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An Efficient Protocol for Large-Scale Plantlet Production from the Apical Meristem of *Musa Paradisiaca* L. cv 'Nendran

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Abstract

Banana is the most popular commercial fruit crop grown in more than 132 countries throughout the world. India is the most productive, producing 26.2 million tons of fruits in 2008, with a yield of 3,698 kg/ha . In vitro micropropagation has played a key role in clonal propagation of banana for obtaining large numbers of homogenous plants and breeding of plantains and bananas . Regeneration via a callus phase is a less desirable for in vitro plant recovery due to high frequencies of genetic aberrations .Plant multiplication from meristems, shoot tips, and other tissues without intermediate callus formations is therefore more desirable, enabling higher frequencies of plants which are genetically stable and homogenous. Among several varieties of banana cultivated in Kerala, the Nendran variety occupies the first choice among Keralites as the fruit is in good demand in the State. The present study was carried out to develop a rapid multiple shoot production for large scale cultivation of this variety.

Keywords: Banana, *In vitro* propagation, apical meristem, multiple shoot initiation, plantlet production.

Introduction

Banana, Musa spp. (Musaceae), is the most popular commercial fruit crop grown in more than 132 countries throughout the world. India is the most productive, producing 26.2 million tons of fruits in 2008, with a yield of 3,698 kg/ha (INIBAP 2000; FAOSTAT 2008). Banana provides nourishment and a well-balanced diet to millions of people around the globe and contributes to livelihood through crop production, processing, and marketing (Singh, 2002). Current world production of bananas is estimated at 97.5 million tonnes per year covering 10 million ha (Kalloo, 2002; Singh, 2002). India is the largest producer of banana with a total annual production of 16.91 million tonnes on 0.49 million ha, contributing 26% of the world production and 37% of the national fruit production (Samuel and Singh, 2002).

In vitro micropropagation has played a key role in clonal propagation of banana for obtaining large numbers of homogenous plants and breeding of plantains and bananas (Vuylsteke *et al.* 1997; Kalimuthu *et al.* 2007). Regeneration via a callus phase is a less desirable for in vitro plant recovery due to high frequencies of genetic aberrations (Martin

et al. 2006; Ray *et al*. 2006). Plant multiplication from meristems, shoot tips, and other tissues without intermediate callus formations is therefore more desirable, enabling higher frequencies of plants which are genetically stable and homogenous (Resmi and Nair 2007). Among several varieties of banana cultivated in Kerala, the Nendran variety occupies the first choice among Keralites as the fruit is in good demand in the State. The present study was carried out to develop a rapid multiple shoot production for large scale cultivation of this variety.

Materials and Methods

Elite suckers of Nendran variety collected from Mallappalli, Pathanathitta District were used as the explant source. The pseudostem was removed and the apical meristem dissected out. Apical meristem was surface-sterilized using mercuric chloride (0.1%, w/v) for 8 minutes, followed by three independent rinses of 5 min each in sterile distilled water. These were then cultured in Murashige and Skoog (MS) basal medium (Murashige and Skoog 1962) and also in modified MS media code named as MU media (Fig.1).

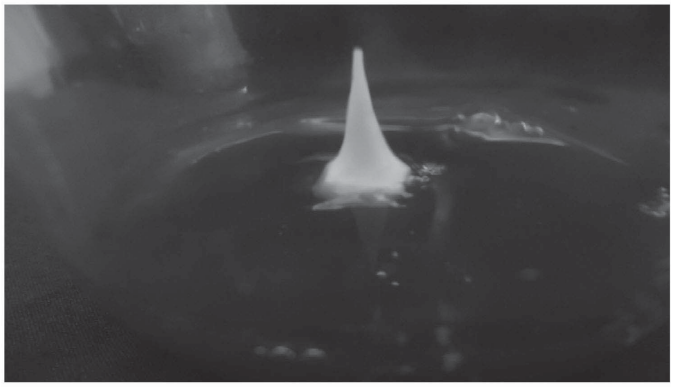

Fig 1: The Apical meristem

The MU medium contained similar concentrations of micro nutrients and vitamins as in MS medium. The macronutrient concentration was modified as follows,

