International Journal of Agriculture, Environment and Biotechnology
Citation: IJAEB: 8(4): 943-950 December 2015
DOI Number: 10.5958/2230-732X.2015.00107.2

Horticulture

High Frequency *In Vitro* Cloning of Banana (*Musa acuminata*) cv. Grande Naine

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Paper No.: 390 Received: 14 Jan 2015 Accepted: 29 Dec 2015

Abstract

A study was carried out at the Biotechnology-cum-Tissue Culture Centre, OUAT, Bhubaneswar, India, to standardise a viable protocol for the "High frequency *in vitro* cloning of Banana (*Musa acuminata*) cv. Grande Naine" during the year 2014. This has helped to determine the best media compositions for shoot multiplication and rooting of the variety Grande Naine, so as to get optimum results with a minimized cost of production. The result revealed that MS medium supplemented with 5.0 mg/l BAP and 1.0 mg/l IAA was found to be a most potent combination and produced a significantly higher number of shoots/explants (4.47) after 30 days of culturing. The same combination recorded significantly higher number of shoots/explants, i.e., 5.97 and 6.13 in 1st and 2nd sub-culturing respectively.MS medium fortified with 2.0 mg/l BAP and 1.0 mg/l IBA was found to be ideal for the early shoot elongation attending height of 3.52 cm with more number of leaves/plants (4.2) and produced more number (5.4) of longer roots (4.47 cm) roots after 30 days of inoculation. Approximately 1308 number of Tissue culture plantlets/explant can be produced by following this protocol.

Highlights

- Protocol for the "High frequency *in vitro* cloning of Banana (*Musa acuminata*) cv. Grande Naine" was developed.
- Approximately 1308 number of Tissue culture plantlets/explant can be produced by following this protocol.

Keywords: Tissue culture, inoculation, multiplication, proliferation

Bananas (*Musa* spp.) are the 4th most valuable food commodity after rice (*Oryza sativa*), wheat (*Triticum aestivum*) and milk. It is considered as a poor man's crop in tropical and subtropical countries (Jain and Swennen, 2004). Bananas have a worldwide production of 74 million t/year, 34% being from Africa (Swennen *et al.* 1991). It is the world's most widely distributed fruit, eaten either raw or cooked and may be processed into Starch, Chips, Puree, Beer, Vinegar, or dried products. Banana fruits have a very high content of potassium (K) and a wide K/Na ratio, imparting a protective effect of K against excessive Na intake in diets (Srinivas *et al.* 2006).

Almost all modern edible parthenocarpic banans come from two wild species –*Musa acuminata* and *Musa balbisiana*. Grande Naine (also spelled Grand Nain) is a banana cultivar of *Musa acuminata*. It translates from French meaning "Large Dwarf" (Randy , 2007). Tissue culture technology offers many advantages besides being pest and disease free. Compared to conventional planting material, tissue culture plants give higher yield, and earlier and more efficient sucker production (Dharamapalan *et al.* 2013). Tissue culture plants are uniform, allow for mass production in relatively short periods of times, and are available all-year round:

important criteria for commercial farming. Rapid and easy mass production also allows for facilitated distribution of improved cultivars, and can compensate for planting material shortage (George and Manuel, 2013). However, during the early transplanting stages banana tissue culture plantlets need higher levels of care and attention than conventional planting material. The high-value crop var. Grande Naine (*Musa acuminata*) is propagated vegetative through mother rhizomes and suckers. The rate of multiplication is very slow as a plant produces only 4-5 suckers in a year. The underground structures are exposed to natural disasters, pests and pathogens, and thus the risk of spreading infection is more. Besides, higher cost of rhizomes production and tedious method of transporting high volume of planting material are constraints faced by the grower. Considering the problems associated with this important crop, it is essential to find out an alternate method of propagation for by-passing the slow rate of multiplication, reduce the cost of transportation and bulking up true-to-type disease available stocks of high yielded new cultivars within a shorter period. Keeping in view the importance the study, has been made to find out the Impact of plant bioregulators on shoot multiplication, proliferation and standardization of media supplements for shoot elongation and root formation.

