

*Full Length Research Paper*

# Micro propagation of banana (*Musa sp.*) cv. Agnishwar by *In vitro* shoot tip culture

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## Abstract

The present study was to investigate the best plant growth regulators for shoot proliferation and multiplication; and to determine the optimum concentrations of phytohormones for shoot tip culture of banana cv. Agishwar by observing the effect of different culture conditions during induction. A micro-propagation protocol for banana (*Musa sp.*) cv. Agnishwar was established by using shoot tip culture. Shoot tips obtained by removing leaf sheaths from sucker were cultured aseptically in MS (Murashige and Skoog) medium supplemented with different concentrations of cytokines viz. 6-benzylaminopurine (BAP), kinetin (kin), N<sub>6</sub>-(2-isopentyl) adenine (2iP) for multiplication of shoot and auxins viz. Indole-3-butyric acid (IBA),  $\alpha$ -naphthalene acetic acid (NAA) for induction of root. Maximum multiplication (95%) was obtained in MS medium containing 4.0 mg/l BAP. The highest average number of shoots for each explant (5.9) was found in MS medium fortified with 4.0 mg/l BAP while maximum elongation of shoot (4.9cm) was observed in MS medium having 5.0 mg/l BAP. IBA at a concentration of 1.0 mg/l was found most suitable for rooting of shoot. The rooted shoots were acclimatized and successfully transferred to plastic pots. After hardening, they were transferred to the main field and the survival rate was around 90%. This protocol might be used for the massive *in vitro* production of the plantlets of banana cv. Agnishwar.

**Keywords:** Micro propagation, shoot tips, fresh sucker, tissue culture, MS medium.

## INTRODUCTION

Banana is one of the most important fruits in the world, both as a staple food as well as a major export commodity for many tropical and subtropical countries. The extensive basic work on the *in vitro* propagation of banana (Kodym and Zapata, 1999; Nandwani et al., 2000) had led to the technological development of *in vitro* mass production of different cultivars. Plant tissue culture techniques can potentially overcome some of the factors

Limiting traditional approaches to banana improvement.

On the basis of year round availability, popularity and production banana is considered to be the number one fruit in Bangladesh but the average yield of banana is about 17 t/ha that is far below the average world yield which is nearly 50 t/ha. Banana occupies second position in terms of area of cultivation among the fruits comprising 42% of the total fruit production in the country and its financial return as a crop is higher compared to other fruits and field crops (Banana Research Project, BAU, 1988). Banana not only contributes directly to the food security but is also a significant source of income generation of small holder farmers. Increased banana production can play an important role in Bangladesh. An increase in the yield on banana is possible by using

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disease free high yielding variety, modern technology of production as well as post harvest management. The combination of mutation breeding and *in vitro* culture has been suggested as an alternative approach for banana improvement (Novak et al., 1990).

Banana is generally propagated vegetatively through suckers. But the traditional method is laborious; time consuming and not very efficient as far as production of homogenous plant is concerned (Banaerjee and De Langhe, 1985). Only 5 to 10 suckers can be obtained from a plant per year in conventional method. Furthermore, banana production sometimes becomes seriously affected by different diseases (Rahman et al., 2004). As a result, banana productivity decreases and the yield become very poor.

To overcome this problem, production of saplings using *in vitro* culture techniques could be an effective for production of planting materials of bananas. A large number of uniform disease free plants can be produced from a single plant or even a small plant tissue (explants) showing good genetic potential in this method (Martin et al., 2006) and plant multiplication can be continued throughout the year irrespective of seasonal variation (Rahman et al., 2004). Tissue cultured plants grow vigorously, establish more quickly and take a shorter time to bunch emergence and harvest. Tissue culture technique produce 39% higher yield than conventional sword suckers (Farahani et al., 2008).

The application of tissue culture and rapid propagation method for banana production continues to become more widely used in both developed and developing countries. Tissue culture techniques can be applied not only to increase propagation rates but also to modify the germ plasm itself. The goals of this study are (i) to examination the effect of different culture conditions on shoot tip culture, (ii) to find out the best plant growth regulators for shoot proliferation and multiplication of banana cv. Agnishwar, (iii) to obtain optimum concentrations of IBA or NAA for root induction of banana cv. Agnishwar and (iv) to develop a Micro propagation system for large scale production of banana.

'Agnishwar' is an important banana cultivar, can be found many places of Bangladesh. Throughout the investigation shoot tips of field grown suckers of banana (*Musa* sp.) cv. Agnishwar were used for shoot and root induction. Different concentrations of cytokinins (BAP, 2iP and Kin) and auxins (IBA and NAA) were used in MS medium for best multiple shoot proliferation from the explants and root from shoots. The suitable medium for shooting was MS medium supplemented with 4.0 mg/l BAP and 1.0mg/l IBA for rooting.

