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IN VITRO MICROPROPAGATION OF *MUSA ACCUMINATA L.*

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ABSTRACT

***AIM OF STUDY:** The present investigation was aimed at reducing the cost of production of micro propagated Musa accuminata L. **MATERIALS AND METHODS:** The surface sterilization treatment with sodium hypo chloride + ethanol + mercuric chloride for 4-6 hrs outside the laminar air flow cabinet followed by 0.1% $HgCl_2$ for five minutes in laminar air flow cabinet registered best sterilization of banana shoot tip explants with minimum contamination of the cultures. Of various treatment combinations, Murashige and Skoog (MS) medium+ BAP 5.00 mg/l with resulted in maximum establishment of cultures. For root initiation, half strength MS medium supplemented with different levels of BAP (1.0, 2.0, 5.0 mg/l) was used. **RESULT**: Sucker were surface sterilized with $HgCl_2$ (0.1%) for 6 min which have minimum contamination with maximum culture establishment. MS medium + BAP 5.00 mg/l gave maximum (2.78) shoots. The highest root length (8.58, 8.76 and 10.22 cm) was recorded at 4-6 days. Survival rate of the plantlets were found to be 34.4% during primary hardening. The plantlet was segregated based on the morphological growth into normal (97.26%), tall (2.1%) and dwarf (2.6%) plants, this condition of abnormality is might be due to all the plantlets were subjected to the secondary hardening with garden soil, sand and red soil in the ratio of 1:1:1 in polybags and all the plantlets showed 100% survivability. **CONCLUSION:** This tradition of in vitro mass propagation method appears to satisfy the increase in demand for disease free and healthy planting materials of banana for commercialization.*

KEY WORDS

Benzyl Amino Purine, Musa Accuminata L., Micropropagation, Murashige and Skoog (MS) medium.

INTRODUCTION

Banana (*Musa accuminata L.*) belongs to the musaceae family. Banana provides millions of people throughout the tropic and subtropics with staple food and accounts for one of the most widely exported fruits in the world. Presently, banana is grown in around 150 countries across the world on an area of 4.84 million ha, producing 95.6 million tonne [1].

Micropropagation means the multiplication of the initial plant material via shoot induction caused by cytokines. The mode of action on plants expressed by different cytokines can be different, influenced by the type of cytokine, the genotype of the plant, the age and physiological status of the cultured tissues [2].

Moreover, the shoot multiplication cycle is very short (2-6 weeks), each cycle resulting in an exponential increase in the number of shoot and plants multiplication can be continued throughout the year irrespective of the season. To minimize the above-mentioned problem, micropropagation could be an alternative for propagation of planting materials for banana. In this method, over a million of plant can be grown from a small or even a microscopic piece of plant tissue within a year [3]. Propagation process either by increasing the number of end-products or improving the

quality of plants thus improving the chance of acclimatization. Interest is focused nowadays onto natural substances, which exerted positive influence on the growth of different plants in field trial.

Often the *in vitro* plants submitted to MS medium with different concentration of BAP (benzyl amino purine). This work, only florogolicinol was used to observe the development behaviour of the *in vitro* banana plant, because in the literature was observed that we can evidence responses to the formation of shoots in several species [4].

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 germplasm itself [5].

For commercialization, it is necessary consistent supplies of good quality bananas are produced to meet the increasing demand. The high rate of multiplication of genetically uniform, pest and disease-free planting material can be achieved through tissue culture. Tissue culture approaches would also allow for off-season production of fast growing plants compared with conventionally propagated once [6].