Materials and Methods

The investigation was carried out at the biotechnology-cum- Tissue Culture centre, OUAT, Bhubaneswar. Healthy and disease free plants of Banana *(Musa acuminata*) cv. Grande Naine was maintained in the Mother Gene Block of Biotechnology-cum- Tissue Culture Center, OUAT, Bhubaneswar, for conducting *in vitro* research and commercial plantlet production in the laboratory. The explants for this experiment were taken from a pre-established $1/8^{th}$ initiated shoot culture of var. Grande Naine maintained in the Tissue Culture Laboratory. The chemicals used for the present study were analytical reagents of excel R grade of Merck (India), Qualigen Fine Chemicals, and Himedia Laboratories Ltd. (India). Auxins, Cytokinins, Myo-inositol and Fe-EDTA were from Sigma (USA) and Agar from Himedia Lab Ltd (India). For the preparation of MS culture medium (Murashige and Skoog, 1962) required quantities of macronutrients, micronutrients, Fe-EDTA, vitamins and plant bioregulators were taken from the stock solution and required quantity of sucrose dissolved in water was added fresh to the medium. The pH of the solution was adjusted to 5.7+ 0.1 using 0.1N NaOH or 0.1 N HCL. Then volume was made up to 1 liter with distilled water. Agar (0.6% w/v) was added to the medium boiled and poured to the culture bottles and capped. Capped culture bottles containing culture medium were autoclaved for the 20 minutes at 121°C and 15 Psi pressure. The autoclaved medium was kept in a laminar air flow bench for cooling. All the glassware were dipped in the detergent solution for overnight and washed under running tap water. They were rinsed with distilled water and then dried in an oven for 2hrs at 150°C. Forceps, petridishes and scaples were thoroughly cleaned with isopropanol or rapped with paper and kept in a clean sterilized in autoclave at 15 psi and 121°C for 20 minutes. The working chamber of laminar air flow cabinet was wiped with isopropanol. Filtered air (80-100 cft/min) to ensure that particles do not settle in working area was blown for 5min. The sterilized materials to be used (except living tissue) were kept made the chamber and exposed to UV light for 30 minutes. While working, filtered air was continuously passed the laminar air flow cabinet. The culture were kept at 25±2°C in an air conditioned room with a 16 hours light period (3000-3200 lux) supplied by fluorescent tubes and 80% relatively humidity. The explants (preferably with 1 shoot initial)were carefully transferred to the MS medium containing a different concentration of cytokinins BAP (2.5,3.0,3.5,4.0, 4.5,5.0,5.5 and 6.0 mg/l) and in combination with Auxin IAA (0.5 and 1.0 mg/l) for shoot proliferation study for one month. Then the shoots were sub-cultured twice at an interval of one month. The observation was recorded on a number of multiple shoot explant and length of shoot. Shoot in the multiplication media were severed to 1 cm height and were carefully transferred to MS medium containing different concentrations of BAP2.0,2.5, 3.0,3.5,4.0 and 4.5 mg/l in combination with IBA (0.5 and 1.0 mg/l) and BAP (2.0 and 4.0 mg/l) with GA_3 (0.5 and 1.0 mg/l) and IBA (0.5 and 1.0 mg/l). Three replications per treatment and three culture bottles per replication were marked for observation of 19 and 17 different treatments. All the experiment were designed in completely randomized design (CRD) and replicated thrice. The data observed were subjected to statistical analysis as suggested by Gomez and Gomez (1984). The analysis of variance (ANOVA) table was prepared. The treatment effects were tested by F-test at 5% level of significance. The critical difference at 5% level was calculated for comparing the treatment means.

Results and Discussion

Shoot proliferation in the present course of the investigation was carried out to visualize the Impact of plant bioregulators on shoot proliferation and standardization of media supplements for shoot

Table 1: Impact of plant Bioregulators on shoot proliferation of banana cv Grande Naine