## MATERIALS AND METHODS

### Plant materials

Fresh suckers of banana were collected from the orchard

grown plants located in the Plant Biotechnology and Genetic Engineering Division (PBGE), Institution of Food and Radiation Biology (IFRB), Atomic Energy Research Establishment (AERE) at Savar, Dhaka. The suckers were chopped off about 3-5 cm length and washed thoroughly under running tap water for 10-15 min.

### Determining *in vitro* proliferation ability

Shoot tips were prepared by trimming corm and outer leaf sheaths from the suckers. These shoot tips were collected in a beaker containing tap water, then brought to the laboratory and washed thoroughly with tap water, then washed with detergent mixed with Tween 80 (Sigma) and finally with distilled water for three times. They were then made into 6 explants and put in a sterile conical flask. These shoot tips were treated with 0.1% HgCl<sub>2</sub> solution for different periods (6, 8, 10 and 12 min) to determine time period for contamination free culture. They were then washed with sterile distilled water for 12 min. Treatment of shoot tips with 0.1% HgCl<sub>2</sub> for 10 min was found suitable for sterilization and finally washed thoroughly with sterile distilled water under aseptic conditions in a laminar air flow cabinet. All explants were placed on autoclaved Murashige and Skoog (MS medium, 1962) medium with different concentrations of cytokinins (1, 2, 3, 4 and 5mg/l) and auxins (0.5, 1 and 2mg/l). The pH of medium was adjusted to 5.8 prior to autoclaving.

The culture jars containing explants were incubated in a temperature controlled growth chamber. 15 jar, replication based on explants were incubated at 26±2°C with 16 hr photoperiod (approximately 2000 lux) provided by cool white fluorescent tubes. The materials were sub-cultured at a regular interval of four weeks into same medium to produce multiple shoots.

The regenerated plantlets after developing sufficient root system were deemed ready to transfer in soil. The plantlets were carefully removed from the culture vessels. The roots of the plantlets were gently washed under running tap water to remove agar attached to the roots. Immediately after washing they were transferred to small polythene bags containing a mixture of normal soil, sand and compost in 1:1:1 ratio. The plantlets with pots were covered with polythene bags to check sudden desiccation. The inner sides of this bag were sprayed with water at every 24 hr to maintain high humidity around the plantlets. The polythene bags were gradually perforated to expose the plantlets to the outer normal environment and subsequently removed after seven days. They were finally transferred into the field.

### Data analysis

The data were taken at six week intervals after subculture. There were 15 jars for each treatment. All

**Table1.** Effects of different concentrations of cytokinins for shoot proliferation and shoot multiplication in banana cv. Agnishwar

Cytokinin	Conc. (mg/l)	Shoot proliferation (%)	No of Shoot/explants	Shoot Length(cm)	No of Shoot/explants
<b>BAP</b>	1	69	2.6±0.28	2.8±0.53	1.73±0.18
	2	67	3.3±0.47	2.6±0.37	0.99±0.24
	3	80	2.8±0.53	3.5±0.45	1.49±0.32
	4	95	5.9±0.45	4.8±0.64	2.61±0.17
	5	88	5.7±0.63	4.9±0.76	2.46±0.44
<b>2iP</b>	1	56	2.8±0.42	2.2±0.32	0.87±0.29
	2	62	2.6±0.27	2.6±0.27	2.69±0.38
	3	74	3.4±0.34	3.8±0.66	1.49±0.47
	4	79	4.1±0.52	3.5±0.43	1.95±0.46
	5	83	4.8±0.37	3.6±0.69	1.15±0.19
<b>Kin</b>	1	46	1.6±0.22	1.9±0.43	0.44±0.24
	2	66	3.2±0.36	3.5±0.37	2.32±0.34
	3	68	3.4±0.38	2.3±0.18	1.58±0.19
	4	60	2.9±0.19	2.7±0.39	1.32±0.28
	5	57	2.6±0.34	2.4±0.25	0.74±0.15

\* Data were collected after 6 weeks of culture. (±) standard error.

date were measured average value of 12 replicates and standard error (±). Results were subjected to ANOVA using Statistical Packages for Social Sciences (SPSS version 18.0) and the means separated using least significant differences (LSD) at 5% level of significance.

