

Different Light Filters Alter Shoot-Multiplication During Micropropagation of Banana

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Received Date: 12-12-2018

Published Date: 15-3-2019

Abstract

Micropropagation is routinely carried out under controlled culture conditions with fluorescent white light for all plant species. *In vitro* multiplication of banana was carried out under different shades of light including red, blue, yellow and green along with normal fluorescent white light as control. Maximum shoot-multiplication was achieved in cultures provided with yellow light. This was higher by 103 % as compared to the control.

Keywords: micropropagation, banana, culture condition, light

Abbreviations

BA- Benzyladenine, IAA- Indole-3-acetic acid, IBA- Indole-3-butyric acid, MS-Murashige and Skoog's, PPFD-Photosynthetic Photon Flux Density, SE- Standard Error, TDZ- Thiodiazuron

Introduction

Banana is cultivated worldwide over an area of 4.8 million hectares, with an annual production of

99 million ton per annum (Anonymous 2010). The Cavendish variety, Grand naine, is a high yielding variety that produces bunches having 10 to 12 hands with 175-225 fruits (Anonymous 2012) and shows resistance to heavy wind currents due to its relatively small habit structure (Ploetz et al. 2007). The demand for tissue cultured banana plantlets increases at the tremendous rate of 25-30 % per annum in India (Anonymous 2005). This is due to the fact that tissue cultured banana provides disease-free planting material, synchronized harvest, earlier fruiting and higher yield than those produced by conventional means (Kahangi 2010).

For micropropagation protocol, the best rate of proliferation is highly desirable. In present study, the rate of shoot-proliferation was studied to find out the best suited light (colour) for shoot-proliferation.

Materials and Methods

Suckers of Grand naine variety of banana (*Musa paradisiaca* Linn.) were collected from Gujarat Green Revolution Company, Umareth, Gujarat in the month of June. They were washed under running tap-water (30 min) and trimmed to remove outer scales. The washed suckers were pretreated (20 min) with a mixture of 0.05 % of Carbendazim (Bavistin) and 0.1 % of activated charcoal on gyratory shaker (100 rpm). After thorough washing with distilled water (3 times) the apical shoots were isolated (Vora and Jasrai 2011a) for apical shoot growth and multiplication was carried out in MS medium (Murashige and Skoog 1962) containing 22.72 μ M BA, 3 % sucrose and 0.8 % Agar-Agar (Cronauer and Krikorian 1984) in culture bottles (10X5 cm). All the cultures were maintained in a culture room with 25 \pm 1 $^{\circ}$ C temperature under 16 h photoperiod (giving approx. 55 μ mol/m²/s). Monochromatic lights of different colours were provided to the cultures during 6th to

8th subculture-cycle of *in vitro* shoot-proliferation using cellophane films of different colours viz red, blue, yellow and green. Cultures provided with normal cool white fluorescent light were used as the control, simultaneously. The shoot-multiplication data was recorded after 4 weeks of incubation in terms of increase in number of shoots, shoot-length and number of leaves. Each treatment had 6 replicates and experiment was repeated 3 times. The data was analyzed for variance using a one-way ANOVA test (Zar 2003), followed by Tukey's multiple range tests at

$p \leq 0.05$. Rooting of *in vitro* derived shoots was achieved in 1/2 strength MS medium with 3mg/l IBA. Acclimatization of generated plantlets was carried out as reported earlier (Jasrai et al. 1999).

Results and Discussion

The maximum shoot-multiplication of banana was noted under yellow light (Table-1). The proliferation was higher by 103% as compared to the white fluorescent light- control (Fig. 1A)

Moreover, increase in length of shoot was also found maximum in the cultures provided with yellow light (fig 1B). This was 130% higher than the control. Number of leaves on the longest shoot was found maximum in the cultures with blue light. Under green and red light, increase in number of leaves was least (Table-1). In addition to this, yellowing of leaves was observed in the cultures provided with green light. Cultures kept in complete dark showed 35% decrease in number of shoots, 65% increase in length of shoot and 16% increase in number of leaves as compared to the control. The cultures kept in dark showed yellowish white etiolated

Shoots, with folded leaf or a pale yellow leaf at the tip (fig. 1C). In contrast, in different cultivars of banana, higher proliferation rates were recorded in dark when TDZ was used (Makara et al. 2010).

Table-1: Effect of different coloured light on *in vitro* shoot-proliferation of banana*

| Light | No. of shoots (Mean + SE) | Shoot-length(cm) | No. of leaves |
|----------------|------------------------------|------------------|---------------|
| Control | 2.60 ± 1.29 bc | 1.3 ± 1.04 a | 2.0 ± 1.41 a |
| Red | 0.30 ± 0.05 b | 1.8 ± 0.70 a | 0.6 ± 0.08 b |
| Blue | 1.00 ± 1.07 a | 2.0 ± 1.87 a | 2.6 ± 1.61 a |
| Yellow | 5.30 ± 1.20 c | 3.0 ± 0.57 b | 2.0 ± 0.00 a |
| Green | 0.30 ± 1.05 b | 1.0 ± 1.00 a | 0.6 ± 0.07 b |
| Dark | 1.69 ± 1.08 a | 2.1 ± 1.02 a | 2.3 ± 1.95 a |

*Data represents Mean ± SE and values followed by different letter(s) in a column are significantly different at $p \leq 0.05$ by Tukey's test

