

ISSN 1682-296X (Print)  
ISSN 1682-2978 (Online)



# Bio Technology



**ANSI***net*

Asian Network for Scientific Information  
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

RESEARCH ARTICLE

OPEN ACCESS

DOI: 10.3923/biotech.2015.41.46

## Suitability of Bud Manipulation Technique as an Alternative to Tissue Culture in the Production of Suckers for Plantains and Bananas

J.N. Buah and J.W. Tachie-Menson

Department of Crop Science, University of Cape Coast, Cape Coast, Ghana

### ARTICLE INFO

#### Article History:

Received: November 21, 2014

Accepted: January 14, 2015

#### Corresponding Author:

J.N. Buah,

Department of Crop Science,

University of Cape Coast,

Cape Coast, Ghana

### ABSTRACT

The suitability of bud manipulation technique as an alternative to tissue culture in the regeneration of healthy planting material for farmers was investigated from May 2013 to August 2013, at the University of Cape Coast. The bud manipulation technique was carried out where the apical meristem of the corm was cut through to remove apical dominance effect. One hundred and twenty corms from sword suckers were used for each of the techniques, forty for each of the three cultivars (2 plantain cultivars and 1 banana cultivar). The tissue culture protocol was used for the *in vitro* culture. Generally, the explants produced more shoots *in vitro* compared to the bud manipulation technique. The highest number of shoot, 23 per explant under the *in vitro* technique was from the plantain cultivar Apantu whilst 15 shoots per explant were produced from the same cultivar, with the bud manipulation technique. The same trend was observed with the shoot height. Tissue cultured shoots had a maximum mean shoot height of 25 cm from Apantu whilst bud manipulation technique produced plantlets with maximum mean shoot height of 14 cm. The three cultivars performed better with tissue culture and of the three cultivars, Apantu had a better performance. Even though tissue culture produced more shoots compared to the bud manipulation technique, the number of shoots, shoot height and percentage survival of plantlets from the bud manipulation makes the bud manipulation technique a suitable alternative to tissue culture since it is farmer friendly and less expensive.

**Key words:** Bud manipulation, tissue culture, Apantu, Asamienu, Dwarf Cavendish

### INTRODUCTION

The genus *Musa* comprises over 50 species of both edible and non-edible cultivars with some of them having numerous subspecies (Faturoti *et al.*, 2002). The edible species are commonly known as plantains (cooked before eating) and bananas (served without cooking). Plantain and banana cultivars evolved by natural hybridization between the two species *Musa acuminata* (contributing genome A) and *Musa balbisiana* (contributing genome B) (Heslop-Harrison and Schwarzacher, 2007; Ngomuo *et al.*, 2014).

Bananas and plantains (*Musa* spp., L.) are important staple foods for nearly 400 million people in many developing countries, especially in Africa where bananas and plantains provide more than 25% of food energy requirements for over 70 million people. The pulp of a ripe banana is essentially a

sugar-rich, easily-digested food. The cooked banana is nutritionally similar to the potato. Bananas consist of about 70% water, 27% carbohydrate, 1.2% protein and 0.3% fat. In energy terms, each gram provides one calorie. The fruit is considered a good source of Vitamins A, B1, B2 and C (Arvanitoyannis *et al.*, 2008a).

The nutritional qualities of bananas and plantains have given the crops a huge market demand and this requires large healthy and uniform plantations for the sustainable export of the crop (Roux *et al.*, 2001; Ray *et al.*, 2006), which raises the need for large quantities of planting materials. The limitation however is that plantains and bananas have a slow sucker production rate and also produces them at different times, giving non-uniform production. This results in inadequate number of planting materials, which are also non-uniform for commercial production.

This has led to the need to use other protocols to ensure the production of large numbers of uniform and healthy suckers. For commercial production and tissue culture technique has been one of such techniques for the mass propagation of banana and plantains *in vitro*.

