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Full Length Research Paper

In vitro micropropagation of *Musa sapientum L.* (Cavendish Dwarf)

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A complete protocol for micropropagation of *Musa sapientum* using shoot meristems was developed. Multiple shoots were induced in vitro from shoot meristems. Murashige and Skoog's medium supplemented with BAP and NAA (3.0 + 0.2 mg/l, respectively) was found to be most suitable combination. Further multiplication of shoots required habituation of cultures up to 3 passages of 21 days each on the same medium after establishment of culture and initiation of shoot buds. Thereafter 3-fold multiplication rate was achieved during every subculture. For rooting the shoots were excised and transferred to same medium. Rooted plantlets were then transferred to primary and secondary hardening and grown in the green house. These hardened plants have been successfully established in soil.

Key words: Micropropagation, regeneration, plantain, Musa sapientum, shoots, meristems.

INTRODUCTION

Bananas and plantains **(**Musa spp.) are the most important and most widely grown fruit crops in India. Banana is a good source of carbohydrates and proteins and other vitamins and minerals. Many pest and diseases threaten production and have resulted in the application of high quantities of pesticides with serious consequences for the environment. Because of its high degree of sterility and polyploidy of the edible varieties (Stover and Simmonds, 1987), classical breeding is difficult. In order to augment conventional breeding and to avoid constraints imposed by some pests and pathogens, transgenic and in vitro approaches are being considered (Tripathi, 2003). Mass propagation of selected genotypes, somoclonal variation techniques, genetic engineering and other biotechnological applications can be utilized for banana improvement and are based on reliable plant regeneration protocols. Several researchers have reported the regeneration of Musa spp. via micro propagation (Cronauer and Krikorian, 1986; Jarret, 1986; Diniz et al., 1999; Nauyen, and Kozai, 2001; Krishnamoorthy et al., 2001; Kagera et al., 2004; Roels et al., 2005; Madhulatha et al., 2004).

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Even though many reports are available on in vitro propagation, the protocols are complicated. Here, we reported a very simple economical, rapidly multiplying and highly reproducible protocol for large scale micropropagation.

MATERIALS AND METHODS

Sword suckers of selected elite plants were collected and the pseudo stem at lower part containing meristem was selected as explant. It was washed in running tap water for 15 - 20 min. The ensheathing leaf bases were removed from the pseudo stem leaving the young leaves around the meristem. The explant material was kept in an ascorbic and citric acid solution (1.0:1.5, w/v) for 30 min and 5 min in 1% Domestos solution. Subsequently they were briefly rinsed with 70% ethanol, followed by 1% mercuric chloride solution for 10 min. After washing thrice in sterile distilled water, the explants were inoculated aseptically in MS Medium (Murashige and Skoog, 1962) containing 30-g/l sucrose and gelled with 8 g/l of Diffco bacto agar. The MS medium was variously supplemented with 6-benzyl amino purines (BAP), and α naphthalene acetic acid (NAA) in various combinations as shown in Table 1. For rooting of shoots, the well grown shoots were sepa-rated and transferred to MS medium containing the same concen-tration of BAP (3.0 mg/l) and NAA (0.2 mg/l). The pH of the medium was adjusted to 5.8 before autoclaving and has maintained at 24 ± 1°C under 16 h cool white, fluorescent light (4000 lux) (Dooley, 1991). To overcome the problem in hardening and acclimatization, the *in-vitro* raised plants were transferred from bottles to net pots and kept

Table 1. Effect of different concentrations of plant growth regulators on in vitro shoot proliferation from shoot tip explants of banana.

Concentration of BAP + NAA in MS medium (mg/l)	No. of explants inoculated	Percentage of explants forming shoots	No. of shoots per explant
0.5 + 0.2	20	45	1
1.0 + 0.2	20	65	1
2.0 + 0.2	20	60	1
3.0 + 0.2	**20**	**95**	**3**
4.0 + 0.2	20	75	1
5.0 + 0.2	20	45	1
Basal medium	20	40	1

in groups in mist chamber maintained at (80 – 90%) humidity. The humidity was gradually reduced and plantlets were kept outside the mist house. After 20 days, they were transferred to polybags which were filled with various potting mixtures containing garden soil, sand and red soil in the ratio of 1:1:1. The hardened plants were then transferred to the field.

