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Effect of various light-emittimg diodes on growth and photosynthetic pigments of banana (*Musa acuminata*) CV. grande naine *in vitro* plantlets

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Abstract
In vitro culture of plant species most commonly uses fluorescent lamps as a light source. Other sources of illumination, LEDs (Light-emitting diodes) have proven to be more efficient for *in vitro* culture. In the conducted experiment, the effect of various LEDs on the *in vitro* morphogenesis, proliferation of shoots, growth & rooting of Banana (*Musa acuminata*) cv. Grande Naine was observed. For this purpose, 5 different sources of light were tested under a 16-h photoperiod: fluorescent lamps (Fl), white (W), red (R), blue (B), and B/R (1:1). The proliferation rate was higher with R LEDs compared with Fl light, although shoots have a lower length under R LEDs. Under B/R LEDs, maximum shoot elongation was obtained. LEDs did not enhance the rooting of shoots but increased the photosynthetic pigments content under B/R, which contributed to the acclimation process of *in vitro* plantlets. Our results revealed that the spectrum of different light sources produced different effects during the *in vitro* cloning of *Grande naine*.

Keywords: Acclimation, *In vitro* culture, LEDs and Morphogenesis

Introduction
Banana (*Musa sapientum*) is an important fruit crop in India and provide staple food for hundreds of millions of people. The banana is one of the oldest fruits known to mankind. The global production of banana is around 102028.17 thousand tons, of which India contributes 29.19% (Post Harvest Profile of Banana, 2015). Banana is a large perennial herb with leaf sheaths that form the trunk like pseudostem. India is the world's largest producer of Banana with 13.90 million tonnes followed by Uganda (10.14 million tonnes) (Sahoo *et al*., 2015) [14]. Cultivars of *Musa acuminata*, Grande Naine or G-9 means "Large Dwarf" (Randy, 2007) [13]. The demand for banana in general and G-9 variety in particular had been on the rise throughout the world as it is one of the most commonly cultivated bananas and a source of commercial Cavendish bananas.

During present investigation, in vitro culture proliferation and root induction response in *Grand naine* as affected by various LEDs (Fluorescent lamps, red, white, blue and combination of blue and red) at the same light intensity was studied to determine the efficacy of this promising radiation sourcells, tissue and organ cultures (Abidi *et al*., 2013) [1]. Different light quality influences the plant development which refers to the different wavelengths reaching a plant's surface (Johkan *et al*., 2010) [6]. The major energy sources for photosynthetic CO2 assimilation in plants are Red (R) and blue (B) lights, hence they have the greatest impact on plant growth. During present investigation, *in vitro* culture proliferation and root induction response in *Grand naine* as affected by various LEDs (Fluorescent lamps, red, white, blue and combination of blue and red) at the same light intensity was studied to determine the efficacy of this promising radiation source.

Materials and Methods
Plant materials and growth conditions: The Grand naine explants (preferably with 1-2 shoots) from already established cultures in the tissue culture laboratory were inoculated to the MS medium containing 4 mg/l BAP and 2 mg/l Kinetin for shoot proliferation and 1/2 MS media with 1 mg/l IBA was used for root induction. After inoculation, the culture were kept at 25±20 ^{0}C in an air conditioned room with a 16 hours light period (160 µmol m−2 s−1) supplied by fluorescent tubes and 80% relatively humidity (Al- amin *et al*., 2009) [2].

Light treatments: LED lighting systems were used to control light quality. Light treatments for banana explants, proliferation and differentiation included Fluorescent lamps (Fl), red LEDs (R), blue LEDs (B), white LEDs (W) and red + blue LEDs (B/R) with photon flux density (PPFD) being set at 160 µmol m-2s-1. The experiment was independently performed three times for a randomized design of growth conditions and measurements representing the means of 15 plants (three reps consisting of five plants each) were taken.

Plant growth parameters: Banana explants were sampled after 4 weeks of light treatment after reaching an optimum stage of shoot proliferation, and root induction. Explants of each replicate and 3 replicates for each light treatment were randomly selected for growth analysis. Number of shoots per explants, shoot length and number of leaves per shoot, number of roots per explants, root length and number of leaves per root were measured.

