International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2017; 5(5): 2107-2110

Received: 24-07-2017
Accepted: 25-08-2017

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Effects of Sub-culturing on *In Vitro* Shoot Multiplication in Banana Cultivar "Grand Naine"

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Abstract
Present study describes the effects of sub-culturing on *in vitro* shoot multiplication in plantlets of banana *cv.* Grand Naine growing on basal MS and CHU media fortified with different concentrations of BAP and Kinetin. Maximum number of shoots was regenerated on full-strength basal MS or CHU medium when fortified with 2.0 mg/L BAP, whereas, the minimum number of shoots recorded on both culture media with 3.0 mg/L Kinetin. The results reported in the current study conclude that the successive behavior of *in vitro* shoot multiplication with the sub-culture cycles indicate the continuity of higher shoot multiplication rates at commercial point of view for banana cultivation.

Keywords: Culture media, Cytokinin, *Musa*, Shoot multiplication

Introduction
Banana (*Musa paradisiaca* L.) is a large herbaceous perennial monocotyledonous and monocarpic fruit crop plant. It is a member of family *Musaceae* of order *Scitamineae*. "Apple of Paradise" *i.e.* Banana governs its antiquity in Indian sub-continent from the ancient periods of Ramayana and Kautillya's Arthashastra. Supportive evidences of its mythological importance can also be seen in painting and sculptures of Ajanta and Ellora caves (600 BC). The edible banana is believed to have originated in the tropical regions of South East Asia from *M. accuminata* and *M. balbisiana*. India is considered to be one of the centers of origin of *M. paradisica* as *M. balbisiana* is of Indian origin (Stover and Simmonds, 1987) [1]. It is interwoven in Indian cultural heritage as its plant along with leaves and fruits are auspicious in all the festive occasions, be it a social function or worship of God. Banana is conventionally propagated through vegetative means and rarely by seeds (especially for banana breeding, ornamental types and wild species). In the recent years, tissue culture techniques especially micropropagation have been standard methodology for commercial banana plantations, primarily because of the advantage of starting with disease-free planting materials (Cronauer and Krikorian, 1984; Jarret *et al.,* 1985; Ali *et al.,* 2011; Keshari B, Pradhan, 2016) [2, 3, 4, 5]. The rate of shoot multiplication under *in vitro* conditions is a major factor that is critical for micropropagation efficiency in any plant species. The studies on the effects of different plant growth regulators on high frequency *in vitro* shoot multiplication during sub-culture cycles seems to be essential requirements for the reliability of an efficient micropropagation protocol. Therefore, the present study was undertaken to evaluate the effects of sub-culture cycles on *in vitro* shoot multiplication rates in banana cultivar "Grand Naine".

Material and Methods
Plant Materials
In the present study, healthy and disease-free sword suckers excised from field grown banana *cv.* Grand Naine were used as explants for aseptic *in vitro* establishment of plantlets on culture media under optimal growth conditions. The present study was carried out at the Tissue Culture Laboratory, Department of Horticulture, Sardar Vallabhbhai Patel University of Agriculture & Technology, Modipuram, Meerut, Uttar Pradesh, India during 2015-16.

Culture Establishment and Shoot Multiplication
For aseptic culture establishment, the excised sword suckers were washed thoroughly under running tap water for 30 min and rinsed for a minimum of three times by sterilized distilled

water and then the outer layer was removed carefully with the help of a sharp stainless steel knife. After that, the sword suckers were soaked in 100% Clorox and few drops of Tween-20 [Hi-Media, Mumbai, India] solution for 15-20 minutes under the hood of laminar air flow. All explants were rinsed with pre-sterilized double distilled water for three to four times followed by soaking subsequently in 0.1% mercuric chloride [Qualigens, Mumbai, India] for 2 min, 0.1% bavistin [Biostadt India Limited, Mumbai, India] for 2 min and 70% ethanol [Hi-Media, Mumbai, India] for 2 min. Finally, all the suckers were rinsed by pre-sterilized double distilled water for three to four times. After that, the explants were trimmed with the help of a fine surgical blade and cultured on basal MS (Murashige and Skoog, 1962) [6] media fortified with the different concentrations of cytokinins *i.e.* BAP and Kinetin [Hi-Media, Mumbai, India] to investigate the effects of sub-culturing on *in vitro* shoot multiplication in banana *cv.* Grand Naine. After explant establishment on culture media, enough mother stock of multiplied shoots was generated on basal MS media with 2.0 mg/L BAP, 100 mg/L myo-inositol [Hi-Media, Mumbai, India] and 30 g/L (w/v) sucrose [Hi-Media, Mumbai, India] for sub-culturing experiments. The culture media pH was adjusted to 6.0 with few drops of either 1*N* NaOH [Merck, Mumbai, India] or 1*N* HCl [Qualigens, Mumbai, India] prior to the addition of 0.8% (w/v) bacteriological grade agar [Hi-Media, Mumbai, India]. All the required glasswares such as test tubes, jam bottles and distilled water were autoclaved at 15 psi pressure and temperature of about 121°C for 20 minutes. The hood of laminar air flow was wiped with 70% ethanol [Jiangsu Huaxi International Limited, China] followed by sterilization with UV radiation for minimum 30 minutes prior to explant surface sterilization, preparation and inoculation on culture media. All inoculated explants were incubated in a culture room at 25±1ºC with ~60% relative humidity and illuminated by white fluorescent tube lights providing the light intensity of 4000 lux for 16h/8h light/dark photoperiods. Culture contamination and plantlet survival was carefully observed regularly at weekly time intervals and any types of contaminated cultures were discarded immediately.

