*Plant Archives* Vol. 15 No. 1, 2015 pp. 307-314 *ISSN 0972-5210*

# EFFECT OF GROWTH HORMONES IN THE MICROPROPAGATION OF BANANA CV. MATTI

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## Abstract

Banana is an important fruit crop of the family musaceae. In Kanyakumari District, many varieties of banana are cultivated. Matti is one among them which fetches good price in the market and farmers called, it as money spinner. This study carry out the micro propagation of banana cv. Matti. The sword suckers of three months old were used as explants. The explants sterilised thoroughly and MS media supplemented with the cytokinins like BAP, kinetin, zeatin and adenine sulphate in different concentration (0.5 to 5.5 mg/l) were used for the multiple shoot generation and shoot growth. Among the tested cytokinins BAP in medium concentration performed well and higher concentration of all tested cytokinins showed declining effect. Generated shoot initials were transferred to the rooting media after three sub cultures. For rooting MS media was supplemented with different levels of IBA. Rooting was highly promoted in the medium concentration of IBA. After 115$^{th}$ day of inoculation the plantlets were transferred to green house for hardening and subsequently planted in the field after 30 days of acclimatization.

***Key words :*** Banana, cytokinin, auxin, sword suckers.

## Introduction

Banana is an edible fruit produced by a large herbaceous plant of the genus *Musa*. It is the most interesting economic plant and is believed that Agasthyamalai range is one of the centres of its origin. The specific name pardisiaca comes from the hypothesis, which made banana figure in the story 'Eve of Paradise'. They are considered to be one of the most important source of energy in the diet of people living in tropical humid regions. Banana is a stenothermic plant cultivated in hot and wet regions and bear fruit in all seasons. Banana is not only the world's most popular fruit; it has carved an indelible niche for itself in popular culture. Banana is considered as fourth most important food in the world after rice, wheat and maize. India occupies the third place in annual production of banana. Banana (*Musa* sp.) an important fruit crop of the family *Musaceae* is widely grown in developing countries. Banana is the second largest fruit crop after citrus (Swennen and Rosales, 1994). Banana originated from the South east Asian region, where the greatest diversity of edible bananas are found (Stover *et al*., 1985). The banana plant is threatened by numerous pathogens and pests therefore, the creation of new banana varieties resistant to the disease and pests with good fruit quality is highly (better control of ripening, taste, shape, colour, pulp rigidity etc.) essential for its improvement.

Tissue culture propagation of banana has gained attention due to its potential to provide genetically uniform pest and disease free planting material. *In vitro* propagation of banana through shoot tip has been demonstrated successfully (Cronauer and Krikorian, 1984; Banerjee 1985; Jarret *et al*., 1985). Commercial production of micro propagated banana is now common in many countries and it is estimated that 25 million plants are produced worldwide each year. In general micro propagated banana plants establish faster, grow more vigorously have more uniform production periods and produce higher yields than conventional suckers (Hawang *et al*., 1984). The propagation of banana is practised by vegetative means, because it is one of the most conspicuously sterile crops of the world (Rowe, 1984). Moreover, the rate of propagation of *Musa* plants by these methods is rather low since only 4 or 5 plants per year from single sucker can be obtained whereas 1000

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plants are required for one acre plantation. The low multiplication ratio of banana has promoted intensive research for rapid propagation methods and attention has been drawn to the possibility of using aseptic culture techniques, which facilitates quick multiplication in higher quantity.

## Methodology

Three months old sword suckers of the banana cv. Matti were detached from the healthy parent plant. After removing the outer sheaths of the sucker, the shoot tips with uniform size were used as explants for the *in vitro* culture.

