2006

Micropropagation and Commercial Utilization of Elite Genotypes of Banana (Musa Spp.)

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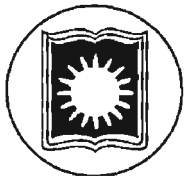
MICROPROPAGATION AND COMMERCIAL UTILIZATION OF ELITE GENOTYPES OF BANANA (*MUSA* SPP.)



THESIS SUBMITTED FOR THE DEGREE
OF
DOCTOR OF PHILOSOPHY
IN THE
INSTITUTE OF BIOLOGICAL SCIENCES
UNIVERSITY OF RAJSHAHI, BANGLADESH.

BY
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June, 2006

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DEDICATED TO

MY

PARENTS

DECLARATION

I do hereby declare that the whole work submitted as a thesis entitled "**Micropropagation and commercial utilization of elite genotypes of banana (*Musa* spp.)**" in the Institute of Biological Sciences, University of Rajshahi, Rajshahi, for the degree of **Doctor of Philosophy** is the result of my own investigation and was carried out under the supervisions of Dr. M. A. Bari Miah, Professor, Institute of Biological Sciences and Dr. M. Monzur Hossain, Professor, Department of Botany, University of Rajshahi, Rajshahi. The thesis has not been submitted in the substance for any other degree.

MHRahman 29-6-06

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CERTIFICATE

This is to certify that **Md. Habibur Rahman** has been working under our supervisions. We are pleased to forward his thesis entitled "**Micropropagation and commercial utilization of elite genotypes of banana (*Musa* spp.)**", which is the record of bonafied research carried out at the Plant Biotechnology Laboratory, Institute of Biological Sciences, University of Rajshahi. He has fulfilled all the requirements of the regulations relating to the nature and prescribed period of research for submission of thesis for the award of Ph.D. degree.

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ABSTRACT

In the present investigation *in vitro* clonal propagation of six important banana cultivars *viz.*, Sabari, Mehersagar, Ranginsagar, Jahaji, Agniswar and Binathuri and subsequent field evaluation of microclones for commercial exploitation were explored. The present study also includes callus induction from male flower culture of five banana cultivars (*viz*, Sabari, Mehersagar, Ranginsagar, Jahaji and Agniswar) followed by somatic embryogenesis, plant regeneration from somatic embryos and subsequent field evaluation of embryo derived plants for exploring possible occurrence of somaclonal variations.

For micropropagation shoot-tips of one-two months-old suckers were surface sterilized with 0.1% $HgCl_2$ for 15 min followed by 3-4 times washing with sterilized distilled H_2O. For the establishment of primary culture the shoot-tips (5×8 mm with 2-3 pairs of leaf primodia together with 3 mm of rhizomatous base) of six banana cultivars were cultured onto agar gelled MS medium supplemented with different concentrations and combinations of BAP and KIN. The cultures were inoculated at 25±1°C under cool florescent white light (2500-3000 Lux). *In vitro* culture response during initial establishment was found to be influenced with genotypes and culture media formulations. Among the various growth regulators formulations 5 mg/l BAP were found to be most effective for Sabari, Mehersagar, Jahaji, and Binathuri. Whereas, BAP 8 mg/l was found most effective formulation for the primary establishment of cv. Agnishwar.

For rapid shoot multiplication the primary cultures were transferred onto agar gelled MS medium supplemented with different combinations and concentrations of BAP, KIN, IAA, IBA and coconut water (CW). The primary cultures of all banana cultivars were induced multiple shoot proliferation in most of the culture media formulations. However, both genotypes and media formulations showed marked effect on the multiple shoot proliferation. Among the various media formulations 0.5 mg/l BAP + 0.5 KIN mg/l + 13% coconut water (cw) for cultivars Mehersagar, Ranginsagar and Jahaji. For Sabari 5 mg/l BAP + 5 mg/l KIN+ 13% coconut water (CW), for Agniswar 8 mg/l BAP + 1 mg/l KIN and for Binathuri 3.5 mg/l BAP + 0.5 mg/l KIN

+ 13% coconut water (cw) were most effective media formulation for inducing multiple shoots proliferation. Among the six banana cultivars cv. Sabari (AAB genome) was the most responsive genotypes whereas Binathuri (ABBB) showed the lowest response to micropropagation.

In vitro multiple shoot proliferation of all banana cultivars was found to affected by numbers of consecutive subculture. Rapid multiple shoot proliferation was noticed upto 7th subcultures and then the shoot proliferation rate was started to decline.

Bulbous structure was noticed to develop during repeated subculture in culture medium containing high concentration KIN (5 mg/l) that showed prolific multiple shoot proliferation. *In vitro* shoot multiplication was also affected with number of subcultures.

Root induction from the microshoots of all six banana cultivars was achieved by culturing them onto auxin supplemented rooting media (1/2 MS). Among the various rooting media formulations, 1 mg/l IBA with or without activated charcoal was the most effective for the induction of roots from banana microcutting.

The micropropagated clones of all banana cultivars after being properly acclimated were cultivated in the field with sucker derived plants as control. Micropropagated plants of all cultivars showed significantly superior performances for yield (bunch wt/plant) and yield contributing characters over sucker derived plants.

Immature male flowers of cultivars Sabari, Mehersagar, Ranginsagar and Agniswar were surface sterilize by dipping in ethanol (70%) for one min and cultured onto agar gelled MS medium supplemented with different concentration and combination of 2,4-D, BAP, IAA, NAA and 1 mg/l biotin. The cultures were incubated in dark at 25±1°C for 4-7 months. The cultures were monitored periodically and after the emergence of somatic embryos, the culture dishes were transferred to light (2000 lux, 16 h/day). The callus with fully developed heart and torpedo shaped embryos were transferred to germination medium that consisted of MS inorganics + Morel vitamin and supplemented with different concentration of BAP (0.1-1.0 mg/l) with 2 mg/l IAA. Induction of callus from the male flowers and subsequent somatic embryogenesis were found to vary with culture media formulations and also with

banana genotypes. Among the different media formulations 4 mg/l 2,4-D + 1 mg/l each of IAA, NAA and biotin was found most effective medium formulation for callus induction and somatic embryogenesis. Among the five cultivars male flowers of Sabari was found to show the highest response to callus induction and subsequent embryo formation. Somatic embryos when individually transferred to germination media, were continued their root and shoot differentiation and eventually developed to miniplant. Somatic embryos of all five banana cultivars were dedifferentiated into plantlets. However, extent of plantlet regeneration was also found to vary with BAP concentrations and banana genotypes. Among the three BAP concentrations 0.5 mg/l was found most effective in embryo germination. The highest degree of somatic embryo germination (70%) was noticed in cv. Sabari.

The embryo derived plantlets after proper acclimatization were transplanted to field and were grown to maturity along with shoot-tip culture derived plants as control. The embryo derived and shoot-tip culture derived plant were assessed for the occurrence of somaclonal variation using different morphological and yield related characters such as dwarf off-type plants, leaf, stem colour, phyllotaxy, inflorescence abnormality, bunch wt./plant, no. of hands/bunch and no. of fingers/hand. Occurrence of somaclonal variation as manifested from the presence of various abnormalities in morphological characters, was very high among the somatic embryo derived plant than shoot-tip culture derived plants. The extent of incidence of somaclonal variation among the somatic embryo derived plant was found to vary with genotypes. The highest incidence of somaclonal variation was noticed in cv. Agniswar followed by cv. Sabari. Somatic embryo derived plants of all banana cultivars at maturity also exhibited wide range of variation in yield (bunch wt./plant) and yield contributing characters.

The results depicted above elucidated that shoot-tip culture used for the micropropagation of six banana cultivars could be effective in minimizing the incidence of somaclonal variation. In addition, the high incidence of somaclonal variations generated through somatic embryogenesis may be useful as an alternative breeding tool for widening genetic basis for the improvement of banana cultivars.

ACKNOWLEDGEMENT

The author expresses his profound appreciation, indebtedness and deepest gratitude to honourable teachers and research supervisors Professor Dr. M.A. Bari Miah, Institute of Biological Sciences and Dr. M. Monzur Hossain, Professor, Department of Botany, University of Rajshahi for their wise guidance, encouragements and valuable suggestions throughout the research work and the preparation of this dissertation.

I am grateful to professor Dr. M. Altaf Hossain, Vice-Chancellor, Professor Dr. M. Mumnunul Karamat, Provice-chancellor, former Director, Professor Md. Sohrab Ali and Present Director Professor Dr. Md. Wahedul Islam, Institute of Biological Sciences, University of Rajshahi with gratitude for their kind co-operation and help during the course of study.

Grateful appreciation and thanks are also to Professor Dr. A K M Rafiul Islam, Department of Botany, University of Rajshahi, Dr. M. Abdus Salam and Dr. M. Mahbub Hassan professor, Department of Zoology University of Rajhahi, Dr. Aladdin Pramanik, Associate Professor, Dept. of Medical Biochemistry and Biophysics (MBB), Karolinaska Institute, Stockholm, Sweden. M. Masud Reza, Professor, Dept. of Biochemistry, Bangladesh Agricultural University, Mymensingh. Dr. Parvez Hassan, Associate Professor, Institute of Biological Sciences, University of Rajshahi, Dr. M. Shahidul Alam, Associate Professor, Department of Botany, University of Rajshahi, Dr. K H Emamul Haque, Associate Professor, Dept. of Geology and Mining, Rajshahi University. Dr. Biswanath Sikdar, Associate Professor, Department of Genetics and Breeding, University of Rajshahi, Dr. M. Al-Amin, Principal scientific officer, Bangladesh Agriculture Research Institute (BARI), Dr. Ara Fatun Nahar Chowdhury, Scientific Officer, Bangladesh Council of Scientific and Industrial Research (BCSIR) for their help and valuable suggestions during research period.

I would like to extend my sincere thanks and gratitude to Dr. M. Mofizuddin Mollah, Assistant Professor, Department of Botany, Rajshahi College and Dr. Nasima Yesmin Chowdhury, Assistant professor, Department of Zoology Rajshahi College for their fruitful suggestion, encouragement and moral supports.

I am thankful to Governing Body of Matiranga degree College, Matiranga, Khagrachori for granting me study leave to accomplish the research work. I would like to express my gratefulnes to our honourable President of College Governing Body (G.B.) and Chairman Chittagong Hill Tracts Development

Board, Govt. of the People's Republic of Bangladesh, Wadud Bhuiyan M.P. I am also thankful to all the members of College Governing Body specially to Mr. Abu Yousuf Chowdhury for his constant help during my research period.

I also express my heartiest thanks to Principal Abul Hossain, former acting Principal Md. Abul Hossain Patwary, all of my colleagues specially to Mohammed Alamgir, Senior Lecturer in Physics, Mr. Prodip Kumar Das, Senior Lecturer in English and Mr. Subash Kirthi Chakma Demonstrator in Biology, Matiranga degree college, Khagrachori for their encouragement during the course of study.

Thanks are due to former Ph. D. fellow Dr. Naid Mohammad Shamsul Huda, Associate Professor, Department of Botany, Dhaka College, Dr. Nurunnahar Khatun, Dr. N. A. Siddique former Ph. D. fellow, Md. Ziaur Rahman, M. Phil Fellow, Md. Emdadul Haque, M.Phil Fellow, Md. Mahbubur Rahman, Ph.D fellow, Rubaiyat Sharmin Sultana, Ph.D Fellow, Sharmin Shahnewaj, Ph.D. fellow, Siddika lutfun Naher, M. Phil Fellow, M. S. K. Zilani, M. Phil Fellow, Fazlul Haque, Ph.D. Fellow, U. M. Rahela Khatun, Deputy Registrar (Lab.), Nasreen Begum, Senior Research Demonstrator, Fazilatun Nessa, Assistant Librarian and Late Md. Nur Hossain, Senior computer operator, Institute of Biological Sciences, University of Rajshahi for their sincere help and encouragement. I would like to extend my thanks to all my friends and colleagues, well wishers and research students of Tissue Culture, Molecular and IPM Laboratory, Institute of biological Sciences for their help during the course of study.

The author deeply remembers his father, mother, brothers, sisters, brother in laws and all relatives for their encouragement and spiritual support. Thanks also to my wife Nighat Afroz Mun Mun, sons Md. Rafid Redwan and Ahmed Habib been Tanzim for their all kinds of help, patience, encouragement, cooperation and inspiration during the whole time of this research work.

The author

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Chapter I

GENERAL INTRODUCTION

1.1. BANANA: BOTANICAL ASPECT

1.1.1. Origin and Distribution

Modern bananas and plantains are originated in the South Asian and Western Pacific regions where their inedible, seed-bearing, diploid ancestors can still be found in the natural forest vegetation (Stover and Simmonds 1987, Price 1995, Robinson 1996), Simmonds and Shepherd (1955), relying on their expertise in banana genetics and cytotaxonomy, concluded that bananas are originated in South East Asia with Malaysia as the center of diversity for dessert banana cultivars.

Edible bananas (*Musa spp.* Eumusa series) originated within the region from the centre of origin and diversity of edible banana in the section Eumusa have been detailed by Stover and Simmonds (1987) to wild progenetors, *Musa acuminata* and *Musa balbisiana*, producing a series of diploids, triploids and tetraploids through natural hybridization. Additionally, man has selected for parthenocarpic (banana varieties). Plantain evolved in Southern India with a secondary centre of *Musa* diversity in West Africa.

The distribution of edible bananas and plantain outside Asia is thought to be started from the 16th to the 19th century, the Portuguese and Spaniards carried bananas all over tropical America. Dutch, British, French and German traders also played a role in the distribution of the popular banana cultivars 'Gros Michel' and the cavendish group to West Africa, Latin America and Caribbean. Now, banana cultivation is distributed throughout the many warmer and even in some cold countries of the world.

1.1.2. Morphological Features of Banana

Vegetative Features – Bananas are large herbs, often apparently tree-like in appearance and the "stem" sometimes semi-ligneous; "stem" unbranched and covered with leaf sheaths that are rolled round each other, concealing the short conical axis. Stem modified into an underground rhizome, the aerial stem from between the leaves elongates to form the inflorescence. Leaves large, alternate or

distichous (*Ravenala*) or spirally arranged; entire or lacerated due to the effect of wind; with a strong midrib from which numerous parallel veins run to the margins.

Floral Features – Inflorescence a spike or panicle or sometimes capitate, subtended by spathaceous bracts that are brilliantly coloured, coriaceous or semisucculent and cymbiform. Flowers bi- or unisexual; when unisexual, the plants monoecious with staminate flowers in the upper bracts and the pistillate ones within the lower bracts, zygomorphic, epigynous. Perianth of 6 segments, mostly in 2 series; tepals unequal in size and shape free or variously connate. Stamens 6, all fertile in *Ravenala* and 5 fertile ones with a staminode in *Musa*; anthers bithecous. Linear, basifixed, dehisce by longitudinal slits; filaments distinct and filiform. Gynoecium syncarpous, tricarpellary, ovary inferior, trilocular, placentation axile, ovules numerous per locule; style 1, filiform, stigma capitate or trilobed. Fruit a 3-celled capsule or a berry, seeds endospermous, often arillate; embryo straight.

1.1.3. Genome Composition, Diversity and Varieties

Musa acuminata (diploid, $2n = 22$, Genome A) has its center of diversity in and near Malaysia where fertile forms with small, inedible fruits grow wild in the rain forests. In this area sterile, parthenocarpic, seedless diploids (genomic formula AA) and hardier autotriploids with larger berries (AAA) were selected and propagated vegetatively by man because they had edible fruits. These clones, which were the first cultivated bananas, were dispersed from their centre of origin, and some of them reached the centre of diversity of wild *M. balbisiana* (diploid, $2n = 22$. Genome B) in India. *Musa balbisiana* has inedible fruits, but natural diploid (AB) and allotriploid (AAB and ABB) hybrids with edible fruits arose following natural crossing with *M. acuminata,* and were selected, propagated vegetatively and eventually dispersed world-wide. Hybrid clones with the B genome (there are no cultivated BB diploids or BBB autotriploids) are hardier and more tolerant of drier climates than AA or AAA clones of *M. acuminata,* they are less susceptible to diseases, and have starchier fruits; these are the plantains of the present day. Some diploid AA and AB clones, and one ABBB tetraploid clone are cultivated, but all the important bananas and plantains in the world today are triploids. The triploids were dispersed from their centres of origin into Asia and the Pacific Islands in ancient times, but not until the

fifth or sixth centuries A.D. did they reach East Africa, from where they spread across the continent to Sahara Desert to Central Africa and eventually to West Africa. The Portuguese introduced the crop to the Canary Islands from West Africa. It was unknown in the New World in pre-Columbian times, but was soon introduced after the European arrival there.

The classification of edible banana plants derived from the *Eumusa* section is based mainly on the recognition of two single sources for most varieties: *Musa acuminata* (A genome) and *M. balbisiana* (B genome) (Cheesman 1947, 1948, Dodds and Simmonds 1948). Simmonds and Shepherd's (1955) classification associates with the ploidy level (2n = 2x. 5x or 4x) with a different contribution of genomes of two species. The following groups are now recognised in edible bananas: diploids AA, and BB; triploids AAA, AAB, ABB and BBB; and tetraploids AAAA, AAAB, AABB, ABBB. Wild bananas can similarly be dominated and they are conveniently marked BB and AA (Swennen and Rosales 1994). Therefore, the cultivars are diploid, triploid and tetraploid. Triploid cultivars are generally numerous, diploids are somewhat less and tetraploid forms very rare. Genome composition and corresponding center of origin of banana are shown in **Table 1.1.**

Table 1.1 Centres of origin and diversity in the Eumusa series of edible bananas (A = *M. acuminata;* B = *M. bulbisiania*)

Group	Centres of origins and diversity
AA	The primary centre is Malaysia, Papua New Guinea is the centre of greatest diversity. The secondary centre is Coastal East Africa (many somatic mutations).
AAA	The primary centre is Malaysia. The secondary centre is the highlands of East Africa (many somatic mutations).
AB	A small group of Indian origin.
AAB	The primary centre in India with a minor group originating from eartern Malaysia. The secondary centre is in the Pacific Island (somatic mutations).
ABB	The major centre is in India with possibly a minor centre in Eeastern Malaysia.
ABBB	Several clones known from South East Asia and Papua New Guinea.

After Stover and Simmonds (1987)

1.1.4. Species and Cultivars

The banana belongs to the family Musaceae. There are only two genera viz. Ensete and Musa with about 50 species in the family. Ensete is an old declining genus which probably originated in Asia and spread to Africa. It has about 6-7 species of which *E. verticosa* is reported to be grown in Ethiopia as a food crop. The generic name of *Musa* is derived from the Arabic word Mouz.

Several Latin names have been used till recently in the botanical nomenclature of the banana. Three of earliest employed were *Musa paradisiaca*, *M. cavendishii* and *M. sapientum*. These are no longer favoured and have been superseded by a genome nomenclature for cultivars in recognition of their derivation from two wild species: *Musa acuminate* and *Musa bulbisiana*. Edible bananas have 22, 33 or 44 chromosomes; the basic haploid number is 11, so that these cultivars are, respectively, diploid, triploid and tetraploids. Triploid cultivars are generally the most numerous, diploid some what less and tetraploid forms are very rare. The most commonly used mathods for classification of bananas is the scoring method based on 15 morphological characteristics described by Simmonds and Shepherd (1955) and later by Stover and Simmonds (1987).

The best-known bananas of commerce all over the world belong to the pure Acuminate AAA group, but the Balbisiana genome is associated with greater drought hardiness and resistance to disease. It is not surprising, therefore, the hybrids of AB, AAB and ABB constitution are better adapted to monsoon areas with marked dry seasons, these bananas show wide variability and are grown chiefly for local consumption in India and Uganda. The AA and AAA bananas are cultivated mainly in areas where rainfall is equally distributed through out the year or where water for irrigation is readily available.

The main groups of bananas on the basis of their importance are: Highland bananas (AAA) and other ABB cooking bananas (24%), Cavendish for export (13%), Cavendish for local consumption (28%), plantain (AAB) 21%, Gross Michel and other dessert bananas (AAA/AAB) 14%.

There are about 300-recorded cultivars of banana and this large number is due to synonyms. In a multilingual country like India more confusion prevails as one variety exists under different local names. Conversely, a single name donates different cultivars in various localities. With recent advances in morphotaxonomy, chemotaxonomy, DNA fingerprinting and data banking, it should soon be possible to overcome the problems associated with Musa terminology (Amtzen, 1994).

1.1.5. Climates and Soils

The major banana growing areas of the world are geographically situated between the Equator and latitudes 20° North and 20° South. Banana is essentially a humid tropical plant, coming up well in regions with a temperature range of 10°C to 40°C and an average of 23°C (Madhava Rao, 1984). Among the climate variables, temperature, frost occurrence, light/solar radiation and rainfall distribution, relative humidity and wind velocity largely influence the growth, morphology and productivity of banana.

Banana can be grown from sea level to an altitude of 1200 metres. The crop has a high water demand and 25 mm per week is regarded as minimum for satisfactory growth. An average annual rainfall of 2000 to 2500 mm evenly distributed throughout the year is considered satisfactory. The crop cycle becomes longer in the cool subtropics than in warmer areas and under normal growing condition new leaf emergence in banana ceases when temperature falls below 16°C and growth stops below 14°C (Turner and Lahav, 1983; Rabinson and Anderson, 1991). Wind can cause different types of damage in a banana plantation. Wind velocity of more than 50 kms/h cause serious damage to banana plantation (Simmonds, 1959). In banana areas where plantations are normally irrigated, drought periodically reduces or removes the source of irrigation water, resulting in plant damage. Bananas and plantains with the AAB genome are more drought tolerant than AAA clones and the ABB cooking bananas are the most drought tolerant of all. Low temperatures (below 16°C) in the subtropics can cause fruit distoration and failure of the bunch to emerge from the pseudostem (Blake and peacock 1966; Robinson 1982). The rate of emergence of new leaf increases as photoperiod increases from 10-14 hours (Allen *et al*, 1988). In banana (CVS williams and Gros Michel) at light flux density of 800-1000 μ mol m^{-2} S^{-1}. The rate of photosynthesis has been recorded highest (Kallarachal *et al*; 1990; Brun, 1961).

It can grow well in slightly alkaline soils, but saline soils with salinity exceeding 0.05 per cent are unsuitable. Banana can be grown well in a pH range of 6.5-7.5. Alluvial and volcanic soils are the best for banana cultivation.