Here, we have attempted a very simple, economically, rapidly multiplying and highly reproducible protocol for large scale micropropagation. Our present work demonstrates plant regeneration from virus free sucker explants of triploid banana variety and feasibility of recovering and converting to plantlets is also studied [7]. The present study was undertaken with the following

OBJECTIVES

1. To selection of explants for tissue culture of Musa accuminata L.
2. To prepare Murashige and Skoog's medium at 121°c for 2 hrs
3. To standardization of multiple shoot induction through different plant growth regulators from Musa accuminata L.
4. To study the effect of BAP and IAA growth regulators on in vitro meristem culture and shoot proliferation of banana
5. To standardization of root induction through different plant growth regulators of Musa accuminata L.
6. To develop a micro propagation system for large scale production of banana

MATERIALS AND METHODS

A) Collections of Plant Material

The major plant source for this study was *Musa accuminata L. sp.* The plants were collected from nursery garden at Sundarakkottai village, Mannargudi (TK), Tiruvarur Distirct, Tamil Nadu, India.

B) Surface sterilization of the explants

The suckers' explants were carefully transported to the laboratory with the help of sterile polypropylene bags [8]. The roots and outer leaves were removed until the shoot measured 3.0-3.5cm in length and 1.0-1.5cm width at the base. Surface sterilized with 70% ethanol, 0.1% mercuric chloride solution and diluted tween-20 solution. After that, the explants were rinsed about three to four times with sterile distilled water.

C) Preparation of explants

The explants were excised with the help of sterile forceps and blade. The stems were cut into 3.0-1.5 cm sized segments and care was taken for preparation of MS medium [9].

D) Inoculation of explants

The surface sterilized meristem tissue block were then prepared under Stereomicroscope by removal of outer tissue of meristem with the help of sterile scalpel under aseptic condition inside the laminar airflow cabinet until the length reached to3.0-1.5cm [10].

E) Establishment medium

In establishment stage, all explants cultured on a MS medium containing salts, vitamins and sucrose and without any hormones [11].

F) Proliferation medium

The basal nutrient medium containing MSsalts and vitamins was used with Naphthalene acetic acid (NAA) and Benzyl amino purile (BAP) Explants were sub cultured to the fresh medium every 4 weeks. Finally, excised single shoot from multiple shoots were transferred to the fresh medium for root induction [4].

G) Shoot proliferation

The shoot proliferation media contained full strength MS salts and vitamins with various levels of BAP (0, 2, 4 and 8µm) in combination

with NAA (0, 0.05, 0.25 and 0.5µm). Each treatment involved 5 repeats with 5 explants [12]. Number of axillaries shoots, and number of new leaves were recorded after 21 days for three subsequent subcultures and the averages were calculated.

H) Rooting Medium

To establish root proliferation, green and normal adventitious shoots from shoot proliferation cultures were excised and cultured on MS medium containing full and half strength of MS salts. Cultures were maintained at 22°c in a culture room with a 16 hrs photoperiod light [13].

I) Root Initiation

Shoots were cultured on shoot elongation medium (MS mineral salts and vitamins without hormones) for 21 days prior to rooting treatments. Each treatment involved 5 repeats with 5 explants (25 explants). After 21 days, number of roots and their lengths were recorded and data for different concentrations of MS media (full, 1/2 and 1/4) and state of media (semi- solid and liquid) were recorded [14].

J) Regeneration of plants from *in vitro* proliferated buds (shoot differentiation)

In vitro proliferated micro shoots were separated with the help of sterilized scalpel and trimmed the lower base. Photoactive shoots into define size and each of the micro- shoots was placed on culture medium, supplemented with BAP for shoot differentiation.

K) Primary hardening

To overcome the problem in hardening and acclimatization, the *in vitro* raised plantlets were transferred from bottle to net pots and kept in groups in mist chamber maintained at 80-90% humidity [15]. The humidity was gradually reduced, and plantlets were kept outside the mist house.

L) Secondary hardening

After 40 days, primary hardened plants with good root ball and shoots were transferred to poly bags which were filled with various potting mixtures such as garden soil [16].

