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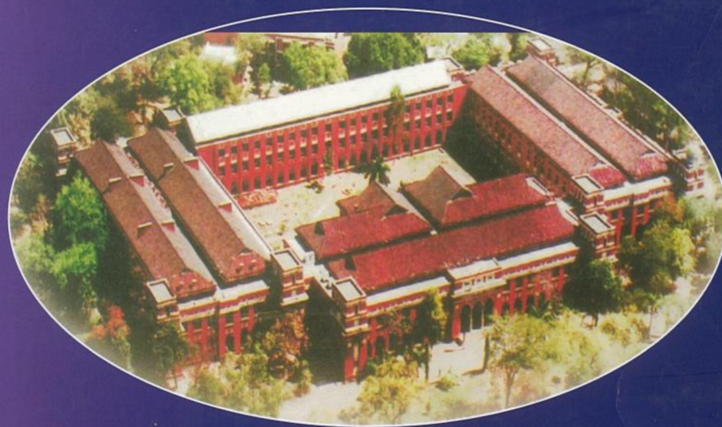

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pounds needs to be explored vigorously. Amongst all the varieties certain resistant varieties are favoured for the use as rootstock. *Citrus limon* is found to be resistant against *Phytopthora* spp., an important citrus pathogen. So it was studied for its inhibitory effect on common pathogens, which can be considered as indicators of community hygiene. The organisms, which are opportunistic pathogens and cause of minor ailments, were selected for the study. The aqueous extract of the leaves of *Citrus limon* exhibited significant activity against *Escherichia coli, Staphylococcus aureus, Candida albicans* and *Bacillus subtilis*, resulting in inhibition zones. The reduction in the growth of the pathogens increased with the increase in the concentration of the leaf extract. Thus Citrus varieties, which have been known since ancient times, as important health-maintaining fruits can be further explored for their antimicrobial properties. Such novel metabolites can be considered for use as a part of integrated approach to face the problem with organic farming as a promising option, and also promoting eco-friendly form of biopesticide in the corridor of health care units.

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IN-VITRO MICROPROPAGATION OF *MUSA SPP.* (G-9), STAGE FIRST INITIATION.

Saoji, A.A., Moghe, R.P. AND Patil L.W.

***Dept. of Botany, Institute of Science, Nagpur**

Abstract

Plant tissue culture is a new technology of Biotechnology for multiplication of *Musa* plants. Meristem of *Musa spp.* (G-9) was growing on Murashige and Skoogs medium under aseptic controlled condition. The MS medium was supplemented with 3.0 mg/lit BAP + 0.2 mg/lit NAA. It shows better result for initiation stage.

The explants were become turgid and turn green within 3-4 weeks. Each explant produces 3 shoots.

Key Words – Micropropagation , Meristem, *Musa spp.*

*****

Comparative study of different manuar on growth & leaf protein in Kanva 2/M-5 variety of Morus sp. in Nagpur region.

Dinesh B. Dhawane

Centre for Sericulture and Biological

Pest management Research (C.S.B.R.) Nagpur

Abstract

Proper soil management for soil health is the pre-requisite for achieving higher productivity in sericulture. When mulberry is grown continuously in the same field, years after years for commercial exploitation, the status of macro and micro-element in the soil are deplected at a faster rate. Therefore it becomes necessary to maintain the soil fertility for optimum production of leaf.

In the present work, experiments were conducted to study the effect of farmyard Manuar (FMY), Vermicompost and silkworm rearing waste on the growth, leaf yield, leaf protein on Kanva – 2/M5 Mulberry variety in Nagpur region.

Results showed that height of plant, sprouting, number of leaves per branch, inter nodal distance and leaf protein content were increased in vermicompost treated plant than FMY and silkworm rearing waste.

This information will be helpful to obtain better growth of mulberry and high cocoon yield and will prove to be cost effective for the resource poor farmers.

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Mangrove Vegetation, *Andaman*

IN VITRO MICROPROPAGATION OF MUSA SAPIENTUM L. (CAVENDISH DWARF)

Saoji, A. A., Patil , L.W. & Moghe, S.
Department of Botany, Institute of Science, Nagpur

ABSTRACT

A complete protocol for micropropagation of Musa sapientum using shoot meristems was developed. Multiple shoots were induced in vitro from shoot meristems. Murashige and Skoog's medium supplemented with BAP and IAA (4.0 + 0.2 mg/l, respectively) was found to be most suitable combination. Further multiplication of shoots required habituation of cultures up to 8 passages of 28 days each on the same medium after establishment of culture and initiation of shoot buds. Thereafter 8- fold multiplication rate was achieved during every subculture. For rooting the shoots were excised and transferred to same medium. Rooted plantlets were then transferred to primary and secondary hardening and grown in the green house. These hardened plants have been successfully established in soil.