1.1.6. **Diseases and Pests of Banana**

Diseases and pests are increasingly limiting factors in smallholder and export production, and can cause catastrophic losses (Jones, 2000a). *Musa* diseases and pests are significant problems worldwide. Diseases affects all portions of the plants, are caused by fungi, bacteria and viruses. Important diseases of banana are listed in **Table 1.2.**

Table 1.2. Major pests and diseases of bananas and plantains

Diseases/Pests	Causal organisms	Succeptible cultivars	Distribution
Fungus diseases			
Panama diseases or Fusarium wilt of banana	*Fusarium oxysporum f. sp. cubense*	Gros Michel, Rasthali/Sabari, Poovan/Champa/Cheni Champa, Silk, Pome, Pisang Awak, Bluggoe, Monthan, Jahaji, Agniswar/Red banana, Dudhsagar/ Green red and Karpuravalli	Widespread throughout Africa, Asia, Australia, West Indies, North Central and South America.
Sigatoka leaf spot	*Mycosphaerella musicola*	Cavendish, Rasthali/Sabari, Mysore group, Nendran, Robusta, Poovan/Champa/Cheni Champa, Gros Michel, Mehersagar Jahaji, Ranginsagar, Rasthali/Sabari, Lacatan and Pisang masak hijau	Widely distributed throughout the banana growing regions of the world with the exception of Egypt, Israel and The Canary Islands.
Viral diseases			
Banana bunchy top virus	*Potyvirus of flexuous* and *rod shaped particle*	Dwarf bananas are very susceptible to this disease; Jahaji, Ranginsagar, Agniswar, Mehersagar and Sabari	Widely distributed throughout the banana growing regions of the world
Banana streak virus (BSV)	*Virus*	Cavendish clones, Poovan, Pome, Matti, Agniswar/ Red banana	Southeast Asia.
Banana bract mosaic virus (BBMV)	*Virus*	Cavendish clones, Pisang Awak, Nendran, Pome types, Poovan, Agniswar, Monthan and many ABB clones	Philippines, Mindanao, India, Bangladesh, Sri Lanka, Vietnam and Western Samoa.
Cucumber mosaic virus (CMV)	*Virus*	Cavendish clones, Poovan (Mysore), Agniswar and Pome	Present in most areas where bananas are grown. The disease is serious in the Cameroon.
Bacterial diseases			
Bacterial wilt or Moko disease	*Pseudomonas solanacearum*	Bluggoe (ABB)	Central and South America, Caribbean, Philippine and

Diseases/Pests	Causal organisms	Succeptible cultivars	Distribution
			Indonesia
Bugdok disease	*Pseudomonas solanaccarum*	Saba and caraba	Philippine.
Pests			
Banana rhizome weevil	*Cosmopolites sordidus* (Germ)	Nendran, Poovan, Rasthali/ Sabari/ Pometypes, Malbhog, Agniswar/Red banana, Cavendish and Binathuri/Klue teparod	Africa, Southeastern Asia, Australia, Brazil, Pacific Ocean Islands and Southern USA.
Pseudostem borer/ Banana stem –boring weevil	*Odoiporus longicollis*	Most of the commercial cultivars of banana, viz. Nendran, Agniswar/Red banana, cavendish, Poovan, binathuri/ Klue teparod.	Southeast Asia.
Nematode			
Burrowing nematode	*Radopholus similis*	Cavendish, Red banana/Agniswar, Rasthali/Sabari, Poovan/Cheni Champa / Champa, Nendran, Pome types.	Probably in majority of banana growing regions.
Spiral nematode	*Helicotylenchus multicinctus*		
Root lesion nematode	Pratyloenchus coffeae		
Root-knot nematode	*Meloidogyne incognita*		
Aphids			
Banana fruits and leaf scarring beetle	*Pentalonia nigroner vosa* *Calaspis hypochlora*	Poovan/Champa/Cheni Champa, Red banana/Agniswar, Nendran etc. All cultivars i.e. Sabari, Kabri, Champa, Jahaji	All banana growing regions.
Thrips	*Thrips florum*	Cavendish group, Plantain group, Silk and Monthan	All banana growing regions.

1.1.7. Banana and Plantain in Bangladesh

About 20 banana cultivars are grown in Bangladesh with regional preferences. On the basis of the uses banana may be divided in to two groups.

Dessert Banana: It can be further divided in to three groups.

a) **Seedless Banana:** Amritsagar, Sabari, Ranginsagar, Mehersagar, Agniswar, Kanaibashi etc.

b) **Few Seeded Banana:** Champa, Chini Champa, Kabri, Genasundari, Dudhsagar, Binathuri etc.

c) **Seeded Banana:** Tula aita, Sungi aita, Nchiallah, Botur aita etc.

Plantain: Anaji, Verer doag, Choal pous, Kadali, Behula, Mondira, Biyerbati, Kapasi, Hathajari etc. are important.

Important banana and plantain varieties grown in Bangladesh are given in **Table 1.2.** and distribution of these varieties are shown in map (**Figure 1.1**).

Table 1.3 Important Banana cultivars grown in Bangladesh

Sl No.	Cultivars	Genomic group
Dessert types		
1	Amritsagar	AAA
2	Jahaji/Singapuri/Kanaibashi/Kabulee/Panjabi/Garing Kola	AAA
3	Ranginsagar	AAA
4	Mehersagar/Nepali/Giant Governor	AAA
5	Agniswar/Surjamuki/Lal Kela	AAA
6	Dudhasgar	AAA
7	Sabari/Malbhog/Anupam/ Mortoman/Manik	AAB
8	Chini Champa	AB
9	Champa	AAB
10	Binathuri/Thursara/ Klue teparod	ABBB
11	Kabri Kola /Gine Kola/Shomoy Kola/Bangla Kola/Vath Kola/Shail Kola/Monua/Kanthali/ Modhna Kola/Sache Kola	AAB
12	Genasundari/Gera Kala	ABB
13	Goma Aita/Buitha Kola	BBB
14	Botur Aita	BBB
15	Nehyalla	BBB
16	Shaggi Aita	BBB
Plantain types		
17	Anajikola /Kacha Kola	ABB
18	Barardog	ABB
19	Dhalpoush	ABB
20	Kadali	ABB

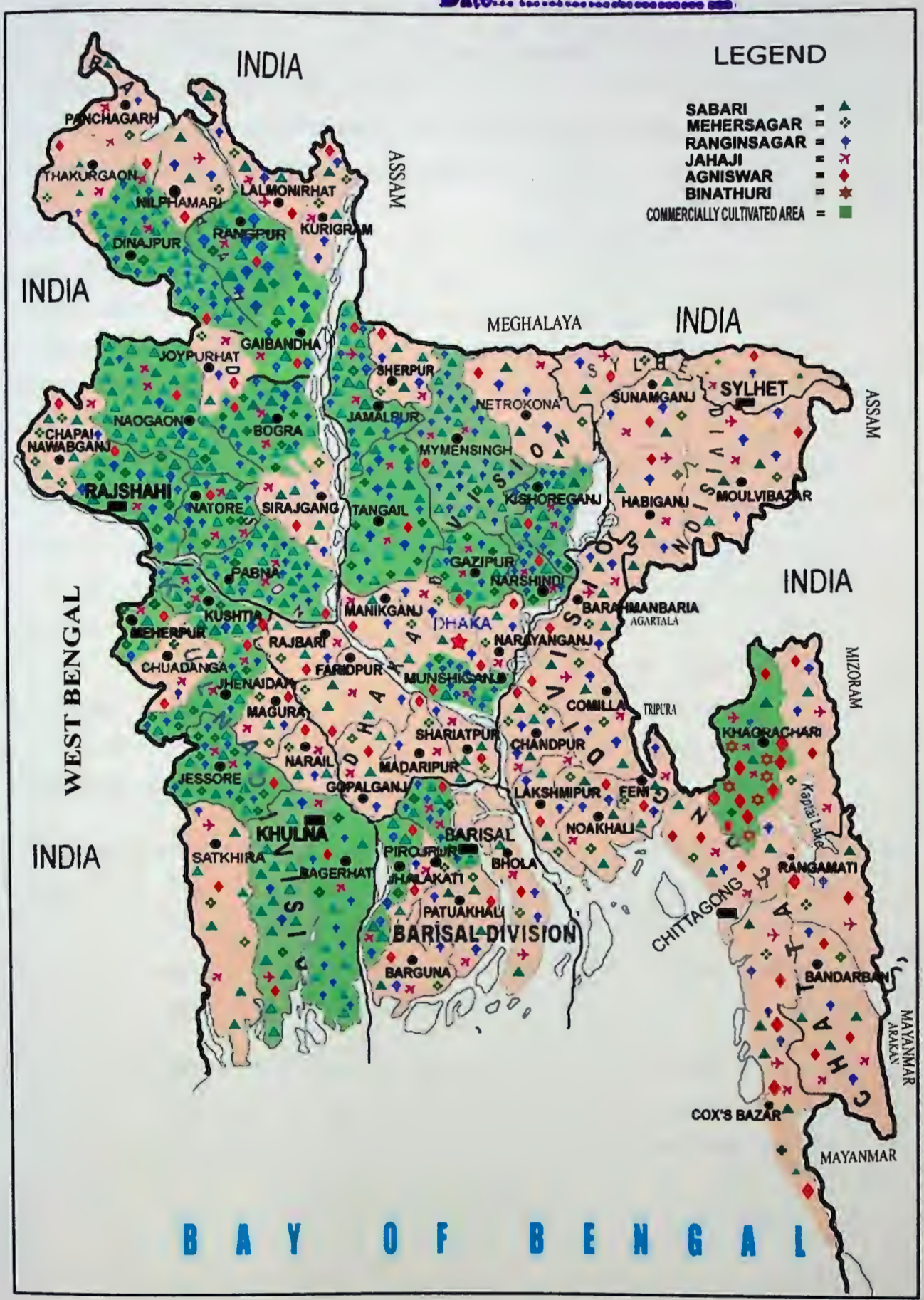


Figure 1.1 Distribution of six banana cultivars in Bangladesh

1 1.8. Uses of Banana

Banana and plantain (Musa spp.) are widely grown in Bangladesh with great socio-economic significance, interwoven in the cultural heritage of the country. In India banana by virtue of its multiple uses, is popularly known as 'kalpataru' (a plant with virtues). It is true that all parts of the plant, including the leaves, pseudostem. flower bud and corm can be used in one way or another.

As a Food Crop: Today bananas and plantains are of course best known as a food crop. The fruit has a high content of carbohydrates with some fats and proteins. Being a rich source of vitamin C and minerals, it makes healthy and salt-free diet. Sweet, dessert bananas are generally eaten fresh as a fruit, while cooking bananas and plantains are boiled, steamed, fried or roasted. Ripe bananas, as well as being eaten out of the hand, may be used in salads, breads, as a garnish for meats or mashed with spices for making chutneys and sauces. Bananas and plantain are available in most tropical household compounds and are readily acceptable and digested by children. In the equatorial belt of Africa, stretching from kilimanjaro to the Atlantic, plantain and cooking bananas are the main staple food; daily consumption here may surpass 4 kg per head. The importance of the crop as a staple food reaches a peck in Uganda where average consumption is 243 kg/cap/year, but the crops is also extremely important in parts of Rwanda, Burundi and Tanzania. In Uganda, the staple food 'matooke' which made from bananas, is eaten daily and the crop has great cultural and social significance.

Bananas and plantain are also made into flour by drying and grinding the green fruit. Such flour is said to be more digestible than cereal flour. Similarly, banana powder for use in confectionery, is made from the ripe fruit. Powder can be used as baby food and in the manufacture of chocolate and biscuits. In the Philippines, bananas are used to produce ketchup which is sold commercially.

Alcohol Production: In parts of East Africa where there is no several dry season (Uganda, Rwanda, Burundi, Zaire and Northern Tanzania), brewing of ripe bananas to make a beer with a low alcohol content has long been an important social function among numerous tribes. (Stover and simmonds 1987).

Non-Fruit Food: According to Simmonds (1966), the use of corms, shoots and male buds as food is widespread in Africa and Asia but not in the Western hemisphere. The banana "heart" can be removed from the centre of pseudostem after harvest. This can be cooked and is like celery, with a texture and test similar to bamboo shoots. Male bud after the removal of outer bracts, can also be cooked as vegetables. In South-East Asia, the male buds from wild *Musa* species, particularly *Musa balbisiana* are commonly eaten.

Animal Feed: Large quantities of reject bananas are often available for animal feed, especially in banana exporting countries. Both cattle and pigs relish ripe bananas, but as an animal feed, bananas are mainly used for feeding pigs. Banana meal as also been used in poultry diets. Banana and plantain pseudostems are also fed either fresh or chopped and ensiled, to cattle and pigs.

1.1.9. Banana Breeding: Scope and Limitation

Breeding of banana is a difficult excercise due to complexities resulting from perthenocarpy, sterility, polyploidy and vegetative propagation. As the degree of sterility is particularly high in edible varieties, breeding of banana is complicated, difficult and time comsuming.

Conventional breeding programmes have been operating since 1922 in Trinidad and 1924 in Jamaica. Another breeding programme was started in Honduras in 1959 by the United Fruit Company. Centre de cooperation internationale en recherché agronomique pour le developpement (CIRAD – FLHOR) (France) had set up a programme in 1983 for genetic breeding of banana for export or local consumption, backed by the Commission of the European Union. The CIRAD Banana breeding programme was based on the improvement of the diploid varieties for the synthesis of triploid varieties. This is as follows: obtaining pure (homozygotic) diploid fertile strains: creation of F_1 diploid elite (heterozygotic) hybrids by crossing these pure strains with each other as with natural varieties, F_1 hybrids may be monospecific origin (*M. acuminata*) or interspecific origin (*M. acuminata* × *M. balbisiana*); doubling of the cromosomes of these hybrids by treating with colchicine to obtain tetraploid plants (4x = twice 2x); lastly the synthesis of triploid hybrid (3x) by crossing this tetreploid (4x) plant with another pure diploid strain (2x). Currently

there is still an ongoing conventional banana breeding programme in Honduras operating under the Fundacion Hondurena de Investigacion Agricola (FHIA). During the 70 years that such breeding work has been carried out, many hybrid progeny have been produced and tested widely, but it is apparent that no hybrid has been bred to date that could be confirmed as being commercially acceptable. However, in 1989, the breeders at FHIA released a tetraploid hybrid (FHIA – 01 or Goldfinger) which has proved most promising in recent tests from disease and nematodes resistance criteria. It is a cross between prata Ana (AAB) from Brazil and the diploid breeding time SH 3142 (AA) from FHIA. In Trinidad, the first artificially produced hybrid banana $IC_1$was a tetraploid and was highly resistant to Panama wilt. It was evolved by crossing Gros Michel (AAA) with a wild-seeded diploid *M. acuminata*. Other hybrids IC_2, S19, J 1877 and Bodles Altafort produced subsequently, were also similarly unacceptable. A few hybrids have also been developed in banana of these H_1, H_2, CO_1 are promising.

After several years of diploid pollen parent breeding in Honduras, to improve pest and disease resistance and horticultural characteristics, a burrowing nematode resistant diploid named SH 3142 was chosen and crossed with Highgate. A tetraploid progeny of this cross (SH 3436) was selected 1982 and this had many good qualities to merit widespread testing for commercial use. The recently released 'Gold finger' (SH 3481, FHIA – 01) is also a tetraploid progeny of the pollen parent SH 3142. Currently, seven Honduran tetraploid hybrids (FHIA – 01 to FHIA – 07) are included in the International *Musa* Testing Programmes (IMTP) of the International Network for the Improvement of Banana and Plantain (INIBAP) in Honduras, Costa Rica, Colombia, Nigeria, Cameroon and Burundi. At the same time, high yielding and black sigatoka resistance tetraploid plantain hybrid such as 'TMP × 548 – 9' have been breed by International Institute of Tropical Agriculture, Nigeria (IITA) in Nigeria and these are showing potential for he disease-ravaged plantain areas of tropical Africa (Vuylsteke *et al.* 1993).

1.1.10. **Role of Biotechnology in Banana Improvement**

During the 1980s and early 1990s much work has been conducted and reported on alternative methods of breeding new cultivars and hybrids of banana and plantain.

One alternative comprised the selection of mutants from organized meristems of banana cultured *in vitro*. The production of such natural mutants during *in vitro* multiplication of banana meristems is called 'somaclonal variation'.

Another, more difficult, route to breeding new banana cultivars is by somatic embryogenesis from callus or single cell cultures, and by gene transfer and protoplast fusion to produce somatic huybrid cells. In general, these alternative methods of breeding bananas offer greater potential than the conventional route because (i) the need to use 'Highgate' in all crosses is bypassed, (ii) the long seed-to-seed cycle is eliminated, (iii) genetic diversity can be increased, (iv) the number of potential new mutants or hybrids is much higher than with seed, (v) costs are reduced and (vi) genetic 'fingerprinting' techniques can differentiate and identify new cultivars very accurately.

Induced mutation breeding enhances the possibility of altering genes by exposing plant parts containing shoot meristems to chemical or physical mutagenes. The main advantage of mutation induction in a vegetatively propagated crop is the ability to change one or more undersirable characters without altering the desirable features. This is not possible with conventional breeding. According to Novak (1992) optimum doses are 25 Gy for diploids, 35 Gy for AAA triploids, 40 Gy for AAB and ABB triploids, and 50 Gy for AAAA tetraploids.

Mutation breeding with dessert bananas is being undertaken in Queensland (Smith *et al.* 1990) where thousands of irradiated plants were established for possible resistance to race 4 of *Fusarium* wilt. Seven promising selections from these are being further evaluated in wilt-infected sites. A similar programme is being conducted jointly by the FAO and the International Atomic Energy Agency in Austria where an early-floweing mutant of AAA 'Grand Nain' was produced by irradiation.

It has become evident that procedures involving the successful regeneration of plants from somatic embryos, cells or protoplasts and somatic hybridization, offer a greater potential to genetically improve bananas and plantains. Somatic embryogenesis and plant regeneration have been reported only in the case of zygotic embryos from seeded wild species. More recently, Novak (1992) reported that embryogenic callus

was induced from basal leaf sheaths and rhizome tissue from AA, AAA and ABB clones, when placed in liquid medium with zeatin.

The recent success achieved with the protoplast culture protocol has opened up the possibility of DNA or gene transfer into isolated *Musa* protoplasts prior to their regeneration into whole plants. An efficient system for transforming bananas is now becoming a reality with modern genetic engineering. The essential components of such a system are (i) an efficient DNA transfer system (bacterium-mediated transfer, electroporation or particle propulsion), (ii) a suitable selection criterion for DNA transfer (virus resistance, weevil borer resistance or fungal resistance) and (iii) an efficient plant regeneration system from isolated protoplsts. The development of this system should be given high priority in banana research programmes together with rapid and reliable methods of screening *Musa* germplasm for assessing the degree of resistance achieved in the transformed progeny.

Bananas are an unusual agronomic crop since only clones selected from nature are currently cultivated. Commercial banana clones are vegetatively propagated, parthenocarpic triploids and are sexually sterile. Consequently, the standard technique of plant breeding based upon sexual crosses can not be utilized for banana improvement (Simmonas and Shepherd, 1955). So far all the varieties of banana under cultivation are natural selections. Man made breeding effects have not been very fruitful. Biotechnological approaches are particularly appropriate for this crop. Conventional breeding of banana is hampered by long generation time in this crop and sterility as well as triploidy of most cultivated varieties. These are the major obstacles of successful breeding programmes for registance against the major diseases caused by fungi (*Mycosphaerella* spp., *Fusarium* spp.), viruses (bunchy top, bract mosaic and streak viruses) and nematodes (*Radopholus similis pratylenchus* spp., etc). Recent development in biotechnology offers opportunities for controlling diseases in banana, too. At the advent of plant biotechnology there are a good number of avenues such as micropropagation, somatic embryogenesis, embrhyo culture, anther culture, protoplast culture and genetic transformation of which potential research may laid for improving banana cultavars:

1.2. OBJECTIVES

Considering the limitations of conventional banana breeding and scope of biotechnology in banana improvement, the present work was undertaken with following specific objectivies:

1. Standardization of suitable culture medium for the establishment of primary culture using shoot-tip as explant for six cultivars of banana.

2. Standardization of culture media and culture methods for rapid clonal multiplication through shoot-tips culture.

3. Acclimatization, transplantation and field evaluation of micropropagated banana plants.

4. Standardization of culture protocol for the induction of somatic embryo from immature male flower culture and subsequent plant regeneration from somatic embryos.

5. Acclimatization, transplantation and field evaluation of somatic embryo derived plants for finding out the occurrence of somaclonal variations.

Chapter 2

MICROPROPAGATION AND FIELD EVALUATION OF SIX BANANA CULTIVARS

2.1 INTRODUCTION

Recent advantages in biotechnology for crop improvement have had a great impact on bananas and plantains cultivation. The micropropagation of shoot-tip *in vitro* is the most common application of Biotechnology in Agriculture (Dale 1990). This technique is currently used in many countries for rapid propagation of disease free planting material for distribution on a large scale. As because the advantages of tissue culture propagation is that the shoot multiplication cycle is very short (2-6 weeks) and multiplication can be carried out through the year irrespective of the season. The apical meristem or shoot-tip culture technique is very efficient for rapid clonal micro-propagation. Thus, development of a shoot-tip culture technique for micropropagation of *Musa* has been received considerable attention when Ma and Shii (1972) for the first time were able to developed shoot multiplication technique. Shoot-tip culture is a basic technique for *Musa* propagation, conservation and movement of germplasm (De Langhe 1984, Willium 1987, Letz and Jaiswal 1991). The basic protocol has been improved by Cronauer and Krikorian (1984a,b) and has been successfully applied to rapid propagation of dessert AAB and AAA bananas, cooking ABB bananas and limited extent to AAB plantains and 'Silk" and 'Pome' AAB dessert bananas. An excellent guide to *Musa* micropropagation and *in vitro* conservation was developed by Vuylsteke (1989).

The first report on *in vitro* multiplication of edible banana and plantain was on the AAA genotypes in the early 1970 (Ma and Shii 1972, Berg and Bustamante 1974). Since then the number of reports have increased to include the AA and AAB genotypes (Cronauer and Krikorian 1984a, Vuylsteke and De Langhe 1985, Novak *et al.* 1990) and the BBB group (Damasco and Barba 1984, Jarret *et al.* 1985).

Like other plants, micropropagation in banana requires four different successive stages viz., (i) establishment of primary culture, (ii) rapid shoot multiplication, (iii)

induction of root in microcuttings and (vi) transplantation of plantlets to *ex vivo* condition. Several methods have been developed for micropropagation of banana by different groups of scientists. A brief review of the reports dealing with various aspects of banana micropropagation is described below.

Ma and Shii (1972), and Dore Swamy *et al.* (1983) developed decapitation of apical dome for the proliferation of new shoots. Destruction of the central growing point allows axillary buds to develop which are normally suppressed through apical dominance (Cronauer and Krikorian 1986a). Wong (1986) reported that multiple shoot formation could also be achieved when the apical dome was not destroyed and a cytokinin source was included in the medium.

Cronauer and Krikorian (1984b) noticed that established cultures from excised shoot tips of banana cv. Grand Naine multiply rapidly. They raised single shoot on semisolid medium and shoot clusters in liquid medium. Individual shoot was induced to form multiple shoot clusters by splitting the shoot longitudinally through apex. Shoot multiplication rate was the maximum at 5 mg/l BAP.

The *in vitro* morphogenetic response of banana is controlled by the culture medium and is strongly influenced by genotype. Banerjee *et al.* (1986) reported that shoot formation and rate of proliferation appear to be genotype dependent. They also found considerable difference between the rates of shoot proliferation in different cultivars.

Khatri *et al.* (1997) developed meristematic tip with two pairs of leaf primordia from clones of dessert banana (*Musa* spp.) viz GN60A, SH3362, William Highgate and Basrai were evaluated for *in vitro* propagation. They reported that clone GN60A produced highest tillers followed by clone SH3362, with lowest number of tillers produced by clone Basrai when the proliferation cultures were established in MS medium with 20μ M BAP. Shoots were easily rooted on MS medium with sucrose.

Bondok *et al.* (1987) conducted an experiment on production of virus free "Hindi" banana plants utilizing meristem tip culture and thiouracil treatments. Explants were cultured on Murashige and Skoog (1962) mineral salt medium with or without the

antiviral compound (thiouracil). Plantlets were assayed for virus by serological diagnosis.

Devi and Nayar (1993) carried out an experiment by using shoot-tips from 1 and 3 months old suckers of *Musa paradashca* cv. Nendran in liquid MS medium supplemented with various growth regulators and coconut water (CW) 15%. After 2-3 weeks they observed that one-month old suckers produced 7-8 shoots/explant compared with 9-16 explants from 3 months old suckers.

Bhaskar *et al.* (1993) studied the effect of three types of explant viz. shoot-tip, eye bud and floral apex of banana (cv. Red banana) cultured on semisolid MS medium supplemented with various growth regulators. For all the three types of explant, the shortest time taken for culture establishment (9-12 per explant) was obtained on medium supplemented with 10 mg/l BA regardless of NAA concentration.

