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Effect of Red, Blue, Green LEDs on the Germination and Seedling Growth of Rice

M. N. Hoque[1]*, M. Z. Islam[2], M. Rahman[1], B. Biswas[1] and N. Mahmud[1]

[1]Department of Agriculture, Bangabandhu Sheikh Mujibur Rahman Science and Technology University, Gopalganj-8100
[2] Bangladesh Rice Research Institute, Regional Station Gopalganj-8100
*Corresponding email: shikshatoroo@gmail.com

Abstract

The present investigations attempted to study the effects of red, blue and green LEDs effect on seed germination, first leaf length, first leaf blade length, root length, seedling fresh and dry weight. Seeds of five rice varieties were germinated and seedlings were grown under dark and red, blue, green LEDs lighting system (6 h photoperiod and 18 h dark) set at 20 to 24± 2 °C for day and night respectively and 70 ± 2 % relative humidity in a control chamber for 14 days (starting 3 days after plated for germination). The result showed that germination percent increased significantly in BRRIdhan52 and BRRIdhan75 under red-blue-green LEDs than dark. First leaf length increased in dark than LEDs treatment. Longest leaf (10.42 cm) was recorded in BRRIdhan86 in dark and under LEDs in BRRIdhan75 (5.70 cm). First leaf blade length was highest in BRRIdhan52 (2.56 cm) under LEDs treatment and in dark BRRIdhan67 had the longest (2.48 cm). Root length was also increased significantly in studied rice varieties. Though, longest root was found in dark in BRRIdhan71 (6.69 cm) and under LEDs, BRRIdhan71 had the longest root (6.35 cm). Seedling fresh and dry weight were highest under red-blue-dark LEDs treatment. BRRIdhan86 had the highest and BRRIdhan67 had the lowest seedling fresh and dry weight with a range of 0.323g to 0.451g and 0.037g to 0.052g respectively than dark condition. Root length showed positive significant correlation with seedling fresh weight (r = 0.876) and seedling dry weight (r = 0.873). Whereas seedling fresh weight showed complete correlation (r = 1) with seedling dry weight. Response index was negative for first leaf length in the studied rice varieties. Highest response index (35.93) was observed for seedling fresh and dry weight in BRRIdhan75 and BRRIdhan86.

Key words: Germination, LEDs, Rice, Response index, Seedlings

Introduction

Light is used by plants as an energy source for photosynthesis. Plants also use light as an environmental signal, and respond to its intensity, wavelength, and direction. Plant photoreceptors, which include phytochromes, cryptochromes, and phototropins, detect light and trigger a variety of physiological reactions. Providing appropriate amount and quality of light intensities from light source to photosynthetic organ is a major challenge for plants (Dong *et al.,* 2014; Samuoliene *et al.,* 2013).

Visible light wavelength ranges from 380 to 780 nm. The most absorbed light by chlorophylls and other pigments in higher plants is red light (640 - 660 nm) and blue light (430 - 450 nm), whereas green light (500 - 600 nm) is the least (Abboud *et al.,* 2013). Many researchers have demonstrated the beneficial effects of red, blue, green light alone or mixed lighting, but rarely considering the duration and intensity of green light and the response of cereal crops (Zhang *et al.,* 2016).

The light spectrum of the growth environment has a big impact on plant development and physiology, and blue light is involved in a lot of plant activities as phototropism, photo-morphogenesis, stomatal opening, and leaf photosynthetic functioning (Whitelam *et al.,* 2007).

Plants absorb 90% of blue and red light (LEDs) (Terashima *et al.,* 2009), indicating that blue and red light have a major influence on plant development and physiology (Olle *et al.,* 2013). On the other hand, Green light has been shown to be harmful to physiological and developmental functions (Folta *et al.,* 2007). Many research on plant growth and development have been published on a variety of crops grown in deficiency/efficiency or with a combination of red and blue light at different wavelengths (Fan *et al.,* 2013; Lin *et al.,* 2013). Photosynthesis is more similar in plants cultivated under blue light than in plants grown under red light (Buschmann *et al.,* 1978); Leong *et al.,* 1984).

Light emitting diodes (LEDs), because of its desirable properties such as minimal mass, safety, and durability, have been proposed as a light source for controlled environment agriculture facilities and space-based plant growth chambers (Yano *et al.,* 2012; Kim *et al.,* 2013; Yori *et al.,* 2001).