Key words: Micropropagation, regeneration, plantain, , shoots, meristems.

INTRODUCTION

Bananas and plantains (Musa spp.) are the most important and most widely grown fruit crops in India. It is a good source of carbohydrates and proteins and other vitamins and minerals. Mass propagation of selected genotypes, somoclonal variation techniques, genetic engineering and other biotechnological applications can be utilized for banana improvement and are based on reliable plant regeneration protocols. Several researchers have reported the regeneration of Musa spp. via micro propagation (Cronauer and Krikorian, 1986;; Diniz et al., 1999; Nauyen, and Kozai, 2001; Krishnamoorthy et al., 2001; Kagera et al., 2004; Roels et al., 2005; Madhulatha et al., 2004Tripathi,2003). Even though many reports are available on in vitro propagation, the protocols are complicated. Here, we reported a very simple economical, rapidly multiplying and highly reproducible protocol for large scale micropropagation.

MATERIALS AND METHODS

Suckers of selected mother plants were collected and meristem was selected as explant. It was washed in running tap water for 15 - 20 min. The ensheathing leaf bases were removed from the pseudo stem leaving the young leaves around the meristem. The explants material was kept 5 min in 1% Teepol solution. Subsequently they were briefly rinsed with 70% IPA, followed by 0.12% mercuric chloride solution for 12 min. After washing thrice in sterile distilled water, the explants were inoculated aseptically in MS Medium (Murashige and Skoog, 1962) containing 30-g/l sucrose and gelled with 5 g/l of agar. The MS medium was variously supplemented with 6-benzyl amino purines (BAP), and Indol- acetic acid (IAA) in various combinations as shown in Table 1. For rooting of shoots, the well grown shoots were separated and transferred to half MS medium containing IBA (2 mg/l). The pH of the medium was adjusted to 5.8 before autoclaving and has maintained at 25oC under 16 h cool white, fluorescent light (4000 lux) . To overcome

the problem in hardening and acclimatization, the *invitro* raised plants were transferred from bottles to net pots and kept in groups in mist chamber maintained at (80 – 90%) humidity. The humidity was gradually reduced and plantlets were kept outside the mist house. After 20 days, they were transferred to polybags which were filled with various potting mixtures containing garden soil,sand and red soil in the ratio of 1:1:1. The hardened plants were then transferred to the field.

Table-1 Effect of different concentration of plant growth regulator on in vitro shoot proliferation from shoot tip explant of banana.

Conc. Of BAP + IAA IN MS medium (mg/lit)	No of explant inoculated	% of explants forming shoots	No of shoots per explant
0.5 + 0.2	20	45	1
1.0 + 0.2	20	65	1
2.0 + 0.2	20	60	1
3.0 + 0.2	20	75	1
4.0 + 0.2	**20**	**95**	**3**
5.0 + 0.2	20	45	1
Basal medium	20	40	1

RESULTS

The shoot tip explants were inoculated on MS medium with six different combinations of BAP and IAA. Among the various treatments, the effective results were obtained from the combinations given in the Table 1. After few days the explants swell and turn green and produce shoots within 4 weeks. The shoot proliferation was best in BAP + IAA (4.0 + 0.2 mg/l). The poor response of shoot initiation ability was noticed both in first (0.5 + 0.2 mg/l of BAP + IAA) and last (5.0 + 0.2 mg/l BAP + IAA) combinations of the growth regulators. Subculturing of the shoots for multiplication on the same medium induced multiple shoots. After two or three subculture, the clump formation occurs. The proliferating axillary buds were well defined, pale green and 0.5 to 1.0 cm long with bulbous base and pointed tips. A three fold increase in multiplication was seen by 4-5 weeks. Further transfer in the same medium resulted in three to four fold ratio at every subculture cycle. The explants cultured for 10-12 weeks in MS with BAP (4.0 mg/l) and IAA (0.2 mg/l) medium, which had attained the stage of vigorous proliferation, consisting of 9-10 shoots were divided in to smaller clumps. Each clump consisting of 5-6 shoots was transferred to the same medium. On the same media, the dwarf shoots recovered to normal growth with more number of axillary shoots. At the same time 2-3 vigorously growing shoots from each clump elongated with expanded leaves by three weeks. These shoots had healthy transfer in the same

