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Research Article

In vitro propagation of Banana (*Musa sp* - Rasthali variety) from sword suckers for its commercial production

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Banana is the fourth most important fruit crop, in the world as well as in India. *In vitro* propagation of bananas provided excellent advantages, including a high multiplication rate, physiological uniformity and the availability of disease-free material all round the year, faster growth in the earlier stages in comparison with conventional plants. An efficient micro propagation method has been developed in banana plants using the sword sucker explants cultured on MS medium with different phyto hormonal supplements for shoot and root proliferation. The shoot proliferation was found best (80%) in the MS medium containing Benzylaminopurine (BAP) 2.0 mg/l. Maximum percent of adventitious root formation was observed in half strength MS medium supplemented with Indole butyric acid (IBA)1.5mg/l and Napthalene acetic acid (NAA) 1.0mg/l. After three weeks, *in vitro* grown plants were transferred to the poly-cups containing 1:1 ratio of soil and sand respectively for hardening and then transferred to garden in which showed 75% survival efficiency. In the present study a simple two step protocol was established using MS medium with 2.0mg/l for shoot proliferation and 1.5mg/l IBA + 1.0 mg/l NAA for rooting in banana. This protocol might be used for the massive *in vitro* production of the plantlets of banana.

Keywords: MS medium, sword sucker, micro propagation, BAP, NAA

Banana contributes to the food security of millions of people in the developing world. It is the world's most widely known and distributed fruit, eaten either raw or cooked and processed food products. Banana is rich in energy (128kcal/100 g), vitamin C and A (Chandler, 1985), high content of potassium and sodium (Srinivas, 2006). Banana has also been found effective against colorectal cancer (Deneo-Pellegrini H, 1996), breast cancer (Zhang CX, 2009) and renal cell carcinoma (Rashidkhani B, 2005). Among the major producers, India alone accounts for 27.43 % (26.2 million tons) followed by Philippines, producing 9.01 million tons and China, Brazil and Ecuador, with production ranging from 7.19 to 8.21 million tons. Current world dessert banana production is approximately 67 million tons per year. But, only 20% of it enters the world trade (Fao stat, 2011). Bananas are fast growing herbaceous perennials arising from underground rhizomes. The fleshy stalks or pseudo stems by upright concentric layers of leaf sheaths constitute the functional trunk. The true stem begins as an underground corn which grows upwards, pushing its way out through the center of the stalk 10-15 months

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after planting, eventually producing the terminal inflorescence which will later bear the fruit and each stalk produces one huge flower cluster. There are two types of suckers, sword suckers – with a well developed base, pointed tip and narrow leaf blades and water suckers, which are small, less vigorous, broad leaved and emerge in clumps. Natural regeneration of cultivated bananas through suckers is very slow due to hormone-mediated apical dominance of the mother plant. A plant produces only 5-20 suckers during its life time of 12-14 months. For accelerating the propagation rate, suckers with growing buds or cut rhizomes called 'bits' and 'peepers' are used. Several good bits, each with a centrally placed germinating eye can be cut from an unbunched rhizome after trimming the roots. Selection of appropriate mother plant for raising new propagules either through *in vivo* or *in vitro* methods is important. Commercial application of banana by *in vitro* culture usually use shoot multiplication technique can increase the rate of seedling production and improve the seedling quality such as uniformity and being true to parental type. The average rate of shoot formation produced by this technique was 4-5 shoot per monthly subculture (Priyono, 1993). Most of the organized cultures, especially the shoot tips maintain strict genotypic and phenotypic stability under tissue culture conditions (Bennici A, 2004). The somaclonal variation corresponds to natural phenotypic variation of banana cultivar which was enhanced *in-vitro* (Vuylsteke and Swennen, 1990) and the rate of variation was not affected by medium composition or by the rate of multiplication (Reuveni, 1993). The multiple shoots of banana and plantain could be produced from sliced meristems on either an agar or in liquid medium (Cronauer and Krikorian, 1984). The plants transplanted from NAA-supplemented medium developed a higher number of adventitious roots per plant than those transplanted from NAA-free medium. But, only 20% of them had lateral roots. On the contrary, 80% of the plants transplanted from NAA-free medium had lower number of adventitious roots than those transplanted from NAA-supplemented medium (Buha, 1998). The aim of this study was to standardize the protocol for *invitro* regeneration of shoots and roots from sword sucker by regulating their growth with different concentration of phytohormones that enhances its growth at different growth duration.

Materials and methods

Source of plant material:

Healthy sword suckers of elite banana plant (Rasthali Variety) was chosen as explants and were collected from Salem, TamilNadu for mass propagation by plant tissue culture technique.