## RESULTS

The results of the study showed that shoot tips obtained from the sword suckers of banana cv. Agnishwar were cultured onto MS medium supplemented with 1.0, 2.0, 3.0, 4.0 and 5.0 mg/l of BAP, 2iP, and Kin for induction of multiple shoot. After two weeks of culture for organogenesis, all culture was refreshed on same media.

The percentage of shoot proliferation under various concentrations of BAP was found in the range of 67 to 95%. 2iP was found in the range of 56 to 83% and 46 to 68% in Kin (Table 1). Out of three concentrations, BAP at a concentration of 4.0 mg/l yielded best response (produced significantly,  $P < 0.05$ ) on shoot proliferation (Figure 1A) which accounted to 95%. Kin at a concentration of 1.0 mg/l yielded lowest response on shoot proliferation, which was 46%.

During the culture period, the number of shoots increased gradually in all the media supplemented with different concentrations of BAP, 2iP and Kin viz. 1.0, 2.0, 3.0, 4.0 and 5.0 mg/l (Table 1). The best and lowest response for shoot formation was observed in MS medium having 4.0 mg/l BAP ( $P < 0.05$ ) (Figure 1B and C) and 1.0 mg/l Kin respectively. However, the highest average number of shoots per explant was found 5.9 under BAP at a concentration of 4.0 mg/l and the lowest was found 1.6 under Kin at a concentration of 1.0 mg/l (Table 1).

The length of shoot in different concentrations of BAP, 2iP and Kin is as shown in Table 1. The highest shoot length (4.9 cm) was recorded ( $P < 0.05$ ) in 5.0 mg/l BAP

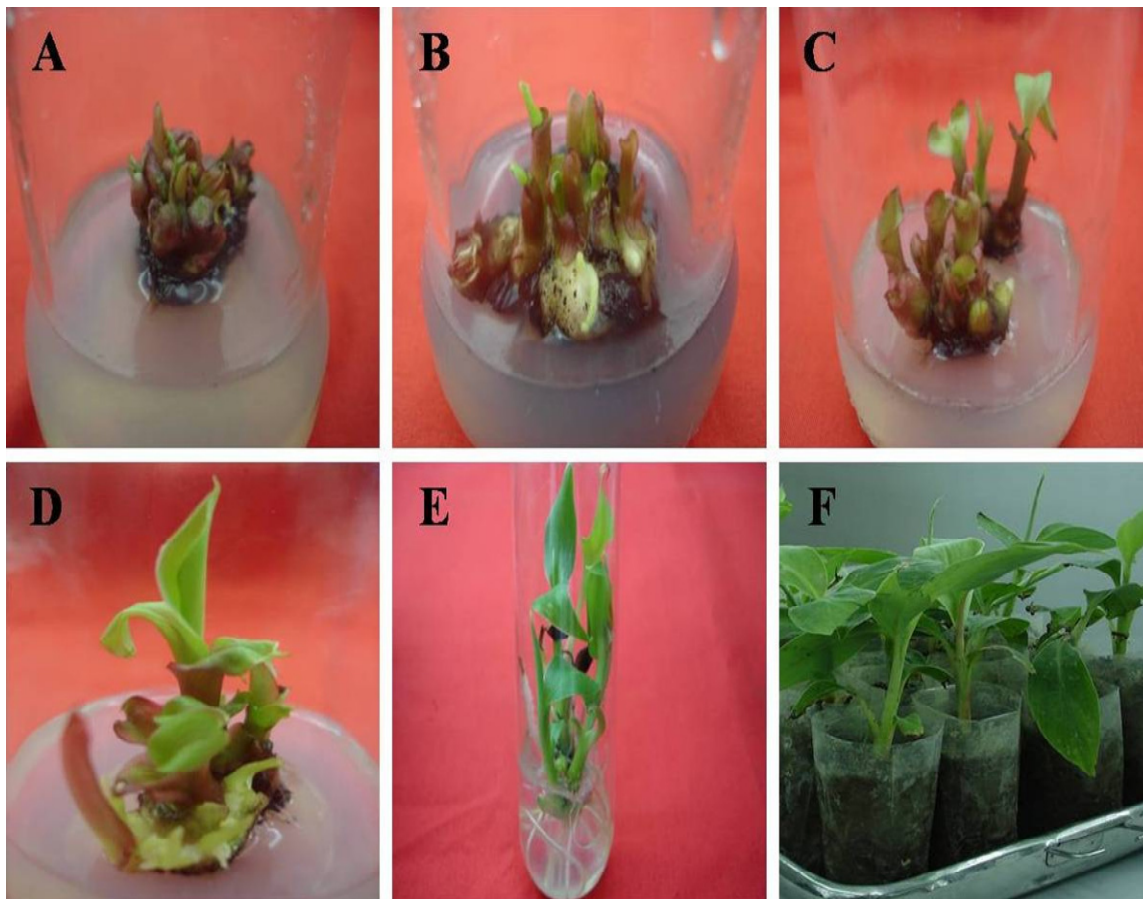
while the lowest shoot length (1.9 cm) was found in 1.0 mg/l Kin. Although the shoot length increased gradually with time in all treatments, the rate of increase was faster in BAP compared to 2iP and Kin (Figure 1C).

