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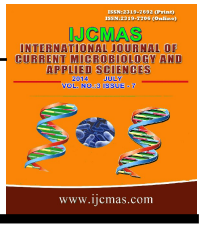

Original Research Article

In vitro propagation of *Musa* sp (Banana)

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A B S T R A C T

Experiments were conducted for the standardization of *in vitro* culture technique for the mass propagation of *Musa* sp is one of the important fruit crops. Shoot tips were used as explants and they were cultured on Murashige and Skoog (MS) medium supplemented with different concentrations of BAP, Kin and IAA both in individual and in combined form for shoot inductions and the best results were obtained from MS medium supplemented with BAP+IAA at the concentration of 3.0mg/l and 0.5mg/l respectively. Best root formation of *in vitro* developed shoots could be achieved on halt strength MS medium supplemented with IBA at concentration 1.0mg/l. The *in vitro* developed plantlets were transferred to pot and they were grown in greenhouse for hardening and finally they were planted in the open field. Around 87% of plants were successfully established in natural field condition.

Keywords

Musa sp; *in vitro* Propagation; MS medium; BAP; Kin; IAA; IBA; NAA

Introduction

Bananas and plantains are giant perennial herbs and provide an essential food source for more than 400 million people throughout the developing countries of the tropics and the subtropics [1]. It is the most important and most widely grown fruit crop in the world. It ranks as the fourth major crop after rice, wheat and maize and is considered as a poor man's apple in tropical and subtropical countries [2, 3]. Generally, banana cultivars are good sources of carbohydrates, proteins, vitamins and minerals. As the banana cultivars are having high degree of sterility and polyploidy the conventional breeding methods are difficult in banana improvement. Many pests and diseases are also threatening the good production of banana cultivars. In Oder to augment conventional breeding and to avoid constraints imposed by pests and pathogens, transgenic and *in vitro* approaches are being considered [4]. Though several *in vitro* approaches have been made on many banana cultivars for their overall improvements the present attempt aims at the formation of simple and cost effective protocol for the *in vitro* propagation of the banana cultivar studied.

Materials and Methods

Source of Explants

For this study, the field grown *Musa* sp were

collected from the Agriculture department, Annamalai University, Annamalainagar, Chidambaram and South India. Explants were collected from shoot tip for shoot initiation.

Surface sterilization

After excision, the surface sterilization, the explants were primarily rinsed in tap water for 30 minutes followed gently rinsed with 70% ethanol for 60 seconds and with 5% sodium hypochloride solution for 10 minutes. After each step of sterilization, the explants were washed with sterile double distilled water for three times. Further sterilization procedures were carried out in laminar air flow chamber by using 0.1% $HgCl_2$ for 5 minutes. The explants were then rinsed five times with sterile distilled water.

Inoculations

After complete sterilization, the explants were inoculated in MS medium [5], supplemented with cytokinins and auxins used either singly or in combination. The pH of the medium was adjusted to 5.8 and prior to autoclaving, 0.7% agar (Himedia, Mumbai) was added to the medium. Then, autoclaving was done at 121ºC and 15psi for 20 minutes.

Establishment of culture

After inoculation, the cultures were maintained at a temperature of 25±2ºC with a photoperiod of 16hrs per day. Lighting of $80\mu Em^{-2}s^{-1}$ was supplied by using cool and white fluorescent tubes (Philips India Ltd.). Various types of plant growth regulator, viz. BAP, Kin and IAA were added with MS medium either alone or in combination for better shoot induction (Table 1). The MS medium without adding of plant growth regulator was served as control. After 35-40 days of culturing, the multiple shoots were separated into pieces and the separation was done at the base of multiple shoots and they were transferred to 500ml culture bottle containing 50ml of the same kind of medium to get a more number of shoots. For root initiation, the shoots were transferred to half strength MS medium supplemented with IAA, IBA and NAA.

Hardening

Healthy plantlets with 4 to 5cm long roots were individually removed from the culture tubes. After washing their roots carefully with tap water, plantlets were transplanted into 10cm diameter plastic pots containing a mixture of sand, soil and vermicompost (1:1:1) and they were placed in the greenhouse for hardening. The plants were watered with half-strength MS salts solution every week and covered with a polythene bag for 2 weeks. Afterwards, the hardened plants were gradually transferred to 20cm pots containing pure garden soil and kept in the field for developing into mature plants.