Das *et al.* (1998) cultured shoot-tip sections of the four banana cultivars Martaman, Kanchkala, Giant Governor and Singapuri on modified MS medium supplemented with NAA 0.5 mg/l and BAP 0.5 mg/l. In general, Kanchkala (*Musa*, ABB group) showed the best explant survival as well as growth response. Martaman (*Musa*, ABB group) also showed good growth but comparatively poor explant survival. Cultivars Singapuri and Giant Governor, both belonging to the Dwarf Cavendish (*Musa*, ABB group) showed a moderate response to the micropropagation technique.

Gupta (1986) reported that heat therapy and mersitem culture were good for rapid clonal propagation of mosaic disease free banana plants.

Shoot multiplication rate for micropropagation of banana varies with the concentration of BAP present in MS basal media (Sun 1985, Jarret *et al.* 1985, Silayoi *et al.* 1986, Namaganda 1994). Kunlayanee *et al.* (1990) observed that shoot multiplication in 10 mg/l BAP produced more plantlet during culture of banana shoot-tips. Banerjee and Sharma (1988) observed that the rate of multiple shoot formation was higher in AAA triploid than in AA diploid clones. De Gomez and De Garcia (1994), Domingues *et al.* (1995) also observed shoot multiplication was highest in medium supplemented with 5 mg/l BA.

Cronauer and Krikorian (1985b) subsequently isolated and cultured the terminal floral apices of Dwarf Cavendish banana in the modified MS medium supplemented with a (5 mg/l) and 10% (v/v) coconut water. The determinate floral buds were transformed to multiplying vegetative shoot system from which rooted plantlets were obtained using NAA (1 mg/l) and activated charcoal (0.025%).

Balakrishnamurthy and Rangasamy (1988) reported that terminal male flower buds of the varieties Robusta and Montana when cultured on MS medium supplemented with 30 g/l sucrose, 0.8% bactoagar and 0, 2.5, or 5.0 mg/l BAP, all the floral apices survived. But proliferation of buds was observed only in culture medium containing BAP at 2.5 and 5.0 mg/l for both the varieties.

Gomez and Garcia (1994) isolated apical meristems and cultured on MS medium supplemented with 0.5 mg/l cysteine, 30 g/l sucrose, 8 g/l agar and 0.5 or 1.0 mg/l BA during the initiation stage and with 5.0 or 10.0 mg/l BAP and/or 0.25 or 0.5 mg/l BA at the multiplication stage. They found no differences among the cultures during the initiation stage with or without BA in the medium. But shoot proliferation was highest in medium supplemented with 5.0 mg/l BA.

Multiple shoots were induced from shoot-tip culture on MS medium supplemented with BA (2 mg/l) by Ganapathy *et al.* (1995). Domingues *et al.* (1995) observed that explant of 1 cm long and 0.7 cm diameter obtained from banan cv. Maca gave the highest number of buds on nutrient solution containing 5 mg/l BA for 45 days.

Sharma *et al.* (1997) developed a highly efficient *in vitro* shoot multiplication method by culturing shoot-tip explant on MS semi-solid medium supplemented with IAA 4.0 mg/l, IBA 10.0 mg/l and Adenine hemisulphate 200.0 mg/l. A maximum of 46.4 mean shoots were also obtained from their culture.

Vani and Reddy (1999) developed shoot-tips culture from healthy suckers of banana (*Musa* spp.) varieties dwarf Cavendish, Amruthapani, Tella Chakkerakeli and Robusta by and inoculated on MS medium supplemented with 6 mg/l BAP, 2 mg/l IAA and 200 mg/l adenine sulphate.

Gubbuk and Pekmezci (2001) reported meristem culture technique for *in vitro* propagation of banana cv. Dwarf Cavendish, Grand Naine, Petit Naim, Poyo, Williams and Basrai. They reported that different thidiazuron (TDZ) concentrations in combination with IAA were used in the propagation stage where TDZ concentrations of more than 2.5 μM/l did not effect the number of shoots and shoot quality.

Ranjan *et al.* (2001) reported that the highest percentage of explants (shoot-tip) responded to shoot initiation on MS media supplemented with 8 mg/l BA, 3 mg/l IAA and 150 mg/l adenine sulphate. The maximum number of shoots were 34.0, 32.3 and 30.3 for Dwarf Cavendish, Alpan and Batisa respectively, after 12-14 weeks of culture. The number of shoots per explant decreased with application of 9 mg/l BA.

Hirimburegama and Gamage (1996) investigated the performance of 10 banana cultivars (groups AAA and AAB and ABB) for their ability to shoot multiplication. In their study, the highest shoot multiplication rate was observed in Binkhel (AAA). It was also observed that subculturing enhanced shoot multiplication especially after the second subculture.

Fitchet and Winnaar (1988) reported that *in vitro* shoot-tip culture of the cultivars Dwarf Cavendish and Williams gave the best development when cultured on media supplemented with a mixture of IBA (2 mg/l), NAA (2 mg/l), kinetin (5 mg/l), adenine sulphate (160 mg/l), sodium phosphate (340 mg/l) and activated charcoal (5 g/l).

Jarret *et al.* (1985) reported the formation in banana of structures similar to those described by Cronauer and Krikorian (1983, 1984), who obtained globular proembryos in bananas and plantains in medium supplemented with 2, 4, 5-T that were morphologically similar to their zygotic counterparts.

Zamora *et al.* (1989) found that micropropagated plants of 'Lakatan', 'Bungulan' and 'Saba' grew faster had higher yield and early to sucker and flower. Hwang (1986) found that about 3% of the Cavendish plantlets derived from meristem culture were variants.

In tissue culture, rooting of banana plantlets is very important. A separate root induction phase is essential for rooting of banana shoots before transferring them into

soil. Various auxins at different concentrations are capable of root induction in micropropagated banana plantlets. For rooting of banana and plantain Novak *et al.* (1989) and De Langhe *et al.* (1985) used ½ MS + 1μ M IBA. Banerjee *et al.* (1986) found that regenerated shoots were rooted subsequently in MS solid medium with half strength of macro-salts plus 0.2 mg/l IBA in banana and plantian. Dore Swamy *et al.* (1983) used MS + 5.0 mg/l IBA, Berg and Bustamante (1974) and De Guzman *et al.* (1976) used 1 mg/l NAA for rooting in dessert banana.

Habiba *et al.* (2002) observed that half strength MS supplemented with 2.0 mg/l IBA was the best for root induction in the regenerated shoots. Gubbuk and Penkezci (2001) reported that the use of 1μM IBA or NAA/l with MS medium was sufficient in terms of rooting. Vani and Reddy (1999) reported that the shoots were rooted on MS medium containing 2.0 mg/l BAP, 2.0 mg/l IAA and 0.1% activated charcoal and eventually established in soil. Devi and Nayar (1993) reported that roots were induced within 4-5 days of culturing single shoots on MS medium containing 0.25% charcoal and 0.1 μM IBA. The plantlets were potted in vermiculite 2-3 weeks after rooting and were successfully transplanted to the fields within 3 months. Murali and Duncan (1991) observed that basal medium supplemented with 1.0 mg/l IBA induced root in micropropagated shoots of banana. Cronauer and Krikorian (1984b) obtained rooted plantlets by treating with NAA (1.0 mg/l) and activated charcoal (0.02%).

Fitchet and Winnaar (1987) reported that 4 weeks old shoot induced more roots in semisolid rooting medium of IBA, NAA, kinetin and activated charcoal. They also observed that rooted plantlets were successful established on soil in 3 weeks. Gupta (1986) reported that regeneration of shoot clusters and subsequently rooted plantlets, from meristem tip took 10 to 12 weeks on BAP and kinetin enriched MS medium.

Jasari *et al.* (1999) found that well-developed plantlets could be hardened without greenhouse facilities for their establishment in the field. The results showed that average 92% of the plantlets survived hardening with the protocol developed.

Cronauer and Krikorian (1984a) reported that *in vitro* induced rooted shoots of four banana cultivars were successfully established on pot mixture containing soil

and vermiculite at 1:1 ratio and also stated that survival from culture vessel to soil was 100%.

Oliveira *et al.* (1997) carried out an investigation on evaluation of commercial micropropagation of banana and observed that losses of *in vitro* grown plantlets due to contamination were 18 and 40.60% in two cultivars Nanicaao and Grand Naine, respectively whereas losses during acclimatization were 2% in 80 days (60 days covered with polythene bags and 20 days outdoors) when rooted plantlets were transferred to the medium containing organic matter: sand: soil (1:1:1).

A comparative study conducted by Kawit *et al.* (1993) on the performance of tissue culture propagated bananas and conventional sucker planting bananas of 16 cultivars in Thailand. The results pointed out that tissue culture plants could be used in commercial banana production in Thailand with some advantages.

Gupta (1986) reported that the survival of plantlets on transfer from *in vitro* cultures to soil was more than 95%. He also noted that the meristem-derived plants grew faster and facilitated early harvesting compared to conventionally propagated suckers.

There is a evidence that TC plants are larger than CON plants in the first crop cycle, according to work in Israel (Israeli *et al.* 1988) and Australia (Daniells 1988, Drew and Smith 1990) and highest yields (Prodeep *et al.* 1992). However, in Costa Rica, Arias and Valvera (1987) found that CON plants were larger, and in Taiwan, Hwang *et al.* (1984) found no difference. In term of yield potential, there is wide spread agreement that TC plants produce larger bances than CON plans in first crop cyclic. These increases were in the order of 2% (Hwang *et al.*, 1984), 5% (Arias and Valverde 1987), 7% (Daniells 1988), 10% (the summer planting of Israeli *et al.* 1986) and 17% (Drew and Smith 1990).

Damsco and Barba (1984) also found that 'Saba' plants derived from *in vitro* grew normally under field conditions. Moreover, Zamora *et al.* (1989) found that micropropagated plants of 'Lacatan', 'Bungulan' and 'Saba' grew faster, had higher yield and early to sucker and flower. Gupta (1986) observed that meristem

derived plants grew faster and had shorter harvesting period from 3 months to 1.5 months due to uniform growth. In the Philippines, De Guzman *et al.* (1980) reported that micropropagated plants of Lakatan were found to have similar characteristics in terms of vegetative growth, flowering, suckering and fruiting under field conditions. Robinson (1989), Robinson and Anderson (1990) and Espino *et al.* (1992) reported early flowering in tissue culture raised plants.

2.2 OBJECTIVES

General objectives of this part of study was to develop effective micropropagation protocols for six banana cultivars *viz.*, Sabari, Mehershgar, Ranginshagar, Jahaji, Agniswar and Binathuri commercially cultivated in Bangladesh. The specific objectives of this study are as follows:

1. Standardization of suitable culture medium for the establishment of primary culture using shoot-tip as explant for six cultivars of banana.
2. Standardization of culture media and culture methods for rapid clonal multiplication through shoot-tips culture.
3. Acclimatization, transplantation and field evaluation of micropropagated banana plants.

2.3. MATERIALS AND METHODS

2.3.1. Plant Materials

The following six cultivars of banana (*Musa* spp.) were used as the experimental materials in the present investigation.

Cultivars	Genome
1. Sabari	AAB
2. Mehersagar	AAA
3. Ranginsagar	AAA
4. Jahaji	AAA
5. Agniswar	AAA
6. Binathuri	ABBB

Shoot-tip explants from healthy grown 2-3 months old suckers were used to establish initial culture for micropropagation.

2.3.1.1. *Description of six banana cultivars*

Sabari: Musa (AAB) group- syn. Malbhog, Anupam, Mortman, Manik. (**Figure A, Plate 1**). It is the choicest table banana for its tasty, good sour-sweet blended and pleasant flavoured fruits of Bangladesh. The plant is tall reaching a height of 2.5-3.0 m and can be identified by the yellowish-green stem with brownish blotches, reddish margins of the petiole and leaf sheath. Leaves are pale green with wax coating on under surface, attaining 150-175 cm length and 60-75 cm width. Crop takes about 13-15 months to come to harvest with bunches weighing 15-18 kg each. It has about 6-8 hands with 85-120 fruits. Fruits are yellowish green throughout their development, but turn pale yellow and golden yellow on ripening. Fruits are medium-sized, about 10 cm long and 10 cm in girth at the middle, very lightly curved, skin thin, skin peels off easily from the pulp, pulp soft, cream coloured, taste exceedingly sweet. Its cultivation is decreasing due to susceptibility to dreaded disease Panama wilt. The other demerits are easily dropping of ripe fruits from the bunch, formation of hard lumps in the pulp and a physiological disorder fruit cracking. In the market fruit fetches double the price of other varieties.

Mehersagar: Musa (AAA) group- Syn. Giant Governor, Nepali, Singapuri. (**Figure B, Plate 1**).

Plant is medium-tall, reaching a height of 2.2-3.0 m above the ground level, circumference 60-75 cm with a strong pseudostem. Due to shout pseudostem it does not broken down by storm and stress, even tree containing bunch does not require any support. It has about 10-11 hands with 150-200 fruits. The plants bear bunches weighing 25-30 kg. Plants comes to harvesting 11-12 months of planting. Fruits have bunt tips and are greenish at ripeness but ripended during the winter season develop yellow colour. Female phase has 8-10 hands each with 16-24 fruits arranged in two rows. Fruit is tall in size, about 15-23 cm long, 11-15 cm in girth. Pulp is tasty and very sweet with a good aroma. The keeping quality of ripe banana is not good, so in the market its price is less. Industrial labour, hospitalized patients and poor people are the consumers of this banana. It is somewhat less hardy and less tolerant of poor soil conditions. The cultivar is susceptible to sigatoka leaf spot but resistance to panama disease.

Ranginsagar: Musa (AAA) group. (**Figure C, Plate 1**).

This is an important commercial banana for northern region of Bangladesh. Plants is semi-dwarf, reaching a height of 1.65-1.90m with a strong pseudostem and so less susceptible to wind damage. The variety is having increasing commercial importance because of the law height and more susceptible to drought and adverse soil conditions. Ranginsagare are shorter than that of Amritsagar but yield is greater than that of Amritasagar. Female phase has 8-11 hands with 140-200 fruits. Fruits are large sized about 15-25 cm long, 10-15 cm in girth. The average bunch weight is about 25-30 kg. Dark green fruits turn bright yellow upon ripening. Fruits harvested about 11-12 months after planting. Ripe banana can not be preserved for long time in summer but in winter its preservation capacity is high. Plants are very susceptible to sigatoka leaf spot, but immune to panama disease. Though it is susceptible to leaf spot disease although it is not serious in Bangladesh.

Jahaji: Musa (AAA) group- syn. Singapuri, Kanibashi, Kabuli/Garing Kola. (**Figure D, Plate 1**).

This is an introduced variety, which has now cultivated only at the homestead gardens throughout the country. Plant is very dwarf, extending 1.5 m above the ground level. Pseudostem is stout with brown black large blotches of conspicuous size; attaining a girth of 75-80 cm. Leaves are clustered at the crown with short

internodes. The bunches are huge, yielding about 8-12 hands at regular intervals and 150 or more fruits and weighing about 25-30 kg. Fruit is long about 15-20 cm, 10-15 cm in girth, skin think and greenish, pulp soft, white to pale yellow in colour, taste sweet with a delightful aroma. Keeping quality not quite good. The greenish colour of the fruit is retained to some extend even after ripening but fruits ripened during the winter season develop colour. It is susceptible to bunchy top and leaf spot disease but resistant to panama wilt. It bears well over a wider rang of conditions and is better adapted to growth in cool climates than any other clone.

Agniswar: Musa (AAA) group- Syn. Surjamuki/ Lal Kela/ Red banana. (**Figure E, Plate 1**)

This is a rare variety, grown on a commercial scale only in few localities; thrives best at higher elevations between 800-1300 m, in the plains it is poor yielder. The colour of the pseudostem, petiole, midrib and fruit peel is purplish red. Its pseudostem is bold, robust and 3-3.5m. Bunch is oriented at 45° angle to the pseudostem, female axis has 5-8 hands each with 12-16 fruits. It is a shy-yielder, bunches weighing 20-22 kg each have 70-120 fruits but has the potential to yield 25-30 kg/bunch under favourable conditions. Fruits are medium sized, slightly curved about 15-19 cm long, about 10-14 cm in girth with a blunt beak and thick skin, pulp is sweet orange yellow coloured and with a pleasant aroma. It has long duration of cropping (15-16 moths). This cultivar was grown only in backward garden. Of late, it is cultivated commercially in Munshigong, Khagrachori and Rangamati districts. It is susceptible to Panama, bunchy top and nematodes but resistance to leaf spot.

Binathuri: Musa (ABBB) group-syn. Thursara/Klue teparod. (**Figure F, Plate 1**)
This is one of the several natural tetraploid bananas. It is a robust plant, immune to panama disease and leaf spot and it may be recorgnized by the massive blunt grea fruits and tendency to have no male axis. The male axis when present is well developed; when absent, is completely absent, being apparently trasnformed in toto in to the last female hand. One Thai name ('Klue plihai' – "hide flower") refers to this behavior. 'Binathuri' probably originated in Indochina and now occurs mainly in Thailand and Burma, where it is used for sweet meats, as the fresh flesh has a disagreeably fibrous spongy texture. In Bangladesh the variety is a recent introduction and people strongly suggest Burma as the source. Plant is

tall, reaching a height 3.90 – 4.25 m, circumference at base 70-90 cm. Female phase has 7-9 hands each with 12-16 fruits. The fruits/bunch vary from 100-120 and weighing about 18-22 kg. Fruit large, about 23-25 cm long, 16-18 cm in girth, very slightly curved at the middle and fetches high price in the market. Crop cycle is completed in 12-14 months.

2.3.1.2. *Soruces of banana cultivars* : The planting materials of cultivars Sabari, Mehersagar, Ranginsagar and Jahaji were collected from villages Biraldah and Baneshware of Puthia Upazilla under Rajshahi district and Shibgonj and Mokamtola under Bogra district. Cultivars Agniswar and Binathuri were collected from the dense hilly region of Khagrachari district. These two cultivars of banana are in the way of extinction and considered to be endangered. Binthuri banana is big in size and very sweet. Agniswar is a rare variety and grown on a commercial scale only in few localities of hilly area (Tholibari under Ramgar Upazilla of Khagrachari District).

Plate 1

Photographs showing six different cultivars of banana used as explants sources in present investigation.

Figures:

A. Sabai
B. Mehersagar
C. Ranginsagar
D. Jahaji
E. Agniswar
F. Binathuri

Plate 1

2.3.2. Other Materials

Nutrient Medium : In the present investigation MS basal salt fomulation (Murashige and Skoog, 1962) was used to conduct all of the experiemnts.

Plant Growth Regulators (PGRs) : The following plant growth regulators were employed for the present investigation:

Auxins :

Indole – 3- acetic acid (IAA)

Indole – 3- butyric acid (IBA)

α-Naphthaleneacetic acid (NAA)

2,4-dichlorophenoxyacetic acid (2,4-D)

Cytokinins :

6-Benzylaminopurine (BAP)

6-Furfurylaminopurine (KIN)

Sterilizing Agents : In the present investigation Mercuric chloride ($HgCl_2$) used as sterilizing agent. Tween-80 and Teepol were used as detergent and surfactant.

Equipments : Culture vessels such as test tubes (125×25m), conical flasks (250, 500 and 1000 ml), separating funnel, pipette, forceps, cotton, spirit lamp, needle, sharp blade, stero microscope, electrical balance, autoclave, laminar air flow machine etc. were used in the present experiment.

2.3.3. Methods

2.3.3.1. *Preparation of culture media:* The methods involved in the present investigation are described under the following steps:

The first step in the preparation of culture media was the preparation of the stock solution. The various constituents of media were prepared into stock solutions for ready use during the preparation of medium. As different media constituents were required in different concentrations, separate stock solutions of macronutrients, micronutrients, organic compounds, Fe-EDTA (iron-stock), vitamins and amino acid, growth regulators (auxin, cytokinin and gibberellin) etc. were prepared.

Stock solution A (macro nutrients)

The stock solution A was made up to 10 times the final strength of the medium in 1,000 ml of distilled water. At first 10 times the weight of the salts present per litre of the medium was weighed accurately, dissolved once at a time and sequentially in 500 ml of distilled water and then made up to 1,000 ml. The stock solution was stored in deep freeze at 4°C in a plastic bottle.

Stock solution B (micro nutrients)

Stock solution B was made up to 10 times the final strength of the medium in 100 ml of distilled water as described for the stock solution A. This stock was filtered and stored in a refrigerator at 4°C in a plastic bottle.

Stock solution C (iron stock: Fe-EDTA)

It was made 10 times the final strength of the medium in 100 ml of distilled water. Here, two constituents, $FeSo_4$ and Na_2-EDTA, were dissolved separately in distilled water and were chelated for 24 hours at 58°C by placing it in an incubator. Then the two solutions were mixed and volume was made up to 100 ml by adding distilled water. The pH of the solution was adjusted at 5.8 and after filtering it was stored at 4°C in refrigerator wrapping by a black paper.

Stock solution D (vitamins and amino acids)

The following vitamins and amino acids were used in the present investigation.

Pyridoxine HCl (vitamin B_6)

Thamin HCl (vitamin B_4)

Nicotinic acid (vitamin B_3)

Glycine

Myoinositol (inositol)

Ten times each of the above mentioned vitamins and amino acids were dissolved separately in distilled water for stock preparation. They were mixed and the volume was made to 100 ml by additional distilled water. The stock solution was stored in a refrigerator at 4°C.

Stock solution E (growth regulators)

In addition to the nutrients, it is generally necessary to add one or more growth regulators such as auxin, cytokinin and gibberellic acid to the media to support good growth of tissues and organs (Bhojwani and Razdan, 1983).

The following growth regulators were used in the present investigation.

Table 2.1 Above growth regulators were dissolved in appropriate solvent as shown against each of them

Growth regulators	Amount taken (mg/l)	Dissolving solvent (ml)	Final volume of the stock solution with DDW (ml)	Strength of the stock solution (mg/1 mg)
2,4-D	10	70% ethanol 0.5 ml	100	0.1
NAA	10	0.1 N KOH 1 ml	100	0.1
IBA	10	0.1 N NaOH 1 ml	100	0.1
IAA	10	0.1 N NaOH 1 ml	100	0.1
BAP	10	0.1 N NaOH 1 ml	100	0.1
KIN	10	0.1 N HCl 1 ml	100	0.1

To prepare stock solution 10 mg/l of any of the growth regulators was taken in a clear test tube and dissolved in required volume of appropriate solvent. Final volume of the solution was made up to 100 ml by adding distilled water. Thus stock solutions of all the growth regulators were prepared and stored at 4°C.

Prepation of coconut water

Green coconut with soft endosprms were used for its water. A hole was drilled through one of the germinating pores. Pour out the liquid. Filter it through filter paper and store in the freezer.

Preparaton of one litre MS medium

The following steps were followed to prepare one litre of any of the above mentioned culture emdium.

(i) 100 ml of stock solution of macro nutrients (Stock A), 10 ml of stock solution of micro nutrients (Stock B), 10 ml of stock solution of Fe-EDTA (Stock C) and 10 ml of stock solution of vitamins (Stock D) were added to 750 ml of ditilled water and mixed well.

(ii) Different concentrations of hormonal supplements were added either singly or in different combinations to the above solution and were mixed throughouly. Since each hormonal stock solution contained 10 g of the chemical in 100 ml of solution, the addition of 10 ml of any hormonal stock solution to made one liter of medium results in 1 mg/l concentrations of that hormonal supplement.

(iii) The whole mixture was then made up to 1000 ml with further addition of distilled water.

(iv) The pH of the constituting medium was generally adjusted to 5.7 using a pH meter with the help of 0.1N KOH or 0.1N HCl whichever as necessary.

(v) Sucrose 30 g for MS basal medium was dissolved in 100 ml of mixed components.

(vi) The culture media were gelled with agar for supporting of plant materials. In the present investigation 7-8 g of agar (Caroline Biological Supply Co.) was added for one liter of medium. Then the whole mixture was heated in microven for 5 minutes, the agar was melted completely and making a clear solution. Care should be taken at the time of heating that the solution not to be evaporated any away.