RESULTS AND DISCUSSIONS

The study demonstrated the feasibility of propagating banana *in vitro.* The effect of different treatments of sterilant used either singly or in combination for the surface sterilization of explants. Mercuric chloride (0.1%) for 6 minutes gave the best sterilization of explants, recording less (5%) contamination and the highest establishment (95%). Sterilization of explants with sodium hypochlorite alone was found to be ineffective resulting in very high contamination (55 to 100%). On the other hand, considerable reduction in the rate of contamination (10%) with 85% survival of explants was obtained by treating the explants with sodium hypochlorite (5%) for 10 minutes after rapid rinsing with ethanol (70%) for 30 s and mercuric chloride (0.1%) for 6 minutes **(Figure 4).** Since the report of [17] on possibility to multiply orchids by micropropagation and later its commercial application, there has been an increasing interest in the development and application of tissue culture techniques as an aid or alternative means of vegetative propagation of horticultural species.

The meristematic shoot tip explants were inoculated on MS medium with eight different combinations of BAP and IAA. Among the various treatments, the effective results were obtained from these eight combinations. After 2 weeks, explants got swelled changed colour to green and produced shoots let. In the micropropagation, the organs and tissues are carried through a sequence of steps in which differential culture and environmental conditions are provided [18]. Sub-culturing of the initially established shoots was done to the liquid media with same hormonal supplements which induced multiple shoots. After five sub-culturing, the clump formation occurs and the capability of culture to further divide gradually becomes low **(Table 1)**. After 3 weeks, threefold increase in multiplication was seen table1.It is evident from the table that sub culturing after 25 days (D3) gives maximum number of shoots per explant (2.74) which was significantly higher than other days subculture. Whereas, sub culturing after 30 days recorded 4.62 cm of longest shoot followed by sub culturing after 25 days (4.58 cm), this value was significantly higher than all other treatments **(Figure 1).** A number of treatments were employed with half strength of MS medium, supplemented with different concentrations of BAP for efficient root induction. In

Eucalyptus citriodra only NAA (0.2 mg/l) induced rooting [19].

Effect of BAP on rooting response of *in vitro* raised microshoot of banana is present. It is evident from the data that cent per cent rooting was recorded with all the six treatments examined **(Figure 2).** Number of roots per shoot was highest in treatment R6 (MS + 5.0 mg/l BAP) i.e.10.22 which was significantly higher than all other treatments tested. Length of longest root was also maximum in treatment R6 (6.23 cm) which was at par with treatment R5 (6.10 cm) and both of these treatments recorded significantly higher root length than other treatment **(Table 2)**. For the induction of roots in *in vitro* raised shoots of banana, half strength MS + 1.0 mg/l BAP was used. whereas auxin free MS medium was also applied for rooting of banana microshoots [20].

The fully developed plantlets were *in-vitro* hardened for 25 days in half strength MS media, then transferred to different potting media under Green House conditions. After 30 days, the plantlets in the different potting media were analyzed **(Figure 3).** Another study [21] described that treatment of shoots of Pignusstrobus in an upright position with 50 µM IBA for 8 days followed by culture in half strength MS medium with 3% sucrose in the light produced the most rooting (50% at 3 months).

The normal plants were in good health, green, with alternate leaf arrangement, broad leaf area, roots ending led in the growth medium and formed a root ball from young roots. The normal plantlets growth was 97.26% during hardening and result.

Tall plants were approximately 2-3 times taller than the normal plants, tapering and with pointed leaves; internodes distance more as compared to the normal ones. Three plants were obtained as taller one and the percentage of occurrence is 2.0%.

Dwarf plants were most difficult to identify during primary hardening, this dwarf plants were identified mostly towards the end of secondary hardening.

