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EFFECT OF ADENINE SULPHATE, AGAR AND LIGHT ON *IN VITRO* MULTIPLICATION OF BANANA CV. GRAND NAINE (AAA)

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ABSTRACT
In the current study, the effect of few important factors such as adenine sulphate, agar and light/dark regime affecting multiplication ratio has been researched in Banana Cv. Grand Naine using modified MS medium. Among various levels (20, 40, 60 and 80 mgL^{-}) of adenine sulphate tested, cultures on medium containing 40mgL^{-} adinine sulphate showed highest multiplication ratio of 2.3 times along with well formed shoots in 22-25 days of incubation. In the agar levels (5.5, 6.0, 6.5, 7.0, 7.5 and 8.0 gmL^{-}) tested, cultures on medium gelled with 6gL^{-} of agar showed highest multiplication ratio of 2.5 times in 22-25 days. Further, in the four light/dark regimes (Light (16/8), Dark, Light/dark and Dark/light) tested, cultures incubated under light showed highest multiplication ratio of 2.5 times in 22-25 days with strong shoots without blackening of base. However, cultures incubated in dark showed a multiplication ratio of 2.0 in 15-18 days of incubation but explants were brittle in nature and found difficult to handle during subculture to next cycle. These results would be helpful in increased production of planting material through *in vitro* multiplication of Banana cv. Grand naine or can be used in further studies for standardization of new protocols.

INTRODUCTION

Bananas and plantains (*Musa* spp.) are important crops in the global fruit industry. Banana accounts for approximately 22 per cent of the world fresh fruit production and is ranked as the second most important fruit crop and makes 39.8 per cent of Indian fruit production (Anon., 2012). The banana cultivars Robusta, dwarf cavendish and grand naine of Cavendish subgroup (*Musa* spp. 'AAA') of banana are very popular because of high yield, wide market acceptability and high economic returns per unit area. Of late, banana is overtaking other fruit crops in production and productivity. Consequently, there is huge demand for quality planting material of the mentioned banana cultivars and major part of the requirement is being met through *in vitro* multiplication, a modern biological technique. Apart from the higher and faster multiplication rates, micropropagated banana plants have many advantages like regular availability of planting material, earliness, synchronized blooming and comparatively higher yields. Hence, there is huge scope for increase in production of good quality planting material of banana. Though several researchers have reported the regeneration of *Musa* spp. *via* micropropagation (Robert *et al*., 2013; Roels *et al*., 2005; Venkatachalam *et al*., 2007), the protocols are not commercially viable due to low multiplication ratio. It appears, identification of healthy mother stocks, standardization of protocols with high multiplication ratio, contamination check, skilled operators, efficient hardening techniques etc., are challenges still faced by banana tissue culture industry. Furthermore, inclusion of adenine sulphate (Lohidas and Sujin, 2015), addition of optimum level of agar (Kacar, 2010) for solidification of culture medium and incubation under proper light/dark regime (Nisyawati and Kariyana, 2013; Makara, 2010) has played major role in few of earlier studies on *in vitro* multiplication of banana. In spite of this, more and more studies on finding out effect of such factors would help in better understanding of their impact and also helps for inclusion of optimum levels of them for standardization of best *in vitro* multiplication protocol in banana. In view of this, in the present study, an effort has been made to find out the effect adenine sulphate, agar and light/dark regime on *in vitro* multiplication ratio in Banana Cv. Grand Naine.

MATERIALS AND METHODS

The explants (clumps having 2-3 shoots obtained from established aseptic culture in our commercial laboratory) were inoculated aseptically as per methodology given by Uzaribara *et al*., (2015) in MS Medium (Murashige and Skoog, 1962) containing 30-gL- sucrose and gelled with 8 gL- of tissue culture grade agar (Biolab india Pvt. Ltd, India). The MS medium was supplemented with 6-benzyl amino purines (BAP) at 4 mgL-. The pH of the medium was adjusted to 5.8 ± 0.1, 30-35ml of medium was dispensed into pre sterilized 250ml glass bottles, autoclaved in steam sterilizer (Nat steel Pvt. Ltd., India) at 121°C and 15lbs for 18 minutes and stored in media storage room at ambient temperature for 7-10 days before usage. To study the effect of factors, various levels of 1) adenine sulphate (Himedia) at 20, 40, 6 and 80 mgL- , 2) agar (tissue culture grade, BioLab India Ltd) at 5, 5.5, 6.0, 6.5, 7.0, 7.5 8.0 gL- and 3) light and dark incubation treatments [light- 16/8 hour light/dark regime; dark- subculture to subculture kept in complete

darkness, light/ dark: 15 days in 16/8 hour light regime and 15 days in complete darkness; dark-light: 15 days in complete dark and 15 days 16/8 hour light regime] were imposed and observations were recorded on health of explants, multiplication ratio (calculated based on number of bottles of cultures obtained from subculture of one bottle of culture, clump size was maintained as mentioned above) and number of days taken for sub culture. Cultures were incubated at 24 ± 1°C under 16 hour cool white, fluorescent light (4000 lux) and 6 hours darkness (Dooley, 1991).The statistical analysis is carried out using ANOVA for finding out significance of treatments.