The number of leaves produced in different concentrations of BAP, 2iP and Kin is as shown in Table 1. The average number of leaves produced in different concentrations of BAP, 2iP and Kin ranged from 0.44 to 2.69. The maximum number of leaves (2.69) was produced ( $P < 0.05$ ) under 2.0 mg/l 2iP while the lowest number was found under 1.0 mg/l Kin. Though the number of leaves increased with time in all treatments, the increasing rate was faster in BAP compared to 2iP and Kin (Figure 1D).

The regenerated shoots were cultured in half strength of MS medium supplemented with different concentrations of auxin (Table 2) and 3% sucrose for healthy root development. Two auxins namely IBA and NAA were supplied in half strength of MS medium. Although few roots developed from multiple shoots during culture, but these roots are not so healthy for future growth and development of plantlets. So multiple shoots were separated and were allowed to grow on half MS medium supplemented with 0.5, 1.0 and 2.0 mg/l each of IBA and NAA for production of healthy as well as enough roots for proper growth of plantlets. All the treatments responded differently to produce rooting. IBA was found better than NAA in rooting of shoot in banana cv. Agnishwar.

The percentage of shoots responded to produce rooting ranged from 67 to 96 in various concentrations of IBA and NAA (Table 2). IBA at a concentration of 1.0 mg/l showed highest response (96%) in rooting (Figure 1E) while NAA at a concentration of 0.5 mg/l produced lowest response (67%) in rooting of shoot.

Regenerated shoots were cultured in half strength of MS medium supplemented with different concentrations



**Figure 1.** *In vitro* shoot tip culture of banana cv. Agnishwar. (A) Initiation of proliferations of shoot tip (explants) in banana cv. Agnishwar cultured in MS medium fortified with 4 mg/l BAP. (B) The best response for shoot formation was observed in MS medium fortified with 4 mg/l BAP. (C) The best length of multiple shoot from shoot tip explants in banana cv. Agnishwar culture was found in MS medium supplemented with 4 mg/l BAP. (D) Production of multiple shoot in banana cv. Agnishwar cultured in MS medium fortified with 4 mg/l BAP. (E) Rooting of *in vitro* regenerated shoots was higher in banana cv. Agnishwar cultured in MS medium supplemented with 1.0 mg/l IBA. (F) Acclimatized plantlets in polythene bag containing normal soil, sand and compost.

**Table 2.** Effects of different concentrations of IBA and NAA on root induction in *in vitro* regenerated shoots.

Auxin	Conc.(mg/ml)	% of Shoot rooted	No of roots/Shoot*	Root length(cm)*
IBA	0.5	83	7.4±0.67	2.26±0.59
	1.0	96	8.6±1.16	3.69±0.34
	2.0	88	5.4±1.65	2.29±0.39
NAA	0.5	67	3.8±1.35	1.61±0.26
	1.0	76	5.8±0.67	2.38±0.45
	2.0	79	5.2±0.51	2.79±0.19

\* Data were collected after 5 weeks of culture. Average values of 12 replicates and std. error ( $\pm$ ).

of IBA and NAA (0.5, 1.0 and 2.0 mg/l). All the media showed satisfactory root induction. 1.0 mg/l IBA produced highest average number of roots per explant was recorded to be 8.6. The lowest number of roots per explant was recorded in medium having 0.5 mg/l NAA. Moreover, it was recorded that root length ranged from 1.61 cm to 3.69 cm under above three concentrations of

IBA and NAA. Highest length of root (3.69 cm) was achieved under 1.0 mg/l IBA and the lowest under 0.5 mg/l NAA (Table 2). From the overall result of rooting, it is revealed that IBA at a concentration of 1.0 mg/l is suitable for *in vitro* rooting in excised shoot of banana.

After initiation of roots, plantlets were taken out from the growth chamber and kept under normal room

temperature for about three days in contact with normal atmospheric temperature and light. After three days, the plantlets were taken out from culture vessels and washed with running tap water.

