±0.002	0.106±0.013	0.009±0.004	0.009±0.004	1.000±0.190
4	Yellow Light	0.013±0.006	0.178±0.015	0.016±0.005	0.019±0.008	1.768±0.215
5	Green Light	*	*	*	*	*

FW-Fresh Weight, DW- Dry Weight, each value is shown as mean ± standard deviation

Table 2: Changes in Mobilization efficiency and vigor index of seedlings of *V. unguiculata* under different light treatments

Treatments	Mobilization Efficiency (ME)	Vigor Index (VI)
Natural Light	224.29±4.786	3.49
Red Light	610.41±8.324	4.41
Blue Light	172.09±3.509	3.56
Yellow Light	163.38±2.575	1.49
Green Light	*	*

Each ME value is shown as mean ± standard deviation

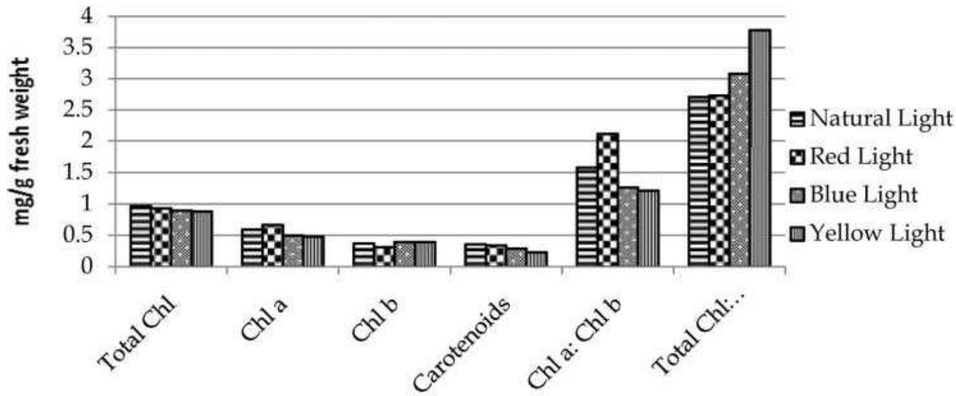

Fig. 2. Changes in the photosynthetic pigments of Cowpea seedling leaves grown under different light treatments

Variation was recorded in mobilization efficiency (ME) and vigor index (VI) in seedlings of cowpea (*Vigna unguiculata* L.) obtained in different light treatments and both ME and VI were highest under red light followed by yellow light (Table 2). Under green light, both ME and VI could not be determined due to absence of well differentiated hypocotyl. Contrary to these findings, Jala (2011) reported that seedling vigor index and germination index were highest under yellow light, followed by red light.

The seedlings formed under different lights showed differences in the quantity of photosynthetic pigments (Figure 2). Total chlorophyll and chlorophyll b were recorded highest in natural light whereas chlorophyll a was highest in red light in comparison to other treatments. Natural light also recorded maximum synthesis of carotenoids followed by red, blue and yellow light, respectively. Green light had no measurable pigments because of insufficient growth and development of seedlings. In some instances, green light may function by informing the plant of photosynthetically unfavorable conditions, allowing plants to adjust their compositions and physiology to the available light quality. The chlorophyll a:b ratio also varied in different light treatments. In red light, chlorophyll a:b ratio was maximum (2.12 mg/g) whereas total chlorophyll : carotenoid ratio was highest (3.78 mg/g) in yellow light in comparison to other lights. Saebo *et al.* (1995) reported that red light is important for the development of the photosynthetic apparatus (plastid differentiation) of plants and a combination of red and blue light is important in the formation of chlorophyll. The use of red-LED light to drive photosynthesis has been widely accepted due to fact that red wavelengths (600–700 nm) are efficiently absorbed by photosynthetic pigments (Sager and McFarlane, 1997) and the same is evident from the present results. Similarly, it has been found the highest inhibition of all the assimilating pigments in spruce (*Picea abies* L.) plantlets when exposed to green LEDs (Burescu, 2015). In a recent study, He *et al.* (2017) suggested that the appropriate combination of red- and blue-LED light enhances plant growth and photosynthetic capacities of *Mesembryanthemum crystallinum* compared to red- or blue-LED alone.

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