RESULTS AND DISCUSSION

Tissue cultured plantlets showed less variability, time taken for flowering and recorded an increase in yield compared to plants from suckers (Sheela and Nair, 2001). Hence, production and supply of large scale good quality planting material through tissue culture is need of the hour which is possible through assessing various factors affecting *in vitro* multiplication.

Effect of adenine sulphate

Adenine sulphate is a building block of life, which involves in synthesis of Protein, DNA and RNA. The effect of adenine sulfate is known in the tissue cultures at many plant species and types of vegetal tissues, effect that is superior in combination with a balanced dose of cytokinine and auxine (Zapartan, 2001). In the present study, various adenine sulphate concentrations used in culture medium significantly affected the multiplication ratio of Banana cv. Grand nine. In range of 20-80 mgL- of adenine sulphate included in culture medium, 20mgL- found suboptimal (Fig. 1A), 40 mgL- was found optimum to show highest multiplication ratio of 2.3 times, better plant health and number to taken less (25-28 days) number of days for subculture (Table 1, Fig. 1B) and further increase in adenine sulphate to 60 and 80 mgL- did not improve any of these parameters. In various earlier studies, adenine sulphate was used in range of 0-150 mgL- and it was found beneficial in enhancing multiplication ratio in Banana (Deo and Pradhan, 2015). Our results are in line with other studies with respect to effect of adenine sulphate on banana *in vitro* multiplication (Venkatachalam *et al*., 2007).

Effect of agar

Agar is a solidification agent used in tissue culture medium. Kaçar *et al*. (2010) has reported that the agar strength in culture medium was very important to obtain high multiplication ratio in banana. In the present study, various agar concentrations used for solidification of culture medium significantly affected the multiplication ratio of Banana cv. Grand nine. Among different levels of agar used, 5.5 mgL^{-} was not enough to obtain proper solidification and 7 mgL^{-} and above imparted little hardens than required in the culture medium which led

Table 1: Effect of adenine sulphate on *in vitro* multiplication of Banana cv. Grand Naine

ADS (mgL-)	Average Multiplication ratio	Health of tissue	Duration for subculture
20	1.3	+	30-33
40	2.5	+ + +	22-25
60	2.0	+ +	25-28
80	1.6	+ +	28-30
SEM	0.03	-	-
CD (0.01%)	0.10**	-	-

+: poor, + +: good, + + +: very good; ** -Highly significant

Table 2: Effect of concentration of agar in medium on *in vitro* multiplication of Banana cv. Grand Naine

Agar(mgL-)	Average Multiplication ratio	Strength	Duration for subculture	Plant Health
5.5	2.5	Thin	22-25	+ +
6.0	2.3	Optimum	22-25	+ + +
6.5	2.2	Optimum	25-28	+ + +
7.0	2.0	Optimum	25-28	+ +
7.5	1.8	Hard	28-30	+
8.0	1.3	Hard	30-32	+
SEM	0.02	-	-	-
CD(0.01%)	0.07**	-	-	-

+: poor, + +: good, + + +: very good; ** -Highly significant

Table 3: Effect of light on *in vitro* multiplication of Banana cv. Grand Naine

Incubation	Average Multiplication ratio	Growth	Duration for subculture	Plant Health
Light	2.5	+ +	22-25	+ + +
Dark	2.0	+ + +	15-18	+ + +
Light-dark	1.6	+ + +	18-20	+ +
Dark-light	2.2	+ +	20-22	+ +
SEM	0.02	-	-	-
CD(0.01%)	0.05**	-	-	-