Well-rooted plantlets were transferred to poly bags containing garden soil, sand and compost in the ratio 1:1:1 to investigate the survivability of transplanted plantlets in the net house. The polythene bags were covered with a plastic film to retain moisture and gradually acclimatized to outdoor condition (Figure 1F). The final survival rate of the transferred plantlets to soil was 90% and their growth in such condition was satisfactory. Mortality of the 10% plantlets occurred during transplantation of plantlets due to shifting between containers, injuries to the root system and excessive evaporation.

## DISCUSSION

Tissue culture technique involves the establishment of different cell or tissue under a suitable culture condition, *in vitro* cell proliferation and subsequent regeneration of plants (Vasil et al., 1982). Failure in most plant cell or tissues to get whole plants *in vitro* is due to lack of proper techniques and insufficient knowledge about nutrient media and other physical and chemical conditions, which are essential for proper growth of cells, tissues and organs (Johri, 1982). In banana, the most widespread used technique for propagation is reproduction by taking actively growing piece of stems called as micro cuttings for *in vitro* micro propagation under varying concentration of different cytokinins and auxins) (Mendes *et al.*, 1999; Wojtania and Gabryszewska, 2001; Vuylsteke and Ortiz, 1996). The present investigation was carried out for the induction and proliferation of multiple shoot from shoot tip and subsequent rooting. Different concentrations of cytokinin and auxin were supplied with MS medium for shoot and root induction.

However, some report showed that MS medium with 0.5 mg/l BAP +0.05 mg/l NAA +10% 9(v/v) CW was most optimum for maximum number of shoot regenerated from sucker explants (Akbar et al., 2003); longest shoot in banana when cultured on MS medium added with 25  $\mu$ M BAP was obtained (Khanam *et al.*, 1999). MS medium (Habiba and Reza, 2002) in concentration with 4 mg/l BAP + 2.0 mg/l NAA + 13% CW was optimum for highest number of shoot regeneration in banana from sucker explants whereas others (Azad and Amin, 2001) developed a medium for regeneration of banana from excised floral apices which was MS +2.0 mg/l BAP+ 1.0 mg/l kin +1.0 mg/l IAA +15% CW. In our observation, the best regeneration of shoots observed in the concentration of MS medium containing 4.0 mg/l BAP and the best shoot proliferation response accounted to 95%. Moreover, the highest average number of shoots was found to be 5.9. The maximum of 4.9 cm shoot length was recorded in

5.0 mg/l BAP. The different results obtained by different authors might be due to differences of genotypes and explants used.

A number of treatments were employed with half strength of MS medium, supplemented with different concentrations of auxin for efficient root induction. In *Eucalyptus citriodra* only NAA (0.2 mg/l) induced rooting (Gupta et al., 1981). The best rooting (up to 100%) from *Springcrest* was obtained by culture on half-strength MS medium with 0.15 mg/l NAA [120]. Another study (Chesick *et al.*, 1991) described that treatment of shoots of *Pignusstrobus* in an upright position with 50  $\mu$ M IBA for 8 days followed by culture in half strength MS medium with 3% sucrose in the light produced the most rooting (50% at 3 months). Efficiency of IBA in root induction was also observed in grape (Chakravorty et al., 1986). For the induction of roots in *in vitro* raised shoots of banana, half strength MS + 1.0 mg/l IBA was used (De Langhe, 1985) whereas auxin free MS medium was also applied for rooting of banana microshoots (Cronauer and Krikorian, 1984). In this study, 1.0 mg/l IBA supplemented MS media showed maximum number of roots/shoots.

The regenerated plantlets were established in small poly bags containing garden soil, sand and compost and acclimatized to outdoor conditions. Efforts were made to establish complete plantlets with sufficient root systems in poly bags. The result of this study for 90% plantlets was established successfully in the field condition. However, previous studies (Sharma and Thorpe, 1990) showed that complete plantlets of *Morusalba* were successfully established (100%) in the field condition. The variation of viability among plantlets with earlier study was due to mortality during handling procedure.

## CONCLUSION

In conclusion, we established suitable media with different composition of phytohormones for Micro propagation of Banana cv. Agnishwar: (1) for multiple shoot regeneration directly from shoot tip, the suitable medium was MS + 4.0 mg/l BAP, (2) for shoot elongation, the suitable medium was MS + 5.0 mg/l BAP and (3) for rooting  $\frac{1}{2}$  MS + 1.0mg/l IBA. Results of this study indicate that the cultures showing higher rate of multiplication in the first two or three subcultures continued this behavior in the next sub-culturing. Therefore, from the commercial point of view, these media composition might be applied for large scale propagation of healthy and disease free banana cv. Agnishwar plant.

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