Banana and plantain can be propagated aseptically in the laboratory through tissue culture techniques. *In vitro* micro-propagation eliminates all sucker-transmitted pests and diseases, with the exception viruses (Buah *et al.*, 2010). The benefits of the tissue culture technique in the mass propagation of planting materials is however not sustainable to the farmers in developing countries due to the cost of producing these *in vitro* plantlets (Gitonga *et al.*, 2010). Apart from the fact that it is expensive, it is also delicate and hence cannot easily be carried out by the farmers on their own (Gitonga *et al.*, 2010).

The International Institute of Tropical Agriculture (IITA) has been investigating alternative means of producing clean planting material as a way of coming up with alternatives that are comparable to tissue culture and macro propagation has been considered as suitable alternative. Macro-propagation is a relatively easy technique that is carried out in a shed or even in the field (Baiyeri and Aba, 2005; Schill *et al.*, 2000). It consists of generating suckers from clean planting material by removing the apical dominance. Macro-propagation can be classified into two categories: Field-based techniques, based on complete or partial decapitation and detached corm techniques, practiced in a shed and this method could be an excellent option for producing low cost quality planting material in large quantities and also reduces the cost of producing planting material (Gitonga *et al.*, 2010) and has the potential of producing 50-60 shoots per sucker in 4-5 months.

This is a simple method because of the ease of the protocol for plantlet regeneration. Macropropagation is achieved by two methods and could be adopted either in the field conditions (*in situ*) or in the nursery (*ex situ*). It involves decapitation, decortication and hardening. A more easily adopted technology of propagation is the bud manipulation method, which is less expensive. The question is whether the bud manipulation can produce as many healthy and uniform plantlets as the tissue culture within a given time.

The purpose of this study therefore, is to assess the potential of the bud manipulation technique as a suitable alternative to the tissue culture technique for the mass propagation of healthy planting materials for bananas and plantains for the sustainable supply of planting materials.

## MATERIALS AND METHODS

The research was conducted between May 2013 to August 2013 at the Biotechnology Laboratory and the experimental farm of the University of Cape Coast, Ghana. Plant materials for the experiment were obtained from a farmer's field at Jukwa in the Cape Coast Metropolis in the Central Region of Ghana. Two cultivars of plantain

(Apantu and Asamienu) and one cultivar of banana (Dwarf Cavendish) were used for both the bud manipulation and the tissue culture experiments.

**Bud manipulation:** A humid chamber of 2×1×1.1 m dimension was constructed using the protocol of Faturoti *et al.* (2002).

For the bud manipulation, sword suckers were removed from a well-watered field. The base of the suckers were cleaned of soil debris and washed under running water. The roots were removed together with any dead tissues to reduce nematode infestation (Baiyeri and Aba, 2005). The materials were further screened for viruses by the Polymerase Chain Reaction (PCR) method by Thomson *et al.* (1996) to ensure that the plant materials were free from virus infestation. The leaf sheaths were removed until the apical meristem was almost exposed. The apical meristem was then destroyed by making a cross-like (+) incision unto the collar of the explant with a sharp knife. A 5 mm stick was inserted into the incision. The explants were then treated with insecticide (kuzitherin) at 30 mL/6 L of water. Wood ash was used as a fungicide at 500 g/6 L of water against soil borne insects and diseases. They were then air dried for 30 min after which the corms were planted in a propagator 3 cm deep in the sawdust, 30 cm apart and covered fully with sawdust. The buds were then watered immediately after planting. Each of the three cultivars had 40 corms as replications thus having a total of 120 corms for the bud manipulation method. Corms were placed in a completely randomized fashion and labeled clearly for identification. The materials were watered until sprouting started after 14 days. Sprouts that were rooted were separated from the main corm and planted in polybags filled with rich top soil under partial shade.

The removal of the sprouts was done weekly up to the 16th week after planting the corms. Sprouts were finally transplanted into nursery beds and data was taken on the mean number of sprouts per corm, mean plantlet height, mean number of leaves, percentage survival after transplanting and chlorophyll content before and after field nursery.