**Sterilization of explants**

After surface sterilisation, the explants were rinsed with distilled water for four times. Before trimming the explants into optimum size for inoculation, the explants were continuously transferred to sterile filter paper placed over sterile petri dishes to remove moisture from the surface. To find out the effective sterilization, six treatments were imposed as follows:

- $T_1$ – Mercuric chloride 0.1% for 10 minutes
- $T_2$ – Absolute ethanol 95% for 45 seconds
- $T_3$ – Sodium hypochlorite 1% for 10 minutes
- $T_4$ – Absolute ethanol 95% for 30 seconds + mercuric chloride 0.1% for 10 minutes
- $T_5$ – Absolute ethanol 95% for 30 seconds + sodium hypochlorite 1% for 10 minutes
- $T_6$ – Absolute ethanol 95% for 30 seconds + sodium hypochlorite 1% for 10 minutes and inoculating the explants in media supplemented with 250-ppm filter sterilised streptomycin sulphate.

After sterilisation based on the said procedures the explants were placed in the laminar flow chamber and the UV light have been switched on for one minute for the final check of pathogens and ensure the explants were complete pathogen free.

**Method of culture adopted in this study**

Selection of sucker
(3 months old sword sucker cv.Matti)
↓
Isolation of clean shoot tip
↓
Clearing of explant with distilled water
↓
Sterilization
(95% Ethanol, 0.1% Mercuric Chloride & 1% Sodium hypo Chloride)
↓
Inoculation of shoot tips in MS Medium with required hormones
↓ 20 Days
Sub culture I
↓ 20 Days
Sub culture II
↓ 20 Days
Sub culture III
↓ 20 Days
Rooting of shoot lets
↓ 35 Days
Hardening of plantlets
↓ 30 Days
Field Planting

**Standardisation of media for multiple shoot formation**

To establish cultures for the initial shoot initiation different concentration of cytokinins, BAP, kinetin, zeatin, adenine sulphate were supplemented with MS media in 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0 and 5.5 mg/l concentrations. The days taken for greening of explant, days taken for multiple shoot initiation, number of shoot initials per explant and length of the longest shoot were recorded.

**Standardisation of Media for Rooting**

Auxin is very much essential for the initiation and growth of roots in the aseptic culture. In the first experiment for rooting the basal MS media was supplemented with indolebuertic acid (IBA) in 0.5, 1.0, 1.5, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0 and 5.5 mg/l concentrations. On 115$^{th}$ ay the following parameters were recorded; days taken for root initiation, number of roots produced per shootlet, and length of the longest root were recorded.

## Results and Discussion

Kanyakumari district is an important biodiversity hot spot having many medicinally important and rare plants. There are many varieties of banana are cultivated in the plains of this district. Matti, which is one among them fetches good price in the market because of its medicinal and nutritional values. In the present investigation, sword suckers of matti were selected for *in vitro* studies. This study primarily aims at developing an exclusive simple and fast protocol for micropropagation. Experiments were

taken up to find effective sterilization procedure, standardize culture establishment for shoot proliferation, elongation and rooting of the shootlet.

**Effect of sterilization**

The explants collected from field grown bananas usually harbor many microorganisms and other impurities. It is essential to eliminate this before inoculation. In the present study, it was observed that higher and effective sterilization could be achieved with the combination of sterilents than single sterilents. The maximum survival (90.33 ± 0.58) of explants after inoculation was obtained when the explants were sterilized with 95 per cent alcohol for 30 seconds followed by one per cent sodium hypochlorite for 10 minutes and 250 ppm streptomycin sulphate. Maximum mortality (53.01 0.82%) was observed when the explants were sterilized only by 95% alcohol (table 1).

Similar results were recorded in the earlier findings of Krikorian and Cronauer (1984), Wong (1986), Vuglsteke and Oritz (1996) and Navarro *et al*. (1997), who observed that the combination treatment of ethyl alcohol with 15 per cent sodium hypochlorite to be superior for establishment of various sword suckers they have tested.