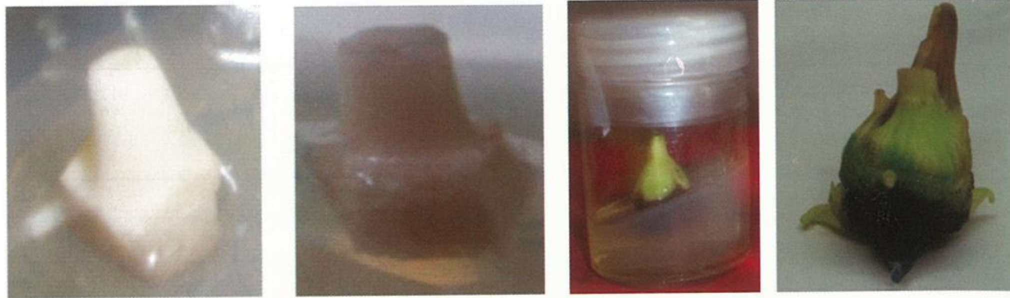
medium giving rise to a three to four fold increase in proliferating clumps and 3- 4 elongated shoots with root initials from each clump. The elongated shoots were excised and cultured separately in same fresh medium to encourage formation of long shoots broad leaves and basal roots. The basal tufts of rooting were observed in 100 % of the transferred shoots. The proliferating clumps were transferred to fresh medium for further multiplication. The elongated shoots with roots (about 5-6 cm) were transferred to primary hardening. The well developed healthy in vitro rooted plantlets were washed thoroughly in running tap water and planted in soil rite, a commercially available sterile potting mix in net pots and hardened in a shade house under 90-95% relative humidity (RH) for

8-10 days.

They were gradually transferred to plastic pots or poly bags. A survival rate of 90-95% was achieved during the hardening. After 20-25 days, the hardened plants were transferred to field.

DISCUSSION

Cronauer and Krikorian (1984) reported that multiple shoots of banana and plantain could be produced from sliced meristems either on agar or in liquid medium. But in the present investigation, shoot meristems were cultured on agar (semisolid) medium only. Irrespective of the medium composition, this observation confirms the report by Mante and Tepper (1983) regarding multiple shoot formation on an agar medium by Abaca meristem slices. Six different combinations with various concentrations of BAP and IAA were used in this study to analyze the shoot initiation and shoot multiplication capacity of the MS medium. 4.0 mg/l of BAP and 0.2 mg/l of IAA of medium showed good results both for shoot initiation and multiplication (Table 2). Vessey and Rivera (1981) reported root formation , 50 days after shoot development. In contrast Berg and Bustamante (1974) noted that it needed 2-3 months for root formation. However, in the present study, These shoots had healthy transfer in the same medium which gave rise to a three to four fold increase in proliferating clumps and 3- 4 elongated shoots with root initials from each clump. The *in vitro* raised plantlets were successfully established in the potting medium and field. Many of the previous reports on banana micropropagation used more than one type of media for initiation, multiplication and rooting (Cronauer and Krikorian, 1986;; Diniz et al., 1999; Nauyen and Kozai, 2001; Krishnamoorthy et al., 2001; Kagera et al., 2004



A B C D

(A & B –Banana explant) (C & D– Initiation stage)

E F G H

(E & F Pre-multiplication stage) (G & H Multiplication stage)

I J K L

(I – Rooting stage) (J- Primary hardening) (Sec. hardening) (L field condition)

Table 2. Effect of different concentrations of plant growth regulators on in vitro shoot multiplication from excised shoots of primary culture.

Concentration of BAP + IAA (mg/l)	No. of excised shootsinoculated per bottle	No. of multiple shoots per explants
0.5 + 0.2	4	1
1.0 + 0.2	4	1
2.0 + 0.2	4	2
3.0 + 0.2	4	2
4.0 + 0.2	**4**	**5**
5.0 + 0.2	4	1
Basal medium	4	0

In the present study very simple one step protocolis reported using MS with BAP and NAA for initiation, multiplication, and elongation and rooting of banana. The protocol raised in the present attempt could be used for the massive in vitro production of the plantlets of the banana.