## Tissue culture method

**Plant materials and explant preparation:** The banana cultivar, Dwarf Cavendish (*Musa accuminata*) and two plantain cultivars Asamienu and Apantu were used as the explant source. Forty suckers were selected for each cultivar. Plant materials were taken from sword suckers that had been grown in the open field at a farmer's farm at Jukwa, a village in the Cape Coast Metropolis under good watering regime. Prior to washing with running tap water, the roots and the top of the shoots were trimmed off. The sheaths that formed the pseudostem were carefully removed to reduce the size of the material to about 4 leaf sheaths. They were then sterilized with 70% ethanol for 3 min and washed 3 times in sterilized distilled water (Buah *et al.*, 2010). More leaf sheaths were then removed aseptically in a clean bench until

about two leaves covered the shoot meristem. This process was followed by sterilization with 1% sodium hypochlorite solution containing a drop of polyoxyethylenesorbitan monolaurate (Twenty) for 5 min with occasional shaking and there after washed three times with sterilized distilled water. Prior to their inoculation on the medium, each shoot tip (about 1 cm) was longitudinally divided into two halves and again sterilized with 1% sodium hypochlorite (NaClO) as above for 1 min.

**Media composition:** MS medium (Murashige and Skoog, 1962) supplemented with 4.5 mg L<sup>-1</sup> 6-benzylaminopurine was used. Thirty grams (30 g L<sup>-1</sup>) sucrose and 6 g L<sup>-1</sup> agar were used because it had been the optimal sucrose concentration from previous work with *Musa* species (Buah *et al.*, 2010). The pH of the media was adjusted at 5.8 before autoclaving for 15 min at 121°C. The explants were inoculated into the various media and kept under a temperature of 26°C, 16 h photoperiod with an intensity of 3000 Lux and a relative humidity of 60%. The initial sub-culturing was done 4 weeks after placing the explants in the media and subsequently at 2 weeks interval. During subculturing, materials with multiple shoots were separately removed and placed into different vessels. In all, seven subcultures were done during which data were taken on mean number of sprouts per corm, mean number of roots per plantlet, mean plantlet height, mean number of leaves, percentage survival after transplanting and chlorophyll content before and after field nursery.

**Determination of chlorophyll pigment:** Leaf samples from plantlets produced from the two methods of propagation were taken for the estimation of total chlorophyll content according to the method of Razani *et al.* (2012) and the amount of chlorophyll was expressed as mg g<sup>-1</sup> fresh weight of sample. Data was analyzed with Genstat version 7.1 for analysis of variance and Excel 2007 for the plotting of graphs (Hilbe, 2007).

## RESULTS AND DISCUSSION

**Shoot formation:** The total number of shoots produced per plant and the rate of sprouting (number of shoots produced in a given period of time) are key parameters to determine the efficiency of a mass multiplication protocol and this is important if large number of suckers are to be produced commercially. The result in Fig. 1 shows that the tissue culture technique produced significantly more number of shoots than the bud manipulation technique for all the cultivars considered. Apantu produced 25 and 15 shoots per corm under the tissue culture and bud manipulation methods, respectively and this was significant compared to the number of shoots produced by Asamienu and Dwarf Cavendish under the two propagation methods. Asamienu produced the least number (14 shoot per corm) under the bud manipulation method.

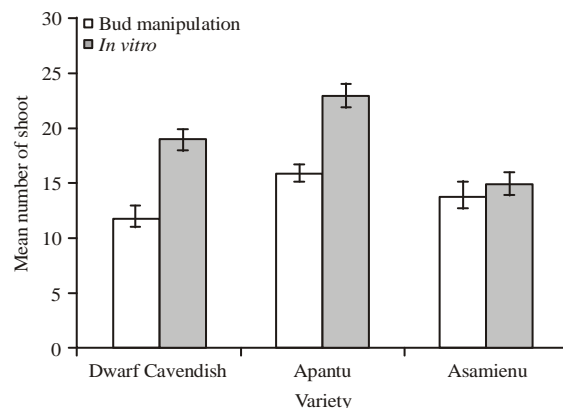


Fig. 1: Mean number of shoots from *in vitro* culture and bud manipulation techniques