**Basal medium for culture**

The establishment of a culture after inoculation requires a suitable environment for the cells to divide rapidly and undergo the desired morphogenetic processes. Culture establishment is greatly influenced by the nature of growth regulator used. Synthetic auxins are generally used along with cytokinins as the basal medium to generate better response. Auxins are required for initiation of cell division and expansion while cytokinins have a greater role in shoot induction and plant regeneration (George and Sherrington, 1994). This investigation found that MS media is the suitable basal media for banana shoot tip culture.

**Effect of cytokinins on shoot initiation**

In the present investigation, the cytokinins benylaminopurine (BAP), kinetin, zeatin, adenine sulphate were tested with different concentrations (table 2). Among the tested cytokinins BAP performed well in shoot initiation compared to the others. In all the tested cytokinins better results were obtained in a medium concentration and in higher concentrations a declining effect was recorded. Maximum number of shoot initials (8.66 $\pm$ 0.58/explant) were observed in the basal MS media and was supplemented with 2.0 mg/l of BAP and kinetin whereas zeatin and adenine sulphate showed the maximum shoot initials per explant in 2.5 mg/l.

The result of the present investigation corroborate following the reports.

Shirani (2010) reported that scalp produced by kinetin treatments was small compared to BAP treatments for banana cultivars. Rahman *et al*. (2002) reported that BAP was effective in shoot proliferation than other cytokinins.

Sholi *et al*. (2009) suggested that *Musa* cultivars require different levels of plant growth regulators; BAP is more effective in multiple shoot generation.

Cytokinins such as benzylaminopurine (BAP) and kinetin are generally known to reduce the apical dominance and induce both axillary and adventitious shoot formation from meristamatic explants in banana and the most established banana shoot tip culture system was achieved by using BAP as a supplement to basal media (Jafari *et al*., 2010).

Abeyaretne and Lathiff (2002) states that BAP is widely used to increase the multiplication rate for plantlets and concurrently control its mutagenic effect so as to decrease the percentage of morphologically abnormal plantlet formation and at the same time high concentration of BAP may lead to produce off type plantlets. They reported that 2-3 mg/l of BAP with basal media is an advisable concentration for banana shoot tip culture.

Bhosalo *et al*. (2011) reports that multiple shoot initiation occurs after 60 days of inoculation in shoot tip culture of banana variety Basari, the frequency of bud formation doubled and the fresh weight increased about four times higher in media with BAP at 3 mg/l were compared to media supplemented with 7 mg/l of BAP.

Rahman *et al*. (2004) reported that in the micropropagation of banana cv. Basari, good number shoot proliferation occurs in MS media with BAP up to 5 mg/l and it declined when the concentration of BAP was increased.

Venkatachalam *et al*. (2007) reported that the number of shoots increased with an increasing concentration of BAP up to 22.2 µM with higher levels of BAP (33-44.4 µM) a reduction in number of shoots regeneration occurred.

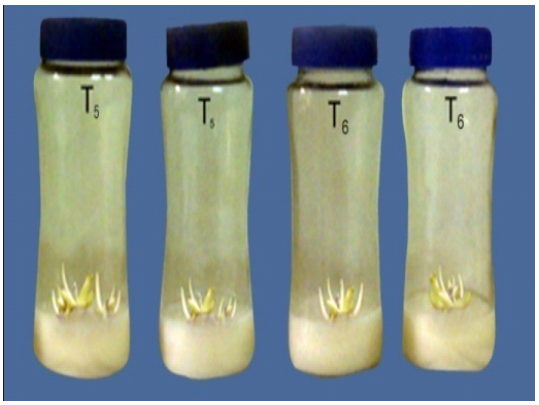
Gubbuk and Pekmezei (2004) and Bairu *et al*. (2008) indicated that 22.2 µM of BAP is the optimum treatment for most banana tissue cultures. Also there are many reports based on inhibitory effects of high concentrations of BAP on shoot multiplication rate.