Explant preparation and disinfection:

Sword suckers were carefully removed from field grown fruiting Banana plants (Rasthali) and washed thoroughly in tap water and diluted solution of detergent teepol. All traces of teepol and the extraneous rhizome tissue were carefully chopped with a stainless steel knife. Trimmed suckers were soaked in solution of 0.5% Bavistin (a fungicide) for 6 to 8 hours. Shoot tips containing rhizome tissue that measures 2.0 to 3.0 cm in length were isolated. Surface – sterilized with chlorine-saturated distilled water for 15 to 20 min and 1.0% mercuric chloride for 5 to 7 minutes. Further operations are carried out under a laminar flow chamber. All traces of chlorine are removed by washing several times with autoclaved, sterile distilled water. The sterilized shoot tip explants, were handled using sterile stainless steel scalpels. Cut surfaces of the rhizomatous tissue and leaf bases are further trimmed, so that shoot tips finally contain at least 6 to eight overlapping

leaf bases enclosing auxiliary buds. The explants measures 1 to 2cm were selected for inoculation. It was then immersed into the sterile solid medium present in the culture vessel.

Media preparation:

Murashige and Skoog (MS) medium was used as the basal medium. Sucrose at 3% (w/v) was added into the mixture and a growth regulator has been added before pH adjustment. The pH of the medium adjusted to 5.7±0.1 with 0.1 M HCl and 0.1 M NaOH followed by addition of 0.8% (w/v) agar. Then the medium was autoclaved at 121^{o}C, 15 psi for 15 min. After autoclaving, a total of 30mL of the sterile medium were poured into sterilized bottle in the laminar flow and allowed for solidification. The bottles were sealed prior to the initiation of treatment.

Shoot proliferation:

The explants were cultured on MS medium supplemented with different concentrations i.e. 0.5, 1.0, 1.5, and 2.0mg/l of Benzyl Amino Purine (BAP). Percentage of explants showing shoot proliferation and total number of shoots per explants were considered as parameters for evaluating this experiment. Later the shoot proliferation was observed from days of incubation, proliferating cultures were sub cultured again in same initial medium in order to increase budding frequency. After another 4 weeks of incubation, the regenerated plant was transferred to medium contains different PGR (1%GA_3) for shoot elongation.

Shoot propagation and Acclimatization

After formation of shoots, up to 5cm, the plantlets were then transferred to similar media with the same concentration of growth hormone under aseptic condition and again incubated. Serial sub culturing with certain time interval results in multiple shoot formation. Newly formed shoots measuring 5-7cm in length were excised individually from the parent explants and transferred to rooting media. The growth regulators NAA, IBA were used in combination/separate in various concentrations from 0.5, 1.0, 1.5 and 2.0 mg/l was added to half strength MS medium. The observations on development pattern of roots were made throughout the entire culture period. Data were recorded after 4 weeks of culture.

After 20 days of root formation on rooting media, the plantlets were shifted for acclimatization. Pots were kept ready filled with garden soil, compost and sand in the ratio of 1:1:1. Then, the plants were transplanted into the pots with special care. The plant was kept in surface chamber for initial periods and then, to the glass house having 80% humidity and 31°C temperature for 10 days. Then it was taken to shade house with less humidity. After 45 days, the plants were transferred to the soil.

Results

The explants started to show the signs of shoot proliferation after a weak of culturing. All explants gave aseptic culture. Plants were free from fungal as well as bacterial contamination. The shoot proliferation at different concentration of BAP (0.5- 2.0 mg/l) was recorded. The higher concentration of BAP showed good response compared to lower concentration of BAP. Shoot proliferation on medium with 2.0mg/l BAP gave the best response (Table 1, Fig 1A, B and C). Medium having the concentrations of 1.5mg/l IBA, 1.0mg/l of NAA and 2.0mg/l of BAP was found to be the best medium for shoot and root proliferations in half strength MS medium (Table 2,Fig1D and E). The small rooted shoots were transferred from *in vitro* conditions to plastic pots and placed under net to keep the environment wet and shade. Survival of plantlets was observed after one month of acclimatization (Fig 1F).

Discussion

The present study implies that, for shoot proliferation the growth regulators like Auxin and Cytokin in influences the process seriously. The regeneration of shoots from the explants were observed within 7 days of incubation and the roots were observed after 7 days of incubation under controlled conditions i.e. at 24±2ºC and 16 hours photoperiod with light. The maximum shoot and root generation observed in the concentration of MS medium + 1.5 IBA+1.0 NAA was observed and the optimum root multiplication with maximum of 1.3cm root length. MS medium supplemented with 5.0mg L^{-1} BAP+5.0mg L^{-1} KIN+13% coconut water were found to be the optimal media composition for maximum number (5.8±0.154) of shoot regeneration from sucker explants reported by Rahman *et.al* (2005). MS medium supplemented with 1mg/L of IBA rooted earlier that was reported by Buah *et.al* (1998). Noor Aziah and Khalid (2002) used higher concentration of BAP for regeneration, using whole meristems and scalps as explants. In the present study a simple two step protocol was established using MS with BAP for shoot initiation, multiplication and IBA+NAA for rooting in Banana using sword sucker as explants.