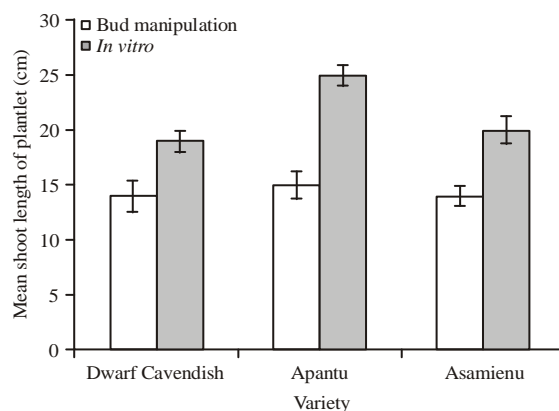


Fig. 2: Mean shoot height of plantlets from *in vitro* culture and bud manipulation techniques

Similar trend was observed with the mean shoot height and number of leaves per shoot. Mean shoot height ranged from 25 cm for Apantu and 20 cm for Asamienu for *in vitro* plantlets and from 15 cm for Apantu, 13 cm each for Dwarf Cavendish and Asamienu plantlets regenerated from bud manipulation technique (Fig. 2). The difference in plantlet height for *in vitro* and bud manipulation plantlets was significant. Under the *in vitro* technique, the cultivar Apantu had significantly taller plantlets compared to the Cavendish and Asamienu cultivars. The differences in height among three cultivars under the bud manipulation method was however insignificant.

Leave production was also significantly high under the tissue culture technique compared to the bud manipulation technique, with Apantu and Asamienu producing more leaves 12 and 11, respectively whilst the Cavendish cultivar produced 10 (Fig. 3).

More buds sprouted from the tissue culture technique compared to the bud manipulation technique, which agrees with the finding of Arias (1992), Arvanitoyannis *et al.* (2008a) and Khalil *et al.* (2002) who reported that tissue culture

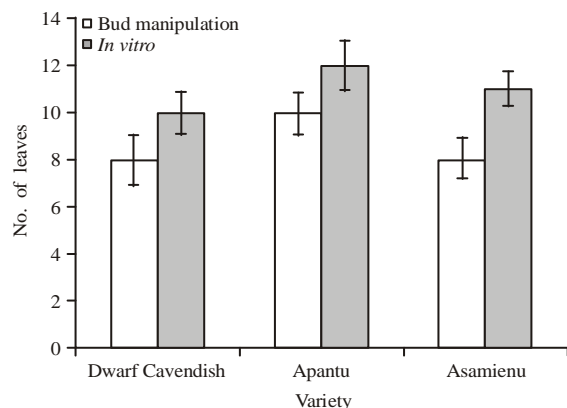


Fig. 3: Mean number of leaves per plantlet produced from *in vitro* and bud manipulation

produced significantly higher number of shoots than bud manipulation technique. The significantly high shooting of the three cultivars under the tissue culture method could be due to the addition of Benzylaminopurine (BAP) to the tissue culture medium. It was possible that the added BAP to the medium was readily available to the explants under *in vitro* conditions. Jafari *et al.* (2011), Dhanalakshmi and Stephan (2014) and Arvanitoyannis *et al.* (2008a, b) have all reported that BAP and Kinetin are known to reduce apical dominance and induce both auxillary and adventitious shoot formation from meristem explants in banana especially under *in vitro* conditions. Apical dominance is also reduced in bananas and plantains when the apical meristem is destroyed and this releases the other auxillary buds to sprout (Ngomuo *et al.*, 2014). The incision which was made into the apical meristem as part of the protocol of the bud manipulation technique resulted in the removal of the apical inhibitions on the development of auxillary buds into shoots in the bud manipulation technique. These auxillary meristems are commonly the source of bud formation in nature especially when the apical meristem is damaged. Several researchers such as Burrows (1989), Cline (1994), Wickson and Thimann (1958) and Mateille and Foncelle (1988) have reported that the arrest of apical dominance is essential for plantlet multiplication in bananas. Even though the destruction of the apical meristem in the bud manipulation method removed the effect of apical dominance, it is probable that the exogenous BAP in the MS medium enhanced the removal of apical dominance better.