RESULTS

The shoot tip explants were inoculated on MS medium with six different combinations of BAP and NAA. Among the various treatments, the effective results were obtained from the combinations given in the Table 1. After few days the explants swell and turn green and produce shoots within 4 weeks. The shoot proliferation was best in BAP + NAA (3.0 + 0.2 mg/l), followed by the next combination of BAP + NAA (4.0 + 0.2 mg /l). The poor response of shoot initiation ability was noticed both in first (0.5 + 0.2 mg/l of BAP + NAA) and last (5.0 + 0.2 mg/l BAP + NAA) combinations of the growth regulators.

Subculturing of the shoots for multiplication on the same medium induced multiple shoots. After two or three subculture, the clump formation occurs. The proliferating axillary buds were well defined, pale green and 0.5 to 1.0 cm long with bulbous base and pointed tips. A three fold increase in multiplication was seen by 4-5 weeks. Further transfer in the same medium resulted in three to four fold ratio at every subculture cycle.

The explants cultured for 10-12 weeks in MS with BAP (3.0 mg/l) and NAA (0.2 mg/l) medium, which had attained the stage of vigorous proliferation, consisting of 9-10 shoots were divided in to smaller clumps. Each clump consisting of 5-6 shoots was transferred to the same medium. On the same media, the dwarf shoots recovered to normal growth with more number of axillary shoots. At the same time 2-3 vigorously growing shoots from each clump elongated with expanded leaves by three weeks (Figure 1). These shoots had healthy transfer in the same medium gave rise to a three to four fold increase in proliferating clumps and 3- 4 elongated shoots with root initials from each clump. The elongated shoots were excised and cultured separately in same fresh medium to encourage formation of long shoots broad leaves and basal roots. The basal tufts of rooting were observed in 100 % of the transferred shoots. The proliferating clumps were transferred to fresh medium for further multiplication. The elongated shoots with roots (about 8-9 cm) were transferred to primary hardening. The well developed healthy in vitro rooted plantlets were washed thoroughly in running tap water and planted in soil rite, a commercially available sterile potting mix in net pots and hardened in a shade house under 90-95% relative humidity (RH) for 8-10 days. They were gradually transferred to plastic pots or poly bags. A survival rate of 90-95% was achieved during the hardening. After 20-25 days, the hardened plants were transferred to field.

DISCUSSION

Cronauer and Krikorian (1984) reported that multiple shoots of banana and plantain could be produced from sliced meristems on either an agar or in liquid medium. But in the present investigation, shoot meristems were cultured on agar (semisolid) medium only. Irrespective of the medium composition, this observation confirms the report by Mante and Tepper (1983) regarding multiple shoot formation on an agar medium by Abaca meristem slices. Six different combinations with various concentrations of BAP and NAA were used in this study to analyze the shoot initiation and shoot multiplication capacity of the MS medium. 3.0 mg/l of BAP and 0.2 mg/l of NAA of medium showed good results both for shoot initiation and multiplication (Table 2).

Vessey and Rivera (1981) reported root formation occurred 50 days after shoot development. In contrast Berg and Bustamante (1974) noted that it needed 2-3 months for root formation. However, in the present study, These shoots had healthy transfer in the same medium gave rise to a three to four fold increase in proliferating clumps and 3- 4 elongated shoots with root initials from each clump. The in vitro raised plantlets were successfully established in the potting medium and field

Many of the previous reports on banana micropropagation used more than one type of media for initiation, multiplication and rooting (Cronauer and Krikorian, 1986; Jarret, 1986; Diniz et al., 1999; Nauyen and Kozai, 2001; Krishnamoorthy et al., 2001; Kagera et al., 2004). In the