KNO3	-	2050 mg/l
CaCl2.2H2O	-	200 mg/l
MgSO4.7H2O	-	120 mg/l
KH2PO4.H2O	-	40mg/l

These media were supplemented with different concentrations of plant growth regulators like 6-Benylaminopurine (BAP), kinetin and 2, 4-dichlorophenoxyacetic acid (2,4-D) individually and also in combination with phloroglucinol. The effect of activated charcoal was also studied. In all treatments, agar at 8.0 g/l was added as the gelling agent and sucrose at 30 g/l as the carbon source. The media was steam sterilised at 121 C for 20 minute. The sterile solid media was stored at 20 ± 2 C. Each treatment was replicated two times with 12 cultures for each replication. Cultures were incubated at 22±2°C at a relative humidity of 60–80%, with a light intensity of 55 ì mol m”2 s”1 using cool white fluorescent lamps (Phillips, Kolkata, India) and a photoperiod of 8:16 h light and dark, respectively.

After culturing for 15 d on basal medium, the apical meristem was subcultured into MS and MU media containing different concentrations of PGRS. The expanded shoot clusters were subdivided into pieces (~1.5 cm in size) and sub cultured onto fresh medium of the same type for further multiplication. The multiple induced shoots were separated into clump sizes of two to three shoots per clump and sub cultured at intervals of 30 d, thereafter onto the same medium type.

The separated shoots were then transferred rooting media containing different concentrations of auxins and cytokinins.The percentage of rooting and average number of roots per shoot was recorded .The rooted shoots were carefully removed and transferred to a soil mixture (sterile sand/top soil , 1:2) for hardening in plastic pots (26.5 cm diameter×23.0 cm height); maintained for 10 d in the specially constructed cabinet maintained at a temperature of 25±2°C, relative humidity of 60–70%, light intensity of 55 ìmol m”2 s”1, and a photoperiod of 16:8 h light and dark; and then transferred to the green house for further establishment.

Results

The explants expanded, became white in colour within 15 days of inoculation in the Modified MS named as MU basal media, while in MS basal media this initial response was observed after 30 days. So in all further experiments MU media were tried. The responded explants in basal media were transferred to MU media supplemented with PGRs and additives .

Among the various concentrations of cytokinins tried maximum multiple shoot initiation was observed in MU medium supplemented with 3.0 mg/l KIN . A maximum of 20 shoot clusters per explants was observed at this concentration. Addition of 10 mg/l Phloroglucinol along with KIN hastened the multiple shot initiation process. In the study it was observed that 0.1 % w/v charcoal promotes growth.