The majority of studies examining the effects of blue light (blue LEDs) on the leaf or whole plant have compared the response to a broadband light source with the response to blue deficient light (Matsuda *et al.,* 2008) or compared plants grown under red light alone (Yori *et al.,* 2001; Matsuda *et al.,* 2004). On the other hand, red LEDs emit a narrow spectrum of light (660 nm) that is close to chlorophyll and phytochrome maximum absorbance. Plants have evolved to use a wide spectrum of light to drive photosynthesis, despite the fact that red light components have a lot of potential for use as a light source to drive photosynthesis (Kang *et al.,* 2013). Photosynthesis has been hindered by green LEDs (Sun *et al.,* 1998). Several studies have evaluated the effectiveness and ineffectiveness of green light on plant growth and development (Frechilla *et al.,* 2000).

The quality of seedlings during growth is therefore an important factor in rice improvement and production, especially when rice variety developed in controlled condition. Rice plants grow better under RB lights than under R alone during the vegetative growth stage (Matsuda *et al.,* 2004; Ohashi *et al.,* 2006). Guo *et al.* (2011) cultured rice seedlings using RB LEDs, which were more robust in terms of root number, stem diameter, health index, and soluble sugars than when incubated under other LED spectra.

Little is known on the integrity of combined effect of green, red and blue LEDs, with no experimental evidence available concerning BRRI developed rice varieties. Therefore, in order to apply the findings of previous research to rice seedling quality and production, this study considered that, it is important to investigate the effects of light quality when provided by red, blue, green LEDs systems to meet different purposes.

The objectives of this study was to determine the efficacy of RBG LEDs radiation source in relation to the growth, development and quality of rice grown under various LEDs together at the same light intensity.

Material and Methods

Plant materials and growth conditions

Seeds of *indica* rice (*Oryza sativa* L.) cultivar, BRRIdhan52, BRRIdhan67, BRRIdhan71, BRRIdhan75 and BRRIdhan86 were used in this study (Table 1). Seeds were sterilized with 2% sodium hypochlorite for 20 min, washed extensively with distilled water and then germinated in Petri dishes with wetted filter paper at 24 ± 2 °C in the dark and LEDs. The germination test was conducted using the petridish method with three replicates of 100 seeds. Seeds were soaked in distilled water for 36 h at room temperature and placed on Whatman filter paper no.1. The petridishes were observed every day and the numbers of germinated seeds were recorded at 24 h interval up to 14 days from set up of the experiment.

Table 1. Description of the four rice variety used in the present study

Rice variety	Growing season	Special phenotypic feature	Year of Release, Institution
BRRIdhan52	Aman	Submergence tolerant	2010, BRRI
BRRIdhan67	Boro	Salinity tolerant	2014, BRRI
BRRIdhan71	Aman	Drought tolerant	2014, BRRI
BRRIdhan75	Aman	Uptake 25% less nutrient	2016, BRRI
BRRIdhan86	Boro	Anther culture derivative	2017, BRRI

After 48 h of incubation, uniformly germinated seeds were selected and sand cultured in a natural medium with 1:1 (Organic matter: Sand). Rice seedlings were raised in growth rooms with the LED lighting system set at 20 ± 2 °C and 24 ± 2 °C for day and night respectively and 70 ± 2 % relative humidity under a 6 h photoperiod and 18 h dark.

Light treatments

LED lighting of 12W were used to control light quality. The spectral quality were measured using android apps. Light treatments for rice seed and seedlings included red LEDs (R), blue LEDs (B) and green LEDs (G). Specification of the red, blue and green LEDs light: 12Watt; voltage range 85 – 265 V; Frequency 50 – 60Hz; power factor ≥ 0.5; efficacy ≥ 95lm/W; color rendering index ≥ 80%; total harmonic distribution ≤ 15%; high lumen PF>0.9; 6500k daylight, ambient temperature: -20 °C ~40 °C. Distance between LED lights and seed or seedling were adjusted to get the approximately equal photosynthetic photon flux (PPF). The plants without red, blue and green light irradiation were used as the control *i.e.,* dark.

Data collection

The experiment was independently performed three times with a randomized design of growth conditions and measurements representing the means of 15 plants (three replications consisting of five plants each) were taken. Five seedlings for each replication for combined LEDS (red, blue and green) light treatments were randomly selected for growth analysis.