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INVITRO MICROPROPAGATION OF Brassica oleracea sps.

Lalit Patil and Sonali Kadam

Abstract

An improved effective protocol for Invitro micropropogation of Cauliflower from fractionated & graded curd was presented. The protocol was optimized for the production of more than 1500 plants from one mother curd. Micro shoots were produced in 15 days on Murashige and Skoog's Murashige and Skoog's medium supplemented with BAP and NAA (2+ 0.5mg/l respectively), and transferred to Rooting medium. Fully rooted propagate was transferred to polyhouse in 4-5 weeks and was ready for transfer to the field after 3-5 weeks.

Key words - Micropropagation, curd, explants, Polyhouse

Introduction

Cauliflower is one of the several vegetables belonging to the species Brassica Oleracea, in the family Brassicaceae. It is an annual plant. Cauliflower is low in fat, high in dietary fiber, and Vitamin C, possessing a high nutritional density. Cauliflower contains several phytochemicals that may be beneficial to human health.

For several reasons, cauliflower is an excellent species to demonstrate a micro propagation system in which vegetative shoot cultures are initiated from floral meristem. (Sharma et.al. 2004, Kumar et.al. 1993, Dash et.al. 1995) Consequently the purpose of this laboratory exercise is to demonstrate the establishment of Cauliflower micro propagation system using curd explants. For the development of this protocol, MS medium (Murashige and Skoog 1962) was used supplemented with different conc. of BAP, NAA. PH adjusted to 5.8

Material and Methods

Cauliflower is a member of mustard family Brassicacease. The head or curd consists of the mass of apical meristems within floral Buds. These buds were exposed to a process known as vernalization. These potential floral buds can be devernalized. They were induced to produce leafy shoots by treatment with certain phytoharmones. Thus, cauliflower provides a source of meristem that serves as explants for invitro micro propagation.

Using forceps and a scalpel, cauliflowers curd were cut down into beaker, covered with muslin cloth and tied with a rubber band.

Breaker was placed under tap water for 10 min. Two drops of tween-20 was added into beaker.

After the 10 min. rinse, the explants from the beaker were transfered into disinfecting bottle containing about 100ml of 70% ethanol, beakers were shaken gently for 10 min.

After that ethanol was drained out from a beaker and 100ml of the sodium hypochloride was added. Shaken gently for 15 min. After 15 min. Sodium hypochloride solution was decanted into waste beaker and sterile water added for 5 min.

This process repeated twice. After surface sterilization the buds were placed upright in the culture medium. MS medium was used supplemented with different conc. of BAP, NAA. PH was adjusted to 5.8. Incubated bottles were kept in rack by giving 16 hrs 2000 lux light for 25°C temp.

Table:1 Effect of Different Concentration of plant growth regulator on in vitro shoot proliferation from curd explant of Cauliflower.

Conc. of BAP+IAA in MS medium (mg/lit)	NO. of Explant Inoculated	% of explant forming Shoots	No. of shoots per explants
0.5 + 0.5	20	35	-
1.0 + 0.5	20	45	1
1.5 +0.5	20	60	5
2.0 +0.5	20	70	8
2.5 + 0.5	20	45	3
3.0 + 0.5	20	40	1

Result

The explants were inoculated on MS medium with different conc. of BAP , NAA .The effective results were obtained from the given combinations(Table No.1) .After few days explants turns to green and produces shoot within 3 weeks. The shoot proliferation was best in MS + BAP (2.0 + 0.5 mg/l). The poor response of shoot proliferation ability was noticed in MS + BAP (0.5 + 0.5 mg/l). Sub culturing of the shoots for multiplication on the same medium induced multiple shoots. The elongated shoots were excised and cultured separately in fresh medium to encourage formation of long shoots and basal roots. The elongated shoots with roots were transferred to primary hardening. The well developed in vitro rooted healthy plants were transferred to soil, and hardened in a Shade house under 90- 95 % relative humidity (RH) for 8-10 days.

Conclusion

From our results it appears that a curd is ideal source of explants for in vitro micro propagation of cauliflower. Curd used as explants for rapid and large scale propagation of cauliflower plants. Curd explants regenerated shoots at a high frequency in media MS + BAP (2.0 + 0.5 mg/l).