FIGURE 1: Shoot proliferation

FIGURE 2: Stages of growth in explants

FIGURE 3: Regeneration of plant

FIGURE 4: Effect of surface sterilization treatments on establishment of shoot tip explants of banana

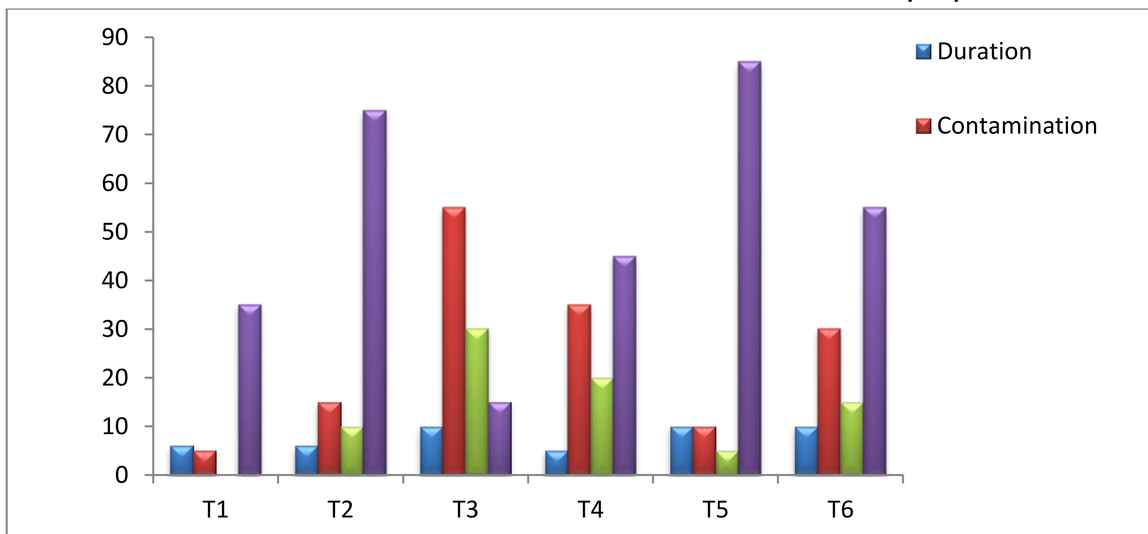

TABLE 1: Effect of sub culturing days (duration of growth cycle) on the establishment, multiplication and growth of the regenerated shoots of banana

S.No	Treatment	Days of sub culturing (Days)	No.of shoots per explant	Length of longest shoot (cm)	Growth of culture
1	D1	15	2.06	3.2	+++
2	D2	20	2.20	4.02	+++
3	D3	25	2.74	4.58	+++
4	D4	30	2.72	4.62	++

+ Poorest, ++ Medium, +++ Good, ++++ Best

TABLE 2: Effect of BAP and medium strength on the induction of rooting of *in vitro* regeneration of banana roots.

Treatment	Media and BAP concentration	Culture rooted (%)	Time taken for root initiation (days)	No. of roots per shoot	Length of longest root (cm)	Growth of culture
R1	½ MS + 1.0 mg/l BAP	100.00	4-6	8.30	4.50	Long, thin and fibrous root
R2	½ MS + 2.0 mg/l BAP	100.00	4-6	8.58	4.60	Long, thin and fibrous root
R3	½ MS + 5.0 mg/l BAP	100.00	4-6	8.76	4.32	Long, thin and fibrous root
R4	MS + 1.0 mg/l BAP	100.00	4-6	7.10	5.22	Long, thin and fibrous root
R5	MS + 2.0 mg/l BAP	100.00	4-6	8.54	6.10	Long, thin and fibrous root
R6	MS + 5.0 mg/l BAP	100.00	4-6	10.22	6.23	Long, thin and fibrous root

CONCLUSION

The present investigation was aimed at reducing the cost of production of micropropagated *Musa accuminata L.* Moreover, the developed protocol showed no problems normally encountered in MS media-based techniques. The study may be extended for different concentrations of other growth hormones. It is visualized that this concept of micropropagation can be utilized for large scale multiplication of the plantlets in which farmers can get true to type plants at lower cost. Therefore, from the composition might be applied for large scale propagation of healthy and disease-free banana.

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