Treatments		Shootproliferation Culturing (1 Month)		1st Subculturing After (1month)		2nd Subculturing After (1month)	
Label	Composition	No of Shoot multiplication	Length of the root (cm)	No of multiples shoot	Length (cm)	No of Multiple Shoot	Length of shoot (cm)
T1	MS	1.07	0.53	1.13	0.67	1.57	0.93
T2	MS+2.5mg/l BAP+0.5mg/l IAA	1.40	0.70	2.10	0.87	2.60	1.47
T3	MS+2.5mg/l BAP+0.5mg/l IAA	1.67	0.77	2.43	1.03	2.83	1.63
T4	MS+3.0mg/l BAP+0.5mg/l IAA	2.13	0.83	2.67	1.10	3.00	1.87
T5	MS+3.5mg/l BAP+0.5mg/l IAA	2.23	0.90	2.83	1.17	3.10	2.10
T6	MS+4.0mg/l BAP+0.5mg/l IAA	3.13	1.03	2.37	1.80	3.77	2.20
T7	MS+4.5mg/l BAP+0.5mg/l IAA	3.30	1.17	4.23	2.10	4.87	2.93
T8	MS+5.0mg/l BAP+0.5mg/l IAA	3.57	1.27	5.43	2.53	5.63	3.50
T9	MS+5.5mg/l BAP+0.5mg/l IAA	3.27	1.27	5.33	2.60	5.47	3.53
T10	MS+6.0mg/l BAP+0.5mg/l IAA	3.20	1.30	5.23	2.70	5.40	3.70
T11	MS+2.0mg/l BAP+1.0mg/l IAA	1.60	0.80	2.20	1.03	2.63	1.60
T12	MS+2.5mg/l BAP+1.0mg/l IAA	1.87	0.83	2.53	1.43	2.87	1.80
T13	MS+3.0mg/l BAP+1.0mg/l IAA	2.43	0.87	2.77	1.63	3.03	2.03
T14	MS+3.5mg/l BAP+1.0mg/l IAA	2.87	0.90	3.03	1.83	3.53	2.53
T15	MS+4.0mg/l BAP+1.0mg/l IAA	3.57	1.17	4.17	2.23	4.03	2.77
T16	MS+4.5mg/l BAP+1.0mg/l IAA	4.10	1.33	4.87	2.33	5.00	3.13
T17	MS+5.0mg/l BAP+1.0mg/l IAA	4.47	1.53	5.97	2.60	6.13	3.83
T18	MS+5.5mg/l BAP+1.0mg/l IAA	4.17	1.60	5.80	2.67	5.97	3.90
T19	MS+6.0mg/l BAP+1.0mg/l IAA	3.77	1.63	5.60	2.73	5.80	4.30
	SC(M) ±	**0.04**	**0.02**	**0.03**	**0.03**	**0.33**	**0.06**
	CD 5%	**0.13**	**0.07**	**0.12**	**0.11**	**0.11**	**0.18**

Table 2: Impact of Plant Bioregulators on shoot proliferation of banana cv Grande Naine

Treatments	Concentration mg/l	Colour of the leaves	Description
T1	MS	Green	Slender plantlets, open leaves
T2	MS+2.5mg/l BAP+0.5mg/l IAA	Whitish green	Normal shoots, slow proliferation
T3	MS+2.5mg/l BAP+0.5mg/l IAA	Pale green	Good shoots
T4	MS+3.0mg/l BAP+0.5mg/l IAA	Light green	Open leaves, good shoots
T5	MS+3.5mg/l BAP+0.5mg/l IAA	Light green	Open leaves, good shoots
T6	MS+4.0mg/l BAP+0.5mg/l IAA	Pale green	Stunted growth rapid
T7	MS+4.5mg/l BAP+0.5mg/l IAA	Pale green	Proliferation
T8	MS+5.0mg/l BAP+0.5mg/l IAA	Light green	Blood shoots
T9	MS+5.5mg/l BAP+0.5mg/l IAA	Green	Open leaves, good shoots
T10	MS+6.0mg/l BAP+0.5mg/l IAA	Light green	Good shoots, Sword Suckers
T11	MS+2.0mg/l BAP+1.0mg/l IAA	Pale green	Open leaves, normal shoots
T12	MS+2.5mg/l BAP+1.0mg/l IAA	Green	Normal shoots
T13	MS+3.0mg/l BAP+1.0mg/l IAA	Green	Slow proliferation
T14	MS+3.5mg/l BAP+1.0mg/l IAA	Green	Normal shoots
T15	MS+4.0mg/l BAP+1.0mg/l IAA	Green	Slow proliferation
T16	MS+4.5mg/l BAP+1.0mg/l IAA	Green	Normal shoots
T17	MS+5.0mg/l BAP+1.0mg/l IAA	Pale green	Open leaves, normal shoots
T18	MS+5.5mg/l BAP+1.0mg/l IAA	Green	Open leaves, normal growth
T19	MS+6.0mg/l BAP+1.0mg/l IAA	Green	Open leaves, normal growth