The improved height and number of leaves per plantlets regenerated under tissue culture technique could again be due to the addition of BAP, which is primarily for shoot formation. Moreover, the humid conditions in the culture vessel as well as the sub culturing on fresh medium had some effect on moisture availability and better diffusion of nutrients in the plantlets. Even though plant materials have some amount of endogenous growth regulators in them, the levels probably were lower therefore the additional BAP was beneficial. The three cultivars showed differences in their ability to produce plantlets. The differences in shooting by different cultivars of

Table 1: Chlorophyll content of *in vitro* cultured plants and plantlets from bud manipulation 5 weeks after nursery

Cultivars	Leaves from <i>in vitro</i> plants	Leaves from bud manipulation plants
Dwarf Cavendish	0.496±0.4	0.680±0.6
Apantu	0.594±0.5	0.704±0.4
Asamienu	0.474±0.5	0.671±0.4

banana have been reported by Gubbuk and Pekmezci (2004) and Baiyeri and Aba (2007). Thus the genotype of a given plant species determines its bud proliferation *in vitro* and this has been collaborated by Baiyeri and Aba (2007), who reported that the ploidy level of bananas influences the size and shooting ability and that there is variable genetic response of *Musa* species to plantlets initiation through macropropagation. We observed that, Asamienu which is a cultivar of plantain produced more shoots which were also taller than the Cavendish. This observation however is contrary to the fact that the B genome is slow in multiplication than the A genome since the Asamienu is AAB and Cavendish is AAA (Hirimburgama and Gamage, 1997).

The chlorophyll content of leaves from *in vitro* cultured plantlets were lower than plantlets produced from the bud manipulation method. Apantu had the highest chlorophyll content of 0.594 and 0.704 mg g<sup>-1</sup> FW among the three cultivars from both *in vitro* and bud manipulation plantlets, respectively (Table 1).

*In vitro* plants are generally known to have low chlorophyll content due to the conditions under which they are grown. The high humidity, high carbon dioxide concentration in the culture vessels, low light intensity and restricted gaseous exchange all contribute to the low chlorophyll content of *in vitro* plants, as also been reported by Kumar and Rao (2012). The high chlorophyll content of Apantu *in vitro* and from the bud manipulation methods could have played a role in the better growth of Apantu among the three cultivars.

**Survival of shoots:** Field survival of the shoots was generally high (above 80%) for both bud manipulation and tissue cultured plants and for all cultivars giving an indication that plantlets from both techniques have high probabilities of surviving after sprouting. It was only with the Dwarf Cavendish where *in vitro* plants had lower percentage survival than those from the bud manipulation (Fig. 4).

Mortality of sprouted shoots may be as a result of disease incidence or the inability of the shoot to tolerate adverse conditions. The high survival rate of plantlets produced from the two techniques makes both of them acceptable for the production of large quantities of plants. Survival rate was about 90% for all cultivars from both protocols except Dwarf Cavendish that recorded 80% survival for plantlets regenerated from tissue culture. Probably because the Dwarf Cavendish shoots were tenderer compared to the local cultivars, which were hardier. The *in vitro* cultured Dwarf Cavendish shoots might therefore require gradual introduction to the natural environment in contrast with the bud manipulation technique which does not need so much a