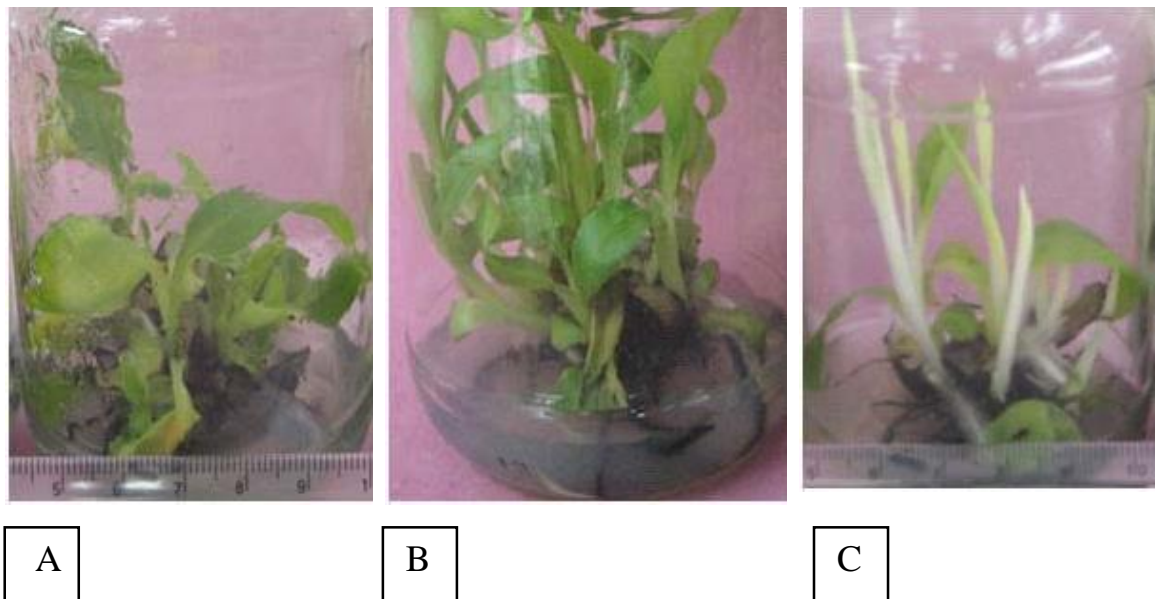


Fig. 1: *In vitro* shoot-proliferation of banana var Grand naine after 4 weeks A. Control, B. Yellow light, C. Dark (bar 1 cm)

Light is one of the most important factors affecting growth and plant regeneration. Each photosynthetic pigment has its own absorption spectrum (Devlin and Witham 1983). Transport of cytokinin depends upon light gradients, which was shown to regulate photosynthetic capacity in tobacco and *Arabidopsis thaliana* (Boonman et al. 2007). Earlier, better elongation growth in terms of shoot was revealed with yellow light (Matsumoto and Caldas 2007). In contrast, blue light treatment improved micropropagation, rhizogenesis and aerial root elongation of hybrid orchid *Cattleya* (Cybularz-Urban et al. 2007). Growth of roots was reported higher (3-4 times) under yellow-filtered light than under unfiltered light in *Arabidopsis* and tomato explants. This was noted due to improved stability of IAA in the medium (Stasinopoulos and Hangarter 1990).

Pollen germination and pollen-tube growth of *Peltophorumpterocarpum* were found to be maximum in blue light, among blue, red, green, yellow and dark conditions (Jha et al. 2011). Moreover, the influence of monochromatic spectral filter on growth and development of potato in tissue culture was reviewed (Seabrook 2005). The pioneering work of many investigators has clearly established the important role of light in the development of cultured plant tissue. The effect

of intensity and quality of light on growth and shoot initiation in tobacco callus was demonstrated (Seibert et al. 1975). Photochemical changes in culture media caused by fluorescent light from 290 to 450 nm and prevented with a yellow filter (Stasinopoulos and Hangarter 1990). Recently, light emitting diodes (LEDs) have been used as an alternative light source for plant tissue culture because of their advantages regarding wavelength specificity and narrow bandwidth with improved organogenesis in *Lilium* (Lian et al. 2002), lettuce (Hoenecke et al. 1992), *Morindacitrifolia* (Baque et al. 2010), *Calanthe* hybrid (Baque et al. 2011). In addition to this, for improving *in vitro* shoot-growth and rooting of *Anthuriumandraeanum*, the use of complex of red plus blue LED was suggested (Aisu et al. 2012). Even for banana, combined LED with red (80 %) and blue (20 %) light was reported to improve *in vitro* growth (Nhut et al. 2002). Moreover, red wavelength LED caused higher frequencies of somatic embryo germination and longer roots in pine than the standard cool white fluorescent light treatment (Merkle et al. 2005). The effect of Chopper-light system producing intermittent light with common fluorescent tubes on *in vitro* shoot-multiplication of *Amelanchierlamarckii* and *Tiliacordata* was studied at three different PPF (Pinder and Oellerich 2007).

Banana is the most important commercial crop, which is being highly proliferated through micropropagation technique. For commercial purpose, higher rate of proliferation as well as cost-effective ways of banana micropropagation (Vora and Jasrai 2012; Vora 2011b) are always favoured by the tissue culture units. In conclusion, proliferation of *in vitro* raised banana shoots can be increased using yellow light.

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