Table 3: Impact of Plant Bioregulator on Shoot elongation and root proliferation of banana cv Grande Naine

Treatments		Shoot Elongation and Root formation		Sub Culture after one month		
Label	Composition	Plant height (cm)	No of leaves	Leaf length (cm)	No of roots/plant	Length of root (cm)
T1	MS	1.25	3.73	1.58	4.23	1.48
T2	MS+2.0mg/l BAP+0.5mg/l IBA	1.37	3.73	1.61	4.33	1.45
T3	MS+2.5mg/l BAP+0.5mg/l IBA	1.43	3.74	1.89	4.73	2.33
T4	MS+3.0mg/l BAP+0.5mg/l IBA	1.64	3.87	1.99	4.83	2.94
T5	MS+3.5mg/l BAP+0.5mg/l IBA	1.93	3.87	2.49	5.23	2.98
T6	MS+4.0mg/l BAP+0.5mg/l IBA	1.77	4.10	2.74	4.33	3.27
T7	MS+4.5mg/l BAP+0.5mg/l IBA	1.58	3.37	2.91	3.53	2.70
T8	MS+2.0mg/l BAP+GA3(0.5 Mg/l)+0.5mg/l IBA	2.14	3.17	3.77	3.47	2.55
T9	MS+2.0mg/l BAP GA3(1.0 mg/l)+0.5mg/l IBA	2.20	3.17	3.30	3.47	2.53
T10	MS+2.0mg/l BAP+1mg/l IBA	3.52	4.20	3.78	5.40	4.47
T11	MS+2.5mg/l BAP+1mg/l IBA	3.64	3.83	3.66	4.67	3.50
T12	MS+3.0mg/l BAP+1mg/l IBA	2.54	3.83	2.66	4.64	3.38
T13	MS+3.5mg/l BAP+1mg/l IBA	2.46	3.83	1.55	4.63	3.37
T14	MS+4.0mg/l BAP+1mg/l IBA	2.39	3.47	0.95	4.63	2.81
T15	MS+4.5mg/l BAP+1mg/l IBA	2.31	3.40	0.93	4.53	2.65
T16	MS+4.0mg/l BAP+GA3 0.5mg/l +IBA 0.5mg/l	2.56	2.70	3.25	4.13	3.40
T17	MS+4.0mg/l BAP+GA3 1mg/l +IBA 1mg/l	2.13	3.20	3.53	2.70	2.15
	SC(M) ±	**0.08**	**0.13**	**0.20**	**0.07**	**0.06**
	CD 5%	**0.24**	**0.36**	**0.57**	**0.23**	**0.19**

Table 4: Impact of Plant Bioregulator on Growth of banana cv Grande Naine Plantlets

Treatments		Colour of the leaves	Description
Label	**Composition**		
T1	MS	Light green	Good growth with good shoots
T2	MS+2.0mg/l BAP+0.5mg/l IBA	Deep green	Luxuriant growth, bold shoots
T3	MS+2.5mg/l BAP+0.5mg/l IBA	Luxuriant green	Good growth with normal shoots
T4	MS+3.0mg/l BAP+0.5mg/l IBA	Light green	Normal shoots
T5	MS+3.5mg/l BAP+0.5mg/l IBA	Light green	Good growth and good shoot
T6	MS+4.0mg/l BAP+0.5mg/l IBA	Luxuriant green	Good growth with normal shoot
T7	MS+4.5mg/l BAP+0.5mg/l IBA	Light green	Good growth with normal shoot
T8	MS+2.0mg/l BAP +GA3(0.5 Mg/l)+0.5mg/l IBA	Light green	Good growth with normal shoot
T9	MS+2.0mg/l BAP GA3(1.0 mg/lit)+0.5mg/l IBA	Green	Good growth with normal shoot
T10	MS+2.0mg/l BAP+1mg/l IBA	Green	Good growth with normal shoot
T11	MS+2.5mg/l BAP+1mg/l IBA	Light green	Good growth with good shoots
T12	MS+3.0mg/l BAP+1mg/l IBA	Light green	Good growth with good shoots
T13	MS+3.5mg/l BAP+1mg/l IBA	Light green	Good growth with good shoots
T14	MS+4.0mg/l BAP+1mg/l IBA	Light green	Good growth with good shoots
T15	MS+4.5mg/l BAP+1mg/l IBA	Green	Good growth with good shoots
T16	MS+4.0mg/l BAP+GA3 0.5mg/l +IBA 0.5mg/l	Green	Good growth with good shoots
T17	MS+4.0mg/l BAP+GA3 1mg/l +IBA 1mg/l	Green	Good growth with good shoots

