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RESEARCH ARTICLE

HIGH FREQUENCY IN VITRO PROPAGATION OF BANANA, CV. KARIBALE

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ABSTRACT

In the present study, an efficient reproducible protocol for high frequency in-vitro propagation of Banana cultivar Karibale was described. An in-vitro protocol for Cv. Karibale was established by using shoot tip culture. The healthy suckers which were collected from the disease free and high yielding mother plants were surfaced sterilized and initiated on standard MS medium supplemented with different combinations and concentrations of Plant growth hormones. Out of tried combinations, maximum multiplication (95%) was obtained in MS medium supplemented with 4 mg/L BAP, 8 mg/L Adenine Sulphate and 0.5 mg/L NAA, while maximum elongation of shoot (4.2cm) was observed in MS medium supplemented with 3.5 mg/L BAP, 6 mg/L Adenine Sulphate and 0.5 mg/L IAA. Then the micro shoots were transferred to in-vitro rooting medium MS medium half strength, supplemented with 1.5 mg/L BAP, 2 mg/L IAA, 1 mg/L IBA and 2 g/L Activated charcoal. Then excellent acclimatization was observed for transferred plantlets with 90% survival rate.

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INTRODUCTION

Uttara Kannada is a district in Karnataka state, India. It has rich biodiversity supporting number of ecological functions as well as livelihood of thousands of peoples in the district. Banana is one of the chiefly available fruits in the Uttara Kannada district. There are number of local verities of banana cultivated in the region; viz., Boodibale, Kembale, Karibale, Metlibale Mysore Metli, Nenibale, Baragibale, Rasabale, Pachebale, Sakkarebale and Shanbale etc. To safeguard sustainable banana production and generate wealth for small-scale farmers, high quality planting material is crucial. Banana is one of the most important fruit crop in the world, both as a staple food as well as a major export commodity for many tropical and sub-tropical countries (Singh, 2002), (Bose, T.K., 1985). 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. 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. 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. Banana is successfully micropropagated through plant tissue culture (Bhatt, P. and Bhatt, D, 2003), (Vuylsteke, D. and De Langhe, E.A.L, 1985), (Cronauer, M.S.S. and Krikorain, 1987).

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MATERIALS AND METHODS

Plant materials and Surface sterilization of explants

Fresh suckers of Kariibale were collected from farmer's fields of Uttara Kannada district with identification. The suckers were chopped off about 5-7 cm length and washed thoroughly under running tap water for 10-15 min. 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 and finally with distilled water for three times. These shoot tips were treated with 0.1% HgCl2 solution for different periods (4, 6, 8 and 10 min) to determine time period for contamination free culture. They were then washed with sterile distilled water for 5 minutes, three times. Treatment of shoot tips with 0.1% HgCl2 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.

Culture conditions and hardening

All explants were placed on autoclaved Murashige and Skoog (MS medium, 1962) medium with different concentrations of cytokinin (2, 2.5, 3, 3.5, 4, 4.5, 5, 5,5 and 6mg/l BAP), auxin (0.5 mg/l NAA) and Adenine Sulphate (0, 2, 4, 6, 8 and 10 mg/l). The pH of medium was adjusted to 5.8 prior to autoclaving. 3% sucrose was supplemented in the media. The culture bottles containing explants were incubated in a temperature controlled growth chamber. 10 bottle, replication based on explants were incubated at 25±2°C with 12-16 hr photoperiod (approximately 2000 lux) provided by white fluorescent tubes. The materials were sub cultured at a regular interval of four weeks into same medium to produce multiple shoots. For *in-vitro* rooting, elongated shoots were transferred to a rooting medium, half strength Murashige and Skoog medium with different concentrations of auxin (1, 1.5, 2, 2.5, 3, 3.5, 4mg/l IAA, 1 mg/L IBA) 1.5 mg/L BAP, and 2 g/L activated charcoal for four weeks maintaining standard culture conditions. After four to five weeks, 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. The plantlets were hardened under poly tunnel for 4 weeks and shade house for 6 weeks.

RESULTS

The results of the study showed that shoot tips obtained from the sword suckers of banana cv. Karibale were cultured onto MS medium supplemented with (2, 2.5, 3, 3.5, 4, 4.5, 5, 5, 5 and 6mg/l BAP), (0.5 mg/l NAA) and Adenine Sulphate (0, 2, 4, 6, 8 and 10 mg/l) for induction of multiple shoot (Figure 1, a).

After two weeks of culture for organogenesis, all culture was refreshed on same media. After four weeks of sub culture, the percentage of shoot proliferation under various concentrations of BAP was found in the range of 65 to 95%. Out of tried concentrations, MS medium supplemented with BAP at a concentration of 4 mg/l+0.5 mg/l NAA and Adenine Sulphate 8 mg/l yielded best response on shoot proliferation which accounted to 95%. BAP at a concentration of 2.0 mg/l yielded lowest response on shoot proliferation, which was 65%. During the culture period, the number of shoots increased gradually in all the media. (Table 1) and (Figure 1, b).

Figure 1: a) Karibale suckers selected for experiment. b) Aseptic culture after one week of inoculation. c) High frequency multiplication. d) Shoot elongation. e) *In-vitro* rooting. f) Hardened plantlets

MS medium supplemented with BAP at a concentration of 3.5 mg/l+0.5 mg/l NAA and Adenine Sulphate 8 mg/l yielded best shoot elongation, which was found to be 4.2 cm in shoot length. While MS medium supplemented with BAP at a concentration of 6 mg/l+0.5 mg/l NAA and Adenine Sulphate 10 mg/l yielded the lowest shoot elongation, which was found to be 2.4 cm in shoot length (Table 1) and (Figure 1, c). The regenerated shoots were cultured on half strength of MS medium supplemented with different concentrations of auxin (1, 1.5, 2, 2.5, 3mg/l IAA), 1mg/l BAP and 2g/l Activated charcoal for *in-vitro* rooting. Although few roots developed from multiple shoots during culture, but those roots are not so healthy for future growth and development of plantlets. IAA at a concentration of 2.5 mg/l was found to be the best medium for *in-vitro* rooting in banana cv. Karibale (Table 2) and (Figure d). Well-rooted plantlets were hardened under poly tunnel for 4 weeks and shade house for 6 weeks. The polythene bags were covered with a plastic film to retain moisture and gradually acclimatized to outdoor condition.

Table 1.

Concentration in mg/L			Response %	Number of shoots/ explants	Shoot length (in cm)
AP	Adenine sulphate	NAA			
2.0	0	0.5	65	4	4.0
2.5	2	0.5	70	4	4.0
3.0	4	0.5	85	8	4.2
3.5	6	0.5	90	9	4.2
4.0	8	0.5	95	10	4.0
4.5	10	0.5	95	10	3.7
5.0	12	0.5	90	8	3.1
5.5	14	0.5	90	9	2.6
6.0	16	0.5	80	8	2.4

The final survival rate of the transferred plantlets to soil was 90% and their growth in such condition was satisfactory.

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). 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 Gabryszweska, 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 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 µM 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, maximum multiplication (100%) was obtained in MS medium supplemented with 3.5 mg/L BAP, 10 mg/L Adenine Sulphate and 0.5 mg/L IAA, while maximum elongation of shoot (3.6cm) was observed in MS medium supplemented with 3 mg/L BAP, 8 mg/L Adenine Sulphate and 0.5 mg/L IAA. The best *in-vitro* rooting was found in MS medium half strength, supplemented with 1 mg/L BAP, 2.5 mg/L IAA and 2 g/L Activated charcoal. Then excellent acclimatization was observed for transferred plantlets with 90% survival rate.

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