Statistical Analysis

All the experiments were conducted in a Complete Randomized Design (CRD) with twelve replications (n=12) per treatment and repeated thrice. The data on shoot multiplication was recorded after each subculture upto four subculture cycles. Each sub-culturing was done after 8-weeks of shoot inoculation on multiplication media. The recorded data were subjected to analyze as per the experimental design. The analysis of variance (ANOVA) of different data records was performed and the data means were compared by using SPSS version 16.0 (SPSS, Chicago, USA) followed by Duncan's New Multiple Range Test (DMRT) at $p < 0.05$

Results and Discussion

Cytokinins have also known to induce high frequency *in vitro* shoot regeneration and multiplication in plants including banana (Buah *et al.,* 2000 & 2010) [7,8]. Present investigation also exhibited the potential of cytokinins to induce high frequency *in vitro* shoot regeneration in studied banana cultivar. The study showed that maximum number of shoots (2.33) was produced in the medium containing 2.00 mg/L BAP followed by 2.00 mg/L Kinetin and the minimum (1.66) was recorded in almost all rest of the treatments at 1st sub-culturing of banana *cv.* Grand Naine. At second sub-culturing, the maximum shoots (7.00) were recorded under the treatment of BAP 2.00 mg/L followed by 5.00 and 4.00 with the treatments of 1.00 mg/L BAP, 3.00 mg/L BAP and 1.00 mg/L Kinetin (both the same) while the minimum (3.00) was noted under 2.00 mg/L Kinetin. Further, at 3rd sub-culturing the maximum shoots (26.33) were recorded under the treatment of 2.00 mg/L BAP followed by 15.66 and 14.66 with the treatments of 1.00 mg/L Kinetin and 1.00 mg/L BAP, however, they were significantly at par to each other. The minimum (9.66) was noted under the treatment of 2.00 mg/L Kinetin. Again, at 4th sub culturing the maximum shoots (117.33) were recorded under the treatment of 2.00 mg/L BAP followed by 102.66 and 50.66 with the treatments of 1.00 mg/L BAP and 1.00 mg/L Kinetin, while the minimum (18.66) was noted under treatment 3.00 mg/L Kinetin under MS medium [Table-1]. Similarly on CHU media, maximum shoots (1.66) were produced in the medium containing 1.00 mg/L Kinetin followed by 1.33 at 2.00 mg/L BAP and 2.00 mg/L Kinetin, while the minimum (1.00) was recorded in almost all rest of the treatments at 1st sub culturing of banana *cv.* Grand Naine. At second sub-culturing the maximum shoots (3.33) were recorded on 1.00 mg/L Kinetin followed by 3.00 and 2.33 with the treatments of 2.00 mg/L BAP, 2.00 mg/L and 3.00 mg/L Kinetin (both the same), while the minimum (1.66) was noted at 1.00 mg/L BAP. Further, at 3rd sub-culturing the maximum shoots (15.66) were noted under the treatment of 2.00 mg/L BAP followed by 14.33 and 12.33 with the treatments of 3.00 mg/L BAP and 2.00 mg/L Kinetin. The minimum (10.00) shoots were produced when BAP used at 1.00 mg/L concentration in culture media. Again at 4th sub-culturing, the maximum shoots (51.66) were recorded under the treatment of 2.00 mg/L BAP followed by 30.00 and 26.00 with the treatments of 1.00 mg/L BAP and 3.00 mg/L BAP, while the minimum (14.00) was noted under treatment 3.00 mg/L Kinetin under CHU medium [Table-2]. The effect of different concentrations of BAP (1.00, 2.00, 3.00 mg/L) and Kinetin (1.00, 2.00, 3.00 mg/L) on CHU media exhibited lowest results as compared to MS media and the BAP concentrations was produced highest shoots/cluster and overall average best results were performed with BAP on both MS and CHU media. The same media concentrations were also produced a considerably good numbers of shoots at 3rd sub-culture at both MS and CHU media. The same pattern of observations were reported by Aish *et al.* (2004) [9], Muhammad *et al*. (2004) [10], Buah *et al.* (2010) [8], Bhosale *et al.* (2011) [11], Imran *et al.* (2012) [12] and Devendrakumar *et al.* (2013) [13].