elongation and root formation in Banana (*Musa acuminate*) cv. Grande Naine. The data presented in Table 1 for shoot multiplication, the study of cultivar Grand Naine revealed that MS medium fortified with 5.0mg /l BAP along with 1.0mg/l IAA, significantly increased the number of multiple shoots per explant (4.47) being lowest in the treatment T1 i.e MS medium alone, while culturing. However, the height of the shoot was significantly maximum (1.63cm) in T19 where MS medium supplied with 6.0 mg/l BAP and 1.0 mg/l IAA. The data remained at par with treatment T18 i.e MS medium supplemented with (5.5 mg/l BAP and 1.0 mg/l IAA) during culturing. In the first sub culturing, significantly a maximum number of multiple shoots per plant (5.97) was observed in treatment T17 whereas MS medium was fortified with 5.0 mg/l BAP and 1.0 mg/l IAA. A number of multiple shoots per explant was minimum (1.13) in T1 (MS medium only). The treatment T17 also enhanced the number of multiple shoots/plant (6.13) and increased the height (3.83 cm) during 2nd sub culturing.

Hence, it has been concluded that the treatment T17 (MS medium + 5.0 mg/l BAP + 1.0 mg/l IAA) was most suitable for the production of multiple shoot during culturing and subculturing. Whereas the treatment T19 (MS medium + 6.0 mg/l BAP + 1.0 mg/l IAA) was most ideal for the increasing height of the shoots. During initial stage of growth MS medium fortified with BAP at higher concentration enhanced the plant height as well as multiple shoot/explant. Lower concentration of BAP affected the plant height in a decreasing manner. The addition of lower concentration BAP fortified medium produced mediocre height plants. During culturing and subculturing plant height and a number of multiple shoots/explant were maximum with inclusion of BAP. MS medium fortified with a higher concentration of BAP and lower concentration of IAA had shown spectacular effect on production of multiple shoots per explant. BAP is considered to be most potent cytokinin and played a vital role for production of multiple shoots per explant in table 1 type of banana cv. Grande Naine. Higher concentration of BAP with lower concentration of IAA produced more number of multiple shoots per explant. During initial stage of growth MS medium fortified with BAP at higher concentration enhanced the plant height as well as multiple shoots/explant. The ability of cytokinin to promote the growth of di-cotyledons has been reported by Murashige (1977). Application of cytokinin to the lateral buds encourages the differentiation of vascular traces (Moore, 1989). The effect of cytokines on breaking the dormancy of axillary buds under *in vitro* condition and proliferation of axillary shoots has been reported in various bulbus plants like Iris, Hyacinth, Lilium and Narcissus (Hussey 1975, 1976). Cytokinin at moderate concentration enhances shoot development; at higher levels it promotes multiple shoots through precocious axillary shoot formation (Ammirato, 1976). Hussain (1995) reported that BAP at

higher concentration produced a maximum number of shoots.