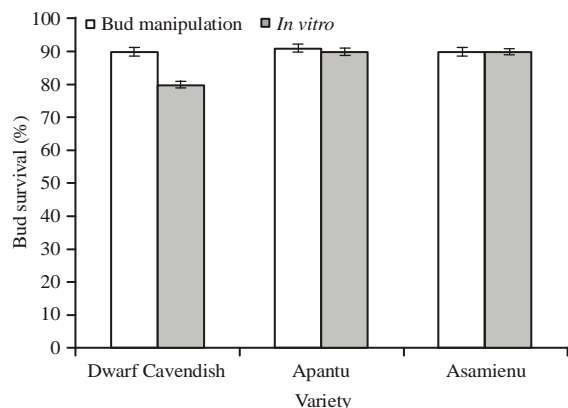


Fig. 4: Percentage survival of plantlets from *in vitro* culture and bud manipulation techniques

gradual process. The attachment of the sprouted buds to the corm as they grow, could have given an added advantage to the bud manipulation plantlets. Blomme *et al.* (2000) has reported that initial growth of naturally regenerated plant can be attributed to the fact that sucker corm is a nutrient reserve which could support growth. Moreover, the fact that the humid chamber conditions were different from the tissue culture conditions could introduce differences in the ability of plantlets from the two protocols in adjusting to field conditions.

### CONCLUSION

Though the three cultivars performed better under the tissue culture method compared to the bud manipulation method in terms of number of shoots, number of leaves and height of shoot, the bud manipulation technique did not do poorly in terms of number of shoots per explant. The high cost of producing plantlets through tissue culture compared to that of the bud manipulation method, places the bud manipulation technique as a suitable alternative to regenerate adequate healthy planting materials for farmers in countries where access to and affordability for tissue cultured plantlets is beyond the farmer.

### ACKNOWLEDGMENTS

We are very grateful to Mr. Anaman for the Technology village, School of Agriculture, University of Cape Coast for helping in setting up the humid chamber and also nursing the corms.

### REFERENCES

Arias, O., 1992. Commercial micro-propagation of Banana. In: Biotechnology Applications for Banana and Plantain Improvement. Inibap, San Jose, Costa Rica, pp: 139-142.

- Arvanitoyannis, I.S., O. Vaitis and A. Mavromatis, 2008a. Potato: A comparative study of the effect of cultivars and cultivation conditions and genetic modification on the physico-chemical properties of potato tubers in conjunction with multivariate analysis towards authenticity. *Crit. Rev. Food Sci. Nutr.*, 48: 799-823.
- Arvanitoyannis, I.S., A.G. Mavromatis, G. Grammatikaki-Avgeli and M. Sakellariou, 2008b. Banana: Cultivars, biotechnological approaches and genetic transformation. *Int. J. Food Sci. Technol.*, 43: 1871-1879.
- Baiyeri, K.P. and S.C. Aba, 2005. Response of *Musa* species to macro-propagation. I: Genetic and initiation media effects on number, quality and survival of plantlets at pre-nursery and early nursery stages. *Afr. J. Biotechnol.*, 4: 223-228.
- Baiyeri, K.P. and S.C. Aba, 2007. A review of protocols for macropropagation in *Musa* species. *Fruit Veg. Cereal Sci. Biotechnol.*, 1: 110-115.
- Blomme, G., R. Swennen and A. Tenkouano, 2000. Assessment of variability in the root system characteristics of banana (*Musa* spp.) according to genome group and ploidy level. *Infomusa*, 9: 4-7.
- Buah, J.N., E. Danso, K.J. Taah, E.A. Abole, E.A. Bediako, J. Asiedu and R. Baidoo, 2010. The effects of different concentrations cytokinins on the *in vitro* multiplication of plantain (*Musa* sp.). *Biotechnology*, 9: 343-347.
- Burrows, G.E., 1989. Developmental anatomy of axillary meristems of *Araucaria cunninghamii* released from apical dominance following shoot apex decapitation *in vitro* and *in vivo*. *Bot. Gazette*, 150: 369-377.
- Cline, M.G., 1994. The role of hormones in apical dominance. *New approaches to an old problem in plant development. Physiologia Plantarum*, 90: 230-237.
- Dhanalakshmi, S. and R. Stephan, 2014. Low cost media options for the production of banana (*Musa paradisiaca* L.) through plant tissue culture. *J. Acad. Ind. Res.*, 2: 509-512.
- Faturoti, B., A. Tenkouano, J. Lemchi and N. Nnaji, 2002. Rapid Multiplication of Plantain and Banana: Macropropagation Technique: A Pictorial Guide. IITA, Ibadan, Nigeria, ISBN: 9789781312175, Pages: 12.
- Gitonga, N.M., O. Ombori, K.S.D. Murithi and M. Ngugi, 2010. Low technology tissue culture materials for initiation and multiplication of banana plants. *Afr. Crop Sci. J.*, 18: 243-251.
- Gubbuk, H. and M. Pekmezci, 2004. *In vitro* propagation of some new banana types (*Musa* spp.). *Turk. J. Agric. For.*, 28: 355-361.
- Heslop-Harrison, J.S. and T. Schwarzacher, 2007. Domestication, genomics and the future for banana. *Ann. Bot.*, 100: 1073-1084.
- Hilbe, J.M., 2007. GenStat 9: A review. *Am. Statistician*, 61: 269-273.
- Hirimburgama, K. and N. Gamage, 1997. Cultivar specificity with respect to *in vitro* micro-propagation of *Musa* sp. (banana and plantain). *J. Hortic. Sci.*, 72: 205-211.

