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Optimizing the effect of plant growth regulators in Micropropagation of banana variety Monthan

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Abstract
Monthan variety of banana is widely cultivated variety for processing. An advantage of micro propagation over conventional techniques is that it helps pave a way towards conservation of endangered species, helps produce disease free plants and also helps in rapidly multiplying stock plant material. This study emphasizes the effect of plant growth regulators on the micro propagation of Monthan variety of banana. The sword suckers of three months old were used as explants. The explants sterilised thoroughly and MS media supplemented with the cytokinins (BAP and kinetin), coconut water and auxins (IBA and NAA) in different concentration (3 to 5 mg/l) were used to study the best the multiple shoot generation from explants and root growth from shoots. Among the tested concentrations the desirable shoot multiplication was found in MS basal with BAP 3 mgl-1+Coconut water 5%. The micro propagated plants were then hardened in greenhouse followed by the acclimatization. This study concluded this concentration of growth regulators can be set as a practicable protocol for the successful regeneration of new plantlets of Monthan variety of banana.

Keywords: Banana, cytokinin, auxin, sword suckers

Introduction
Banana is one of the most important fruit crops of India. It occupies an area of 883.8 thousand Hectares. It supports livelihood of millions of people in India with total annual production of 30.807 million tonnes and a productivity of 34.9 MT/Ha. (Horticulture Statistics Division, Department of Agri. & Cooperation 2017-18). Monthan variety of banana is a widely cultivated variety in Tamil Nadu and is used for processing and vegetable purpose. Monthan is an average heighted robust plant bearing bold, stocky green fruits having a bunch size of 18-20 kg with an average yield of 30-40 tonnes per ha. The pseudostem core is a highly relished vegetable with immense medicinal properties. It has inbuilt desirable qualities like immunity to Banana Bunchy Top Virus (BBTV) diseases, salt tolerance and normal bunch mass even under marginal condition, but it is highly susceptible to Fusarium wilt disease.

The multiplication ratio of banana is however very low and hence, extensive research is being conducted worldwide for rapid propagation methods and attention has been drawn to the possibility of using aseptic culture techniques, which facilitates quick multiplication in higher quantity (Binns, 1994) [1]. The micro propagated banana plants grow more dynamically with uniform production periods and produce higher yields than conventional suckers. Success of in vitro culture depends largely on the choice of nutrient medium, including its chemical composition and physical form (Murashige, 1974) [6, 7]. Several media formulations have been reported for banana shoot tip culture but nearly half of them are modified MS media. The goals of this study is to examine the effect of different culture conditions on shoot tip culture and to find out the best plant growth regulators for shoot proliferation and multiplication of banana so as to obtain optimum concentrations of IBA or NAA for root induction of banana. In this study, the Monthan micropropagation is carried out to propagate plantlets for expansion of area under cultivation and also to produce disease free plants. Since there is demand for Monthan banana for culinary purpose all over the country, its production will have immense advantages to farmers and people to earn better income and relish the banana for cooking (Chen, and Ertl, 1994) [4].

Materials and Methods
The meristem used for the tissue culture work was obtained from the selected healthy developing suckers (weighing 2-3kgs) of about four months of age grown under field conditions. The outer leaves were removed until the shoot measured about 2.0 cm in length and 1.5 cm width at the base of the rhizome. The Sucker was then sterilized using 0.2% Bavistin for 30 min and washing with Tween-20 for 2 mins and then again washed thoroughly for 20 mins in running tap water

and then Streptomycin 0.1% treatment was done and kept overnight. The Explants are sterilized in LAF chamber. The pale white tissue block (l.0 × 2.0 cm) containing meristem and rhizomatous base were taken in a beaker and treated with Sodium hypochlorite 4% for 5 min which is finally trimmed and cultured in bottles containing MS media with the following media components with MS basal.

Murashige and Skoog (1962) medium supplemented with different phyto-hormones as per treatments were used as culture medium for shoot induction, shoot multiplication and maintenance and regeneration of roots from multiplied shoot. Hormones were added separately to different media according to the requirements. The culture tubes with media were then autoclaved at 1.06 kg/cm^2 pressure at 121 ℃ for 25 minutes.

The following are the media compositions

1. Benzyl aminopurine 5 mgl^{-1}
2. BAP 3 mgl^{-1} + kinetin 2 mgl^{-1}
3. BAP 3 mgl^{-1} +Coconut water 5%
4. BAP 5 mgl^{-1} +IAA 1mgl^{-1}

The isolated and surface sterilized explants were directly inoculated to each of the culture tube containing 20 ml of MS medium supplemented with different concentrations of hormones as per treatment and was covered with paraffin.

Incubation: The culture tubes were then transferred to growth room and allowed to grow in controlled environment. A 16-hour light period was maintained with light intensity of 2000 lux for the growth and development of culture.

Subculturing: To control blackening after about one week, the blackish tissues on the explants were removed and the meristematic tissues were, transferred to similar fresh medium. It was repeated 10days interval for about one month to minimize further blackening of the tissues. Initial sub-culturing was done when the explant produced some shoots. For sub-culturing, the entire samples of *in vitro* shoot were cut. It was practiced at the interval of every one month.

Root induction: When the shoots grew about 3-5 cm in length with 3-6 well developed leaves, they were rescued aseptically from the culture tubes and were separated from each other and again cultured on freshly prepared medium containing different combinations of hormonal supplements of 1mg/l of Indole Butyric Acid for root induction.

Hardening: Potting mixture containing ground soil and cow-dung in the ratio of 1:1 was mixed thoroughly and were placed into a 10 × 15 cm polythene bag for growing *in vitro* grown plantlets under *ex vitro* conditions.

Results

Micro-propagation in banana: Regeneration of banana plantlets through meristem culture offers a unique scope of developing disease free planting materials against bunchy top, cucumber mosaic virus and panama wilt. This study emphasized the in vitro culture of meristem in different concentration of hormones. It was observed that there was initiation of shoots from the explant in the MS media supplemented with BAP 2mg/l after which the sub culturing was done. There best combination of multiple shoot formation of explants was obtained in the MS media supplemented with BAP 3 mgl-1 +Coconut water 5%.

Discussion

It was observed that shoot multiplication in Monthan was found in MS basal with BAP 3 mgl-1 +Coconut water 5%. However, Some report showed that MS Media with 0.5 mg/l BAP +0.5 mg/l NAA was considered effective for maximum shoot regeneration. (Akbar *et al.*, 2003) [9] and attained maximum shoot length at a concentration of BAP25 µM. MS media in concentration with 4mg/l BAP+2mg/l NAA was considered optimum for more number of shoot regeneration in banana.

Conclusion

Under this project work course we had undergone the tissue culturing of banana variety Monthan. The stock solutions of MS Media were prepared and suckers were selected from 4 month old plants and sterilized the suckers and treated them with bavistin, teen twenty and anti oxidants. Then inoculation of the explants was done in MS media with different concentrations of BAP in Laminar air flow chamber. We observed initiation of shoots from the explants in all different combinations of plant growth regulators and through this study it was confirmed hat basal medium (MS) supplemented with BAP 3 mgl-1 +Coconut water 5% of is good for multiple shoot generation and growth. It can be further used in future studies of monthan variety of banana.

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