Fig. 1. Multiple shoot formation in the medium

Fig. 2. Healthy plantlets formed from multiple shoot initials

BAP was reported to be in general the most active cytokinin for meristem shoot tip and axillary bud culture of various species (Maharana, 2012; Kar, 2015). The data presented in Table 3 revealed that ½ MS medium supplemented with 2.5mg/l BAP along with 1.0 mg/l IBA significantly enhanced the plant height (3.64 cm) and the data stood at per with T10 (1/2MS +2.0mg/l BAP+1.0mg/l IBA). The lowest plant height was recorded in control (MS Medium only). The increase in concentration of BAP from 2.0mg/l to 3.5mg/l increased the plant height, and further increase inconcentration decreased plant height. It was evident from the data presented in Table 3 that significantly maximum number of leaves per plant (4.2)was recorded in the treatment T10 (½ MS+2.0mg/lBAP+1.0mg/lIBA) and the data stood at par with T6 (MS+4.0mg/l BAP + 0.5mg/l IBA) T5 and T6.The inclusion of Gibberllic Acid to the MS medium fortified with BAP and IBA reduced the number of leaves per plant. Significantly longer leaf (4.3 cm) was recorded in treatment T9(MS +2.0mg/l BAP + 1.0mg/L GA_3 +0.5mg/l IBA), which remained at par with treatment T10 (½ MS +2.0mg/l BAP +1.0mg/l IBA) and T8 (MS +2.0mg/l BAP + 0.5 mg/l GA_3 +0.5mg/l IBA).The data about a number of roots per plant revealed that 1/2 MS medium supplemented with 2.0mg/l BAP +1.0mg/l IBA increased the number of roots per plant (5.4) and it remained at par with the treatment T5 (MS +3.5mg/l BAP +0.5 mg/l IBA).The treatment T10 i.e, 1/2MS medium fortified with 2.0mg/l BAP and 1.0mg/l IBA significantly produced longer roots (4.47cm). Considering all above mentioned characters for shoot elongation and root initiation and it had been concluded that the treatment T10 (1/2Ms +2.0mg/l BAP +1.0mg/l IBA) produced elongated shoots with more number of (4.2) longer leaf (4.3 cm) along with number (5.4) of longer roots (4.47cm). The inclusion of 1.0 mg/l IBA in 1/2MS medium containing 2.0mg/l BAP had shown the tremendous effect on the production of roots as well as elongation of shoots. The present investigation revealed that auxins (IBA) help in better rooting of micro shoots and corroborated with findings of (Kar, 2015). Ancora *et al.* (1981) reported the effectiveness of IBA and NAA in root induction of *in vitro* produced plants. Hussian (1995) reported that lower levels of auxin (0.5 or 1.0 mg/l NAA) induced early rooting. The result obtained in this investigation was in agreement with Tiwari (1997-98), who stated that ½ MS medium supplemented with IBA successfully produced roots. Treatment with auxins stimulates and show an increase in peroxidase activity, as observed by Palai (2001). It was suggested that the auxin entered through the cut surfaces of the proliferated shoots and rapidly absorbed in the cell walls by pH trapping (Rubery and Sheldrake, 1973) and by influx carriers (Delbarre *et al.* 1996), Epstein and Muller (1993) reported that during the process of root induction, there were two major pathways of conversion: oxidation and conjugation. Exogenous auxins applied to micro-cuttings get oxidized, resembled the enzymes involved in the wounding reaction as reported by De Klerk *et al.* (1999). They also indicated that IAA or IBA oxidation caused by non-specific peroxidase was related to the rooting response.

Conclusion

It was concluded that the MS medium fortified with 5.0 mg/l BAP and 1.0 mg/l IAA was most suitable for the production of multiple shoots/explant, i.e., 4.47, 5.97 and 6.13 during culturing, 1st and 2nd subculturing respectively. MS medium supplemented with 6.0 mg/l

BAP and 1.0 mg/l IAA increased shoot height, i.e., 1.63 cm, 2.73 cm and 4.30 cm during culturing, 1st and 2nd subculturing respectively. Half MS medium supplied with 2.0 mg/l BAP and 1.0 mg/l IBA was most ideal for the production of longer plant with a number (4.2) of longer leaf, as well as the number (5.4) of longer roots (4.4 cm). The study thus revealed vital information related to rapid *in vitro* propagation of banana cv. Grande Naine, which can be very useful to the future research workers as well as entrepreneurs for mass production of banana by various commercial entrepreneurs. Approximately 1308 number of Tissue culture plantlets/ explant can be produced by following this protocol

References

Ammirato, P.V. 1976. Hormonal control of tuber formation in cultured axillary buds of *Dioscorea bulbifera* and *D. alata. Plant Physiol* **57** (suppl): 66.