Chlorophyll (Chl) and Carotene (Car) contents: Chl and Car contents were eluted from the second leaf fresh weight (FW) samples (0.01 g) with 5 ml of 80 % acetone at 4 ^{0}C overnight and determined using the methods by Porra *et al* [12] and Holm [5] respectively. Samples were then centrifuged at 13,000 *g* for 5 min. Supernatants were tested to determine the absorbances of Chl *a*, Chl *b* and Car in acetone as measured with a spectrophotometer at wavelengths of 663.6, 646.6 and 440.5 nm respectively. Concentrations (µg g-1 FW) of Chl *a*, Chl b and Car were determined using the following equations:

Chl *a* = (12.25 × OD663.6 – 2.55 × A646.6) × volume of supernatant (ml) / sample weight (g)

Chl *b* = (20.31 × A646.6 – 4.91 × A663.6) × volume of supernatant (ml) / sample weight (g)

Car = [(4.69 × A440.5 × volume of supernatant (ml) / sample weight (g)) – 0.267 × (Chl *a* + Chl *b*)

Statistical analysis: The raw data obtained during the experimental observations were subjected to statistical analysis as per method by Gomez and Gomez, (1984) [3]. 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. All statistical analyses were conducted using SAS 9.2 (SAS Institute; Cary, NC, USA).

Results and Discussion

Plant Growth: The effects of light quality treatments on Grand naine explants were monitored by measuring the changes in no. of shoots and roots per explants, shoot and root length, no. of leaves per shoot and root after 4 weeks of culture (30 days). A factorial experimental design with a completely randomized arrangement was used. Table 1 & 2 shows that all the measured components of growth parameters were significantly different at the 5 % level for the main effects.

Significant differences were seen among the effects of LEDs on *in vitro* shooting of banana explants as shown in table 1. Red LED showed highest number of shoots per explant (9.11) followed by Blue and White LEDs (7.09 and 6.25 respectively). Florescent lamps showed lowest number of shoots per explant (5.40). Highest shoot length and highest number of leaves per shoot was exhibited by B/R LED (2.99 cm and 8.92) followed by Blue LED and White LEDs (2.75 cm, 2.65 cm and 8.13, 655 respectively), whereas, Florescent lamps showed lowest shoot length (1.90 cm) and Red LED showed lowest number of leaves per shoot. Similar results are reported by Silva *et al*. (2014) [15] in sugarcane.

In vitro rooting of banana explants also showed significant differences among various parameters monitored under different light treatment as summarized in table 2. LEDs reduced the number of roots per explant as compared to florescent lamps. Fl showed highest number of roots per explant (10.00) and highest root length (2.13 cm) when compared with W, R, B, and B/R LEDs. Blue LED showed lowest number of roots (1.52) and root length (0.71 cm). Hence, Blue light inhibited the root development in banana explants. This agrees with the reports by Guo *et al* (2011) [4] and Nhut *et al* (2003) [12] but differs from the reports of Liu *et al* [9]. Highest number of leaves per root was calculated in B/R treatment (8.9) and lowest in Red light (5.2).

Photosynthetic pigments: ANOVA was used to uncover the main effects light quality treatment (Fl, W, R, B and B/R) on photosynthetic pigments of Grand naine variety of banana as summarized in table 3. All pigments displayed significant differences ($p< 0.05$) for the main effects.

Pigments content in leaves was influenced by different lighting environments. Total Chl was highest (38.39 µg g-1 FW) under B/R and lowest (1.87 µg g-1 FW) under Fl condition (Table 3). B/R light showed highest value for Chl a and b both (26.42 µg g-1 FW and 11.96 µg g-1 FW) whereas Chl a and Chl b was lowest under Fl (1.19 µg g-1 FW and 0.34 µg g-1 FW). LEDs can be used over florescent lamps for better pigments production in banana explants. Carotene pigment also showed the same results with various LEDs treatments with significant differences. B/R light showed higher production of Car (6.25 µg g-1 FW) and lowest under Fl (0.06 µg g-1 FW). Earlier reports of Guo *et al*. (2011) [4]; Nut *et al*. (2003) [11]; Liu *et al*. (2011) [9]; Johkan *et al*. (2010) [6]; Tanaka *et al*. (1998) [16]; Lee *et al*. (2007) [7]; and Lin *et al*. (2011) [8]. Demonstrated that blue light induces the synthesis of Chl and Car. In our study, light quality also affected photosynthetic pigments in banana explants leaves.