(vii) Fixed volume of above hot melted medium was transferred into culture vessles like test tubes or conical flasks through separating funnel. The culture vessels were plugged with non-absorbent cotton, which were inserted tightly at the mouth of culture tube.

(viii) The culture vessels containing medium were then autoclaved at 15 lb/sp inch presure and at the temperature of 121°C for 20 minutes to ensure sterilization.

Then the vessels with medium were allowed to cool as vertically and marked with a glass marked pen to indicate specific hormonal supplements.

Preparation of sterilent solution: Mercuric chloride ($HgCl_2$) solution at various concentrations, generally 0.1% was used for surface sterilization of plant materials. To prepare 0.1% solution 1 mg of $HgCl_2$ was taken in one litre bottle and dissolved in 100 ml sterilized distilled water. Freshly prepared $HgCl_2$ was always used. Generally $HgCl_2$ solution was prepared 1 hour before use.

2.3.3.2. ***Culture Techniques*:** The following techniques were employed in the present investigation.

Preparation of Plant Explants

About 2-3 months old suckers were collected from field grown plant. The leaves, roots, dust and debris were removed around the suckers before taking them to laboratory. Using large knife suckers were trimmed to a size of approximately 3 cm corm bade with 3 cm pseudostem. Washing with gradual change of distilled water until complete removal of all traces of dirt from the sucker.

Surface sterilization of plant explant

The collected explants were taken into a conical flask and washed thoroughly under running tap water for 30 minutes to remove loose contaminants attached to explants. Then these explants were washed with distilled water containing 3-4 drops savlon to remove dusty substance. This was followed by successive three times washing with distilled water to made the material free from Savlon and Tween-80 or Teepol. Subsequently the materials were transferred to running Laminar-airflow hood. The surface sterilization of explants was carried out in $HgCl_2$ and suspended in different concentrations of $HgCl_2$ for different periods (5 to 18 minutes) according to nature of explants. Materials were washed 5-7 times with sterile distilled water to remove all traces of $HgCl_2$.

Culture inoculation

The sterilized explants were taken in a sterilized tiles. Using sterile tools under running laminar airflow the outer leaf whorls of the pseudostems thus prepared were

removed. The corm base was held with one hand and the leaf whorls were carefully removed with a pointed scalpel and successive sheathing leaf bases were removed by scalpel. The apical meristematic area with one or two leaf primordia attaining 3-4 mm in size remained. Sufficient care was taken to obtain possible contamination free culture. During the incision efficient care was taken also to avoid injury to the explant. Prepared explants were carefully inoculated in culture vessels containing sterilized agar gelled medium. The plugs of the culture vessels were removed inside laminar airflow cabinet in presence of spirit lamp flame. Then the inoculation process was done. Inoculation of explants was made singly per culture vessel. Special care was taken that the explants must touch the medium equally and not dip into the medium.

After inoculation the culture vessels were sealed by plastic cover, cotton plugs or aluminum foil. Finally the culture vessels were labeled by glass marker with inoculation date. Then culture vessels were ready for inoculation.

Culture incubation

The inoculated culture vessels were incubated in a growth chamber having special culture environment. All cultures were gown in the chamber illuminated by 40 W white fluorescent tubes fitted at a distance of nearly 30 cm above culture shelves. The culture room temperature was 25 ± 2° c and light intensity was 2000-3000 lux. Except special need most of the cultures were incubated at 16 hours light and 8 hours dark regime treatment. In special cases the culture vessels were placed in an incubator and maintained at desired temperature of they were placed in a dark chamber in the same room.

Subculture

Subculture for multiplication of shoots: Within 2-3 weeks several growing points became defined in the incised shoot-tips. It was then transferred to similar or different types of media for rapid multiplication. It is important to note here that since the shoot-tips in culture secret blackish colored phenolic substance which inhibit further growth of the explant's, several subcultures were required initially to overcome the problem. After 2-3 subcultures the culture stopped secreting the phenolic substances and the plants showed normal growth. In some of the experiments activated charcoal

at 0.25 percent was also added to the medium as an antioxidant to arrest the exudation of phenols.

Subculture for root induction of regeneration shoots: When the regenerated shoots were 2 to 3 cm in length they were rescued aseptically from the culture vessels and placed in a sterilized petridish. Then the multiple shoots were separated individually and subculture in the freshly prepared medium containing $\frac{1}{2}$ strength of MS salt with different combinations and concentrations of auxins for produced well developed roots. Addition of activated charcoal increases the average root length.

Transplantation of plantlets to pots

When the regenerated plantlets induced sufficient root (3-5 cm in length) they were considered ready to transfer in soil. The plantlets grown inside the culture vessels were brought out the controlled environment of growth chamber. They were then kept in the room temperature for 4-7 days to bring them in contact with normal temperature. The plantlets were then removed carefully from the culture vessels. The roots of the plantlets were gently washed under running tap water to remove agar attached to the root zone. Immediately after that, they were transferred to small pots or polythene bags containing the following mixtures: Garden soil + compost + sand (1:1:1)

After transplantation each of the plantlet was covered with transparent polythene bag to prevent sudden desiccation. The inner side of the bag was sprayed with water everyday to maintain high humidy around the juvenile plants. The polythene bags were gradually perforated to expose the plantlets to the outer normal environment and subsequently removed after 8-10 days. By this time the plantlets established in the soil. Potted plants were kept in shedy place at least for 2-3 months before final transplantation to field. Different stages of field establishment of micropropagated plants of six banana cultivars are illustrated in (**Figures A-C of Plate 8**).

Field preparation, fertilization and nursing of in vitro grown experimental plants

In vitro propagated plantlets and naturally grown suckers of six cultivars of banana were transplanted to field during were transplantated to field during March 2004 to May 2005. The experiment was laid out in randomized complete block design with 3

replications. The blocks were separated by 1.0 m wide drain. The unit plot size was 6 m × 6 m with 2 m × 2 m spacing between rows and between plants. Three weeks before planting, land was ploughed and levelled properly, and 60 cm × 60 cm × 60 cm pits were dug. Micropropagated plants, about 30 – 45 cm in height and 3 months old naturally grown sword sucker of 30 cm in height were also planted as control. The old roots base of the conventional suckers was pruned before planting in the pits. Manures and fertilizers were applied in pit as well as top-dressing as recommended by Karim *et al.* (1989). The good quality well docomposed cowdung, TSP, gypsum and zinc oxide were applied in the pits 10 days before planting. Urea and MP were applied as topdressing in five equal installments from two months after planting and continued upto flowering. Desuckering was done at 15 days interval. Diseased, dried and decayed leaves and plant parts were removed to keep the orchard clean. The male part of buenh was removed shortly after fruit set. Weeding, irrigation, mulching, and propping were done as and when necessary.

2.3.3.3. *Data collection*

In vitro **Grown:** Data were collected using the following parameters and the methods of data collection are given bellow:

1. Percentage of response: Percentage of explant responsed was calculated usign followign formula:

$$\% \text{ of explants sresponded} = \frac{\text{Number of explant formed shoots}}{\text{Total number of explant cultured}} \times 100$$

2. Number of shoots/explant: Number of shoot was counted for each culture 30 days after inoculation and average number of calculated and noted.

3. Shoot length: After 25, 35 and 45 days of culture shoot length was measured in cm scales for each plantlet and average of shoot length was calculated and noted.

4. Root length was measured in Centimeter (cm) from the base to the tip of the roots. Average length of the longest root was calculated using above-mentioned formula.

Field grown: During the field evaluation trial observation were made and the data were on the following parameters:

1. **Pseudostem height:** The height of the pseudostem was measured in cm from ground level to the point of emergence of bunch at monthly interval till harvest.

2. **Base girth of pseudostem:** Pseudostem girth was measured in cm above the ground level at monthly interval till shooting.

3. **Number of functional leaves:** The number of functional leaves was recorded till harvesting. Leaves that are erect and have at least 75% of their area that is green.

4. **Number of suckers:** Number of suckers was counted upto harvest on tissue culture and sucker derived planting materials.

5. **Days to shoot:** Days to shoot i.e. flowering was recorded from planting to emergence of inflorescence. The date of emergence of inflorescence was recorded when it just appear in the heart of the plant.

6. **Days from flowering to harvest:** Days from flowering to harvest was recorded for individual plants.

7. **Total cropping period:** Days from planting to harvesting was recorded as total cropping period. The bunches were harvested when the ridges on the fruit surface changed from angular to round.

8. **Number of hands**: Number of hands were counted for individual bunch.

9. **Number of fingers per bunch:** Number of fingers were counted for individual hand.

10. **Total number of fingers:** Total number of fingers were counted for individual bunch.

11. **Length and girth of fruits:** Length and girth of the fruits were recorded in cm after the first harvest.

2.3.3.4. ***Data analysis*** : Mean and standard error of mean: The mean of different experiments was calculated as mentioned above. The standard errors of mean (SE) were calculated as follows:

$$SE = \sqrt{\frac{S^2}{n}}$$

Where,

S^2 = Sample variance

S = Standard deviation

n = Number of observation

Data collected on different parameter were subjected to statistical analysis according to Duncan's Multiple Range Test (DMRT) as discribed by Steel and Torrie (1960).

2.4. RESULTS

In the present study, shoot-tips of six cultivars of banana from field grown plants were used as explants and the effects of different cytokinins, auxins and coconut water (CW) on shoot regeneration, multiplication and rooting of micro shoots of banana were investigated. The *In vitro* micropropagated plants were transplanted to the field and their performances were evaluated. Details of the results so far obtained from each of the experiments are described under following heads.

2.4.1. Primary Establishment of Shoot-Tip Culture

Morphogenic response of the cultured shoot-tip was appeared to visible after 10-15 days lag period. The shoot-tip commenced new growth through enlargement of their size. Shoot-tips were white in colour at the time in inoculation. However, gradually they turned to greenish along with increase in size. Data on percentage of shoot forming explants and mean length of the largest shoot were recorded. The result on the effect of different cytokinins on culture media to morphogenic response is discussed under separate heads.

2.4.1.1. ***Effect of different concentrations of BAP on primary establishment of shoot-tip culture*:** Shoot-tip explants of six cultivars of banana were cultured onto MS medium supplemented with 7 concentrations of BAP (**Table 2.1**). The data on the primary establishment of explants were scored as percentage of shoot forming explants, number's of shoots/explant, length of the longest shoot and the results are shown in **Table 2.1** and illustrated in the (**Figures A1-C2, Plate 2**) and (**Figure D1-F2, Plate 3**).

Among the seven concentrations of BAP, 5 mg/l was found to be the most effective formulation where most of the banana cultivars resumed new growth. In this formulation cvs. Mehersagar, Jahaji, Sabari, Ranginsagar and Agniswar showed the highest percentage of explant survival. However, the highest percentage of explant survival for cv. Agniswar was recorded onto medium containing 8 mg/l BAP. In general lower concentration of BAP (1-3 mg/l) was found less effective for the primary culture establishment.

Among the six cultivars of banana, cvs. Mehersagar, Jahaji and Ranginsagar were the most responsive genotypes in culture condition. The highest 92% of the explants of these cultivars were resumed new growth after culturing onto 5 mg/l BAP. Cv. Binathuri was found to be the less responsive in the establishment of primary culture (**Table 2.1**).

The shoot-tip explants after resuming new growth started to new mini suckers development that eventually induced to develop new shoots. Numbers of shoots/explant recorded after 25 and 35 days of culture were found to be varied according to genotypes and level of BAP in culture medium. Among the different BAP formulation, 5 mg/l was also found to be the most effective in inducing lateral bud proliferation from the corm of primary established explant. Shoot growth in primary culture was also the best in 5 mg/l BAP formulation. Among the six cultivars, the shoot length was highest for Agniswar recorded onto the same culture medium formulation.

2.4.1.2. ***Effect of different concentrations of KIN on primary establishment of shoot-tip culture*** : Shoot-tip explants of six cultivars of banana were cultured on the MS medium supplemented with 7 concentrations of KIN (**Table 2.2**). The data on the primary establishment of explants in the different KIN supplemented media formulations were scored as percentage of shoot forming explants, number of shoots/explant, length of the largest shoot and the results are shown in **Table 2.2.**

Among the seven concentrations of KIN, 5 mg/l was found to be the most effective formulation where most of the banana cultivars resumed new growth. In this formulation cvs. Mehersagar, Jahaji, Sabari and Ranginsagar showed the highest percentage of explant survival. In general lower concentrations of KIN (1-3 mg/l) were found less effective in the primary culture establishment.

Among the six cultivars of banana cvs. Mehersagar and Ranginsagar were the most responsive genotypes in the culture condition. The highest 75% of the explants of these cultivars were resumed new growth after culturing onto 5 mg/l KIN. Binathuri was found to be the less responsive in the establishment of primary culture (**Table 2.2**).

The shoot-tip explants after resuming new growth induced to develop new mini suckers that eventually induced to develop new shoots. Number of shoots/explants recorded after 25 and 35 days of culture were found to be varied according to genotypes and levels of KIN in culture medium. Among the different KIN formulation 5 mg/l was also found to be the most effective in inducing lateral bud proliferation from the corm of primary established explant. Shoot growth in primary culture was also the best in 5 mg/l KIN formulation. Among the six cultivars, the shoot length was the highest for Agniswar was (1.42 ± 0.087 cm) recorded onto the same culture media formulation.

2.4.1.3. *Effect of different concentrations and combiantions of BAP with KIN on primary establishment of shoot-tip culture:* To find out the synergistic effect of BAP and KIN on the primary establishment of the shoot-tip explants of six banana cultivars were cultured onto MS medium supplemented with 7 different concentrations of BAP and KIN together (**Table 2.3**). The data on the primary establishment of explants were collected on percentage of shoot forming explants, number of shoots/explant and length of the longest shoot. The results on these parameters are summarized and presented in **Table 2.3.**

In all media formulations, a number of cultured shoot-tips resumed new growth. However, depending upon the media formulation the success rate of the explants in starting new growth was found to vary. Among the different concentration of 0.5 mg/l BAP + 0.5 mg/l KIN was found to an effective formulation where most of the banana cultivars resumed new growth. In this formulation cvs. Mehersagar, Jahaji and Ranginsagar showed the highest percentage of explant survival. In Sabari the best medium composition for primary culture establishment was observed in MS + 5.0 mg/l BAP + 5.0 mg/l KIN and the percentage of response was 90%. However, the highest percentage of explant survival for cvs. Agniswar (70%) and Binathuri (60%) were recorded into medium containing 8.0 mg/l BAP and 1.0 mg/l KIN and 3.5 mg/l BAP + 1.0 mg/l KIN respectively. In general, lower concentration of BAP + KIN was found more effective in the primary culture establishment for the cultivar Mehersagar, Jahaji and Ranginsagar.

Among the six cultivars of banana cvs. Sabari, Mehersagar, Jahaji, Ranginsagar were the more responsive genotypes in these culture condition. The highest 80 to 90% of the explants of these cultivars were resumed new growth after culturing 0.5 mg/l BAP + 0.5 mg/l KIN except cv. Sabari and cv. Binathuri was found to be the less responsive in the establishment of primary culture condition **Table 2.3.**

The primary established shoot-tip culture were induced to proliferate multiple shoot through the formation of new mini suckers, number of shoots/explant recorded after 25 and 35 days and length of longest shoot after 35 days were found to be varied according to genotypes and level of BAP and KIN in culture medium. Among the different BAP + KIN formulation, 0.5 mg/l BAP + 0.5 mg/l KIN was found to be the most effective in inducing lateral bud proliferation from the corm of primary established explants. Shoot growth in primary culture was also the best in 0.5 mg/l BAP + 0.5 mg/l KIN formulation. Among the six cultivars, the shoot length was highest for cv. Agniswar recorded onto the 8.0 mg/l BAP + 1.0 mg/l KIN culture medium formulation.

Table 2.1 Effect of different concentrations of BAP in MS medium on *in vitro* establishment of primary shoot-tip culture of six cultivars of banana. Each treatment consisted of 12 explants and the experiments were repeated at least twice

Cultivars	PGR (mg/l) BAP	% of explant induced shoot regeneration	No. of shoots/explant $\overline{X}$ ±SE		Length of the longest shoot (cm) $\overline{X}$ ±SE
			25 days	35 days	35 days
Sabari	1.0	17	1.00 ± 0.000	1.00 ± 0.000	1.30 ± 0.071
	2.0	25	1.00 ± 0.000	1.66 ± 0.272	1.23 ± 0.072
	3.0	33	1.25 ± 0.217	1.75 ± 0.217	1.77 ± 0.108
	4.0	58	1.28 ± 0.171	1.85 ± 0.241	1.85 ± 0.166
	5.0	75	1.66 ± 0.157	2.22 ± 0.305	1.98 ± 0.134
	7.0	67	1.37 ± 0.171	1.75 ± 0.234	1.43 ± 0.088
	10.0	50	1.33 ± 0.192	1.33 ± 0.192	1.20 ± 0.085
Mehersagar	1.0	50	1.16 ± 0.152	1.33 ± 0.192	1.30 ± 0.147
	2.0	67	1.37 ± 0.171	1.50 ± 0.177	1.36 ± 0.170
	3.0	75	1.44 ± 0.166	1.50 ± 0.177	1.44 ± 0.170
	4.0	83	1.63 ± 0.643	2.00 ± 0.223	1.83 ± 0.136
	5.0	92	2.09 ± 0.276	2.27 ± 0.260	2.03 ± 0.143
	7.0	75	1.33 ± 0.157	1.55 ± 0.166	1.28 ± 0.170
	10.0	75	1.22 ± 0.139	1.33 ± 0.157	1.20 ± 0.123
Ranginsagar	1.0	42	1.00 ± 0.00	1.20 ± 0.179	1.40 ± 0.123
	2.0	58	1.00 ± 0.00	1.29 ± 0. 1 71	1.46 ± 0.049
	3.0	75	1.22 ± 0.105	1.33 ± 0.157	1.49 ± 0.098
	4.0	83	1.30 ± 0.145	1.50 ± 0.212	1.75 ± 0.114
	5.0	92	1.45 ± 0.150	1.73 ± 0.226	1.86 ± 0.096
	7.0	67	1.25 ± 0.153	1.50 ± 0.067	1.58 ± 0.118
	10.0	50	1.17 ± 0.152	1.33 ± 0.192	1.50 ± 0.138
Jahaj	1.0	42	1.00 ± 0.000	1.20 ± 0.179	0.78 ± 0.125
	2.0	58	1.14 ± 0.132	1.42 ± 0.187	0.85 ± 0.030
	3.0	67	1.25 ± 2.828	1.62 ± 0.246	0.85 ± 0.085
	4.0	83	1.40 ± 0.155	1.90 ± 0.221	1.08 ± 0.099
	5.0	92	1.54 ± 0.500	2.00 ± 0.223	1.29 ± 0.097
	7.0	75	1.22 ± 0.139	1.55 ± 0.166	0.85 ± 0.074
	10.0	58	1.14 ± 0.132	1.42 ± 0.187	0.91 ± 0.051
Agniswar	1.0	16	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
	2.0	25	1.00 ± 0.00	1.33 ± 0.272	1.23 ± 0.119
	3.0	42	1.00 ± 0.00	1.40 ± 0.219	1.44 ± 0.151
	4.0	50	1.00 ± 0.000	1.33 ± 0.192	1.40 ± 0.139
	5.0	67	1.12 ± 0.117	1.50 ± 0.117	1.56 ± 0.133
	8.0	75	1.33 ± 0.157	1.67 ± 0.222	2.12± 0.162
	10.0	60	1.17 ± 0.152	1.33 ± 0.204	1.60 ± 0.117
Binathuri	1.0	00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.000
	2.0	25	1.00 ± 0.00	1.33 ± 0.292	0.75 ± 0.047
	3.0	25	1.00 ± 0.00	1.33 ± 0.272	0.90 ± 0.125
	4.0	58	1.14 ± 0.132	1.43 ± 0.187	0.97 ± 0.077
	5.0	67	1.25 ± 0.153	1.63 ± 0.246	1.06 ± 0.081
	7.0	42	1.00 ± 0.00	1.40 ± 0.219	0.90 ± 0.098
	10.0	33	1.00 ± 0.00	1.25 ± 0.217	0.85 ± 0.096

Table 2.2 Effect of different concentrations of KIN in MS medium on *in vitro* establishment of primary shoot-tip culture of six cultivars of banana. Each treatment consisted of 12 explants and the experiments were repeated at least twice

Cultivars	PGR (mg/l) KIN	% of explant induced shoot regeneration	No. of shoots/explant $\overline{X}$ ±SE		Length of the longest shoot (cm) $\overline{X}$ ±SE
			25 days	35 days	35 days
Sabari	1.0	08	1.00 ± 0.000	1.00 ± 0.000	0.70 ± 0.000
	2.0	25	1.00 ± 0.000	1.00 ± 0.000	0.86 ± 0.054
	3.0	33	1.00 ± 0.000	1.25 ± 0.217	0.97 ± 0.054
	4.0	50	1.16 ± 0.152	1.50 ± 0.204	1.06 ± 0.045
	5.0	58	1.28 ± 0.171	1.71 ± 0.171	1.18 ± 0.087
	7.0	42	1.20 ± 0.179	1.20 ± 0.179	0.96 ± 0.046
	10.0	33	1.00 ± 0.000	1.00 ± 0.000	0.87 ± 0.041
Mehersagar	1.0	08	1.00 ± 0.000	1.00 ± 0.000	0.70 ± 0.000
	2.0	25	1.00 ± 0.000	1.00 ± 0.000	0.86 ± 0.054
	3.0	33	1.00 ± 0.000	1.25 ± 0.217	0.97 ± 0.054
	4.0	50	1.16 ± 0.152	1.50 ± 0.204	1.06 ± 0.045
	5.0	75	1.28 ± 0.171	1.71 ± 0.171	1.18 ± 0.087
	7.0	42	1.20 ± 0.179	1.20 ± 0.179	0.96 ± 0.046
	10.0	33	1.00 ± 0.000	1.00 ± 0.000	0.87 ± 0.041
Ranginsagor	1.0	17	1.00 ± 0.000	1.00 ± 0.000	0.85 ± 0.035
	2.0	25	1.00 ± 0.000	1.33 ± 0.272	0.90 ± 0.094
	3.0	42	1.20 ± 0.179	1.40 ± 0.219	0.96 ± 0.046
	4.0	67	1.25 ± 0.153	1.63 ± 0.246	1.09 ± 0.065
	5.0	75	1.38 ± 0.171	1.88 ± 0.276	1.25 ± 0.068
	7.0	58	1.14 ± 0.132	1.43 ± 0.187	1.04 ± 0.070
	10.0	33	1.00 ± 0.000	1.25 ± 0.217	0.98 ± 0.074
Jahaji	1.0	08	1.00 ± 0.000	1.00 ± 0.000	0.60 ± 0.000
	2.0	17	1.00 ± 0.000	1.00 ± .000	0.70 ± 0.071
	3.0	17	1.00 ± 0.000	1.33 ± 0.272	0.83 ± 0.072
	4.0	42	1.20 ± 0.179	1.40 ± 0.219	1.04 ± 0.073
	5.0	58	1.50 ± 0.204	1.83 ± 0.281	1.08 ± 0.068
	7.0	25	1.00 ± 0.000	1.33 ± 0.272	0.90 ± 0.125
	10.0	17	1.00 ± 0.000	1.00 ± 0.000	0.85 ± 0.035
Agniswar	1.0	00	0.00 ± 0.000	1.00 ± 0.000	0.00 ± 0.000
	2.0	08	1.00 ± 0.000	1.00 ± 0.000	1.00 ± 0.000
	3.0	25	1.00 ± 0.000	1.33 ± 0.272	1.03 ± 0.072
	4.0	33	1.25 ± 0.217	1.50 ± 0.250	1.23 ± 0.089
	5.0	42	1.40 ± 0.219	1.60 ± 0.219	1.42 ± 0.087
	7.0	25	1.00 ± 0.000	1.33 ± 0.272	1.17 ± 0.072
	10.0	17	1.00 ± 0.000	1.00 ± 0.000	1.10 ± 0.071
Binathuri	1.0	00	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000
	2.0	08	1.00 ± 0.000	1.00 ± 0.000	0.60 ± 0.000
	3.0	17	1.00 ± 0.000	1.00 ± 0.000	0.75 ± 0.035
	4.0	33	1.00 ± 0.000	1.25 ± 0.217	0.83 ± 0.074
	5.0	33	1.25 ± 0.217	1.50 ± 0.250	1.03 ± 0.054
	7.0	25	1.00 ± 0.000	1.33 ± 0.272	0.90 ± 0.047
	10.0	17	1.00 ± 0.000	1.00 ± 0.000	0.80 ± 0.141