De Klerk, G.J., Paffen, A., Jasik, J. and Haralampieva, V. 1999. A dual effect of ethylene during rooting of apple microcuttings. In: Altman, A.; Ziv, M.; Izhar, S., eds. Plant biotechnology and in vitro biology in the 21st century. Dordrecht: Kluwer Academic Press, pp. 41–44.

Delbarre, A., Muller, P., Imhoff, V.G. and Uern, J. 1996. Comparison of mechanisms controlling uptake and accumulation of 2,4-dichlorophenoxy acetic acid, naphthalene-1-acetic acid, and indole-3-acetic acid in suspension-cultured tobacco cells. *Planta* **198:** 532–541.

Dharmapalan, B., Renuka, S., Jisha, S. and Varghese, S. 2013. An efficient protocol for large scale plantlet production from the apical Meristem of *Musa paradisica* L.cv 'Nendran'. *International Journal of Agriculture, Environment and Biotechnology* **6**: 787-792.

Epstein, E. and Ludwig muller, J. 1993. Indole -3 butiric acid in plants occurrence, synthesis, metabolism and transport, *Plant Physiology* **88**: 382-389.

George, P. and Manuel, J. 2013. Low cost Tissue Culture Technology for the regeneration of some economically Important plants for developing Countries. *International Journal of Agriculture, Environment and Biotechnology* **6**: 703-711.

Gomez, K.A. and Gomez, A.A. 1984. Completely Randomized Design. In: Statistical procedures for Agricultural Research, 2nd Edn. John Wiley and Sons, New York, pp. 8-20.

Hussain, C.T.S. 1995. Response of Gladiolus to rapid cloning through in vitro techniques. M.Sc thesis Kerala Agric, Univ, Vellanikkara, Thrissur.

Jain, S.M. and Swennen, R. 2004. Banana improvement: cellular, molecular and mutagenesis approaches. Science Publishers, New Hampshire.

Kar, D.K. 2015. *In vitro* seed culture of interspecific hybrid in Cymbidium Spp. L.M.Sc.Ag. Thesis, OUAT, Bhubaneswar.

Maharana, D. 2012. *In vitro* shoot regeneration in hybrid cultivars of Gerbera (*Gerbera jamesonii* Bollus). MSc Ag Thesis, OUAT, Bhubaneswar.

Murashige, T. 1977. Clonal crops through tissue culture. In: W. Barz *et.al.* (Editors), Plant Tissue Culture and its in Biotechnological Application, Springer-Verlag, Berlin, pp. 392-403.

Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and biosassay with tobacco tissue cultures. *Plant Physiology* **15**: 473-497.

Palai, S.K. 2001. Studies on physiological, biochemical and molecular status *in vitro* culture of ginger (*Gingiber Officinale Rosc*) and turmeric (*curcuma longa L*) Ph.D thesis, Utkal University.

Randy, C.P. 2007. Banana and plantain – an overview with cmphasis on pacific island cultivars species profiles for pacific island agro forestry ver, 1.

Rubery, P.H. and Sheldrake, A.R. 1973. Effect of pH and surface charge on cell uptake of auxin. Natures (Lond.) *New Biology* **244**: 285-288.

Srinivas, C.R., Shanmuga Sundaram, V., Appala Raju, B., Karthick Prabhu, S., Srinivas, C.R., Shanmuga Sundaram, V., Appala Raju, B., Karthick Prabhu, S., Thirumurthy, M. and Bhaskar, A.C. 2006. Achieving asepsis of banana leaves for the management of toxic Achieving asepsis of banana leaves for the management of toxic epidermal necrolysis epidermal necrolysis. *Indian Journal of Dermatology, Venereology and Leprology* **72**(3): 201-202.

Swennen, R., Vuylsteke, D. and Hahn, S.K. 1991. Contribution No. IITA/91/CP/21. Int. Inst. Trop. Agric., Ibadan, Nigeria.

Tiwari, R.S. 1997-1998. Micropropagation of turmeric (*Curcuma longa L*) *Recent Horticulture* **4**: 145-148.