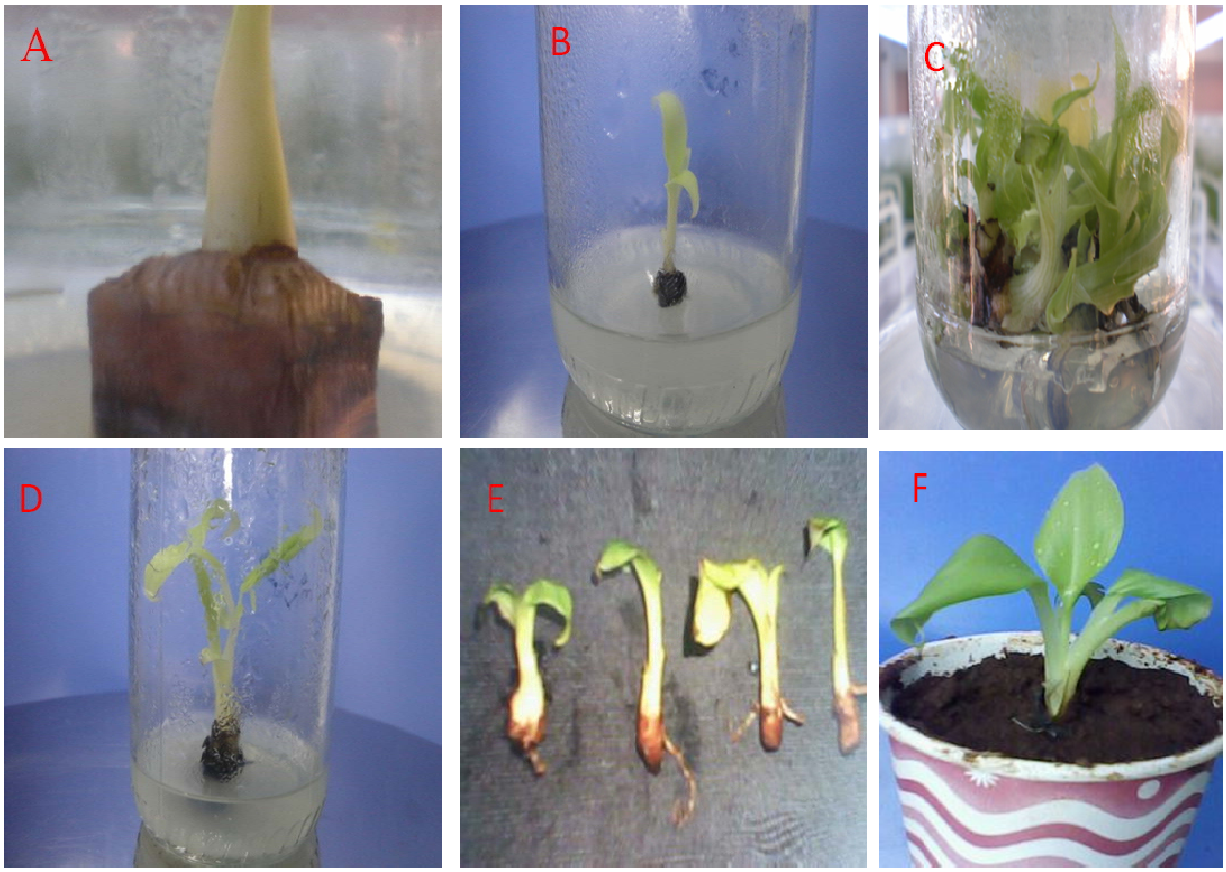

Figure 1: *In vitro* propagation of sword sucker (Banana) (A) Inoculated explants showing shoot formation after a weak of incubation; (B) Shoot Elongation; (C) multiple shoot proliferation Inductions; (D) Root initiation; (E) Root formation; (F) Acclimatization.

Table 1. Effect of different concentrations of 6-Benzyl amino purine (6-BAP) in half strength MS on shoot formation of Banana (sword suckers)

Concentration of BAP (mg/l)	% of explants producing shoots (Mean ± S.E)	Averages of shoots (Mean ± S.E)	Bud elongation
0.5	31.5±1.12	12.75±1.48	+
1.0	51.0±1.87	13.75±1.49	++
1.5	61.0±2.55	15.25±1.51	+++
2.0	82.3±1.92	18.25±1.53	++++

NOTE: + Very less, ++less, +++Moderate, +++ good. *The experiments were repeated and their mean values are calculated.*

Table 2: Effect of different concentrations of IBA, IBA and NAA in half strength MS on rooting of micro shoots in banana (sword sucker)

Hormone concentration (mg/l)	No of roots developed	Root length (cm)	Fresh weight (gm) (Mean ± S.E)
IBA 0.5	1	0.6	0.20±0.020
1.0	1	1.22	0.39±0.015
IBA+NAA 1.5+1.0	2	1.3	0.43±0.015
2.0+1.0	1	1.2	0.31±0.019

The experiments were repeated and their mean values are calculated.

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