Table 2.3 Effect of different concentrations and combinations of BAP and KIN in MS medium on *in vitro* establishment of primary shoot-tip culture of six cultivars of banana. Each treatment consisted of 10 explants and the experiments were repeated at least twice

Cultivars	PGR (mg/l) BAP+KIN	% of explant induced shoot regeneration	No. of shoots/explant $\overline{X}$ ±SE		Length of the longest shoot (cm) $\overline{X}$ ±SE
			25 days	35 days	35 days
Sabari	0.5+0.5	20	1.00 ± 0.000	1.00 ± 0.000	1.05 ± 0.106
	1.0+1.0	20	1.50 ± 0.354	1.50 ± 0.354	1.10 ± 0.071
	2.0+1.0	50	1.40 ± 0.219	1.60 ± 0.219	1.18 ± 0.059
	2.5+2.0	60	1.50 ± 0.204	1.83 ± 0.281	1.08 ± 0.068
	4.0+1.0	70	1.42 ± 0.187	1.85 ± 0.241	1.14 ± 0.040
	5.0+1.0	80	1.50 ± 0.177	2.00 ± 0.250	1.72 ± 0.098
	5.0+5.0	90	1.66 ± 0.157	2.22 ± 0.305	2.43 ± 0.119
Mehersagar	0.5+0.5	90	1.44 ± 0.166	2.11 ± 0.189	2.51 ± 0.180
	1.0+1.0	40	1.25 ± 0.217	1.50 ± 0.250	1.36 ± 0.072
	2.0+1.0	40	1.25 ± 0.217	1.50 ± 0.250	1.40 ± 0.047
	2.5+2.0	60	1.50 ± 0.204	1.83 ± 0.281	1.95 ± 0.126
	4.0+1.0	50	1.40 ± 0.219	1.60 ± 0.219	1.84 ± 0.014
	5.0+1.0	40	1.25 ± 0.217	1.50 ± 0.250	1.52 ± 0.014
	5.0+5.0	70	1.42 ± 0.187	1.85 ± 0.241	2.12 ± 0.127
Ranginsagor	0.5+0.5	80	1.50 ± 0.189	2.00 ± 0.167	2.06 ± 0.154
	1.0+1.0	30	1.25 ± 0.217	1.50 ± 0.250	1.37 ± 0.072
	2.0+1.0	30	1.25 ± 0.217	1.50 ± 0.250	1.37 ± 0.072
	2.5+2.0	50	1.40 ± 0.219	1.60 ± 0.219	1.67 ± 0.125
	4.0+1.0	50	1.40 ± 0.219	1.60 ± 0.219	1.64 ± 0.100
	5.0+1.0	50	1.25 ± 0.194	1.40 ± 0.219	1.68 ± 0.104
	5.0+5.0	70	1.42 ± 0.187	1.85 ± 0.241	2.01 ± 0.142
Jahaji	0.5+0.5	90	1.67 ± 0.157	2.00 ± 0.222	2.00 ± 0.133
	1.0+1.0	40	1.25 ± 0.217	1.50 ± 0.250	1.13 ± 0.027
	2.0+1.0	40	1.25 ± 0.217	1.50 ±0.250	1.20 ± 0.027
	2.5+2.0	50	1.40 ± 0.219	1.60 ± 0.219	1.52 ± 0.118
	4.0+1.0	60	1.50 ± 0.204	1.66 ± 0.304	1.88 ± 0.116
	5.0+1.0	50	1.40 ± 0.219	1.60 ± 0.219	1.58 ± 0.104
	5.0+5.0	60	1.50 ± 0.204	1.66 ± 0.192	1.98 ± 0.112
Agniswar	0.5+0.5	10	1.00 ± 0.000	1.00 ± 0.000	2.47 ± 0.148
	2.0+1.0	20	1.00 ± 0.000	1.00 ± 0.000	1.90 ± 0.071
	3.0+1.0	20	1.00 ± 0.000	1.00 ± 0.000	2.00 ± 0.141
	4.0+1.0	30	1.00 ± 0.000	1.33 ± 0.272	1.97 ± 0.213
	5.0+1.0	40	1.25 ± 0.217	1.50 ± 0.250	2.15 ± 0.135
	8.0+1.0	70	1.42 ± 0.187	1.71 ± 0.265	2.38 ± 0.230
	10.0+1.0	50	1.40 ± 0.219	1.60 ± 0.219	2.32 ± 0.129
Binathuri	0.5+0.5	10	1.00 ± 0.000	1.00 ± 0.000	2.37 ± 0.072
	1.0+1.0	20	1.00 ± 0.000	1.00 ± 0.000	1.90 ± 0.071
	2.0+1.0	20	1.00 ± 0.000	1.50 ± 0.354	1.90 ± 0.212
	2.5+1.0	30	1.00 ± 0.000	1.33 ± 0.272	2.00 ± 0.141
	3.0+1.0	40	1.25 ± 0.217	1.50 ± 0.250	2.36 ± 0.152
	3.5+1.0	60	1.50 ± 0.204	1.83 ± 0.281	2.64 ± 0.154
	4.0+1.0	40	1.25 ± 0.217	1.50 ± 0.250	2.26 ± 0.119

Plate 2

Photographs showing primary establishment of shoot-tip explants of three different banana cultivars cultured in MS semisolid medium supplemented with 5.0 mg/l BAP.

Figures:

A1. Sabari 5 weeks after culture.

A2. Sabari 6 weeks after culture.

B1. Mehersagar 3 weeks after culture.

B2. Mehersagar 4 weeks after culture.

C1. Ranginsagar 4 weeks after culture.

C2. Ranginsagar 5 weeks after culture.

Plate 2

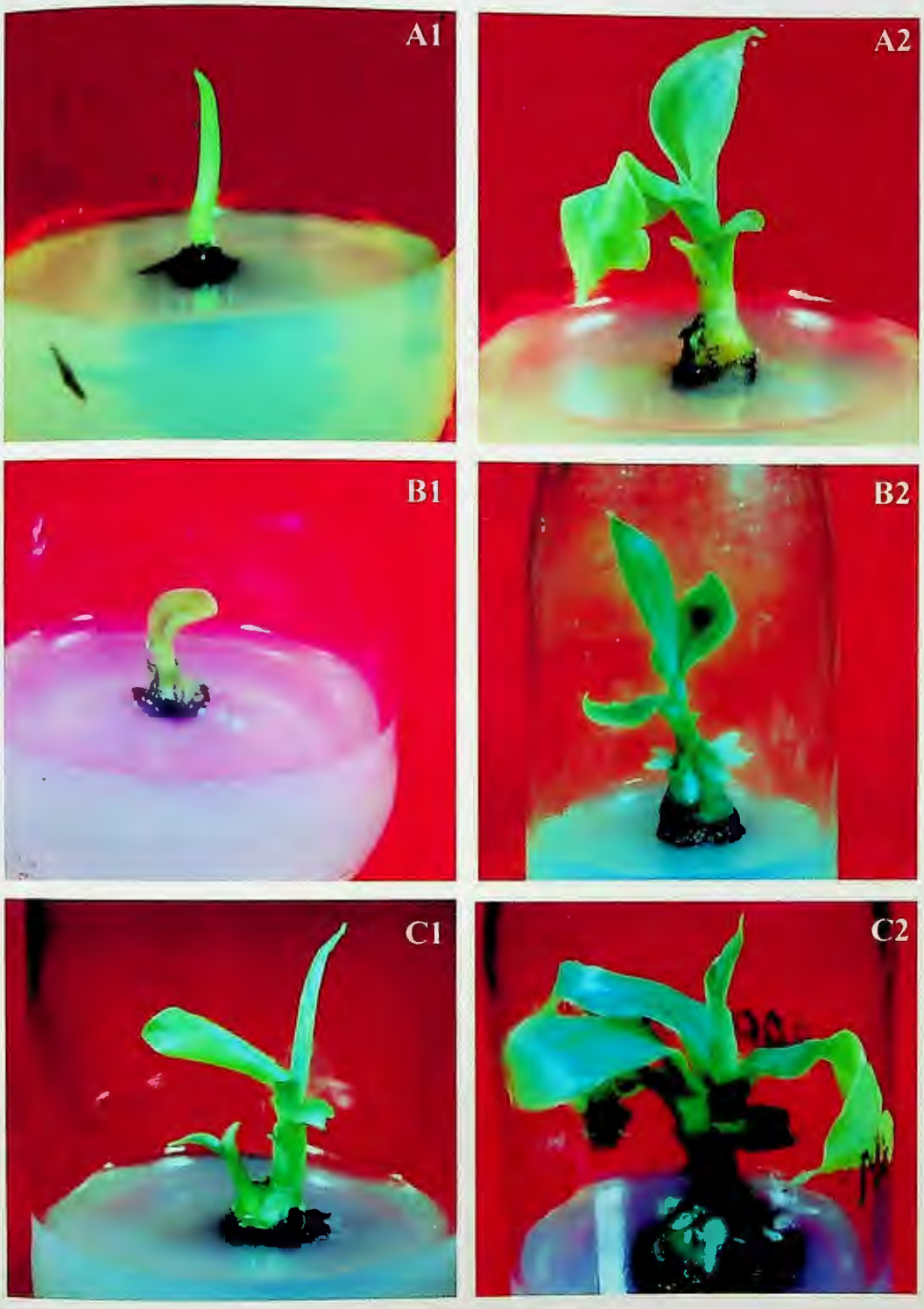


Plate 3

Photographs showing primary establishment of shoot-tip explants of three differe
banana cultivars cultured in MS semisolid medium supplemented with 5.0 mg /
BAP.

Figures:

D1. Jahaji 3 weeks after culture.

D2. Jahaji 4 weeks after culture.

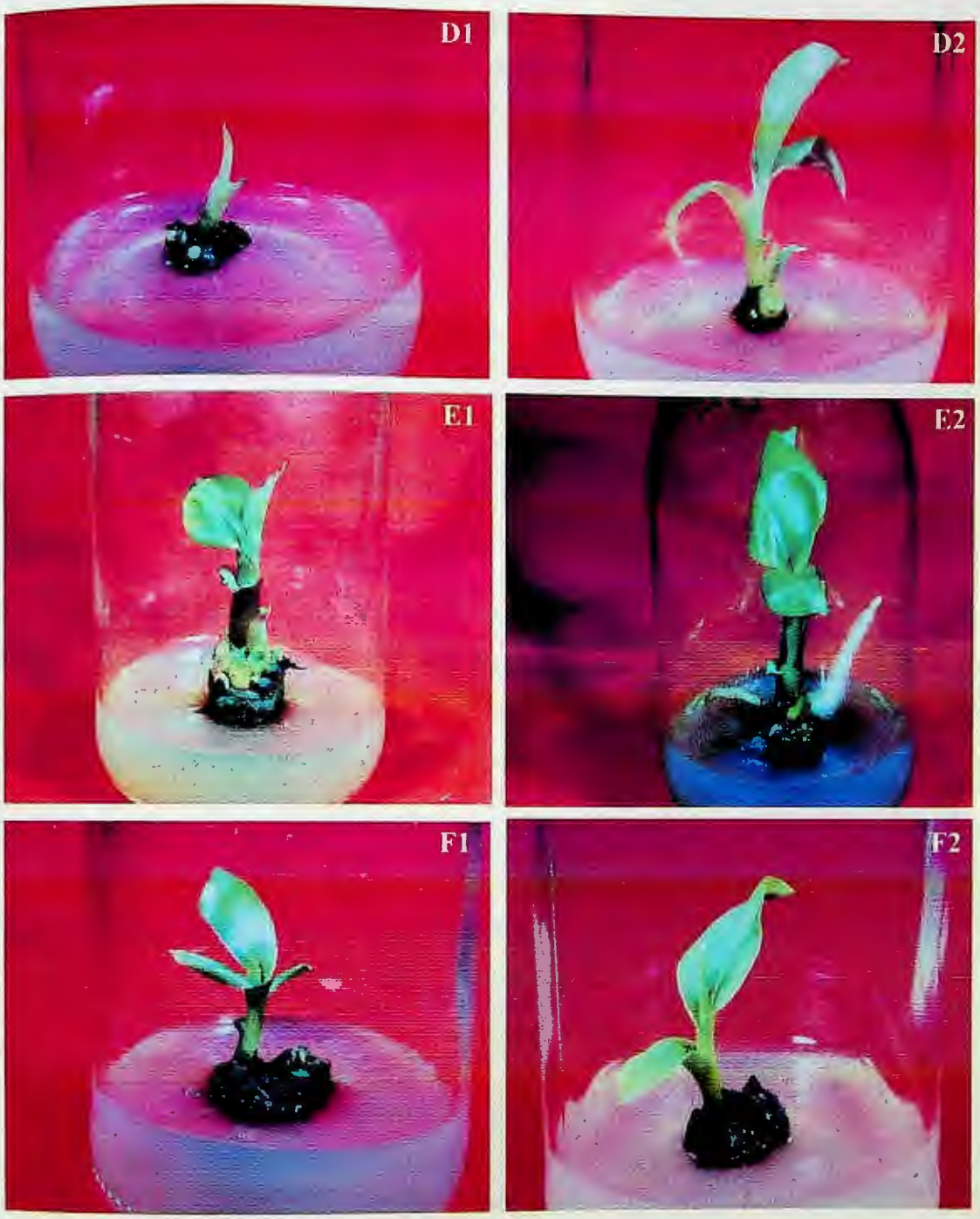
E1. Agniswar 4 weeks after culture.

E2. Agniswar 5 weeks after culture.

D1. Binathuri 5 weeks after culture.

D2. Binathuri 6 weeks after culture.

Plate 3



2.4.2. Multiple Shoot Proliferation

Different plant growth regulators formulations such as BAP, KIN, IAA, IBA Coconat water (CW) were tested in MS medium for multiple shoot proliferation from primary established shoot-tip cultures of six banana cultivars. The effect of different culture media formulations on multiple shoot proliferation are described below under different heads.

2.4.2.1. ***Effect of different concentrations of BAP on the production of multiple shoots:*** Primary cultures established from shoot-tip explants of six cultivars of banana were subcultured onto MS semisolid media supplemented with seven concentrations of BAP (**Table 2.4**). The data on the proliferation of multiple shoots were scored as percentage of response, number's of shoots/explant, length of the longest shoots/culture and the results are presented in **Table 2.4**.

Among the different concentrations of BAP 5 mg/l BAP was found to be the most effective formulation for the induction of multiple shoots for most of the cultivars. In this formulation cv. Sabari showed the highest % of primary culture induced shoot multiplication. However, the highest % of primary cultures showed shoot multiplication for cvs. Agniswar was recorded onto 8 mg/l BAP.

Among the six cultivars of banana cv. Sabari was the most responsive genotypes in culture condition. The highest 90% of the cultivars were induced multiple shoot proliferation after culturing onto 5 mg/l BAP. cv. Binathuri was found to be the less responsive (70%) in the formation of multiple shootlets (**Table 2.4**).

Number's of shoots / explant recorded after 45 days of culture were found to be varied according to genotypes and growth regulators formulation (BAP) in culture medium. Among the different media formulations 5 mg/l BAP was also found to be the most effective in inducing lateral bud proliferation from the corm of the primary culture for most of the cultivars. For cv. Agniswar 8 mg/l BAP was found to be most effective in multiple shoot proliferation. For shoot growth in 5 mg/l BAP was the best PGR formulation for most of the cultivars except cv. Agniswar. Among the six cultivars the shoot length was highest for Sabari recorded onto the same culture medium formulation.

2.4.2.2. ***Effect of different combinations and concentrations of BAP, KIN and IAA on the production of multiple shoots*** : *In vitro* grown shoots of primary culture were subcultured repeatedly for multiple shoot proliferation. For the optimization of the production of multiple shoots different concentrations and combinations of BAP, KIN and IAA in MS semi solid media were used. The data were recorded on the percentage of shoot induced multiple shoots proliferation, number of multiple shoots/explant and length of the longest shoot. Data collected after 45 days of culture and the results are presented in **Table 2.5**.

In vitro grown shoots were individually cultured onto MS semisolid media supplemented with seven concentrations and combinations of BAP, KIN and IAA (**Table 2.5**).

Among the seven concentrations of BAP + KIN + IAA, 5 mg/l BAP + 2 mg/l KIN + 2 mg/l IAA was found to be the most suitable formulation where most of the explants of six banana cultivars induced to develop shoots. In this formulation showed the highest percentage of explant induced multiple shoots proliferation for cvs. Mehersagar, Ranginsagar, Jahaji and Sabari. However, the highest percentage of explant showed induction for cvs. Agniswar and Binathuri were recorded onto medium 8 mg/l BAP + 1 mg/l KIN + 1 mg/ IAA and 3.5 mg/l BAP + 1 mg/l KIN + 1 mg/l IAA respectively.

Among the six cultivars of banana cvs. Mehersagar and Jahaji were the most responsive genotypes in terms of shoot multiplication. The highest 83% of the explants of these cultivars induced multiple shoots proliferation after culturing onto 5 mg/l BAP + 2 mg/l KIN + 2 mg/l IAA. Cv. Binathuri was found to be the less responsive in the formation of multiple shoots (**Table 2.5**).

Number's of shoots/explant recorded after 45 days of culture were found to be varied according to genotypes and level of growth regulators (BAP, KIN and IAA) in culture medium. Among the different media formulation 5 mg/l BAP + 2 mg/l KIN + 2 mg/l IAA was also found to be the most effective in inducing lateral bud proliferation from the corm of primary culture stablished explant except cv. Agniswar (in 8 mg/l BAP + 1 mg/l KIN + 1 mg/l IAA) and Binathuri (in 3.5 mg/l BAP + 1 mg/l

KIN + 1 mg/l IAA). Shoot growth in culture was also the best in 5 mg/l BAP + 2 mg/l KIN + 2 mg/l IAA in cvs. Sabari, Ranginsagar, Mehersagar and Jahaji. Among the six cultivars, the shoot length was highest for Sabari recorded onto the same culture medium formulation.

2.4.2.3. ***Effect of different concentrations of BAP, IBA and IAA on production of multiple shoots*** : Primary cultures established from shoot-tip explants of six cultivars of banana were also subcultured onto MS semisolid medium supplemented with six concentrations and combinations of BAP, IAA and IBA (**Table 2.6**). The data on the proliferation of multiple shoots were scored as percentage of response, number's of shoots/explant, the length of the longest shoot and the results are presented in **Table 2.6.**

Among the different concentrations and combinations of cytokinin and auxins, 4 mg/l BAP + 2 mg/l IAA + 2 mg/l IBA was found to be the most effective formulation for the induction of multiple shoots from most of the cultures of most of the cultivars. In this formulation cv. Shabri showed the highest % of the primary culture induced to develop shoot multiplication. However, the highest % of primary cultures showed multiple shoot multiplication for cvs. Mehersagar, Ranginsagar, Jahaji, Agniswar and Binathuri were recorded onto 4.0 mg/l BAP + 2.0 mg/l IAA + 2.0 mg/l IBA, 8.0 mg/l BAP + 1.0 mg/l IAA + 1.0 mg/l IBA and 3.5 mg/l BAP + 1.0 mg/l IAA+1.0 mg/l IBA respectively.

Among the six cultivars of banana cv. Sabari was the most responsive genotypes in culture condition. The highest 92% of the cultures of this cultivar was induced multiple shoot proliferation after culturing onto 4.0 mg/l BAP + 2.0 mg/l IAA + 2.0 mg/l IBA. cvs. Agniswar and Binathuri were found to be the less responsive in the formation of multiple shoots (**Table 2.6**).

Number of shoots/explant recorded after 45 days of culture were found to be varied according to genotypes and growth regulators formulations. Among the different media formulations 4.0 mg/l BAP + 2.0 mg/l IBA + 2.0 mg/l IAA was also found to be the most effective in inducing lateral bud proliferation from the corm of primary cultures for most of the cultivars. For Agniswar 8.0 mg/l BAP + 1.0 mg/l IAA + 1.0

mg/l IBA and for cv. Binathuri 3.5 mg/l BAP + 1.0 mg/l IAA + 1.0 mg/l IBA were found to be most effective in multiple shoots proliferation. For shoot growth in 4.0 mg/l BAP + 2.0 mg/l IAA + 2.0 mg/l IBA was the best PGRs formulation for cvs. Sabari, Mehersagar, Ranginsagar and Jahaji. Among the six cultivars the shoot length was highest for cv. Agniswar recorded onto the same culture medium formulation.

2.4.2.4. *Effect of different concentrations of BAP, KIN and coconut water (CW) on production of multiple shoots* : Primary cultures established from shoot-tip explants of six cultivars of banana were subcultured onto MS semisolid media supplemented with three concentrations and combinations of BAP, KIN and coconut water (CW) (**Table 2.7**). The data on production of multiple shoots were scored as numbers of shoots/culture and average length of longest shoots (cm)/culture. The results are presented in **Table 2.7** and illusted in (**Figures A1-C2, Plate 4**) and (**Figures D1-F2, Plate 5**).

Among the three concentrations 0.5 mg/l BAP + 0.5 mg/l KIN + 13% CW was found to be the most effective formulation for induction of multiple shoots from most of cultivars. In this formulation cvs. Mehersagar, Ranginsagar and Jahaji showed the highest % of primary cultures induced multiple shoot multiplication. However, the highest percentage of primary cultures showed multiple shoots multiplication for cvs. Sabri, Agniswar and Binathuri were recorded onto 5 mg/l BAP + 5 mg/l KIN + 13% cw, 8.0 mg/l BAP + 1.0 mg/l KIN + 13% CW and 3.5 mg/l BAP + 0.5 mg/l KIN + 13% CW respectively.

Among the various concentration of coconut water, 13% CW were most effective formulation for the production of multiple shoots and 10% CW were less effective formulation (**Table 2.7**).

Number of shoots/explant recorded after 35 days of culture were found to be varied according to genotypes and culture media formulations (BAP, KIN and coconut water) in culture medium. Among the different media formulation 0.5 mg/l BAP + 0.5 mg/l KIN + 13% CW was also found to be the most effective in inducing lateral bud proliferation from the corm of primary cultured for most of the cultivars. For shoot growth 0.5 mg/l BAP + 0.5 mg/l KIN + 13% CW was the best PGR formulation for cvs. Mehersagar, Ranginsagar and Jahahji. Among the six cultivars,

the shoot length (5.5 ± 0.095) was highest for cv. Sabari recorded onto the same culture medium formulation.

2.4.2.5. *Effect of number of subculture on multiple shoots production*

To find out the effect of subculture on the extent of shoot proliferation the primary cultures established from shoot-tip explants of six cultivars of banana were subcultured onto MS media supplemented with BAP at 5.0 mg/l (**Table 2.9**). The cultures were successively subcultured after 5 weeks of intervals on the same medium and continued upto 10^{th} subculture. The data were recorded on numbers of multiple shoots/subculture and the results are shown in **Table 2.9**.