Aish Muhammad *et al*. (2007) reported that the saturation concentration of BAP for banana cv. Basrai was 6.0 mg/l, beyond which multiplication rate started to

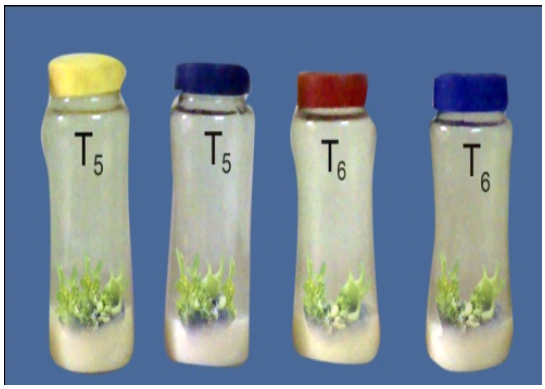
decline.

Higher concentration of BAP and kinetin beyond optimum levels were also reported to cause necrosis and reduction in shoot formation during *in vitro* multiplication of banana cv. Nendran (Madhulatha *et al*., 2004).

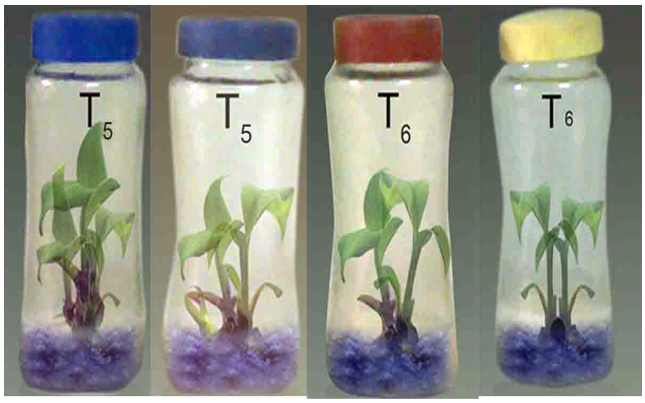
Muhammad *et al*. (2007) reported that media with various concentrations of kinetin, the number of shoots generation increased with increase in kinetin concentration up to a certain level, beyond which number of multiple shoots decreased.




**Multiple shoots generated on 80th day**




**Shoots initiating leaflets**




**Plantlets developed from initiated shoots on 115th day**

### Effect of hormones on rooting

The inducted shootlets were transferred to the rooting media in the 4th sub culture (after 80 days of inoculation).

In the present investigation the basic rooting media (MS) was supplemented with IBA in different concentrations.

This study revealed that early rooting was observed when the basal media was supplemented with IBA. When the IBA supplemented with MS media a good performance of rooting was observed in 2.5 mg/l of IBA. Higher concentrations of IBA showed a retarding effect on rooting (table 3). The following reports supported the above results.

Azad and Amin (2001) obtained sufficient number of roots in the micropropagated shoots of banana cv. bansari in half MS + 2.0 mg/l IBA. Atique Akbar *et al*. (2003) also found similar results in the micropropagation of banana cv. Sagar.

Dihiz *et al*. (2007) reported that consistently more number of roots are produced when MS media is supplemented with moderate concentration of IBA, NAA and activated charcoal.

**Table 1 :** Effect of sterilization in tissue culture of banana.

| **Sterilizing agent** | **Contamination (%)** | **Dried explants (%)** | **Mortality (%)** | **Survival (%)** |
|---|---|---|---|---|
| | 24.66±6.58 | 20.33±0.58 | 45.66±0.58 | 54.33±0.58 |
| Absolute alcohol 95% for 30 second **($T_2$)** | 34.00±0.82 | 18.33±0.58 | 53.00±0.82 | 47.33±0.58 |
| Sodium hypochlorite 1% for 10 in **($T_3$)** | 34.33±0.58 | 11.66±0.58 | 47.33±0.58 | 52.33±0.58 |
| Absolute alcohol 95% for 30 second + Mercuric chloride 0.1% for 30 min **($T_4$)** | 17.66±0.58 | 6.00±0.82 | 25±0.82 | 74.66±0.58 |
| Absolute alcohol 95% for 30 second + sodium hypochlorite 1% for 10 min **($T_5$)** | 11.66±0.58 | 2.33±0.58 | 14.33±0.58 | 85±0.82 |
| Absolute alcohol 95% for 30 second + Sodium hypochlorite 1% for 10 min + 250 PPM Sterilized streptomycin sulphate **($T_6$)** | 00.00±0.00 | 10.00±0.82 | 9.66±0.58 | 90.33±0.58 |