+: poor, + +: good, + + +: very good; ** -Highly significant

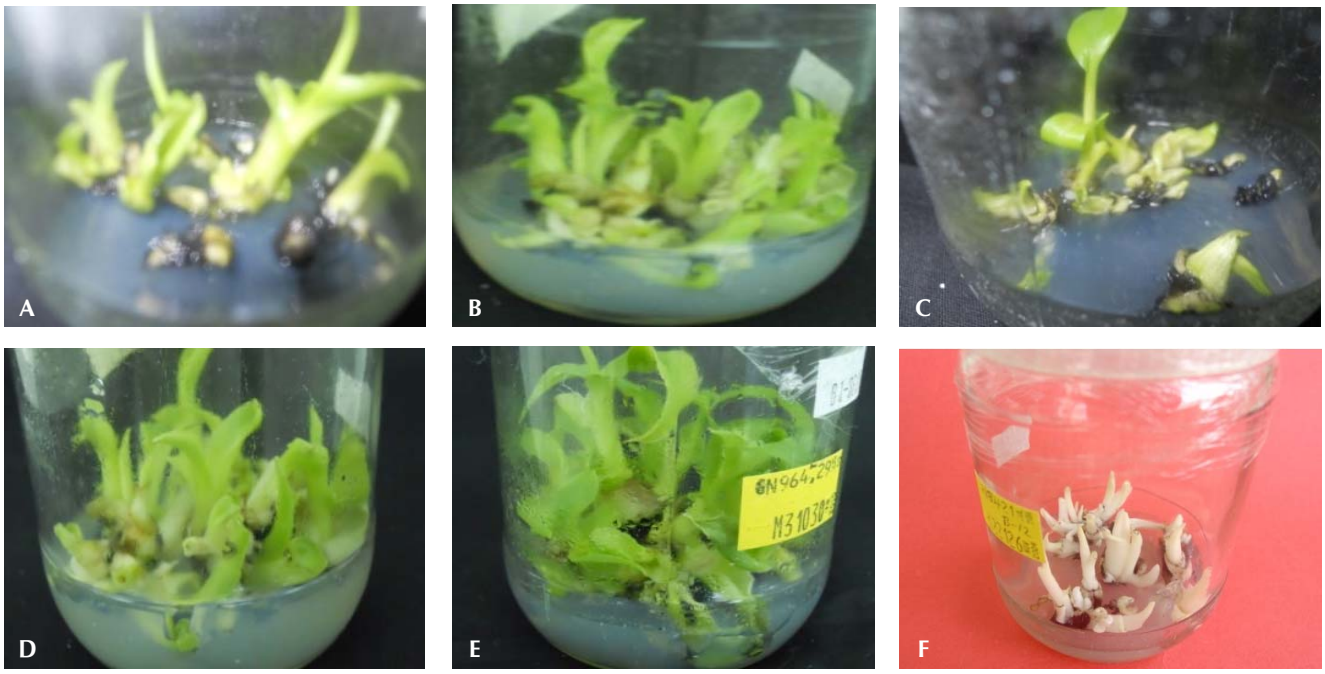

Figure 1: *In vitro* multiplication response of banana cv. Grand Naine cultured on MS medium containing A) 20 mgL- B) and 40 mgL- ADS; *In vitro* multiplication response of banana cv. Grand Naine cultured on MS medium solidified with C) 8 mgL- D) 6 mgL- agar; *In vitro* multiplication response of banana cv. Grand Naine on MS medium incubated in E) Light and F) dark

to blackening of base of explants and reduced multiplication ratio (Fig. 1C). The blackening is may be induced phenolics in the clumps. The growth of was reduced which may be due to hampered uptake of nutrients due to blackening at base. Ghashigaie *et al*., (1991) has reported that the absorption of cytokinin and mineral nutrient from the medium was reduced at high gelling agent concentration. In this study 6 mgL- was found optimum to show high multiplication ratio of 2.5 in 22-25 days with good outgrowth of cultures (Table 2, Fig. 1D). Similar observations have been made in banana *in vitro* studies by Kacar *et al*. (2010). Further, Cronauer and Krikorian (1986) reported that multiple shoots of banana and plantain could be produced from sliced meristems on either an agar or in liquid medium. But in the present investigation, shoot meristems were cultured on agar (semisolid) medium only and it was found that the solidification strength of medium has an impact on multiplication ratio, plant health as well as days taken to subculture.

Effect of light

Light is an essential component for plant growth and it plays an important role in the regeneration of shoots (Makara, 2010). Usually incubation at16 hours light and 8 hours dark regime will be maintained for *in vitro* plant cultures. However, in many earlier studies incubation in different light/dark regimes was advantageous (Dooley, 1991). In the present study, various light/dark regimes significantly affected the *in vitro* multiplication ratio of Banana cv. Grand nine. Among four types of incubation treatments imposed, continuous light incubation (Fig 1E) has shown high multiplication ratio, better shoot growth and health (Table-3; Fig 1F). Cultures incubated in complete dark till next sub culture showed fast shoot growth and less multiplication ratio and other light/dark regime treatments imposed were not advantageous for improving multiplication ratio. Further, shoot height was more in dark incubated cultures. The results of present study are in line with studies on *in vitro* multiplication ratio in banana cv. Dwarf cavendish (Buah, 2016). In the contrary to the results of present study, Makara et al., (2010) reported that dark conditions enhanced shoots growth than light conditions in banana in cultivars Gros Michel, Bwara and Sukalindizi suggesting that banana *in vitro* culture is a photomorphogenically process. Although light may be essential for plant development, darkness is also beneficial for plant morphogenesis. Further, high multiplication ratio under dark condition has also been reported by Nisyawati and Kariyana, (2013). Similarly, In this study also the cultures incubated in dark showed a multiplication ratio of 2.0 in 15-18 days. However, the explants kept in dark were brittle during subculture to next cycle.

In conclusion, the present investigation showed that 40 mgL- of adenine sulphate, 6mgL- of agar and incubation at 16/8 hour light/dark regime are optimum for getting high multiplication ratio in Banana cv. Grand naine. Nevertheless, the results should help directly in production of large scale planting material through *in vitro* multiplication or in further standardization of high multiplication ratio protocol in the same banana cultivar.

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