Germination

Germination was recorded daily and was considered complete once the radicle protruded about >2 mm in length. The experiments were continued for 14 days (Ellis and Roberts, 1981).

Germination per cent

A seed was considered to be germinated as seed coat ruptured, plumule and radicle came out and were >2mm long. Germination count was expressed in percentage. The germination percentage was calculated using the following formula (International Seed Testing Association, 2006).
Germination (%) = (Number of seed germinated/Total number of seed for test) × 100

Measurement of leaf, root and shoot length

Randomly selected five seedlings were taken from each petridish to measure first leaf length, first leaf blade length, root length and shoot length. It was measured with a measuring scale and expressed in centimeters. First leaf length, first leaf blade length, root length and shoot length of the seedlings were measured after 17 days of seed setting (kouio, 2003).

Measurement of fresh weight and dry weight of the seedling

After 17 days of seed setting 15 seedlings of each petridish was wrapped with brown paper and weighed the fresh weight first and then they were died in oven at 70° C for 48 hours and weighed the dry weight. These were measured by four digit balance and expressed in gram.

Response index

Response index (RI) was calculated for showing inhibition and stimulation by different lights on seed germination and seedling growth (Tehrani *et al.,* 2016). RI is calculated as: RI = (T/C - 1) × 100, where, T is the parameter under treatment and C is the parameter under control.

Data analysis

All measurements were evaluated for significance using analysis of variance (ANOVA) followed by the least significant difference (LSD) test at the $P < 0.05$ level. Completely Randomized Design was used to test the variation in rice varieties and growth condition. Simple linear correlation coefficient were calculated to find out the inter relation of the studied characters. All statistical analyses were conducted using MSTAT-C (Statistical analysis software) computer package program (Gomez and Gomez, 1984).

Results and Discussion

All of the studied parameters, including germination percent, first leaf length, first leaf blade length, root length, seedling fresh weight, and seedling dry weight, for five rice varieties: BRRIdhan52, BRRIdhan67, BRRIdhan71, BRRIdhan75 and BRRIdhan86 (Table 2).

Seed germination

Germination in the dark ranges from 70 to 99 percent. Highest germination was recorded in BRRIdhan71 and lowest in BRRIdhan75. Under red, blue and green LEDs, germination percentage ranged from 66 to 98 percent. BRRIdhan67 and BRRIdhan71 had the highest and BRRIdhan86 had the lowest percent of seed germination (Table 2). Earlier investigations showed that light spectrum plays an important role in germination (Tehrani *et al.,* 2016). Simpson (1990) also reported that light inducible germination happens in a certain set of environmental conditions in many plant species, those may be dependent or independent to light. Information on effect of red, blue and green light on rice seed germination is very scars till to date.

First leaf length and leaf blade breadth

In dark first leaf length ranges from 7.88 to 10.42 cm. BRRIdhan86 had the longest and BRRIdhan52 had the shortest. First leaf blade length ranges from 1.93 to 2.48 cm (Table 2). BRRIdhan67 had the most and BRRIdhan75 had the least. Under red, blue and green LEDs, first leaf length ranges from 2.59 to 5.70 cm. BRRIdhan75 had the longest and BRRIdhan52 had the shortest. First leaf blade length ranges from 2.12 to 2.56 cm. BRRIdhan52 had the longest and BRRIdhan86 had the shortest (Table 2). Zhang *et al.* (2016) found highest seedling height in red-blue-green LEDs, which is different in the present study. Rice seedling height inhibition under blue light reported by Xu *et al.* (2020). In seedling stage, rice plants are short and strong under single-wave blue light, and the quality of seedlings is the best. LED red light promotes the growth of seedlings, and the number of seedlings and uniformity are better (Renliang *et al.,* 2016). In the present study first leaf blade length increased under red-blue-green LEDs which agrees with the findings of Zhang *et al.* (2016) where, red-blue-green light increase leaf area in rice seedlings.