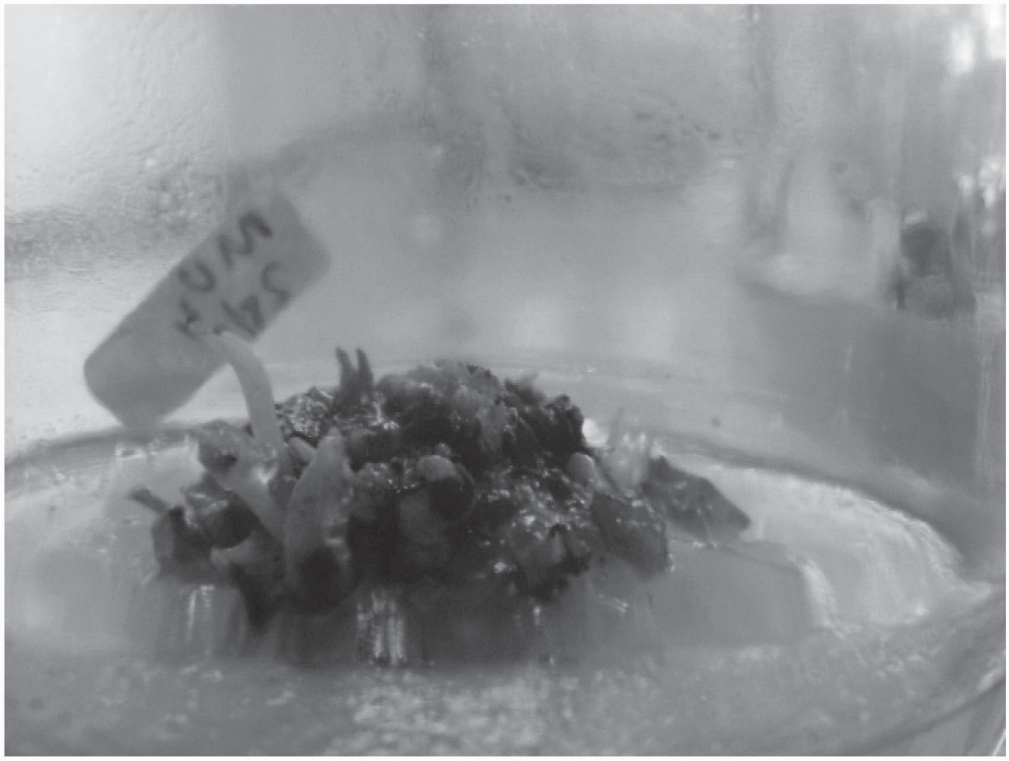

Fig. 2: Initiation of multiple shoot initials in MU media supplemented with 3 mg/l KIN

Fig. 3: Multiple shoot formation in MU medium supplemented with 3 mg/l BAP

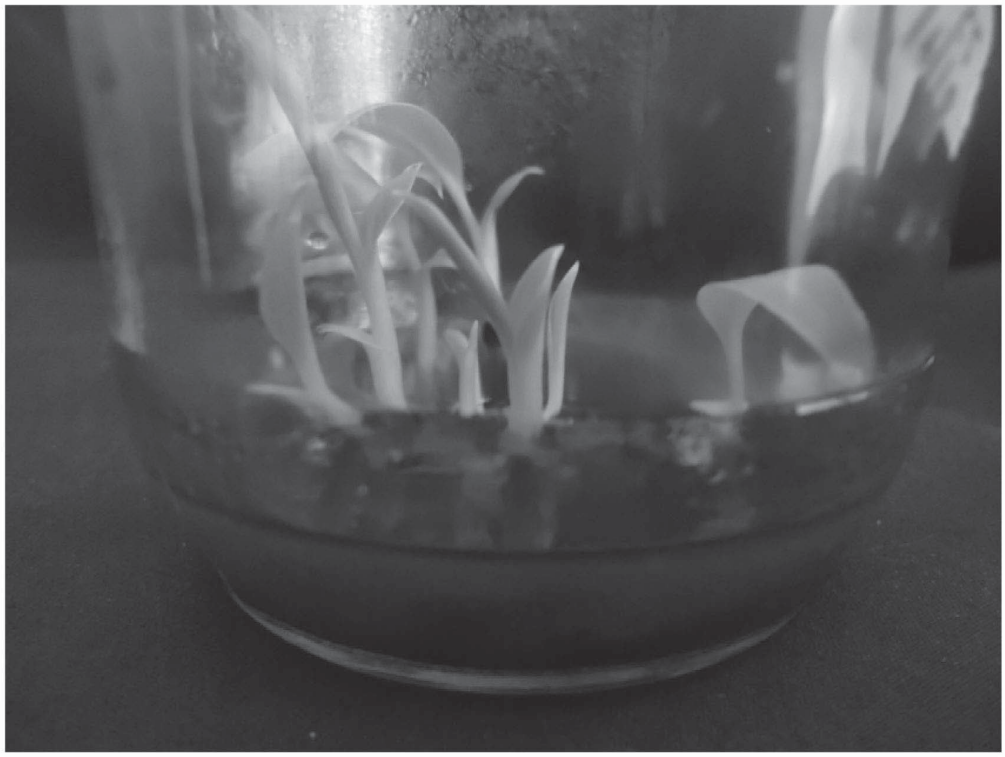

Fig. 4: Multiple shoot formation in MU medium supplemented with 3 mg/l KIN and 10 mg/l Phloroglucinol after 20 days of inoculation