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A – Cauliflower Explant

D- Rooting Stage

B – Multiplication Stage

E – Root development

C – Shooting Stage

F- Primary hardening

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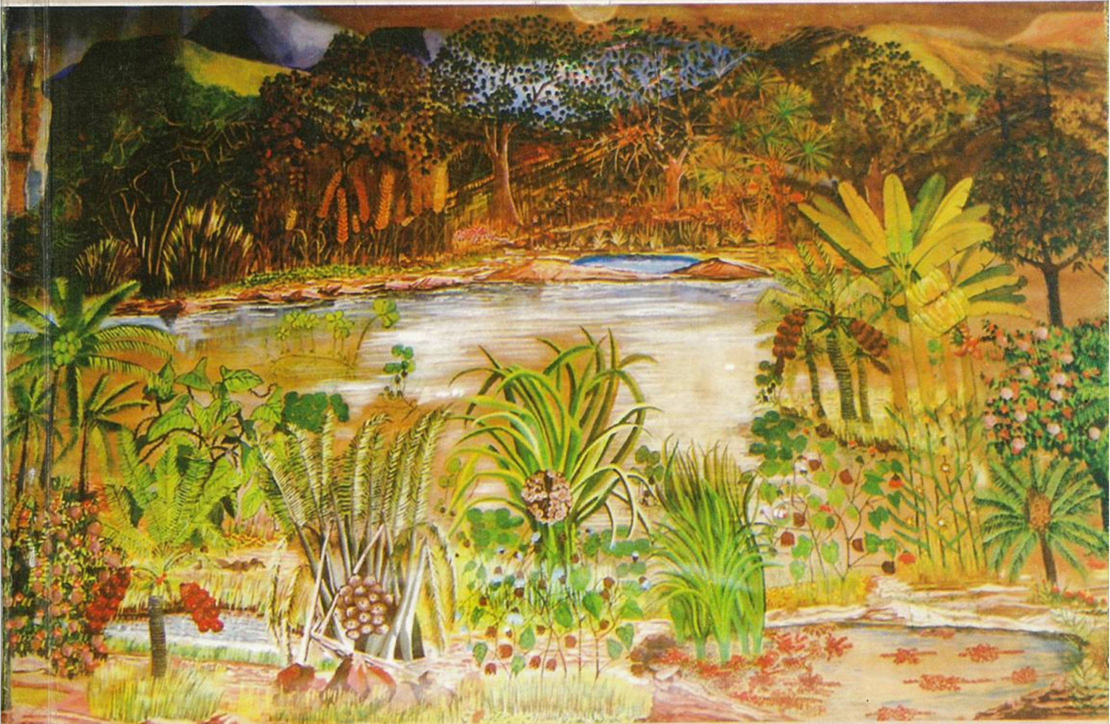
Dr. S.D. Chitaley
93 rd Birth Anniversary Felicitation Volume



Deccan Intertrappean flora of Central India, in uppermost Cretaceous age-restored by S.D. Chitaley (1971)

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LOW COST OPTIONS FOR *IN VITRO* MICROPOPAGATION OF BANANA (CAVENDISH DWARF)

Saoji A.A., Moghe S.S., Patil L.W.

Abstract

A complete protocol for micropropagation of *Musa sapientum* using shoot meristems was developed. Multiple shoots were induced *in vitro* from shoot meristems. MS medium supplemented with BAP and IAA (4.0 + 0.2 mg/lit respectively). For rooting the shoots were put into liquid medium which is supplemented with ½ MS + IBA 2 mg/lit in sterile plastic bags and kept this bags under 90 % shade net for root development and cost reduction.

Key words:- Micropropagation, regeneration, plantain, shoots, explants, meristems, medium

Introduction

Bananas and plantains (Musa spp.) are the most important and most widely grown fruit crops in India. Banana is a good source of carbohydrates and proteins and other vitamins and minerals. Mass propagation of selected genotypes, somoclonal variation techniques, genetic engineering and other biotechnological applications can be utilized for banana improvement and are based on reliable plant regeneration protocols. Several researchers have reported the regeneration of Musa spp. via micro propagation (Cronauer and Krikorian, 1986, Nauyen and Kozai, 2001; Kalimuthu et.al. 2004) Here, very simple economical, rapidly multiplying and highly reproducible protocol is presented for large scale micropropagation. Otherwise, Its a very expensive technique, but we reported a low