The results shown in **Table 2.9** indicate that the multiple shoots proliferation rate was varied with the cultivars and also with subcultures. Average number of multiple shoots/culture after the 7^{th} subculture was become the maximum in cv. Sabari (15.60±1.471) followed by Ranginsagar (11.30±0.467) and the minimum in cv. Binathuri (6.71±0.224). The multiple shoots proliferation rates were increased gradually with the increaseing in the number of subculture upto 7^{th} subculture. However, the average rate of shoot multiplication was started to decline from 8^{th} to 10^{th} subculture. The average rate of shoot multiplication upto 10^{th} subculture was highest (9.82±1.395) in cv. Sabari and the lowest (4.37±0.515) in cv. Binathuri

Table 2.4 Effect of different concentrations of BAP on the production of multiple shoots from primary culture. Ten *In vitro* grown shoots were cultured for each treatment. Data collected after 45 days of culture

Cultivars	PGRs (mg/l) BAP	% of culture induced multiple shoot proliferation	No. of multiple shoots/ explant $\bar{x} \pm SE$	Average langth of longest shoot (cm) $\bar{x} \pm SE$
Sabari	1.0	20	2.50±0.354	2.40±0.071
	2.0	30	2.66±0.272	2.73±0.098
	3.0	70	4.00±0.286	3.00±0.107
	4.0	80	5.25±0.342	4.15±0.145
	5.0	90	6.66±0.314	5.33±0.138
	8.0	70	5.00±0.286	3.94±0.135
	10.0	60	4.16±0.436	3.20±0.105
Mehersagar	1.0	20	2.00±0.3000	2.30±0.141
	2.0	40	2.75±0.451	2.92±0.119
	3.0	60	3.83±0.281	3.30±0.149
	4.0	80	6.00±0.433	4.28±0.168
	5.0	80	5.75±0.293	4.70±0.181
	8.0	70	4.57±0.341	3.72±0.148
	10.0	50	3.80±0.335	3.12±0.129
Ranginsagar	1.0	20	2.00±0.000	2.25±0.035
	2.0	40	2.50±0.250	2.87±0.129
	3.0	70	3.42±0.341	3.22±0.127
	4.0	70	4.00±0.286	3.62±0.157
	5.0	80	5.50±0.306	4.47±1.162
	8.0	60	3.66±0.304	3.41±0.185
	10.0	40	3.00±0.354	4.00±0.096
Jahaji	1.0	20	2.30±0.354	2.35±0.106
	2.0	40	2.75±0.415	2.55±0.075
	3.0	60	3.50±0.204	2.80±0.085
	4.0	70	4.28±0.265	3.04±0.126
	5.0	80	5.00±0.354	3.73±0.056
	8.0	60	3.83±0281	3.00±0.053
	10.0	50	3.80±0.335	2.70±0.106
Agniswar	1.0	20	2.00±0.000	2.90±0.071
	2.0	30	2.33±0.272	2.96±1.713
	3.0	50	2.80±0.335	3.12±0.095
	4.0	50	3.00±0.283	3.16±0.108
	5.0	60	3.66±0.304	3.38±0.119
	8.0	70	4.57±0.341	4.20±0.163
	10.0	60	3.66±0.192	3.33±1.169
Binathuri	1.0	10	1.00±0.000	2.60±0.000
	2.0	20	2.00±0.000	2.85±0.106
	3.0	40	2.50±0.250	2.87±0.096
	4.0	60	3.16±0.281	3.08±0.093
	5.0	70	3.85±0.241	3.45±0.168
	8.0	50	3.00±0.283	2.88±0.091
	10.0	30	2.33±0.270	2.80±0.141

Table 2.5 Effect of different concentrations and combinations of BAP, KIN and IAA on the production of multiple shoots for primary culture. Twelve *in vitro* grown shoot were cultured for each treatment. Data collected after 45 days of culture

Cultivars	PGR (mg/L) BAP+KIN+IAA	% of culture induced multiple shoot proliferation	No. of multiple shoots/explant $\overline{X}$ ±SE	Average length of longest shoot per culture (cm) $\overline{X}$ ±SE
Sabari	3.0+1.0+10.	17	0.00 ± 0.000	2.30 ± 0.141
	4.0+1.0+1.0	33	3.00 ± 0.354	2.52 ± 0.178
	4.0+2.0+1.0	58	3.85 ± 0.315	2.85 ± 0.134
	4.0+2.0+2.0	75	4.77 ± 0.262	3.80 ± 0.215
	5.0+1.0+1.0	42	3.40 ± 0.219	2.70 ± 0.110
	5.0+2.0+2.0	83	5.60 ± 0.405	4.10 ± 0.197
	6.0+2.0+2.0	25	2.66 ± 0.272	2.20 ± 0.125
Mehersagar	3.0+1.0+10.	17	1.50 ± 0.254	2.25 ± 0.106
	4.0+1.0+1.0	25	2.33 ± 0.272	2.56 ± 0.144
	4.0+2.0+1.0	42	3.00 ± 0.283	2.70 ± 0.181
	4.0+2.0+2.0	67	3.75 ± 0.293	3.35 ± 0.141
	5.0+1.0+1.0	50	3.50 ± 0.312	2.38 ± 0.104
	5.0+2.0+2.0	75	4.44 ± 0.355	3.78 ± 0.200
	6.0+2.0+2.0	08	2.00 ± 0.000	1.95 ± 0.106
Ranginsagar	3.0+1.0+10.	17	2.50 ± 0.354	2.10 ± 0.071
	4.0+1.0+1.0	42	3.20 ± 0.335	2.44 ± 0.151
	4.0+2.0+1.0	50	3.67 ± 0.304	2.53 ± 0.185
	4.0+2.0+2.0	75	4.33 ± 0.272	3.34 ± 0.189
	5.0+1.0+1.0	42	3.40 ± 0.219	2.50 ± 0.165
	5.0+2.0+2.0	75	4.67 ± 0.314	3.75 ± 0.227
	6.0+2.0+2.0	17	2.00 ± 0.000	2.05 ± 0.035
Jahaji	3.0+1.0+10.	17	2.00 ± 0.000	2.05 ± 0.035
	4.0+1.0+1.0	33	2.50 ± 0.250	2.30 ± 0.079
	4.0+2.0+1.0	50	3.33 ± 0.304	2.33 ± 0.137
	4.0+2.0+2.0	75	4.11 ± 0.292	3.02 ± 0.166
	5.0+1.0+1.0	50	3.50 ± 0.204	2.31 ± 0.123
	5.0+2.0+2.0	83	4.80 ± 0.276	3.30 ± 0.137
	6.0+2.0+2.0	17	2.50 ± 0.354	1.80 ± 0.141
Agniswar	3.0+1.0+10.	17	2.00 ± 0.000	2.75 ± 0.177
	4.0+1.0+1.0	25	2.37 ± 0.272	2.90 ± 0.170
	4.0+2.0+1.0	42	2.60 ± 0.358	3.02 ± 0.148
	4.0+2.0+2.0	67	3.38 ± 0.303	3.91 ± 0.187
	5.0+1.0+1.0	58	3.00 ± 0.286	3.16 ± 0.140
	8.0+1.0+1.0	76	3.62 ± 0.246	4.08 ± 0.192
	10.0+2.0+2.0	25	2.00 ± 0.000	2.83 ± 0.166
Binathuri	1.0+1.0+10.	08	2.00 ± 0.000	2.20 ± 0.000
	2.0+1.0+1.0	25	2.00 ± 0.000	2.77 ± 0.119
	2.5+1.0+1.0	33	2.50 ± 0.250	2.88 ± 0.129
	3.0+2.0+2.0	58	3.14 ± 0.241	3.57 ± 0.216
	3.5+1.0+1.0	67	3.38 ± 0.303	3.66 ± 0.184
	4.0+1.0+1.0	25	2.33 ± 0.272	2.80 ± 0.129
	5.0+2.0+2.0	08	2.00 ± 0.000	1.90 ± 0.000

Table 2.6 Effect of different concentrations and combinations of BAP, IAA and IBA on the production of multiple shoots from primary culture. Twelve *in vitro* grown shoots were cultured for each treatment. Data collected after 45 days of culture.

Cultivars	PGR (mg/L) BAP+IAA+IBA	% of culture induced multiple shoot proliferation	No. of multiple shoots/explant $\overline{X}$ ±SE	Average length of longest shoot per culture (cm) $\overline{X}$ ±SE
Sabari	2.0+1.0+1.0	33	3.00 ± 0.354	2.57 ± 0.246
	3.0+2.0+2.0	42	3.40 ± 0.219	2.66 ± 0.187
	4.0+1.0+1.0	67	4.37 ± 0.351	2.97 ± 1.052
	4.0+2.0+2.0	92	6.09 ± 0.489	4.14 ± 0.137
	5.0+2.0+2.0	92	5.27 ± 0.388	3.60 ± 0.151
	6.0+2.0+2.0	33	3.75 ± 0.217	2.37 ± 0.292
Mehersagar	2.0+1.0+1.0	25	2.00 ± 0.471	2.10 ± 0.170
	3.0+2.0+2.0	33	3.00 ± 0.354	2.27 ± 0.198
	4.0+1.0+1.0	58	3.42 ± 0.275	2.80 ± 0.245
	4.0+2.0+2.0	83	4.60 ± 0.290	3.80 ± 0.191
	5.0+2.0+2.0	75	4.22 ± 0.262	3.467 ± 0.189
	6.0+2.0+2.0	42	3.00 ± 0.283	2.30 ± 0.139
Ranginsagor	2.0+1.0+1.0	33	3.00 ± 0.00	2.58 ± 0.246
	3.0+2.0+2.0	33	3.25 ± 0.217	2.62 ± 0.219
	4.0+1.0+1.0	67	3.71 ± 0.265	2.72 ± 0.143
	4.0+2.0+2.0	83	5.09 ± 0.395	3.83 ± 0.203
	5.0+2.0+2.0	83	4.80 ± 0.341	3.55 ± 0.170
	6.0+2.0+2.0	25	3.00 ± 0.000	2.48 ± 0.222
Jahaji	2.0+1.0+1.0	25	2.33 ± 0.272	2.10 ± 0.170
	3.0+2.0+2.0	42	3.40 ± 0.219	2.44 ± 0.171
	4.0+1.0+1.0	67	3.62 ± 0.246	2.81 ± 0.111
	4.0+2.0+2.0	83	4.90 ± 0.271	3.43 ± 0.176
	5.0+2.0+2.0	83	4.40 ± 0.253	3.25 ± 0.137
	6.0+2.0+2.0	33	3.00 ± 0.354	2.37 ± 0.207
Agniswar	2.0+1.0+1.0	25	2.00 ± 0.00	2.75 ± 0.177
	3.0+1.0+1.0	25	2.33 ± 0.72	2.83 ± 0.166
	4.0+1.0+1.0	58	3.14 ± 0.241	3.01 ± 0.113
	5.0+1.0+1.0	75	4.22 ± 0.305	4.19 ± 0.197
	8.0+1.0+1.0	75	3.89 ± 0.249	3.75 ± 0.207
	10.0+1.0+1.0	33	2.5 ± 0.250	2.88 ± 0.129
Binathuri	2.0+1.0+1.0	17	2.0 ± 0.000	2.35 ± 0.106
	3.0+1.0+1.0	25	2.33 ± 0.272	2.43 ± 0.191
	3.5+1.0+1.0	75	2.80 ± 0.335	2.94 ± 0.120
	4.0+1.0+1.0	67	3.75 ± 0.234	3.43 ± 0.123
	5 0+1.0+1.0	58	3.43 ± 0.187	3.11 ± 0.208
	6.0+1.0+1.0	25	2.67 ± 0.272	2.76 ± 0.119

Table 2.7 Effect of different combinations of BAP, KIN and coconut water (CW) on production of multiple shoots from primary culture. Ten *in vitro* grown shoots were culture for each tretment. Data were recorded after 35 days of culture

Cultivars	PGRs (mg/l)+CW(%)	% of culture	Average No. of Shoots per culture $\bar{x}$ ±SE	Average length of longest shoots (cm.) $\bar{x}$ ±SE
	5BAP+5KIN +10%		6.2 ± 0.194	4.59 ± 0.110
Sabari	5BAP+5KIN + 13%	100	8.2 ± 0.236	5.5 ± 0.095
	5BAP+5KIN + 10%		7.0 ± 0.173	4.75 ± 0.108
	0.5BAP+0.5KIN +10%		4.1 ± 0.094	2.66 ± 0.072
Mehersagar	0.5BAP+0.5KIN +13%	90	5.8 ± 0.154	3.7 ± 0.075
	0.5BAP+0.5KIN +20%		4.6 ± 0.120	3.12 ± 0.058
	0.5BAP+0.5KIN +10%		4.9 ± 0.114	2.91 ± 0.052
Ranginsagar	0.5BAP+0.5KIN +13%	100	6.0 ± 0.126	3.75 ± 0.073
	0.5BAP+0.5KIN +20%		5.2 ± 0.125	3.19 ± 0.054
	0.5BAP+0.5KIN +10%		3.5 ± 0.120	1.78 ± 0.052
Jahaji	0.5BAP+0.5KIN +13%	100	4.8 ± 0.147	2.55 ± 0.058
	0.5BAP+0.5KIN +20%		3.9 ± 0.130	2.12 ± 0.061
	8.0BAP+1.0KIN +10%		2.9 ± 0.070	3.92 ± 0.110
Agnishwar	8.0BAP+1.0KIN +13%	80	4.9 ± 0.013	5.15 ± 0.107
	8.0BAP+1.0KIN +20%		4.3 ± 0.110	4.39 ± 0.103
	3.5BAP+0.5KIN +10%		2.8 ± 0.075	3.74 ± 0.081
Binathuri	3.5BAP+0.5KIN +13%	70	3.6 ± 0.094	4.30 ± 0.105
	3.5BAP+0.5KIN +20%		3.0 ± 0.092	3.58 ± 0.107

Table 2.8 Effects of subculture on multiple shoots formation of different cultivars of banana. Each treatment consisted of 10 explants and the experiments were repeated twice

Subculture	Number of shoots/explant											
	Sabari		Mehersagar		Ranginsagar		Jahaji		Agniswar		Binathuri	
	Mean ±SE	Range	Mean ±SE	Range	Mean ±SE	Range	Mean ±SE	Range	Mean ±SE	Range	Mean ±SE	Range
1st	2.30±0.247	2-4	2.00±0.283	1-4	1.90±0.221	1-3	1.70±0.232	1-3	1.70±0.202	1-3	1.60±0.210	1-3
2nd	4.20±0.544	2-7	3.30±0.285	2-5	3.4 ±0.128	2-6	2.50±0.158	2-3	2.5 ±0.050	2-3	2.5 ±0.050	2-3
3rd	6.20±0.544	4-10	3.60±0.452	2-7	4.9 ±0.197	2-9	3.10±0.330	2-5	3.2 ±0.117	2-5	2.9 ±0.070	2-4
4th	11.20±0.525	8-14	5.10±0.632	3-9	6.8 ±0.264	3-12	4.10±0.263	3-5	4.9 ±0.145	3-8	4.9 ±0.104	3-7
5th	14.10±1.459	10-25	8.00±0.949	4-14	9.5±0.393	4-17	6.30±0.584	4-8	7.1±0.247	3-12	5.5±0.169	3-9
6th	14.40±1.663	8-22	8.30±0.980	4-14	10.30±1.06	6-13	6.80±0.506	4-8	7.40±0.738	5-10	6.50±0.430	5-9
7th	15.60±1.471	9-22	9.00±0.894	5-15	11.3±0.467	5-20	7.30±0.649	4-11	7.8±0.306	4-14	6.7±0.224	4-10
8th	13.20±1.793	6-20	8.30±0.664	5-13	10.0±0.402	5-18	6.40±0.533	5-10	6.9±0.259	4-12	5.3±0.119	4-8
9th	9.90±1.118	5-17	6.40±0.587	4-10	8.7±0.310	4-15	5.60±0.405	4-8	5.5±0.175	3-8	4.3±0.064	3-5
10th	7.10±0.655	4-11	5.30±0.348	4-8	6.30±0.549	4-10	4.20±0.237	3-5	4.10±0.221	3-5	3.50±0.158	3-4
Mean ($\bar{x}$)	9.82±1.395		5.93±0.735		7.31±0.951		4.80±0.586		5.11±0.655		4.37±0.515	

Plate 4

Photographs showing the effect of BAP + KIN + coconut water (CW) in MS semisolid medium on shoot multiplication of three different banana cultivars.

Figures:

A1. Multiple shoot induction from shoot-tip explants derived from primary culture of Sabari in MS + 5.0 mg/l BAP + 5.0 mg/l KIN + 13% CW 4 weeks after culture.

A2. Multiple shoot induction from shoot-tip explants derived from primary culture of Sabari in MS + 5.0 mg/l BAP + 5.0 mg/l KIN + 13% CW 6 weeks after culture.

B1. Multiple shoot induction from shoot-tip explants derived from primary culture of Mehersagar in MS + 0.5 mg/l BAP + 0.5 mg/l KIN + 13% CW 3 weeks after culture.

B2. Multiple shoot induction from shoot-tip explants derived from primary culture of Mehersagar in MS + 0.5 mg/l BAP + 0.5 mg/l KIN + 13% CW 4 weeks after culture.

C1. Multiple shoot induction from shoot-tip explants derived from primary culture of Ranginsagar in MS + 0.5 mg/l BAP + 0.5 mg/l KIN + 13% CW 4 weeks after culture.

C2. Multiple shoot induction from shoot-tip explants derived from primary culture of Ranginsagar in MS + 0.5 mg/l BAP + 0.5 mg/l KIN + 13% CW 5 weeks after culture.

Plate 4

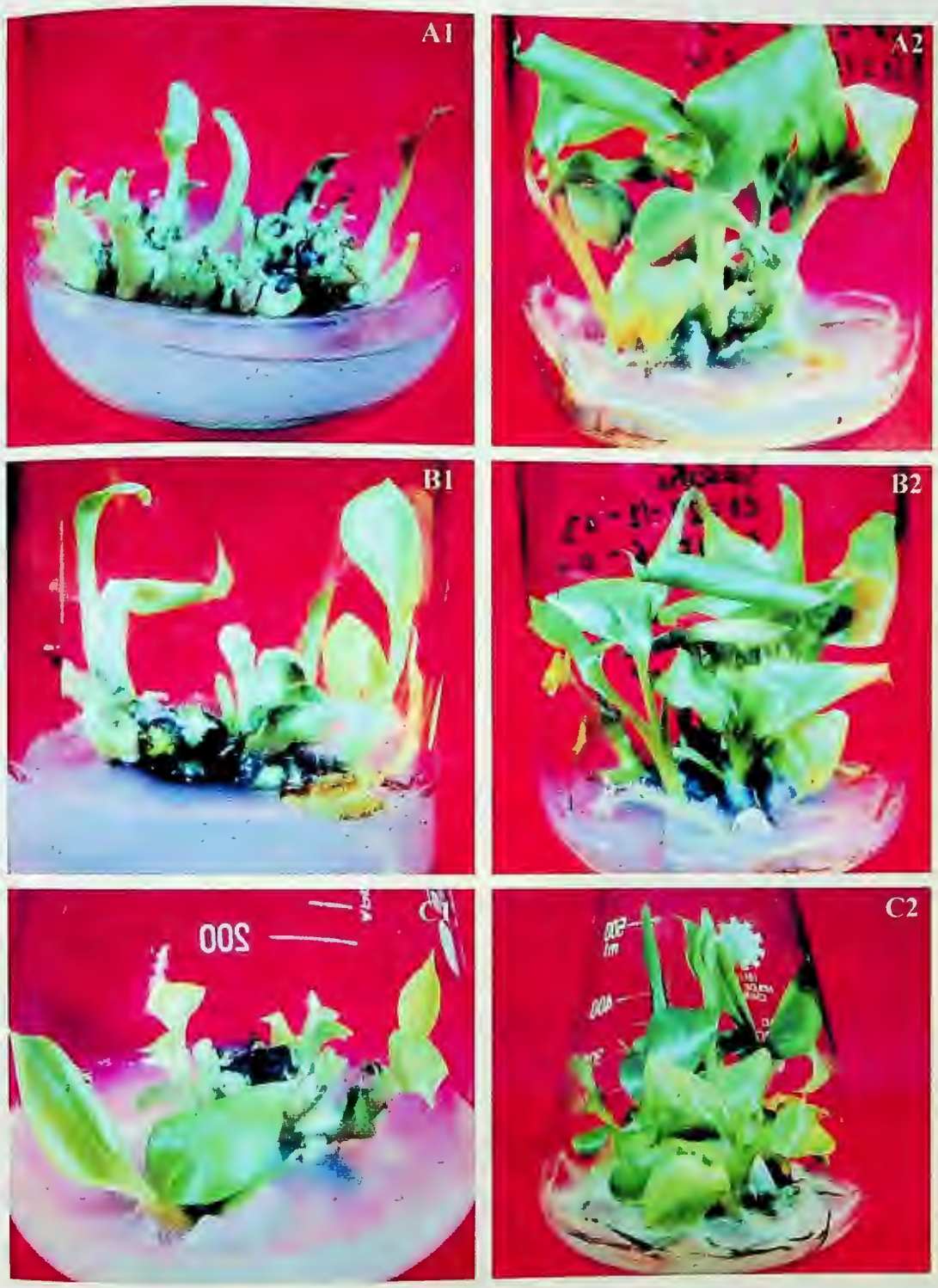


Plate 5

Photographs showing effect of BAP + KIN + coconut water (cw) in MS semisolid medium on shoot multiplication of three different banana cultivars.

Figures:

D1. Multiple shoot induction from shoot-tip explants derived from primary culture of Jahaji in MS + 0.5 mg/l BAP + 0.5 mg/l KIN + 13% cw 4 weeks after culture.

D2. Multiple shoot induction from shoot-tip explants derived from primary culture of Jahaji in MS + 0.5 mg/l BAP + 0.5 mg/l KIN + 13% CW 5 weeks after culture.

E1. Multiple shoot induction from shoot-tip explants derived from primary culture of Agniswar in MS + 8 mg/l BAP + 1.0 mg/l KIN + 13% CW 4 weeks after culture.

E2. Multiple shoot induction from shoot-tip explants derived from primary culture of Agniswar in MS + 8 mg/l BAP + 1.0 mg/l KIN + 13% CW 5 weeks after culture.

F1. Multiple shoot induction from shoot-tip explants derived from primary culture of Binathuri in MS + 3.5 mg/l BAP + 0.5 mg/l KIN + 13% CW 5 weeks after culture.

F2. Multiple shoot induction from shoot-tip explants derived from primary culture of Binathuri in MS + 3.5 mg/l BAP + 0.5 mg/l KIN + 13% CW 6 weeks after culture.

Plate 5

2.4.3. Formation of Bulbous Structure

Shoot-tips of young field grown suckers of six cultivars of banana were inoculated in MS + 5.0 mg/l BAP + 2.0 mg/l IAA, cultured explants induced to develop after 20-25 days (**Table 2.8**). The single shoots were then subcultured in the same medium. After another 20-25 days of first subculture multiple shoots developed. When a cluster of four-five plantlets were formed these were used as explants in this experiment.

Each clusters of plantlets was subcultured in MS medium with different concentrations of KIN. In several concentrations these shoots instead of giving normal plantlets were transformed into bulbous structure. The result of such bulbous structures are presented in **Table 2.8** and illustrated in (**Figures A-F** of **Plate 6**).

It was found that the hard meristematic bulbous structure formation was higher in explant placed onto MS medium supplemented with 5.0 mg/l KIN. Among the six cultivars of banana cv. Sabari was the most responsive (86.67) genotypes in the formation of bulbous structure. In case of Sabari, among the fifteen explants, thirteen are responded to bulbous structure.

At the concentration of 2.0 – 3.0 mg/l of KIN and 8.0 mg/l KIN bulbous structure did not form in case of all six cultivars. On the other hand lowest percentage of bulbous structure formation were found in MS + 6.0 mg/l KIN.