Table 1: Effects of sub-culturing on *in vitro* shoot multiplication in banana plantlets growing on basal MS media fortified with different concentrations of BAP and Kinetin.

Treatments	Total number of shoots produced at 1st subculture	Total number of shoots produced at 2nd subculture	Total number of shoots produced at 3rd subculture	Total number of shoots produced at 4th subculture
BAP 1.0 mg/L	1.667b	5.000b	14.667bc	102.667a
BAP 2.0 mg/L	2.333a	7.000a	26.333a	117.333a
BAP 3.0 mg/L	1.667b	4.000bc	10.333d	20.333c
Kinetin 1.0 mg/L	1.667b	4.000bc	15.667b	50.667b
Kinetin 2.0 mg/L	2.000a	3.000c	9.667d	39.000b
Kinetin 3.0 mg/L	1.667b	3.333bc	12.333cd	18.667c
Gen. Mean	1.833	4.389	14.833	58.111
C.V.	28.748	21.482	11.677	16.987
F Prob.	0.546	0.003	0.000	0.000
S.E.M.	0.304	0.544	1.000	5.699
C.D. @ 5%	--	1.677	3.081	17.561
C.D. @ 1%	--	2.351	4.320	24.619

Data represents here, the mean values of 12 replicates per treatment in three repeated experiments. Each mean values followed by the same letter does not differ significantly according to Duncan's multiple new rang test ($p \leq 0.05$).

Table 2: Effects of sub-culturing on *in vitro* shoot multiplication in banana plantlets growing on basal CHU media fortified with different concentrations of BAP and Kinetin.

Treatments	Total number of shoots produced at 1st subculture	Total number of shoots produced at 2nd subculture	Total number of shoots produced at 3rd subculture	Total number of shoots produced at 4th subculture
BAP 1.0 mg/L	1.000b	1.667d	10.000b	30.000b
BAP 2.0 mg/L	1.333ab	3.000b	15.667a	51.667a
BAP 3.0 mg/L	1.000b	2.667bc	14.333a	26.000bc
Kinetin 1.0 mg/L	1.667a	3.333a	9.667b	21.667cd
Kinetin 2.0 mg/L	1.333ab	2.333bc	12.333ab	20.000cd
Kinetin 3.0 mg/L	1.000b	2.333bc	10.333b	14.000d
Gen. Mean	1.222	2.556	12.056	27.222
C.V.	33.402	26.087	18.131	16.129
F Prob.	0.315	0.110	0.024	0.000
S.E.M.	0.236	0.385	1.262	2.535
C.D. @ 5%	--	--	3.889	7.811
C.D. @ 1%	--	--	5.451	10.950

Data represents here, the mean values of 12 replicates per treatment in three repeated experiments. Each mean values followed by the same letter does not differ significantly according to Duncan's multiple new rang test ($p \leq 0.05$).

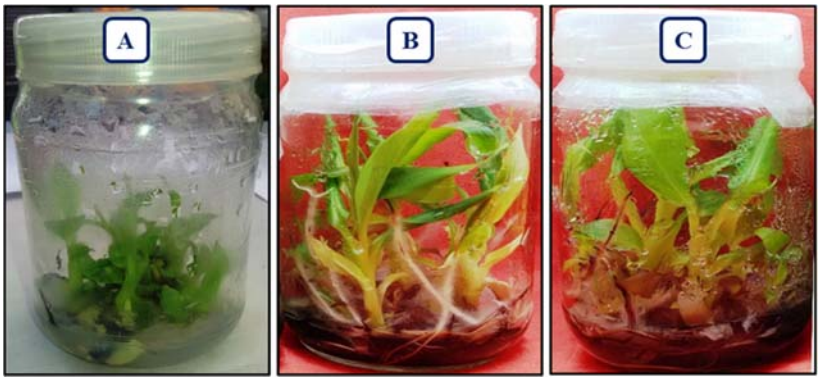

Fig 1: Effects of sub-culturing on *in vitro* shoot multiplication in banana plantlets growing on basal MS media supplemented with 2.0 mg/L BAP. Here, (A) Shoot multiplication after first sub-culturing, (B) Shoot multiplication after second sub-culturing and (C) Shoot multiplication after third sub-culturing.

Fig 2: Effects of sub-culturing on *in vitro* shoot multiplication in banana plantlets growing on basal CHU media supplemented with 2.0 mg/L BAP. Here, (A) Shoot multiplication after first sub-culturing, (B) Shoot multiplication after second sub-culturing and (C) Shoot multiplication after third sub-culturing.

Conclusion

The results reported in the current study conclude that the successive behavior of shoot multiplication with sub-culture cycles indicate the continuity of higher multiplication rates at commercial point of view for banana cultivation.

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