Table 1: Effect of different LED wavelengths on the *in vitro* formation of shoots in Grande naine after 4 weeks of culture.

Light quality	Number of shoots per explant	Shoot length (cm)	Number of leaves per shoot
Fl	5.40±0.21^{bc}	1.90±0.11^{c}	6.19±0.28^{c}
W	6.25±0.75^{b}	2.65±0.58^{ab}	6.55±0.54^{bc}
R	9.11±0.85^{a}	2.05±0.15^{c}	5.24±0.44^{c}
B	7.09±0.51^{b}	2.75±0.26^{ab}	8.13±0.59^{ab}
B/R	5.60±0.32^{bc}	2.99±0.21^{a}	8.92±0.52^{a}

Figures represents the mean±SE. Means with different letters are significantly different; Fl: Florescent lamps, LEDs: W- White, R- Red, B- Blue, B/R- Blue+Red (1:1)

Table 2: Effect of different LED wavelengths on the *in vitro* rooting in Grande naine after 4 weeks of culture.

Light quality	Number of roots per explant	Root length (cm)	Number of leaves per root
FI	10.00 ±0.24[a]	2.13±0.16[a]	6.1 ±0.28[a]
W	4.20±0.25[bc]	1.16±0.07[bc]	6.5 ± 0.54[bc]
R	5.50±0.07[c]	1.33±0.06[b]	5.2 ±0.44[c]
B	1.52±0.06[c]	0.71±0.04[c]	8.1 ±0.59[b]
B/R	*5.20±0.26[bc]*	*1.14±0.12[bc]*	*8.9 ±0.52[bc]*

Figures represents the mean±SE. Means with different letters are significantly different;
Fl: Florescent lamps; LEDs: W- White, R- Red, B- Blue, B/R- Blue+ Red (1:1)

Table 3: Effect of different LED wavelengths on the content of photosynthetic pigments in Grande naine.

Light Quality	Chlorophyll a (µg g-1 FW)	Chlorophyll b (µg g-1 FW)	Chlorophyll a+b (µg g-1 FW)	Carotenes (µg g-1 FW)
Fl	1.19±0.014[f]	0.34±0.14[f]	1.87±0.19[f]	0.06±0.06[f]
W	8.09±2.35[c]	6.51±0.25[c]	14.60±0.11[c]	1.02±0.01[c]
R	5.14±2.31[d]	6.50±0.07[d]	11.64±0.10[d]	0.78±0.01[d]
B	21.31±0.09[b]	2.23±0.06[b]	23.54±0.01[b]	2.55±0.02[b]
B/R	26.42±0.93[a]	11.96±0.26[a]	38.39±0.01[a]	6.25±0.01[a]

Figures represents the mean±SE, Means with different letters are significantly different; Fl: Florescent lamps; LEDs: W- White, R- Red, B- Blue, B/R- Blue+ Red (1:1)

Conclusion

Optimization of environmental factors are of great concern in agricultural production. Because of the long life expectancies of LEDs, their robustness, and compactness, LED lighting systems have the potential to be a very cost-effective technology to adopt. In our study, we investigated effective light quality with sufficient intensity for growing Grand naine variety of banana explants *in vitro*. Different light emitting diodes (LEDs) influenced the growth, morphology, and photosynthetic potential of banana explants. Particularly, blue light in addition to red light increased the shooting and photosynthetic pigments in leaves and may optimize the growth and development of banana in a controlled-climate setting, however further investigation could help in optimizing the culture conditions.

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