- Jafari, N., R.Y. Othman and N. Khalid, 2011. Effect of benzylaminopurine (BAP) pulsing on *in vitro* shoot multiplication of *Musa acuminata* (banana) cv. Berangan. Afr. J. Biotechnol., 10: 2446-2450.
- Khalil, S., K. Cheah, E. Perez, D. Gaskill and J. Hu, 2002. Regeneration of banana (*Musa* spp. AAB cv. Dwarf Brazilian) via secondary somatic embryogenesis. Plant Cell Rep., 20: 1128-1134.
- Kumar, K. and I.U. Rao, 2012. Morphophysiological problems in acclimatization of micropropagated plants *in-vitro* conditions: A reviews. J. Ornamental Horticult. Plants, 2: 271-283.
- Mateille, T. and B. Foncelle, 1988. Micropropagation of *Musa AAA* cv poyo in the Ivory coast. Trop. Agric., 65: 325-327.
- Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiologia Plantarum, 15: 473-497.
- Ngomuo, M., E. Mneney and P.A. Ndakidemi, 2014. The *in vitro* propagation techniques for producing banana using shoot tip cultures. Am. J. Plant Sci., 5: 1614-1622.
- Ray, T., I. Dutta, P. Saha, S. Das and S.C. Roy, 2006. Genetic stability of three economically important micropropagated banana (*Musa* spp.) cultivars of lower Indo-Gangetic plains, as assessed by RAPD and ISSR markers. Plant Cell Tissue Org. Cult., 85: 11-21.
- Razani, M., N.A. Shaharuddin, S. Subramaniam and M. Mahmood, 2012. Effects of TDZ on morphological and biochemical changes of banana plantlets (*Musa* spp.) cultivar mas cultured in temporary immersion bioreactor system. Proceedings of the International Banana Symposium, November 19-22, 2012, Malaysia, pp: 1-37.
- Roux, N., J. Dolezel, R. Swennen and F.J. Zapata-Arias, 2001. Effectiveness of three micropropagation techniques to dissociate cytochimeras in *Musa* spp. Plant Cell Tissue Org. Cult., 66: 189-197.
- Schill, P.F., K. Afreh-Nuamah, C.S. Gold and K.R. Green, 2000. Farmers' perceptions of constraints to plantain production in Ghana. Int. J. Sustainable Dev. World Ecol., 7: 12-24.
- Thomson, K.G., R.G. Dietzgen, J.E. Thomas and D.S. Teakle, 1996. Detection of pineapple bacilliform virus using the polymerase chain reaction. Ann. Applied Biol., 129: 57-69.
- Wickson, M.E. and K.V. Thimann, 1958. The antagonism of auxin and kinetin in apical dominance. Physiologia Plantarum, 11: 62-74.