Table 2. Rice seed germination and seedling morphology as affected by dark and lighting from red, blue, green light-emitting diodes (LEDs)

Variety	Germination (%)	First leaf length (cm)	First leaf blade length (cm)	Root length (cm)	Seedling fresh weight(g)	Seedling dry weight(g)
Dark						
BRRIdhan52	82 ±1.14	7.88 ±0.17	1.99 ±0.01	5.79 ±0.13	0.356 ±0.006	0.041 ±0.0007
BRRIdhan67	96 ±1.13	10.18 ±0.24	2.48 ±0.06	4.10 ±0.18	0.305 ±0.009	0.035 ±0.0001
BRRIdhan71	99 ±0.58	8.43 ±0.34	2.22 ±0.04	6.69 ±0.19	0.405 ±0.008	0.047 ±0.0001
BRRIdhan75	70 ±1.15	8.46 ±0.15	1.93 ±0.03	4.94 ±0.15	0.304 ±0.008	0.035 ±0.0009
BRRIdhan86	80 ±1.14	10.42 ±0.09	2.25 ±0.01	5.48 ±0.10	0.332 ±0.005	0.038 ±0.0005
RBG LEDs						
BRRIdhan52	90 ±1.15	2.59 ±0.00	2.56 ±0.02	5.95 ±0.05	0.348 ±0.002	0.040 ±0.0002
BRRIdhan67	98 ±0.58	4.80 ±0.03	2.38 ±0.03	5.43 ±0.03	0.323 ±0.002	0.037 ±0.0002
BRRIdhan71	98 ±0.33	4.36 ±0.02	1.94 ±0.06	6.35 ±0.31	0.385 ±0.004	0.044 ±0.0004
BRRIdhan75	92 ±1.15	5.70 ±0.02	2.45 ±0.05	4.94 ±0.08	0.377 ±0.002	0.043 ±0.0002
BRRIdhan86	66 ±1.14	5.07 ±0.15	2.12 ±0.07	6.12 ±0.08	0.451 ±0.018	0.052 ±0.0021

Values show Mean ± SE (n=5). All the values were significantly different at $p \leq 0.0001$.

Root length, seedling fresh weight and dry weight

In dark root length ranges from 4.10 to 6.69 cm. BRRIdhan71 had the longest and BRRIdhan67 had the shortest root. Under red, blue and green LEDs, root length ranges from 4.94 to 6.35 cm. Longest root was found in BRRIdhan71 and shortest in BRRIdhan75. According to Zhang *et al.* (2016), higher total root length of rice seedlings was found under red-blue-green light, which was not different in the present study (Table 2).

Seedling fresh weight ranges from 0.304 to 0.405 g and seedling dry weight ranges from 0.035 to 0.047 g in dark. BRRIdhan71 had the highest and BRRIdhan75 had the lowest seedling fresh weight and seedling dry weight. Under red, blue and green LEDs, Seedling fresh weight ranges from 0.323 to 0.4051 g and seedling dry weight ranges from 0.037 to 0.052 g. BRRIdhan86 had the highest and BRRIdhan67 had the lowest seedling fresh and seedling dry weight. The findings of the present study agree with the statement of Zhang *et al.* (2016), who found highest shoot dry weight and dry mass of root in rice seedlings in consecutive years under red-blue-green light LEDs treatment.

Correlation between seed germination and seedling characters

Correlation coefficient was calculated for germination percent, first leaf length, first leaf blade length, root length, seedling fresh weight and seedling dry weight (Table 3). Germination percent showed non-significant correlation with all the seedling characters, among these association there were two negative and two positive. First leaf blade showed negative significant correlation with root length, seedling fresh and dry weight. Root length showed positive and significant correlation with seedling fresh weight ($r = 0.876$) and seedling dry weight ($r = 0.873$). Seedling fresh weight showed complete correlation ($r = 1$) with seedling dry weight. Zhang *et al.* (2016) also reported positive significant correlation for root shoot characters in rice seedlings, which justified the findings in the present study.

Table 3. Correlation between seed and seedling characters in rice varieties.

	Germination (%)	First leaf length (cm)	First leaf blade length (cm)	Root length (cm)	Seedling fresh weight(g)	Seedling dry weight(g)
Germination (%)	**0**	-0.277^{ns}	0.165^{ns}	0.129^{ns}	-0.226^{ns}	-0.208^{ns}
First leaf length (cm)		**0**	0.143^{ns}	-0.469*	-0.062^{ns}	-0.045^{ns}
First leaf blade length (cm)			**0**	-0.726*	-0.849*	-0.849*
Root length (cm)				**0**	0.876*	0.873*
Seedling fresh weight (g)					**0**	1***
Seedling dry weight (g)						**0**

*, ** Indicate significant differences between dark and green LEDs treatment at $p \leq 0.05$ and $p \leq 0.01$level, respectively.
Ns: Not significant at (n - 2) df.