**Table 2 :** Effect of cytokinins with MS medium on shoot initiation.

| No. | Different concentration of hormones | Days taken for Greening of explant | | | | Days taken for multiple shoot initiation | | | | No of shoots initials / explants (On 80th DAI) | | | | Length of the longest shoot in Cm (On 115th DAI) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Benzyl amino purine | Kinetin | Zeatin | Adenine Sulphate | Benzyl amino | Kinetin | Zeatin | Adenine Sulphate purine | Benzyl amino | Kinetin | Zeatin | Adenine Sulphate | Benzyl amino purine | Kinetin | Zeatin | Adenine Sulphate |
| $T_1$ | Control | 18.00 ±0.82 | 18.33 ±0.58 | 17.66 ±0.58 | 17.66 ±0.58 | 77.66 ±0.58 | 78.33 ± 0.58 | 76.66 ± 0.58 | 77.33 ± 0.58 | 4.00 ± 0.82 | 4.00 ± 0.82 | 4.00 ± 0.82 | 4.33 ± 0.58 | 3.06 ± 0.00 | 3.83 ± 0.18 | 3.06 ± 0.08 | 3.66 ± 0.11 |
| $T_2$ | 0.5 | 10.33 ± 0.58 | 10.66 ± 0.58 | 11.33 ± 0.58 | 11.33 ± 0.58 | 69.66 ± 0.58 | 69.33 ± 0.58 | 69.33 ± 0.58 | 70.00 ± 0.82 | 5.66 ± 0.58 | 5.00 ± 0.82 | 6.00 ± 0.82 | 5.03 ± 0.58 | 5.33 ± 0.53 | 4.01 ± 0.16 | 5.33 ± 0.11 | 5.16 ± 0.12 |
| $T_3$ | 1.0 | 9.66 ± 0.58 | 9.00 ± 0.82 | 11.00 ± 0.82 | 10.66 ± 0.58 | 68.33 ± 0.58 | 68.00 ± 0.58 | 68.33 ± 0.58 | 70.00 ± 0.82 | 6.66 ± 0.58 | 6.66 ± 0.58 | 6.00 ± 0.00 | 5.66 ± 0.58 | 6.06 ± 0.58 | 4.05 ± 0.16 | 5.63 ± 0.11 | 5.05 ± 0.12 |
| $T_4$ | 1.5 | 9.33 ± 0.58 | 8.33 ± 0.58 | 10.66 ± 0.58 | 9.66 ± 0.58 | 68.66 ± 0.58 | 67.00 ± 0.58 | 67.00 ± 0.82 | 68.66 ± 0.58 | 7.00 ± 0.82 | 7.00 ± 0.82 | 7.33 ± 0.58 | 6.66 ± 0.58 | 6.03 ± 0.16 | 5.06 ± 0.18 | 6.01 ± 0.14 | 5.93 ± 0.12 |
| $T_5$ | 2.0 | 8.33 ± 0.58 | 7.66 ± 0.58 | 11.00 ± 0.82 | 8.33 ± 0.58 | 65.66 ± 0.58 | 64.33 ± 0.58 | 65.33 ± 0.58 | 63.00 ± 0.82 | 8.66 ± 0.58 | 8.33 ± 0.58 | 7.66 ± 0.82 | 7.00 ± 0.82 | 7.23 ± 0.18 | 5.08 ± 0.08 | 6.04 ± 0.08 | 6.26 ± 0.05 |