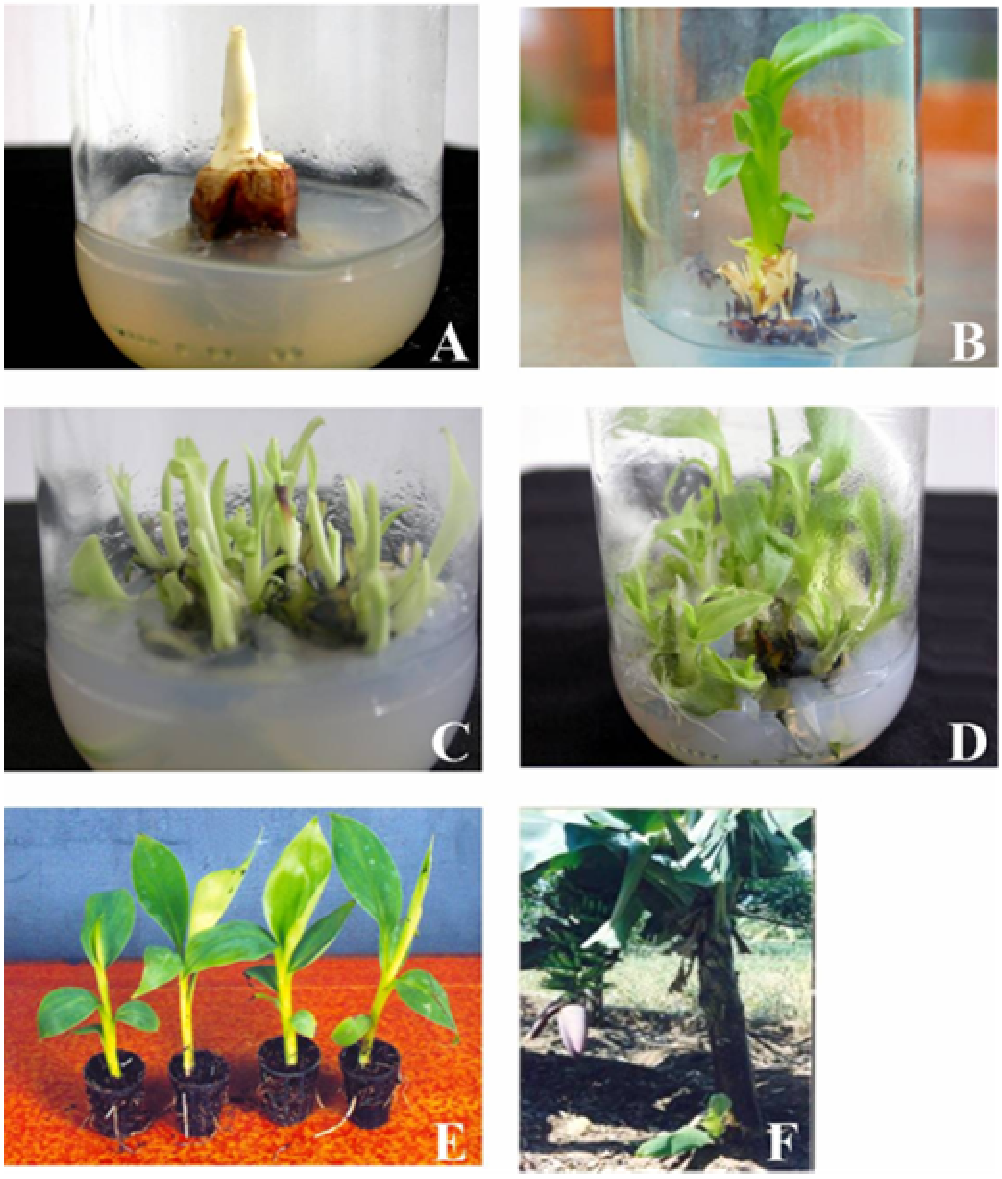

Figure 1. Different stages of Shoot tip regeneration of Musa sapientum. A, Shoot tip inoculation; B, PLBs and young plants; C, D, multiplication stage I and II; E, hardened plant; and F, in vitro raised plant under field condition.

Table 2. Effect of different concentrations of plant growth regulators on in vitro shoot multiplication from excised shoots of primary culture.

Concentration of BAP + NAA (mg/l)	No. of excised shoots inoculated per bottle	No. of multiple shoots per explant
0.5 + 0.2	4	1
1.0 + 0.2	4	1
2.0 + 0.2	4	2
3.0 + 0.2	4	5
4.0 + 0.2	4	2
5.0 + 0.2	4	1
Basal medium	4	-

present study we reported very simple one step protocol using MS with BAP and NAA for initiation, multiplication, and elongation and rooting of banana. The protocol raised in the present attempt could be used for the massive in vitro production of the plantlets of the banana

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