Advanced Studies in Biology, Vol. 8, 2016, no. 4, 173 - 180
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https://doi.org/10.12988/asb.2016.6832

Effect of Red and Blue Light-Emitting Diodes on Germination, Morphological and Anatomical Features of *Brassica napus*

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Abstract

Effect of red and blue light on seed germination, anatomical and ultrastructural features of chloroplasts were studied in oilseed rape (*Brassica napus* 'Modena'). Oilseed rape seeds were germinated in 2, 4 and 8 h light under various light emitting-diodes (LED) treatments: white light (control), red light (638 nm) and blue light (450 nm) in the laboratory. In greenhouse experiment, potted plants were grown under 4, 8 and 12 days of blue and red lights and white light at the background. The photoperiod of 16 h and 28/18 °C day/night temperature and 30 % relative humidity were maintained in the greenhouse. Seed germination percentage under red light increased significantly when compared with the control; however, blue light decreased significantly germination percentage of oilseed rape seeds. Red and blue lights had negative effect on shoot and seminal root growth of oilseed rape in all the treatments. In greenhouse experiment, shoot length, plant biomass, stem diameter, were significantly increased by blue light treatment. It seems blue light treatment induces higher photosynthesis in leaves and produced higher biomass of the whole plant in this treatment than the control or red light treatment.

Keywords: oilseed rape, seedling growth, germination percentage, anatomical features

Introduction

Different light wavelengths influence the growth of plant cells and organs and photosynthetic features (Li, 2010), yield of crops, and their morphological, anatomical and physiological characteristics (Deng *et al*., 2007). Blue light plays a role in a wide range of plant responses, such as leaf photosynthetic process, phototropism, photomorphogenesis and stomatal functioning. Blue light has been involved in the expression of “sun-type” characteristics, such as a high photosynthetic capacity of chloroplasts (Kopsell and Sams, 2013).
Many studies showing blue light effects on the leaf or whole-plant level have compared responses to a white light source with responses to blue-deficient light (Matsuda *et al*., 2008), or compared plants treated with blue or a combination of red and blue lights with plants treated with red light alone. In general, blue light-containing irradiance causes higher biomass production and photosynthetic capacity. LED lights create specific wavelengths and a narrow bandwidth for plant growth when compared with filters with broad-spectrum light sources. Therefore, blue and red LED lights can produce the specific blue or red spectrum more efficiently than blue or red filters with other light sources. Light sources in a broader range than red (600 to 700 nm) or blue (400 to 500 nm) region were used before the usage of light-emitting diodes (LEDs) that were intense enough for experimental planting (Kilic *et al*., 2010). Combinations of red and blue lights produced by LEDs have greater enhancement effect on photosynthetic features than blue-deficient light made by a filter (Matsuda *et al*., 2007; 2008). Therefore it must be investigated, whether plants treated with red light alone suffer a spectral deficiency’ syndrome, which may be improved by blue light and other longer wavelengths.
Oilseed rape (*Brassica napus* L.) is the third most important oilseed crop in the world, following oil palm and soybean. It is an excellent raw material for edible oil and biodiesel production (Velasco and Fernández-Martínez, 2002). Oilseed rape crop by allowing the incorporation of carbon into the soil due to its great amount of crop residues and the earlier sowing of double-crop soybean, can also replace winter cereals in crop rotations. Commonly, rape seed yields are expected to reach 40 to 50 % of wheat yields, but in low potential environments, oilseed rape and wheat yields may match (Rondanini *et al*., 2012). Despite of the good comparative performance of oilseed rape crop non-optimal environments, it is perceived by the producers as a risky crop due to its high yield variability at a global scale (Rondanini *et al*., 2012). One source of oilseed rape yield variability may be associated with changes in the light environment experienced by plants that affect biomass production and its allocation to harvestable grains. Even though the importance of the Brassicaceae family, which includes several crops like oilseed rape, broccoli (*Brassica oleracea* L. ssp. *oleracea* convar. *botrytis* (L.) Alef. var.*italica* Plenck) and radish (*Raphanus sativus* L.), the knowledge of

developmental responses to light quantity and quality during their life cycle is scarce with the exception of the model plant *Arabidopsis thaliana* (L.) Heynh. However, although *Arabidopsis* is an ideal model plant there is a need to validate and confirm the physiological and molecular mechanisms in other species in field conditions.
In this study, the effects of raising plants with different light spectra such as blue and red LED lights were determined on seed germination and growth parameters, of oilseed rape (*Brassica napus* L.) as a model plant.