| $T_6$ | 2.5 | 7.66 ± 0.58 | 10.00 ± 0.82 | 10.00 ± 0.82 | 9.33 ± 0.58 | 63.00 ± 0.82 | 63.00 ± 0.58 | 63.00 ± 0.82 | 65.33 ± 0.58 | 8.33 ± 0.58 | 8.00 ± 0.82 | 7.66 ± 0.58 | 7.33 ± 0.58 | 6.96 ± 0.58 | 5.66 ± 0.05 | 6.56 ± 0.11 | 6.46 ± 0.05 |
| $T_7$ | 3.0 | 10.33 ± 0.58 | 10.66 ± 0.58 | 11.00 ± 0.82 | 9.66 ± 0.58 | 68.00 ± 0.82 | 65.66 ± 0.58 | 67.00 ± 0.82 | 66.33 ± 0.58 | 8.00 ± 0.00 | 7.66 ± 0.58 | 7.33 ± 0.58 | 5.66 ± 0.58 | 6.76 ± 0.12 | 5.01 ± 0.16 | 5.96 ± 0.11 | 6.00 ± 0.08 |
| $T_8$ | 3.5 | 10.66 ± 0.58 | 11.33 ± 0.58 | 12.00 ± 0.82 | 11.33 ±0.58 | 69.33 ± 0.58 | 67.33 ± 0.58 | 69.66 ± 0.58 | 67.00 ± 0.82 | 7.00 ± 0.82 | 7.00 ± 0.82 | 6.66 ± 0.58 | 5.00 ± 0.82 | 6.16 ± 0.12 | 4.83 ± 0.11 | 5.76 ± 0.05 | 5.08 ± 0.08 |
| $T_9$ | 4.0 | 11.33 ± 0.58 | 12.00 ± 0.82 | 12.33 ± 0.58 | 12.00 ± 0.82 | 70.66 ± 0.58 | 68.00 ± 0.82 | 71.00 ± 0.82 | 69.00 ±0.82 | 7.00 ± 0.00 | 6.66 ± 0.58 | 5.66 ± 0.58 | 4.33 ± 0.58 | 5.83 ± 0.12 | 4.53 ± 0.18 | 5.43 ± 0.11 | 5.04 ± 0.08 |
| $T_{10}$ | 4.5 | 12. 66 ± 0.58 | 12.66 ± 0.58 | 12.33 ± 0.58 | 12.66 ± 0.58 | 71.66 ± 0.58 | 69.66 ± 0.58 | 72.66 ± 0.58 | 71.00 ± 0.82 | 5.66 ± 0.58 | 5.00 ± 0.82 | 5.03 ± 0.58 | 4.00 ± 0.82 | 5.33 ± 0.36 | 4.13 ± 0.05 | 5.00 ± 0.17 | 4.96 ± 0.11 |
| $T_{11}$ | 5.0 | 13.00 ± 0.00 | 13.00 ± 0.82 | 13.00 ± 0.82 | 13.00 ± 0.82 | 73.00 ± 0.82 | 72.00 ± 0.82 | 73.00 ± 0.82 | 73.33 ± 0.58 | 5.66 ± 0.58 | 4.33 ± 0.58 | 4.00 ± 0.82 | 3.03 ± 0.58 | 5.01 ± 0.08 | 3.96 ± 0.11 | 4.07 ± 0.08 | 4.08 ± 0.08 |
| $T_{12}$ | 5.5 | 13.66 ± 0.58 | 13.66 ± 0.58 | 13.66 ± 0.58 | 13.66 ± 0.58 | 75.00 ± 0.82 | 75.66 ± 0.58 | 74.66 ± 0.82 | 74.00 ± 0.82 | 4.66 ± 0.58 | 4.00 ± 0.82 | 3.03 ± 0.58 | 3.03 ± 0.58 | 4.83 ± 0.05 | 3.86 ± 0.05 | 4.56 ± 0.05 | 4.08 ± 0.17 |

Each value is the mean of (± SD) of three replication.