The rate of formation of bulbous structure increased rapidly in repeated subcultures. These BS of banana is very good for germplasm preservation and could be utilized for further multiplication and subsequently mass proliferation of ideal plantlets for commercial exploitation.

Table 2.9 Effect of different concentrations of KIN in MS medium on the formation of bulbous structure. Each treatment were consisted of 15 explants and repeated twice

Cultivars	KIN (mg/l)	No. of culture forming BS	Rate of response (%)	Days required for BS formations
Sabari	2.0	-	-	-
	3.0	-	-	-
	4.0	3	20	20.30
	4.5	6	40	20.30
	5.0	13	86	20.30
	5.5	7	46	20.30
	6.0	2	13	20.30
	8.0	-	-	-
Mehersagar	2.0	-	-	-
	3.0	-	-	-
	4.0	2	13	30-35
	4.5	4	26	30-35
	5.0	7	46	30-35
	5.5	3	20	30-35
	6.0	1	06	30-35
	8.0	-	-	-
Ranginsagar	2.0	-	-	-
	3.0	-	-	-
	4.0	3	20	30-35
	4.5	4	26	30-35
	5.0	8	53	30-35
	5.5	3	20	30-35
	6.0	1	06	30-35
	8.0	-	-	-
Jahaji	2.0	-	-	-
	3.0	-	-	-
	4.0	2	13	30-35
	4.5	5	33	30-35
	5.0	9	60	30-35
	5.5	3	20	30-35
	6.0	1	06	30-35
	8.0	-	-	-
Agniswar	2.0	-	-	-
	3.0	-	-	-
	4.0	-	-	-
	4.5	2	13	40-45
	5.0	7	46	40-45
	5.5	3	20	40-45
	6.0	1	06	40-45
	8.0	-	--	-
Binathuri	2.0	-	-	-
	3.0	-	-	-
	4.0	1	06	42-55
	4.5	4	26	42-55
	5.0	5	33	42-55
	5.5	3	20	42-55
	6.0	1	06	42-55
	8.0	-	-	-

Plate 6

Photographs showing formation of Bulbous Structure in different banana cul
Bulbous structures was formed during repeated subcultures in medium supplem
with 5.0 mg/l KIN.

Figures:

A. Sabari.
B. Mehersagar.
C. Ranginsagar.
D. Jahaji.
E. Agniswar.
F. Binathuri.

Plate 6

2.4.4. Induction of Roots on *In Vitro* Regenerated Shoots

The minishoots developed from the multiple shoots were excised individually transferred onto ½ MS medium supplemented with different types of auxins. Data were collected on % of shoots induced root formation, no. of roots/shoot, length of the longest root and the results are presented in **Table 2.10 - 2.13.**

2.4.4.1. *Effect of different concentrations of IBA on induction of roots* : The minishoots were considered 2-4 leaves at the time of transferring onto rooting media. The minishoots subcultured onto different rooting media were continued their growth throw the roots and leaves proliferation and shoot elongation. However, the degree of root growth was found to vary with rooting media formulations.

Among the six concentrations ½ MS+1.0 mg IBA was found to be better effective formulations in which 100% minishoot of all banana cultivars induced to developed roots. (**Figures A-F** of **Plate 7**). Among the six different types of meda formulations the number of roots/shoot was found the highest (7.00±0.245) in Sabari in ½ MS+1.0 mg IBA. The lowest numbers of roots/shoots was recorded for the minishoots cultured onto 5.0 mg/l IBA. The root length (4.84±0.257 cm) was the highest for Sabari recorded onto same cultured medium formulation. The root length was the lowest in without PGR rootting media for all of the six cultivars (**Table 2.10**).

2.4.4.2. *Effect of different concentrations of NAA on induction of roots* : The minishoots developed from the multiple shoots were excised individually and transferred onto ½ MS medium supplemented with six different concentrations of NAA. Data were collected on root growth and the results are presented in **Table 2.11**.

The minishoots were considered 2-4 leaves at the time of transferring onto rooting media. The minishoots subcultured onto different rooting media were continued their growth throw the roots and leaves proliferation and shoot elongation. However, the degree of root growth was found to vary with rooting media formulations.

Among the six concentrations 1.0 mg/l NAA was found to be most effective formulation in which minishoot of all banana cultivars induced to develop root. Among the 7 different types of media formulations the numbers of roots/shoot was found the highest for cvs. Sabari and Mehersagar when the minishoots were cultured onto 1.0 mg/l NAA. The lowest numbers of roots/shoot was recorded for the minishoots cultured onto without PGR and 5.0 mg/l NAA. The root length was the highest (3.54±0.142) for Sabari recorded onto the same culture medium formulation. The root length was the lowest in without PGR rooting media for all of the six cultivars (**Table 2.11**).

2.4.4.3. ***Effect of different concentrations of IAA on induction of roots*** : The minishoots developed from the multiple shoots were excised individually and transferred onto ½ MS medium supplemented with six different concentrations of IAA. Data were collected on root growth and the results are presented in **Table 2.12**.

Among the six concentrations 2.0 mg/l IAA was found to better effective formulations in which 100% minishoot of all banana cultivars induce to develop root. Among the 6 different types of media formulations the numbers of roots/shoot was found the highest (4.80±0.341) in Mehersagar in 2.0 mg/l IAA. The lowest numbers of roots/shoot was recorded for the minishoots cultured onto control and ½ MS+5.0 mg/l IAA. The root length (3.35±0.131cm) was highest for cv. Sabari recorded onto the same cultured medium formulation.

2.4.4.4. ***Effect of different percentage of activated charcoal on induction of roots*** : The minishoots developed from the shoot-tip explants cultured in different media formulations were individually transferred onto ½ MS+1.0 mg/l IBA rooting medium supplemented with different concentrations of activated charcoal (AC) (**Table 2.13**). Data were collected on root growth of cultured minishoots and the results are presented in **Table 2.13**.

Addition of AC with 1.0 mg/l IBA further accenticeated the root development process in banana minishoots. Among the five concentrations activated charcoal (AC) 1.0 g/l found more effective in inducing root as well as shoot growth than the other rooting media. In this formulation minishoots of all of the cultivars induced to develop roots. Among the different concentrations the numbers of roots/shoot was found to be highest (7.40±0.494 cm) in cv. Sabari when the minishoots were cultured onto 1.0 g/l activated charcoal media. The lowest numbers of roots/shoot was recorded for the minishoots cultured onto 1.0 g/l activate charcoal (AC). The root length (5.60±0.170 cm) was highest for the cv. Sabari recorded onto the same culture medium formulation. The root length was the lowest (3.58±0.100cm) at 1.0 g/l activated charcoal rooting media for all of the six cultivars (**Table 2.13**). Activated charcoal increased the average root length but not root number.

Table 2.10 Effect of different concentrations of IBA in $\frac{1}{2}$ MS medium on root induction form *in vitro* grown plantlets of different cultivars of banana. In each treatment 10 explants were cultured in three replication. Data collected after 25 days of culture

Cultivar	Concentraion of IBA (mg/l)	% of shoot rooted	No. of roots/shoot ($\overline{X}$ ±SE)	Length of longest root (cm) ($\overline{X}$ ±SE)
Sabari	0.5	80	4.00 ± 0.250	2.23 ± 0.205
	1.0	100	7.00 ± 0.245	4.84 ± 0.257
	1.5	100	6.60 ± 0.352	4.49 ± 0.202
	2.5	100	5.10 ± 0.386	3.18 ± 0.189
	4.0	70	4.14 ± 0.315	3.05 ± 0.255
	5.0	60	3.16 ± 0.281	1.96 ± 0.096
Mehersagar	0.5	90	3.55 ± 0.228	2.80 ± 0.125
	1.0	100	6.20 ± 0.395	4.01 ± 0.210
	1.5	100	5.40 ± 0.352	3.66 ± 0.225
	2.5	90	4.44 ± 0.277	3.144 ± 0.179
	4.0	70	3.85 ± 0.241	2.60 ± 0.159
	5.0	60	3.50 ± 0.321	2.11 ± 0.140
Ranginsagar	0.5	80	3.25 ± 0.234	2.42 ± 0.205
	1.0	100	4.90 ± 0.359	3.57 ± 0.245
	1.5	100	4.40 ± 0.253	3.31 ± 0.243
	2.5	90	4.0 ± 0.222	3.07 ± 0.204
	4.0	60	3.33 ± 0.192	2.36 ± 0.243
	5.0	60	2.83 ± 0.281	2.13 ± 0.200
Jahaji	0.5	80	3.37 ± 0.303	2.25 ± 0.159
	1.0	100	5.90 ± 0.359	3.95 ± 0.112
	1.5	100	5.30 ± 0.401	3.50 ± 0.164
	2.5	90	4.11 ± 0.292	3.10 ± 0.147
	4.0	60	3.66 ± 0.385	2.85 ± 0.091
	5.0	50	3.20 ± 0.335	2.28 ± 0.158
Agniswar	0.5	90	3.33 ± 0.222	2.63 ± 0.181
	1.0	100	4.70 ± 0.318	3.44 ± 0.292
	1.5	100	4.20 ± 0.276	3.05 ± 0.216
	2.5	70	3.85 ± 0.315	2.74 ± 0.236
	4.0	60	3.33 ± 0.192	2.46 ± 0.209
	5.0	50	3.00 ± 0.283	2.02 ± 0.993
Binathuri	0.5	80	3.00 ± 0.250	2.47 ± 0.190
	1.0	100	4.55 ± 0.302	3.83 ± 0.191
	1.5	100	4.20 ± 0.276	3.40 ± 0.189
	2.5	70	3.71 ± 0.265	2.85 ± 0.249
	4.0	60	3.33 ± 0.304	2.36 ± 0.174
	5.0	40	2.75 ± 0.217	2.07 ± 0.129

Table 2.11 Effect of different concentrations of NAA in $\frac{1}{2}$ MS medium on root induction form *in vitro* grown plantlets of different cultivars of banana. In each treatment 10 explants were cultured in three replication. Data collected after 25 days of culture

Cultivar	Concentration of NAA (mg/l)	% of shoot rooted	No. of roots/shoot ($\overline{X}$ ±SE)	Length of longest root (cm.) ($\overline{X}$ ±SE)
Sabari	0.5	80	3.125 ± 0.276	1.73 ± 0.148
	1.0	100	5.60 ± 0.429	3.54 ± 0.142
	1.5	100	5.10 ± 0.359	3.29 ± 0.153
	2.5	90	4.00 ± 0.314	2.86 ± 0.204
	4.0	60	3.16 ± 0.281	1.55 ± 0.135
	5.0	40	2.25 ± 0.217	1.25 ± 0.056
Mehersagar	0.5	90	3.33 ± 0.314	2.27 ± 0.157
	1.0	100	5.60 ± 0.352	3.09 ± 0.184
	1.5	100	5.10 ± 0.298	2.66 ± 0.152
	2.5	100	4.30 ± 0.285	2.52 ± 0.162
	4.0	70	3.85 ± 0.315	2.20 ± 0.151
	5.0	40	3.00 ± 0.354	1.60 ± 0.117
Ranginsagar	0.5	80	2.75 ± 0.234	2.10 ± 0.133
	1.0	100	4.20 ± 0.237	3.05 ± 0.170
	1.5	100	3.80 ± 0.237	2.86 ± 0.154
	2.5	90	3.44 ± 0.166	2.50 ± 0.137
	4.0	60	2.83 ± 0.281	2.10 ± 0.139
	5.0	40	2.50 ± 0.250	1.70 ± 0.106
Jahaji	0.5	70	3.571 ± 0.275	2.22 ± 0.141
	1.0	100	5.50 ± 0.324	3.39 ± 0.224
	1.5	90	4.33 ± 0.222	3.01 ± 0.182
	2.5	80	4.00 ± 0.177	2.57 ± 0.133
	4.0	60	3.33 ± 0.192	2.23 ± 0.128
	5.0	50	3.00 ± 0.283	1.88 ± 0.080
Agniswar	0.5	80	2.75 ± 0.234	2.23 ± 0.157
	1.0	100	4.00 ± 0.245	3.14 ± 0.242
	1.5	100	3.70 ± 0.247	2.95 ± 0.234
	2.5	80	3.50 ± 0.306	2.66 ± 0.224
	4.0	60	2.83 ± 0.281	2.08 ± 0.148
	5.0	60	2.67 ± 0.304	1.93 ± 0.137
Binathuri	0.5	80	2.63 ± 0.246	2.07 ± 0.138
	1.0	100	3.80 ± 0.237	3.11 ± 0.213
	1.5	100	3.60 ± 0.210	2.92 ± 0.198
	2.5	70	3.14 ± 0.241	2.64 ± 0.252
	4.0	50	2.80 ± 0.335	2.08 ± 0.216
	5.0	50	2.40 ± 0.219	1.80 ± 0.075

Table 2.12 Effect of different concentrations of IAA in ½ MS medium on root induction from *in vitro* grown plantlets of different cultivars of banana. In each treatment 10 explants were cultured in three replication. Data collected after 25 days of culture

Cultivars	Concentration of IAA (mg/l)	% of shoot rooted	No. of roots/shoots ($\overline{X}$ ±SE)	Length of largest root (cm) ($\overline{X}$ ±SE)
Sabari	0.5	60	3.16 ± 0.281	2.06 ± 0.216
	1.0	80	4.25 ± 0.342	3.08 ± 0.164
	2.0	100	4.60 ± 0.253	3.35 ± 0.131
	3.0	70	3.42 ± 0.341	2.20 ± 0.170
	4.0	50	2.80 ± 0.179	1.84 ± 0.201
	5.0	40	2.25 ± 0.217	1.27 ± 0.096
Mehersagar	0.5	70	2.71 ± 0.265	2.32 ± 0.137
	1.0	90	3.55 ± 0.277	2.85 ± 0.163
	2.0	100	4.80 ± 0.341	3.400 ± 0.245
	3.0	70	3.57 ± 0.341	2.47 ± 0.200
	4.0	60	3.00 ± 0.236	2.05 ± 0.194
	5.0	60	2.66 ± 0.192	1.88 ± 0.152
Ranginsagar	0.5	70	2.85 ± 0.241	1.91 ± 0.127
	1.0	90	3.44 ± 0.277	2.41 ± 0.176
	2.0	100	3.90 ± 0.298	2.87 ± 0.202
	3.0	80	3.25 ± 0.234	2.36 ± 0.174
	4.0	60	2.50 ± 0.204	2.03 ± 0.131
	5.0	50	2.40 ± 0.219	1.86 ± 0.008
Jahaji	0.5	60	2.50 ± 0.312	1.85 ± 0.139
	1.0	70	2.71 ± 0.265	2.10 ± 0.141
	2.0	100	4.20 ± 0.237	3.05 ± 0.242
	3.0	60	3.00 ± 0.333	1.95 ± 0.135
	4.0	50	2.20 ± 0.335	1.74 ± 0.078
	5.0	50	2.00 ± 0.283	1.66 ± 0.096
Agniswar	0.5	60	2.50 ± 0.204	2.08 ± 0.148
	1.0	80	2.63 ± 0.171	2.40 ± 0.165
	2.0	100	3.70 ± 0.247	2.83 ± 0.178
	3.0	60	3.16 ± 0.281	2.55 ± 0.173
	4.0	50	2.40 ± 0.219	1.88 ± 0.104
	5.0	50	2.20 ± 0.179	1.72 ± 0.111
Binathuri	0.5	60	2.33 ± 0.192	1.80 ± 0.103
	1.0	70	2.72 ± 0.265	2.26 ± 0.148
	2.0	100	3.50 ± 0.255	2.93 ± 0.185
	3.0	50	3.00 ± 0.283	2.32 ± 0.178
	4.0	50	2.40 ± 0.219	1.92 ± 0.104
	5.0	40	2.25 ± 0.217	1.70 ± 0.106

Table 2.13 Effect of different concentrations of activated charcoal (AC) on the induction and growth of root in micro-cuttings of six cultivars of banana. Along with AC the rooting media was ½MS and fortified with 1 mg/l IBA. Data were collected after 25 days of culture

Cultivars	Concentration of growth regulators IBA+Charcoal (g/l)	% of shoot rooted	Nos. of roots/shoot ($\overline{X}$ ±SE)	Length of longest root (cm) ($\overline{X}$ ±SE)
Sabari	1.0	100	7.20±0.443	5.43±0.473
	2.5	100	7.40±0.494	5.60±0.170
	5.0	100	7.40±0.379	5.33±0.170
	7.5	100	7.10±0.482	5.24±0.146
	10.0	100	6.90±0.479	5.07±0.171
Mehersagar	1.0	100	6.20±0.310	4.92±0.173
	2.5	100	6.30±0.348	5.22±0.138
	5.0	100	6.10±0.411	5.47±0.189
	7.5	100	6.00±0.374	4.39±0.203
	10.0	100	5.80±0.395	3.87±0.165
Ranginsagar	1.0	100	5.00±0.316	4.32±0.158
	2.5	100	5.00±0.221	4.48±0.178
	5.0	100	4.90±0.263	4.17±0.190
	7.5	100	4.80±0.310	3.81±0.153
	10.0	100	4.90±0.443	3.58±0.100
Jahaji	1.0	100	5.90±0.435	4.74±0.150
	2.5	100	6.10±0.457	5.12±0.142
	5.0	100	6.00±0.490	4.67±0.118
	7.5	100	5.80±0.348	4.33±0.115
	10.0	100	5.90±0.435	3.91±0.130
Agniswar	1.0	100	4.70±0.276	4.23±0.146
	2.5	100	4.80±0.247	4.55±0.131
	5.0	100	4.70±0.283	4.18±0.281
	7.5	100	4.60±0.253	3.92±0.128
	10.0	100	4.70±0.318	3.80±0.141
Binathuri	1.0	100	4.50±0.158	4.44±0.156
	2.5	100	4.60±0.292	4.75±0.197
	5.0	100	4.60±0.290	4.42±0.139
	7.5	100	4.50±0.212	4.20±0.118
	10.0	100	4.60±0.290	4.08±0.152

Plate 7

Root induction and plantlet development from microculturing of six banana cultiva
Mini shoots were individually cultured onto rooting medium containing ½ MS+1
mg/l IBA. Photographs were taken 25 days after culture onto rooting medium.

Figures:

A. Sabari.
B. Mehersagar.
C. Ranginsagar.
D. Jahaji.
E. Agniswar.
F. Binathuri.

Plate 7

2.4.5. Field Evaluation

Tissue cultured derived plants (TC) of six cultivars of banana after proper acclimatization were transplanted into the field. Sucker derived plants (SC) near about same age and size of all the cultivars were also planted in the same field as control. Randomized block design with three replications was followed to compare the performance of TC plants in field condition over SC plants. Data were recorded on several agronomic characters such as plant height, base girth, number of functional leaves/plant, length of the longest leaves, breadth of longest leaf, number of sucker 4½ months after planting and the results on these observations are shown in **Table 2.14.**

The results presented in **Table 2.14** exhibit that TC plants after 4½ months of plantation were become superior in terms of a numbers of quantitative characters for most of the cultivars. The plant height for TC plants for most of the cultivars except Ranginsagar were significantly higher than those of sucker derived plants. Plant height of TC and SC plants of cv. Ranginsagars 4½ months after transplantation was not differ significantly. Similarly, the difference of stem girth between TC and SC plants of cvs. Sabari, Ranginsagar and Jahaji was non significant but significantly different for cvs. Mehersagar, Agniswar and Binathuri. For numbers of functional leaves/plant difference between TC and SC plants of cv. Mehersagar was not significant but significant for rest of cultivars. The difference of length of longest leaf between TC and SC plants of cvs. Ranginsagar, Jahaji and Agniswar were non significant but significantly different for cvs. Sabari, Mehersagar and Binathuri. The breadth of longest leaf for TC plants and SC plants for most of the cultivars except Mehersagar were significantly higher than those of sucker derived plants.

The results presented in **Table 2.15** reveal that TC plants at flowering stage were also found superior in four quantitative characters for most of the cultivars. The plant height for TC and SC plants except Mehersagar were significantly higher than those of sucker derived plants. Plant height of TC and SC plants of cv. Mehersagar at flowering stage was not differ significantly. The difference of base girth between TC

and SC plants except cv. Binathuri was significant. The base girth of TC and SC plants of cv. Binathuri at flowering stage was not differ significantly. For numbers of functional leaves/plant the difference between TC and SC plants of cv. Binathuri was also not significant but significant for rest of cultivars. The difference of length of longest leaf between TC and SC plants of cvs. Sabari, Ranginsagar and Binathuri also not significant but significantly different for the rest of three cultivars. The breadth of longest leaf for TC and SC plants for most of the cultivars except Agniswar were significantly higher than those of SC plants. The number of suckers/plant were significantly higher in TC plants than SC plants in case of all six cultivars.

The TC and SC derived plants were grown to maturity. Data were recorded on yield and different yield contributing character viz. numbers of hands/bunch, numbers of finger/hands, total numbers of finger/bunch, fruit length, fruit girth and the results on these observation are shown in **Table 2.16.**

The results presented in **Table 2.16** indicate that the TC plants of most cultivars produced significantly higher numbers of hands/bunch than those of SC plants except cvs. Mehersagar and Ranginsagar. Similarly, significant differences were recorded for number of fingers/hand of TC as compared with the SC plants for cvs. Sabari, Mehersagar and Ranginsagar. The differences for numbers of fingers/hand between TC and SC plants of cv. Jahaji, Agniswar and Binathuri were not significant. Total number of fingers/bunch were significantly higher in TC than SC plants for most of the cultivars except Mehersagar. Both length and girth of fruits were significantly higher in TC plants than SC plants in all of the cultivars.

The result presented in **Table 2.17** on days taken planting to flowering, days taken flowering to harvesting and total crop duration show that the TC plants were earlier in flowering as well as in harvesting than the SC plants and the differences were significant in all of six cultivars. Similarly, the total crop duration of the TC plants was shorter than of SC plants and the differences were significant in all of six cultivars.

The results presented in **Table 2.18** on number of functional leaves/plant at harvesting stage reveal that the number of functional leaves (i.e. those were erect and have at least 75% of their area that is green) at harvest in tissue cultured plants in most of the cultivars and were significantly higher than the SC plants except Agniswar. In Agniswar no significant different was observed between TC and SC plants. The TC planting materials had the highest (5.83) number of functional leaves at harvest in cvs. Jahaji and Agniswar had the lowest (3.88) number of functional leaves at harvest.

Qualitative characters of skin and pulp of ripe banana are presented in **Table 2.19**. Ranginsagar had more attractive fruit skin colour than other. It produced bright yellow skin colour at ripening, while Sabari had yellow, Mehersagar and Jahaji had yellowish green in winter and green in summer, Agniswar had red and Binathuri had light yellow colour.

Flesh colour of Mehersagar, Ranginsagar, Jahaji are bright cream. Agniswar had orange yellow and Sabari had light cream. The fruits of Mehersagar, Ranginsagar, Jahahi and Agniswar produced soft textured, Sabari and Binathuri had partially soft texture. Flavour of fruits of all the cultivars was found acceptable except Mehersagar. The fruits of all the cultivars were good in taste.