Statistical Analysis

All experiments were repeated five times. The effects of different treatments were quantified and the data subjected to statistical analysis using 'standard error of the mean' by using SPSS software [6].

Results and Discussion

Explants selection and shoot induction

In this study, shoot tips were excised from the plant species of *Musa acuminata* as explants sources, and the shoot tips were found best explants for shoot induction on MS medium supplemented with different concentrations of BAP like 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0mg/l or Kin like 0.5, 1.0, 2.0,

3.0mg/l individually. In another way, the MS medium supplemented with the combined form of BAP + Kin and BAP + IAA. In individual form, the BAP concentration above 3.0mg/l level caused poor shoot induction in two explants studied. The more number of shoot induction was observed in two explants studied on MS medium supplemented with BAP 2.0mg/l + IAA 0.1mg/l. When observations were made on the cultures of 35 days period, development of 6 (16.20 ± 0.37, Table 1) shoots were observed from a single shoot tip explants, while 14 (14.00 ± 0.31, Table 1). Frequency of shoot proliferation was increased with the increase of sub culture in the same medium. In early studies, optimum BAP concentrations were found to be 22.2µm by [7, 8] and 2.0mg/l by [9, 10] stated that 44.4 µm BAP reduced shoot multiplication. [11] Stated that shoot proliferation is cultivar dependent

This *in vitro* propagation studies confirmed the importance of plant growth regulators in the initiations of callus, shoot and root on the whole the regeneration of plant. In this way, the two cytokinins namely, BAP used in this study, the BAP proved better one in shoot induction from shoot tip explants. On the other hand, mineral nutrients are being as the basic component of culture media play a vital role in rapid growth of tissue and the quality of morphogenesis of tissue [12].In this study, the synergistic effect of both BAP + IAA at concentration 2.0 and 0.5 mg/l respectively, were found best in regenerating shoot tip explants. Similarly the combination of BAP 2.0mg/l and Kin 0.5mg/l worked well for the shoot proliferation and elongation from the same explants.

Root induction

The micro cutting of *in vitro* proliferated shoots were implanted on half strength MS medium supplemented individually with IAA, IBA and NAA concentration of 1.0, 1.5, and 2.0mg/l for root initiation. Among them, maximum number of root formation (96%) was observed in medium supplemented with (1.0mg/l) IBA (11.80 ± 0.80, Table 2). The root induction was gradually decreased with increasing concentrations of auxin types. No root formation was observed auxin free basal medium. Similar types of results were found by earlier workers in the same species

Acclimatization of regenerated plants

For acclimatization, the plantlets were taken out from the culture tubes when the roots were partially brown in colour and root portion was washed with tap water to remove the attached medium. Then they were transferred to pots containing sand, soil and vermicompost in the ratio 1:1:1 and they were placed in greenhouse for hardening for the period of four weeks.

The use of sufficiently porous substratum that allows adequate drainage and aeration has been recommended for fast acclimatization of *in vitro* regenerated plants [13] and this study also it has been followed. After four weeks under greenhouse, the potted plants were transferred to natural field conditions for better establishment and at the end of this study, around 87% of plants were thrived well in natural situation.