**Table 3 :** Effect of IBA on rooting.

| No | IBA $mg^{-1}$ | Days taken for root initiation after shifted to rooting medium | No of roots produced/shoot let (35 days after shifted in the rooting medium) | Length of the longest root in (cm) (35 days after shifted in the rooting medium) |
|---|---|---|---|---|
| $T_1$ | Control | 18.66±0.58 | 3.66±0.58 | 3.01±0.08 |
| $T_2$ | 0.5 | 13.00±0.82 | 4.33±0.58 | 3.63±0.02 |
| $T_3$ | 1.0 | 12.00±0.82 | 6.66±0.58 | 4.03±0.18 |
| $T_4$ | 1.5 | 10.66±0.58 | 7.00±0.82 | 4.23±0.05 |
| $T_5$ | 2.0 | 10.00±0.82 | 8.66±0.58 | 4.03±0.08 |
| $T_6$ | 2.5 | 08.33±0.58 | 9.33±0.58 | 5.23±0.12 |
| $T_7$ | 3.0 | 10.00±0.82 | 9.00±0.82 | 5.03±0.05 |
| $T_8$ | 3.5 | 13.66±0.58 | 8.33±0.58 | 4.86±0.11 |
| $T_9$ | 4.0 | 14.00±0.82 | 6.66±0.58 | 4.63±0.05 |
| $T_{10}$ | 4.5 | 17.33±0.58 | 5.33±0.82 | 4.43±0.18 |
| $T_{11}$ | 5.0 | 18.66±0.58 | 5.00±0.82 | 4.06±0.12 |
| $T_{12}$ | 5.5 | 19.33±0.58 | 4.00±0.00 | 3.03±0.25 |

The root elongation phase has been found to be very sensitive to auxin concentration, high concentration of auxin inhibit the root elongation and a medium concentration of IBA supports the induction of root (Ganapathy *et al*., 2005).

To initiate rhizogenesis, IBA or NAA are commonly used in the proliferation medium and activated charcoal is added to enhance the rooting while higher concentration of cytokinins and auxins tend to have an adverse effect (Strose *et al*., 2005).

Yamamoto (2000) reported that more roots were produced in the MS with IBA media. Sumaryono *et al*. (2004) recommended 2.5 mg/l of IBA, NAA and 0.5 mg/l activated charcoal for the early rooting of shootlet in the micropropagation banana.

Molla *et al*. (2004) obtained the average 8.28 roots per plantlet on 0.5 mg/l IBA, they got 2.60-5.67 cms range of root length in 0.5 mg/l of IBA.

Rahman *et al*. (2002) reported that the number of roots produced / plantlet increased with increasing concentration up to 2 mg/l IBA and declined thereafter up to 5 mg/l. Among the IBA treatments 2 mg/l was found to be the most effective in rooting of banana plantlets. The highest number of roots / plantlet was obtained in 2 mg/l IBA. The rate of increase in root length was rapid in the media with 2 or 3 mg/l IBA but was slower in the media with 5 mg/l and control.

Rooting of shootlets on 115th day

**Hardening of plantlets**

Acclimatization is necessary in the case of micropropagted plants because *in vitro* plant is not adopted for *in vivo* conditions. On the 115th day of inoculation, the plantlets produced were transferred to the green house. The plantlets were planted in a pot containing sand, soil and vermicompost. It was kepts in the green house for 30 days and then transferred to planting field.

## Conclusion

This study concludes that *in vitro* culture is the best method for the propagation of banana. It is also proved that sword suckers are the good explants for micro propagation of banana. Through, this study it is confirmed

that basal medium (MS) supplemented with moderate amount of BAP and NAA is good for multiple shoot generation and growth. IBA is the growth hormone that promotes root initiation and growth. This study suggests that it is optimum to utilize the micro propagation method to propagate some important varieties of banana which are on the verge of extinction so as to conserve them.

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