MATERIAL AND METHODS

suckers of selected mother plants were collected and the pseudo stem at lower part containing meristem was selected as explant. It was washed in running tap water for 15 - 20 min. The ensheathing leaf bases were removed from the pseudo stem leaving the young leaves around the meristem. The explants material was kept in an ascorbic and citric acid solution (1.0:1.5, w/v) for 30 min and 5 min in 1% Domestos solution. Subsequently they were briefly rinsed with 70% ethanol, followed by 1% mercuric chloride solution for 10 min. After washing thrice in sterile distilled water, the explants were inoculated aseptically in MS Medium (Murashige and Skoog, 1962) containing 30-g/l sucrose and gelled with 8 g/l of agar. The MS medium was variously supplemented with 6-benzyl amino purines (BAP), and naphthalene acetic acid (NAA) in various combinations .For rooting of shoots, the well grown shoots were sepa-rated and transferred to MS1/2 medium containing the same concen-tration of IBA (2.0 mg/l) without agar i.e. liquid medium which is kept in polypropylene bags. The pH of the medium was adjusted to 5.8 before autoclaving and has maintained at 30oC under 90 % green shade net that is a natural hardening. To overcome the problem in hardening and acclimatization, the invitro raised plants were transferred from bottles to net pots and kept in groups in mist

chamber maintained at (80 - 90%) humidity. The humidity was gradually reduced and plantlets were kept outside the mist house. After 20 days, they were transferred to polybags which were filled with various potting mixtures containing garden soil, sand and red soil in the ratio of 1:1:1. The hardened plants were then transferred to the field.

Results

The shoot tip explants were inoculated on MS medium which is supplemented with BAP 4.0 mg/lit and IAA.0.2 mg/lit. After multiplication stage (8 cycle) the clump were transferred in shooting medium for elongation. The last stage of invitro micropropogation in lab was rooting stage. The effective result were obtained from the given table. The cost of the solid medium in glass bottle and its processing was high. This clumps required growth room,light temp for artificial control.Comparitively the cost of liquid medium and its processing cost is very low. After inoculated the clumps in the liquid medium with polybags, this clumps kept directly under shade net.(mist chamber) Here the temp was 30. The rate of rooting in this technique is more than in growth room. There was natural light, humidity. Within 4 weeks the elongated shoots with root (about 5-7 cm) were transferred to primary hardening in a shade house(90%) under 90-95 % humidity for 8 days. They were gradually transferred to plastic polybag (4 X 6" size). A survival rate was 95 %. After 45 days this hardened plants were transferred to field.

Discussion

Cronauer and Krikorian (1984) reported that multiple shoots of banana and plantain could be produced from sliced meristems on either an agar or in liquid medium. But in the present investigation, shoot meristems were cultured on liquid medium. Irrespective of the medium composition, this observation confirms the report by earlier workers. Usually 2-3 months are required for root formation. However, in the present study. Many of the previous reports on banana micropropagation used more than one type of media for initiation,multiplication and rooting (Cronauer and Krikorian, 1986; Nauyen and Kozai, 2001; Kalimuthu et al., 2004.

Present study we reported very simple one step protocol using MS with BAP and IAA for initiation, multiplication,elongation and for rooting- liquid medium with mist chamber for growth of banana.

Acknowledgment

Authors are thankful to the Director Institute of Science and Ankur Seeds Co. for Providing necessary facilities, for carrying out present research work.

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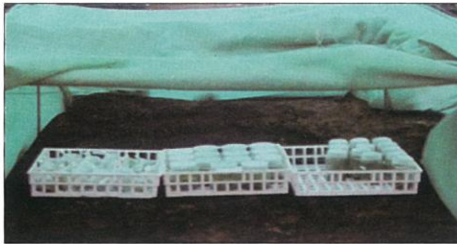
Media type	Cost of one bottle / bag	No of clump inoculate in one bottle / bag	Period of development (weeks)	No of explants got from one clump	Ratio from one clump
MS ½ + IAA Solid medium in glass bottle	Rs 2/-	10	4	25	1 : 2.5
MS ½ + IAA Liquid medium in bag	Rs .5/-	10	3	70	1 : 7.0

Comparatives chart of solid medium with glass bottle to liquid medium with poly bag

A-Explant with solid medium in Glass Bottles

B – Explant with liquid medium in polypropylene bags



C – Bottles / Bags in mist chamber

D – After 3 weeks – developed rooted clump

E- Ratio – 1 : 7

Association for the Improvement in Production and Utilization of Banana (AIPUB)

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Meeting the Challenges in Banana and Plaintain for Emerging Biotic and Abiotic Stresses

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