Materials and Methods

Germination experiment: Seeds of oilseed rape (*Brassica napus* 'Modena') were taken from the Agricultural Research Centre of Khorasan Razavi Province, Iran, in 2014. Germination experiment was conducted in a randomized completely design with four replications in laboratory conditions. One hundred seeds were placed on a filter paper moistened with 5 ml distilled water, in a 9-cm glass Petri dish covered with lid. The treatments were provided by LEDs (LZ400D/H; China) designated as red and blue (2, 4 and 8 h per day), YaHuaNing Co., Nanjing and white (control). The power of every LED lamp treatment was 20 W. Ten LED lamps were used in each treatment. LEDs were operated with the same photosynthetic photon flux density (PPFD; (≈320 µmol m^{-2} s^{-1}). Data on different germination parameters were recorded after initial germination every day until no further germination occurred. The seeds were inspected every day and were considered to be germinated when the seminal root penetrated the seed coat and attained about 1 mm in length. Seminal root and shoot length (cm), were measured on the 7th day. Response index (RI) was calculated for showing inhibition and stimulation by different lights on seed germination and seminal root/shoot growth of oilseed rape.

RI is calculated as:

$$RI = (T/C - 1) \times 100,$$

where T is the parameter under treatment and C is the parameter under control.

Greenhouse experiment: Oilseed rape seeds were sown in vermiculite and germinated under greenhouse conditions. After one week, when the cotyledons had just opened, the plants were subjected to irradiance (4, 8 and 12 days) provided by blue and red LEDs separately with dominant wavelengths of 450 and 638 nm, respectively. LED arrays all set at 300 µmol·m–2·s–1 PPF. PPF was maintained at this level by adjusting the distance to the top of the plants relative to the light source. PPF was measured at the top of the plants using a quantum sensor (LI-189; LI-COR, Lincoln, Neb.). In the greenhouse experiment, plants treated by blue or red lights in addition to outdoor light. Control plants were grown under greenhouse conditions without LEDs. The treatments were arranged in a completely randomized design with four replications. The photoperiod of 16 h and 28/18°C day/night temperature and 30 % relative humidity in the greenhouse were maintained. The plants were irrigated with tap water throughout the experiment, as necessary.

Morphological and anatomical characteristics: After 45 days from sowing (vegetative phase), plants were uprooted; roots and shoots were separated and used for measuring the parameters. Total length of root and shoot and fresh mass of three plants per treatment were measured. For anatomical measurements, stem from ten plants were collected and fixed in FAA50 for 48 h and then kept in 70 % (v/v) ethanol, then dehydrated in a graded ethanol series (30, 50, 70, 96 and 100 %) and embedded in paraplast. Cross-sections (7 μm) were obtained with a rotary microtome, stained and mounted in synthetic resin and then photographed by light microscope ZEISS model 1.25X, CONTAX camera model 167MT (Kyocera, Japan). The following anatomical data were collected:

Statistical Analysis

Data were analyzed using a multifactor analysis of variance. Differences among means were calculated using the least significant difference range test with a family error rate of 0.05 by using the statistical analysis software *SPSS*.

Results

Germination Experiment

The present study revealed that the maximum germination percentage was observed in seeds under 2 h of red light (83 %), followed by 4 h (81 %) and minimum germination was observed in 8 h of blue light (59 %). The maximum negative influence was observed in 8 h of blue light (−21) (Table 1).

Table 1. Effect of different lights on seed germination percentage of oilseed rape

Treatments	Mean germination (%)	Response index (%)
Control	75b	–
Red light (hours)		
2	83 a	10.7
4	81 a	8.0
8	78 ab	4.0
Blue light (hours)		
2	66 c	−12.0
4	62 cd	−17.3
8	59 d	−21.0

Values followed by the same letter within a column are not significantly different at the $p < 0.05$ level.

The effects of lights on seminal root growth showed that maximum seminal root length was in control seedlings (10.25 cm) and minimum under 8 days of blue light (5.21 cm). Red and blue lights have negative effect on seminal root growth of oilseed rape in all the treatments (Table 2). The effects of lights on shoot growth showed that maximum shoot length was observed in the control (7.41 cm) and minimum under 8 days of blue light (4.12 cm). All treatments of red and blue lights have negative effect on shoot growth of oilseed rape (Table 2).