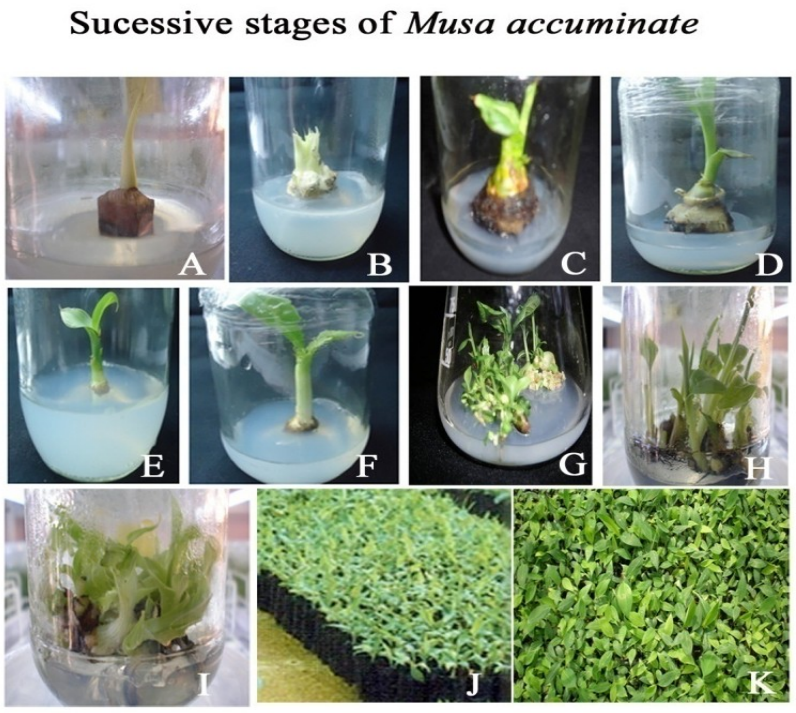
Table.1 Effect of plant growth regulators on shoot induction

Sl. No.	Plant Growth Regulators	Shoot bud (Mean ± SE)
1	Nil	0.20 ± 0.19
2	BAP 0.5	2.60 ± 0.24
3	BAP 1.0	3.60 ± 0.40
4	BAP 1.5	5.00 ± 0.31
5	BAP 2.0	6.25 ± 0.22
6	BAP 2.5	6.50 ± 0.26
7	BAP 3.0	7.25 ± 0.28
8	BAP 4.0	6.15 ± 0.22
9	Kn 0.5	1.90 ± 0.18
10	Kn 1.0	2.15 ± 0.19
11	Kn 2.0	2.75 ± 0.22
12	Kn 3.0	2.50 ± 0.21
13	BAP 1.0 + Kn 0.2	2.75 ± 0.24
14	BAP 1.0 + Kn 0.5	2.90 ± 0.25
15	BAP 2.0 + Kn 0.2	3.35 ± 0.22
16	BAP 2.0 + Kn 0.5	7.50 ± 0.26
17	BAP 1.0 + IAA 0.2	2.80 ± 0.24
18	BAP 1.0 + IAA 0.5	3-10 ± 0.28
19	BAP 2.0 + IAA 0.2	4.00 ± 0.24
20	BAP 2.0 + IAA 0.5	7.85 ± 0.26

Table.2 Effect of different concentrations of IAA, IBA and NAA on root formation

S. No.	Concentrations of auxins (mg/l)	Percentage of root per shoot (%)	No. of roots per shoot (mean ± SE)	Average length of root per shoot (cm, mean ± SE)
	IAA			
1	0.2	72	5.00 ± 0.31	2.40 ± 0.18
2	0.5	87	6.00 ± 0.44	2.94 ± 0.16
3	1.0	93	10.40 ± 0.74	4.00 ± 0.31
4	2.0	70	4.60 ± 0.24	2.32 ± 0.20
	IBA			
5	0.2	76	5.40 ± 0.37	3.00 ± 0.17
6	0.5	88	6.20 ± 0.37	3.80 ± 0.26
7	1.0	96	11.80 ± 0.80	4.80 ± 0.31
8	2.0	67	4.20 ± 0.37	3.06 ± 0.13
	NAA			
9	0.2	57	3.40 ± 0.50	1.96 ± 0.16
10	0.5	64	4.80 ± 0.37	2.40 ± 0.15
11	1.0	70	6.40 ± 0.37	3.60 ± 0.20
12	2.0	48	4.00 ± 0.54	2.16 ± 0.14

Figure.1 The successive stages of *in vitro* propagation of *Musa acuminata*



(A-F) Direct regeneration of shoot from explants
(G) Initiation of multiple shoot formation
(H) Development of more number of multiple shoot
(I) Root formation from regenerated shoot
(J, K) Hardening and establishment regenerated plants

The *in vitro* propagation method developed in this study can serve as a convenient method for large scale, disease free, homogenize development of banana cultivar studied and can help the enhancement of economic benefit of the farmer.

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