(a) Germinated seeds of BRRIdhan52 in dark

(b) Seedlings of BRRIdhan52 grown under red, blue, green LEDs (left) and in dark (Right)

Plate 1. Effect of dark (a) and red, blue, green LEDs (b) on germinated seed and seedling of BRRIdhan52.

Table 4. Response index for growth parameters of 5 rice varieties under red-blue-green LEDs lights.

Variety	Germination (%)	First leaf length (cm)	First leaf blade length (cm)	Root length (cm)	Seedling fresh weight(g)	Seedling dry weight(g)
BRRIdhan52	9.76	-67.20	28.68	2.71	-2.23	-2.23
BRRIdhan67	2.08	-52.89	-4.03	32.34	5.76	5.76
BRRIdhan71	-1.35	-48.29	-12.81	-5.08	-4.97	-4.97
BRRIdhan75	31.43	-32.66	26.58	0.03	23.79	23.79
BRRIdhan86	-17.50	-51.39	-5.92	11.60	35.93	35.93

Response index

Inhibition and stimulation by red, blue and green LEDs on seed germination and seedling growth were assessed through response index in rice varieties (Table 4) (Plate 1). Seed germination was stimulated by red, blue and green LEDs ranging from 2.08 to 31.43 percent. BRRIdahn75 showed highest response and lowest response by BRRIdhan67. Negative response for seed germination was found in BRRIdhan86 and BRRIdhan71. First leaf length was inhibited by red, blue and green LEDs. Response index rages from -32.66 to -67.20 percent. BRRIdhan52 showed highest and BRRIdhan67 showed lowest negative response to light spectra. First leaf blade length was stimulated in BRRIdhan52 (28.68%) and BRRIdhan75 (26.58%). Negative response for first leaf blade length showed by BRRIdhan67, BRRIdhan86 and BRRIdhan71 with a range from -4.03 to 12.81 percent. Root length was stimulated in all the varieties except BRRIdahn71 (-5.08%). Response index for root length ranges from 0.03 to 28.68 percent. Highest growth response showed by BRRIdhan67 and lowest by BRRIdhan75. Seedling fresh weight and dry weight were stimulated in BRRIdhan86, BRRIdhan75 and BRRIdhan67 with a range from 5.76 to 35.93 percent. Highest response was recorded in BRRIdhan86 and lowest in BRRIdhan67. Negative response was indexed in BRRIdhan71 (-4.97%) and BRRIdhan52 (-2.23%) for seedling fresh and seedling dry weight. A number of studies demonstrated that the combination of red and blue light was an effective light source for plant growth (Lian *et al.,* 2002; Nhut *et al.,* 2003; Matsuda *et al.,* 2004, Jao *et al.,* 2005; Ohashi-Kaneko *et al.,* 2006; Johkan *et al.,* 2010). However, it was found that the highest stem width, shoot and root height, dry weight of shoot and root were all observed in RBG (Zhang *et al.,* 2016).

Illuminance is also a key photo-environmental component influencing rice development as well as an important ecological factor influencing photosynthetic rate. Different plants require different levels of illumination. This suggests that combining red and blue LEDs with green light improves stem and root elongation and dry weight accumulation. Kim *et al.* (2004a) also reported a similar result. This could be due to the fact that green light penetrates the plant canopy more than red and blue light, potentially enhancing plant growth via increasing photosynthesis from the lower canopy leaves (Kim *et al.,* 2004a; Terashima *et al.,* 2009). Alternatively, green light could be reversing the effects of blue wave bands on elongation inhibition, causing the leaves to extend further (Bouly *et al.,* 2007; Wang *et al.,* 2015). Therefore, it is concluded the effect of LEDs lighting treatments on rice seed and seedling may be helpful to obtain higher germination and healthy seedlings in controlled environment.

As a result, the optimal illuminance of different rice varieties suitable for different periods can be studied and determined based on the photo-lightness of rice, and then the illuminating irradiance required for the rice daily cycle can be analyzed based on the daily variation of external environmental illuminance. Using rice as an example, researchers discovered that a balanced mix of light quality, illuminance, and illumination time in an artificial light environment is a significant component in improving rice seedling quality, yield, and nutritional content. The use of novel photoelectric technologies to additional plant physiological types deserves more investigation and discussion.

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