Table 2. Seminal root and shoot growth and response index of oilseed rape under different lights

Treatments	Seminal root length cm	Response index %	Shoot length Cm	Response index %
Control	10.25 a	–	7.41 a	–
Red light, hours				
2	9.02 ab	−12.0	6.34 ab	−14.4
4	7.12 bc	−30.5	6.06 ab	−18.2
8	7.03 bc	−31.4	5.64 b	−23.8
Blue light, hours				
2	8.45 b	−17.5	5.26 b	−29.0
4	6.32 c	−38.3	4.92 bc	−33.6
8	5.21 d	−41.9	4.12 c	−44.3

Values followed by the same letter within a column are not significantly different at the $p < 0.05$ level.

Greenhouse Experiment

Treatment of blue light additionally to outdoor light (12 days) induced higher shoot length, root length and plant fresh mass than treatment of red or control light (Table 3).

Table 3. Effects of the different light treatments on morphological features of oilseed rape

Light treatments	Shoot length (cm)	Root length (cm)	Plant fresh mass (g)
Control	43.5 d	37.3 d	0.76 d
Red light, days			
4	55.7 bcd	39.5 d	0.87 cd
8	64.2 b	58.7 b	0.98 c
12	58 bc	56.2 b	1.12 bc
Blue light, days			
4	31.3 e	50.2 c	0.93 c
8	55.4 bcd	66.8 a	2.6 a
12	83 a	69.7 a	1.3 b

Values followed by the same letter within a column are not significantly different at the $p < 0.05$ level.

As shown in Table 4, light spectrum significantly influenced the anatomical features of oilseed rape stem. Significantly, higher stem diameter was observed in the blue light (8 and 12 days) and red light (4and 8 days) treatments, respectively. Blue light (8 and 12 days) induced the highest cortex, stele and meta xylem width of stem. Moreover, red light treatment (12 days) induced significantly higher number of vascular bundles than other treatments (Table 4).

Table 4. Effects of the different light treatments on anatomical features of oilseed rape

Light treatments	Stem diameter mm	Cortex width mm	Stele diameter mm	Metaxylem width μm	Number of vascular bundles
Control	0.13 c	0.035 c	0.013 ab	1.7 ab	9.6 b
Blue light, days					
4	0.17 b	0.04 b	0.011 b	1.8 a	8.2 c
8	0.20 a	0.06 a	0.015 a	1.78 a	8.0 c
12	0.21 a	0.06 a	0.016 a	1.9 a	7.8 bc
Red light, days					
4	0.19 a	0.030 d	0.010 b	1.11 c	9.3 b
8	0.16 b	0.035 c	0.013 ab	1.3 b	9.8 b
12	0.14 c	0.037 bc	0.014 ab	1.3 b	11.2 a

Values followed by the same letter within a column are not significantly different at the $p < 0.05$ level.

Discussion

The results of this study show that, red light increases the germination percentage (Table 1). However, blue light decreases the germination as well as seminal root and shoot length during the experiment period (Table 2). Earlier investigations showed that light spectrum plays an important role in germination and seedling growth. Many studies have revealed that different plant species had optimal growth and development in response to the environmental factors according to the light they received (Li *et al*., 2012). Tang *et al*. (2010) showed that seed germination of some plants occurred under different light spectra. In this experiment, the maximum germination was observed under red light and blue light had negative effect on oilseed rape seed germination. Tang (2008, 2010) reported that seed germination of some important weeds, among them *Chenopodium album* L. increased when treated with red light.

Oilseed rape seeds germination started 3 and 5 days after sowing in red and blue light, respectively. Other studies revealed that the highest germination percentage was found in red light treatments (Tang *et al*., 2010).

Results from Table 2 demonstrated that light spectra influenced the seedling growth of oilseed rape and red and blue lights inhibition of shoot and seminal root

length. Seedling length was the shortest under blue lights when compared with the control and red light treatments. Guo *et al*. (2011) observed similar results in rice seedlings. Also, more inhibitory effects of blue light on seedling growth were reported by several research workers (Abidi *et al*., 2013; Kopsell *et al*., 2013).
Our results do support the conclusion that small amounts of blue light can influence cellular differentiation and maturation of xylem tissues in oilseed rape. Several studies have demonstrated that the addition of small amounts of blue photons to HPS or red light can dramatically alter plant morphometrics (Saebo *et al*., 1995). Our results support the conclusions that low levels of blue light can induce dramatic changes in plant anatomy of oilseed rape.

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Received: September 15, 2016; Published: October 21, 2016