Fig. 5: Healthy plant lets formed from multiple shoot initials

Fig. 7: Hardened plants

Percent root induction was found to be greater from explants cultured on MU medium alone (81.7±1.7%) than in combination with either IAA or NAA or 2,4-D ((1.5 mg l"1) . MU medium without any supplementation with PGRs also produced the maximum number (22.7±4.4) of roots per Explant. The plants were hardened using sterile sand and top soil in the ratio 2: 1

Table1: Influence of various concentrations of BAP on shoot formation

Concentrationmg/l	Number of shoots per explants*	Shoot length (cm)*
0.0	1±0.25	11.3±0.21
0.5	4±0.54	6.5±0.43
1.5	8±0.55	6.0±0.60
3.0	12±1.35	7.5±0.96
4.0	11±0.76	7.0±0.51
5.0	8±0.86	6.9±0.45
6.0	4±0.44	5.2±0.61
7.0	5±0.42	5.4±0.82
8.0	6±0.22	5.6±0.53

*Data (mean ± SE) collected from seven replicates.

Table2: Influence of different concentrations of KIN on shoot formation

Concentrationmg/l	Number of shoots per explants*	Shoot length (cm)*
0.0	1±0.81	11.3 ±0.43
0.5	5±0.53	7.89±0.55
1.5	13±0.94	8.24±1.44
3.0	20±0.86	8.34±1.72
4.0	16±1.10	7.00±1.32
5.0	12±2.20	7.43±1.23
6.0	9±5.67	6.09±0.98
7.0	6±1.24	6.21±0.25
8.0	5±0.21	6.98±0.54

*Data (mean ± SE) collected from seven replicates.

Table 3: Influence of 3mg/l KIN along with different concentrations of Phlorglucinol on shoot formation

Concentration of Phloroglucinol (mg/l)	Number of shoots per nodal explants	Shoot length
0.0	20±0.43	8.34±0.55 .55
4.0	19.5±0.96	9.45±0.66
6.0	19.73±1.13	8.65±1.61
8.0	18.89±0.76	9.77±0.51
10.0	21.23±0.86	8.59±1.35
12.0	20.14±0.65	9.23±0.47
7.0	6±1.24	6.21±0.25
8.0	5±0.21	6.98±0.54

*Data (mean ± SE) collected from seven replicates.

Table 4: Rooting of microshoots of Musa cultivars treated with different auxin types

Auxin type	Rooting (%) *	Mean number of roots per shoot cluster *
Control	20±0.43	8.34±0.55 .55
IAA (1.5 mg/l)	19.5±0.96	9.45±0.66
NAA (1.5 mg/l)	19.73±1.13	8.65±1.61
2,4-D (1.5 mg/l)	18.89±0.76	9.77±0.51

*Data (mean ± SE) collected from seven replicates.

Discussion

Micropropagation has played a key role in banana and plantain breeding programs worldwide (Rowe and Rosales 1996; Vuylsteke *et al.* 1997). Different explants have been tested for propagation of banana and plantain with shoot tips being the most common explant for in vitro propagation of commercial cultivars (Kulkarni *et al.* 2006).

The results of the present investigation revealed that modified MS media (MU media) better results in the initiation , multiplication and rooting stages of culture. Contrary to the report of Kulkarni *et al.*(2004) and Kumar *et al.* (2005) that shows the significance of BAP in inducing multiple shoots in different cultivars of banana , the present study showed that Kin yielded better results.

The results of the present investigation can be utilized for large scale propagation of uniform plantlets for commercial cultivation of banana.

Conclusion

The present study demonstrates that direct regeneration from apical meristems is a rapid and simple method for clonal and mass propagation of Musa cultivar 'Nendran'.

‘ that can be applied on a commercial scale. In this technique, regenerated plants were obtained at an average of 20 shoots per explant. Establishment of micropropagation protocols for local cultivars will provide an impetus to global banana improvement programs.

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