Table 2.14 Mean performance for different morphological characters of micropropagated (TC) and sucker derived (SC) plants of the different banana cultivars after 4½ months of planting

Cultivars	Plant height (cm)			Base girth (cm)			Numbers of functional leaves/ plant			Length of longest leaf (cm)			Breadth of longest leaf (cm)			Numbers of sucker/ plant	
	Tissue culture derived plant	Sucker-derived plant	t-test	Tissue culture derived plant	Sucker-derived plant	t-test	Tissue culture derived plant	Sucker-derived plant	t-test	Tissue culture derived plant	Sucker-derived plant	t-test	Tissue culture derived plant	Sucker-derived plant	t-test	Tissue culture derived plant	Sucker-derived plant
Sabari	128.00 c	106.10 b	*	43.47 c	37.80 c	NS	8.19 c	5.00 b	**	158.80 b	144.20 ab	*	44.81 c	37.60 d	*	1.17 a	-
Mehersagar	115.10 d	98.32 c	*	37.52 d	29.97 de	**	10.02 ab	6.52 a	NS	152.20 c	134.10 bc	*	52.40 b	43.05 bc	NS	1.39 a	-
Ranginsagar	98.66 e	82.50 d	NS	34.30 d	27.68 e	NS	10.03 ab	6.15 a	*	143.80 d	132.10 bc	NS	51.40 b	39.84 cd	**	1.22 a	-
Jahaji	85.58 f	72.49 e	***	35.57 d	30.16 d	NS	10.40 a	6.45 a	*	128.30 e	120.70 c	NS	56.20 ab	47.11 ab	*	1.33 a	-
Agniswar	138.20 b	116.40 a	*	51.10 a	45.41 a	**	8.25 bc	5.75 ab	*	167.00 a	162.10 a	NS	51.02 b	38.98 cd	*	1.33 a	-
Binathuri	147.30 a	118.20 a	**	47.24 b	41.23 b	**	8.56 bc	4.89 b	*	172.80 a	157.20 a	**	58.47 a	49.16 a	*	1.11 a	-
LSD (5%)	5.41	6.56		3.72	2.33		1.69	1.11		6.47	19.77		4.90	4.75		0.26	-

Mean values within a column having the same letter are not significantly different according to Duncan's Multiple Range Test (DMRT) at 5% level of probability.

* = P<0.05; ** = P<0.01; *** = P<0.001; ^{NS} = not significant.

Table 2.15 Mean performance for different morphological characters of micropropagated (TC) and sucker derived (SC) plants of the six cultivars of banana at flowering stage

Cultivars	Plant height (cm)			Base girth (cm)			Numbers of functional leaves/ plant			Length of longest leaf (cm)			Breadth of longest leaf			Numbers of suckers/ plant		
	Tissue culture derived plant	Sucker-derived plant	t-test	Tissue culture derived plant	Sucker-derived plant	t-test	Tissue culture derived plant	Sucker-derived plant	t-test	Tissue culture derived plant	Sucker-derived plant	t-test	Tissue culture derived plant	Sucker-derived plant	t-test	Tissue culture derived plant	Sucker-derived plant	t-test
Sabari	330.50 c	304.70 c	*	83.70 b	75.48 b	*	12.77 ab	10.68 b	*	286.60 c	261.20 c	NS	73.76 b	61.37 b	**	7.52 cd	5.76 ab	*
Mchersagar	241.40 d	222.80 d	NS	72.48 c	61.80 c	*	12.06 b	10.26 b	**	190.60 d	166.70 d	***	73.19 b	64.27 b	*	9.34 a	6.55 a	***
Ranginsagar	184.80 e	171.80 e	**	66.36 c	56.98 c	*	12.01 b	10.07 b	*	192.80 d	157.70 de	NS	80.29 b	66.10 ab	**	7.94 bc	6.21 a	*
Jahaji	159.00 f	150.90 f	*	68.80 c	60.46 c	*	13.53 a	11.66 a	*	162.50 e	143.10 e	**	77.57 b	64.24 b	**	7.84 bc	5.85 ab	***
Agniswar	361.80 b	329.70 b	*	101.40 a	81.81 a	*	9.87 c	8.02 c	*	384.10 a	353.60 a	**	97.48 a	74.13 a	NS	8.41 b	6.69 a	**
Binathuni	422.90 a	390.40 a	*	89.64 b	75.42 b	NS	9.51 c	7.98 c	NS	304.50 b	281.40 b	NS	78.47 b	64.19 b	*	6.81 d	5.19 b	**
LSD (5%)	9.87	12.37		6.74	6.24		1.16	0.89		12.88	15.41		9.61	9.11		0.77	0.94	

Mean values within a column having the same letter are not significantly different according to Duncan's Multiple Range Test (DMRT) at 5% level of probability.

* = Significant at 5% level; ** = Significant at 1% level; *** = Significant at .1% level; NS = Non-significant.

Table 2.16 Mean performance for yield and yield contributing characters of micropropagated (TC) and sucker derived (SC) plants of the different cultivars of banana

Cultivars	Numbers of hand/ bunch			Numbers of finger/ hand			Total number finger/ bunch			Fruit length (cm)			Fruit girth (cm)		
	Tissue culture derived plant	Sucker-derived plant	t-test	Tissue culture derived plant	Sucker-derived plant	t-test	Tissue culture derived plant	Sucker-derived plant	t-test	Tissue culture derived plant	Sucker-derived plant	t-test	Tissue culture derived plant	Sucker-derived plant	t-test
Sabari	7.66 de	6.33 c	***	15.04 b	13.73 c	**	115.30 c	87.04 c	***	16.68 d	12.93 d	**	13.92 bc	11.03 c	***
Mehersagar	8.22 bc	8.33 ab	NS	16.88 a	13.52 c	*	138.90 b	112.60 b	NS	21.60 b	15.38 c	**	13.85 bc	11.17 c	*
Ranginsagar	8.89 a	8.44 a	NS	16.92 a	15.17 b	*	150.40 a	128.90 a	*	21.97 b	14.99 c	***	13.90 bc	11.37 c	**
Jahaji	8.50 b	7.66 b	*	17.33 a	16.38 a	NS	147.30 a	125.60 a	*	16.44 d	14.83 c	*	14.15 b	12.27 b	***
Agniswar	7.39 e	6.38 c	*	16.34 a	13.92 c	NS	120.60 c	88.83 c	**	18.23 c	16.25 b	*	13.69 c	11.58 bc	**
Binathuri	7.89 cd	6.66 c	**	15.29 b	15.86 ab	NS	120.70 c	105.40 b	**	25.82 a	23.29 a	*	18.12 a	16.45 a	***
LSD (5%)	0.36	0.72		1.10	1.11		8.34	10.80		0.85	0.54		0.40	0.69	

Mean values within a column having the same letter are not significantly different according to Duncan's Multiple Range Test (DMRT) at 5% level of probability.

* = Significant at 5% level; ** = Significant at 1% level; *** = Significant at .1% level; NS = Non-significant.

Table 2.17 Comparative study between micropropagated and sucker derived plants for days taken planting to flowering, flowering to harvesting and total crop duration (days) of different cultivars of banana

Cultivars	Days taken planting to flowering			Days taken flowering to harvesting			Total crop duration (days)		
	Tissue culture derived plant	Sucker-derived plant	t-test	Tissue culture derived plant	Sucker-derived plant	t-test	Tissue culture derived plant	Sucker-derived plant	t-test
Sabari	306.00 a	326.80 a	**	100.50 a	119.30 a	*	406.30 a	446.10 a	*
Mehersagar	258.70 b	278.70 c	***	84.51 b	104.80 b	*	343.20 c	383.50 b	**
Ranginsagar	259.00 b	277.70 c	***	82.36 bc	106.50 b	*	341.30 c	384.30 b	**
Jahaji	243.20 b	262.40 d	**	74.99 c	89.71 c	*	318.20 d	352.10 c	**
Agniswar	309.80 a	326.30 a	*	99.12 a	117.80 a	*	408.90 a	443.40 a	**
Binathuri	309.10 a	292.90 b	**	86.74 b	100.70 b	NS	362.50 b	393.70 b	*
LSD (5%)	43.01	8.13		8.06	9.20		12.63	12.95	

Mean values within a column having the same letter are not significantly different according to Duncan's Multiple Range Test (DMRT) at 5% level of probability.

* = Significant at 5% level; ** = Significant at 1% level; *** = Significant at .1% level;
NS = Non-significant.

Table 2.18 Mean number of functional leaves present at harvest of micropropagated and sucker derived planting materials of six banana cultivars

Cultivars	Tissue culture derived plant	Sucker-derived plant	t-test
Sabari	4.39 c	3.72 bc	NS
Mehersagar	5.17 b	3.94 b	*
Ranginsagar	4.95 b	3.94 b	*
Jahaji	5.83 a	4.67 a	**
Agniswar	3.88 d	3.33 c	NS
Binathuri	3.89 d	3.39 c	**
LSD (5%)	0.48	0.42	

Mean values within a column having the same letter are not significantly different according to Duncan's Multiple Range Test (DMRT) at 5% level of probability.

* = Significant at 5% level; ** = Significant at 1% level; *** = Significant at .1% level;

NS = Non-significant.

Table 2.19 Some qualitative characters of ripe fruits of six banana cultivars

Cultivars	Skin colour	Flesh colour	Softness	Flavour	Taste
Sabari	Ivory yellow	Light cream	Partially soft	Acceptable	Very good
Mehersagar	Yellowish green (in winter) Green (in summer)	Bright cream	Soft	Average	Good
Ranginsagar	Bright yellow	Bright cream	Soft	Pleasant	Good
Jahaji	Yellowish green (in winter) Green (in summer)	Bright cream	Soft	Acceptable	Good
Agniswar	Red	Orange yellow	Soft	Acceptable	Good
Binathuri	Light yellow	Light cream	Partially soft	Acceptable	Good

Plate 8

Photographs showing different stages of field establishment of micropropagated plants.

Figures:

A. Rooted plantlets ready for transfer to pot.

B. Potted plants ready for transfer to field.

C. Tissue culture derived banana orchard 7 month after transplantation.

Plate 8

2.5. DISCUSSION

2.5.1. Micropropagation

Banana is one of the most popular fruits of the world which has been under cultivation since time immemorial. The genetic system of *Musa* is extremely complicated. Sterility, interspecific hybrid constitutions, heterogygosity and polyploidy are common in most of the clones. So these can be propagated only by vegetative methods. In Bangladesh bananas are severely affected by diseases such as Bunchy top virus, Cucumber mosaic virus, Panama wilt, Sigatoka leaf spot diseases and Nematodes. Propagation *in vitro* helps in obtaining propagules free from these diseases. The complexity of *Musa* genetics illustrates the need for a more sophisticated system to support conventional breeding programmes. There is a great potential for biotechnology in this crop (Parsely and De Langhe 1987). Shoot-tip culture is a basic technique for *Musa* propagation, conservation and movement of germplasm (De Langhe 1984, Williams 1987, Litz and Jaiswal 1991). In the present investigation attempt was made to make rapid clonal propagation of disease free plantlets of local cultivars of banana through *in vitro* shoot-tip and somatic embryogenesis from male flower bud.

The advantages of *in vitro* micropropagation include higher rates of multiplication, production of clean or disease-free planting material and the small amount of space required to multiply large number of plants. These advantages of micropropagation are particularly relevant to vegetative propagated crops such as banana and plantain (*Musa* spp. L.) planting material derived from micropropagation performs equal to or superior to conventional material (Smith and Drew 1990a, Vuylsteke 1998). Micropropagated plant establish faster, grow more vigorously and have a shorter and more uniform production cycle and yield higher than conventional propagules (Drew and Smith 1990, Robinson *et al.* 1993, Vuylsteke and Ouitz 1996). Hence, *in vitro* shoot-tip culture is a vital technical adjunct to any international *Musa* breeding program for multiplication and dissemination of newly bred clones. Thus development of a shoot-tip culture technique for micropropagation of *Musa* has received considerable attention during the 15 years and since has become well established (Cronauer and Krikorian 1984, Hwang *et al.* 1984, Jarret *et al.* 1985, Smith and Drew 1990, Vuylsteke 1989,

Vuylsteke and De Langhe 1985), shoot-tip culture is simple, easy and applicable to a wide range *Musa* genotypes (Vuylsteke 1989).

Shoot cultures of banana start conventionally from any plant part that contains a shoot meristem i.e. the parental pseudomstem, small suckers, peepers and lateral buds (Vuylsteke 1989). The apex of the inflorescence and axillary flower buds are also suitable explants for tissue culture initiation. Overall, it is important to select explant material from preferably mature individuals whose response to environmental factors is known. Shoot-tip culture is simple, easy and applicable to a wide range *Musa* genotypes (Vuylsteke 1989). A shoot-tip of about 3×5 mm, consisting of the apical dome covered with several leaf primodia and thin layer of corm tissue, is aseptically dissected larger explants have the merit of consisting of a shoot apex bearing more lateral buds (Lee and Hwang 1993) which rapidly develop in to shoots.

The optimal size of the explant depends on the purpose. For rapid multiplication, a relatively larger explant (3-10 mm) is desirable despite its higher susceptibility to blackening and contamination when virus or bacteria elimination is needed, meristem-tip culture is the preferred option. Smith and Murashige (1970) accomplished the first true meristem culture of an isolated angiosperm meristem in to a complete plant. Before that time it was believed that the isolated shoot apical meristem of an angiosperm could not direct its own development but rather, relied on subjacent primodial leaves and stems and tissue (Ball 1946, 1960). Generally, to establish a disease free plant one can culture the apical dome plus two or four subjacent primodial leaves. In banana micropropagation meristem cultures have the disadvantage that they may have a higher mortality rate and an initial slower grown. Meristem cultures have the disadvantage that they may have a higher mortality rate and an initial slower growth. (Strosse *et al.* 2004).

In vitro multiplication by shoot-tip culture having emphasis on indexing and monitoring has great potential for producing pathogen-free planting materials in large quantity. Use of disease-free planting materials will greatly reduce the inoculum sources in the field and they will held to rehabilitate the infected areas. Though no genetic improvement is expected from shoot-tip culture, planting materials obtain

through shoot-tip culture have proved better over traditional planting materials under proper management conditions. It has been observed in a banana plantation that some plants always produce very high bunch masses and many times they are earlier in harvest. These plants may be utilized for *in vitro* multiplication.

For the primary establishment of *in vitro* culture, surface sterilization of the explants (shoot-tips of banana) was essential because of presence of microbial contaminants remain attached to the aerial surface. Various methods have been described to initiate bananas in to aseptic culture (Cronauer and Krikorian 1984, Hwang *et al.* 1984, Dore Swamy *et al.* 1983, Vuylsteke 1989, Wong 1986), however there is still a lack of published data on the relative success and efficiency of the various methods using field collected banana suckers of varying age and condition.

According to adaptation of Cronauer and Krikorians (1984) methods that involved excision of the meristem followed by a single sterilization step of 0.0525% NaOCl with Tween 80 for 5 min and subsequently rinsing 4 times in sterile distilled water.

An adaptation of Wong's (1986) methods that involved a single sterilization step followed by rapid excision to produce a block of tissue (5 mm × 8 mm) containing the apical tip, ensheathing leaf bases and 2-3 mm of basal corm. This methods was similar to that of Drew *et al.* (1989), which also involved surface sterilization for 15 min in 1% NaOCl with Tween 80 and removal of bleached tissue, but also included rinsing of the explant in sterile distilled water before removing tissue. Modification of a double sterilisation technique from Novak *et al.* (1986). Instead of dissecting the apical meristem, a block of tissue (20 mm × 40 mm) was rapidly excised, sterilised in 3.5% NaOCl with Tween 80 for 15 min and bleached tissue removed leaving a block (15 mm × 30 mm) with intact apex, leaf primordia and corm that was resterilised as before for 5 min. The bleached tissue was again removed, without rinsing, to leave a block of tissue (5 mm × 8 mm).

A simple, rapid method where a block of tissue containing the growing point (15 mm × 40 mm) was sterilised in 3.5% NaOCl with Tween 80 for 20 min and rinsed in sterile distilled water.

For sterilization of explants, many scientists used different types of sterilizing agents with different concentrations. There have been many reports on using calcium hypochlorite (Morel and Martin 1955) or sodium hypochlorite (Jones 1986) for surface sterilization of explants. There are also many other reports on using $HgCl_2$ for surface sterilization. It is used as on of the surface sterilant and considered as a potent surface sterilizing agents but its residual inhibitory effect is also greater than the other sterilizing agent commonly used plant tissue culture (Bhojwani and Razdan 1983, Torres 1988). In the present investigation maximum (100%) contamination free cultures were obtained when the explants were treated for 15 min with 0.1% $HgCl_2$. Therefore, 15 min duration was considered as optimum for surface sterilization of shoot-tip explants of banana with 0.1% $HgCl_2$ which was also reported by Hadiuzzaman *et al.* (1997) and Moshiur (1999).

For banana micropropagation, MS-based media (Murashige and Skoog 1962) are widely adopted. Banana tissue culture often suffer from excessive blacking caused by oxidation of polyphenolic compounds released from wounded tissues. These undesirable exudates form a barrier round the tissue, preventing nutrient uptake and hindering growth. The problem of blackening of the cultures can be overcome by a combined effect of the addition of ascorbic acid and frequent transfer (every two weeks) of the tissue to fresh medium. It is also observed that the degree of blackening of the culture medium is considerably reduced after a few subcultures. Therefore, during the first 4-6 weeks, fresh shoot-tips are transfer to new medium every 1-2 weeks. Alternatively, freshly initiated cultures can be kept in complete darkness for one week. Antioxidents, such as ascorbic acid or citric acid in concentrations ranging from 10-150 mg/l, charcoal, pvp solution were used to the growth medium to reduce blacking. Instead of that in the present investigation, it was observed that 3-4 initial subculture were sufficient to bring the secretion to a negligible minimum.

The primary goal of plant tissue culture research is crop improvement. Regeneration of shoots from shoot-tips have the potential to give rise to whole plants by organised growth and development. The ability to regenerate plants from excised shoot-tips is well documented. The technique of splitting shoot-tips is well induce multiple shoot formation has already been used by many other authors (Wong 1986, Cronauer and

Krikorian 1984, Dore Swamy *et al.* 1983, Novak *et al.* 1986, Damasco *et al.* 1985, De Gauzman *et al.* 1976).

Previous reports (Ma and Shii 1972), Dore Swamy *et al.* (1983) on the importance of apical dominance in shoot production from explants of banana are conflicting. Ma and Shii (1972) reported that the destruction of apical dominance by removing the apical domes, was essential for the production of multiple shoot-initials in cv. Cavendish. Dore Swamy *et al.* (1983) later reported that multiple shoot-initials were produced in the presence of apical domes in cv. Robusta. The findings reported here for a range of cultivars support those of Dore Swamy *et al.* (1983) in that the removal of the apical dome is not essential for multiple shoot initiation. Shoot-tip explants with intact apical domes formed both single apical and multiple lateral shoot-initials. However, explants without apical domes formed only multiple lateral shoots. With or without an apical dome, BA stimulated multiple shoot development, as has been shown for other plants in a comprehensive review by Phillips (1975). Cytokinins are involved in the regulation of shoot formation such as in axillary bud development by antogonizing apical dominance (Wickson and Thimann 1958) or in adventive organogenesis (Skoog and Miller 1957).

The finding that banana explants with apical domes have a higher rate of survival has not been observed before, though this has been reported in other crops (Hu and Wang 1983). Explants without apical domes offer no particular advantage. The retention of apical domes in explants is therefore recommended for banana tissue culture. Proliferating meristems from shoot-tip explants may originate from either axillary or advantitious buds (Novak *et al.* 1990).

Ma and Shii (1972, 1974) induced advantitious bud formation and development of buds into plantlets in decapitated banana shoot-tips. Berg and Bustamante (1974) cultured excised meristems of banana *in vitro* up to stage suitable for transplanting to the soil. De Guzman and Co-workers (1980) regenerated buds from irradiated and unirradiated shoot-tips. Vessey and Rivera (1981) used the meristem culture technique for Cavendish banana.

Ma and Shii (1972) have reported that decapitation was necessary for releasing axillary buds from apical dominance. However, in the present investigation decapitation was not necessary.

The ability to regenerate plants from the excised shoot-tips is well documented (Cutter 1965). Shoot-tips will regenerate when cut into 4 to more equal segments (Ball 1952, Sussex 1952). Similar responses obtained with banana suggest that this technique could be used for rapid multiplication of clones and hybrids with desirable qualities.

Apices isolated aseptically cultured shoots respond the quickest, while apices isolated from field-grown plants respond somewhat slower. This variation inresponse may indicate that the production of cytokinin the roots helps to overcome the powerful apical dominance of the banana shoot-tip and that the addition of exogenous cytokinins such as BAP is not always sufficient in establishing a multiplying shoot culture. Nevertheless, we still wish to emphasize that once cultures have been established, a potentially unlimited number of plantlets can be produced.

It has been observed that banana multiplication rate is genotype dependent (Malik *et al.* 2000, Muhammad *et al.* 2000), and highly variable behavior has been observed among clones of the same banana genotype cultured *in vitro* (Israeli *et al.* 1996, Mendes *et al.* 1996). The morphogenetic response of the explants seem to be influenced on one hand by the concentration of BA used in the medium and on the other hand by the genomic configuration of the cultivars. It was also reported that the B genome play a role in the higher proliferative growth of meristematic buds but there seems to be no relation to the apical dominance (Vuylsteke and De Langhe 1985). The observation that the ABB cv. did not show higher proliferation rates than the AAB cvs, although the former has two B genomes can be explained by the fact that the presence of two identical genomes does not mean that a characteristic will be expressed in double intensity. However, the morphogenic response is controlled by the culture medium and is strongly influenced by genotype. For this type of proliferation, bulb-like structures bear numerous minute meristem (Banerjee and De Langhe 1985).

Rapid multiplication *in vitro* was achieved using a BA concentration ten times than in the regeneration medium. Highly proliferative growth of meristem tips was observed, especially in the Acuminata × Bulbisiana hybrids, AAB and ABB genomes. It appeared that the B genome may play a role in the achievement of higher proliferation rates, but there seem to be no relation to apical dominance. The same proliferative growth was obtain in the acuminata cultivars, AA and AAA genomes, by further increasing the cytokinin concentration in the medium (Vuylsteke and De Langhe 1985).

In banana BA is the preferred cytokinin and is usually added in a concentration of 0.1 – 20 mg/l (Banerjee and De Langhe 1985). Higher concentrations of the cytokinin BA tend to have an adverse effect on the multiplication rate and morphology of the culture and should therefore be avoided.

The rate of multiplication depends both on the cytokinin concentration and genotype. In general shoot-tips of cultivars having only A genomes produce 2-4 new shoots, where as cultivars having one or two B genomes produce a cluster of many shoots and buds at each subculture cycle. Approximately 6 – 12 weeks after culture initiation, depending on the initial explant size, new axillary and adventitious shoots may arise directly from the shoot-tip explant. Cluster can be separated trimmed and repeatedly subculture at 4 – 6 week intervals.

For rapid propagation, shoot-regeneration and multiplication is necessary. Workers used different media supplemented with various combinations and concentrations of vitamins and hormones for shoot-tip culture of banana. Although the techniques differ in many ways, the induction of shoot proliferation in cytokinin rich media is common to all procedures (Novak *et al.* 1986).

Cytokinins stimulate the initiation and growth of excised shoots *in vitro*. Consequently cytokinins are usually added to tissue culture media to stimulate auxillary or adventitious shoot production. The synthetic cytokinin BA is the most frequently used compound in commercial micropropagation (Thomas and Blakesly 1987). In *Gerbera* shoot cultures, Blakesly and Lenton (1987) demonstrated that all BA added to the medium was taken up with in 24 days and rapidly metabolised.

Using shoot-tip explants, Wong (1986) successfully obtained proliferating shoot cultures of 22 *Musa* cultivars on media containing BA. Some Rhododendron species have also been cultured media containing BA although 2-isopentenyladenine is more often used (Norton and Norton 1985). BA at high concentration (10 – 15 mg/l) was inhibitory and induced severe leaf necrosis three weeks after treatment. A poor proliferation rate was observed with KIN on 22 cultivars of *Musa* reported by Wong 1986. But KIN stimulated shoot elongation and induced vigorous root growth. An intermediate shoot elongation stage is not necessary in banana tissue culture. No supplementary auxin is required for root growth (Wong 1986).

Shoot did not proliferate if cytokinin was omitted from the medium. The cytokinin BA was found consistently more effective than KIN for shoot proliferation even though the extent of the response was variable with cultivars. Shoot multiplication was reduced in most cultivars at the higher cytokinin levels (10 to 15 mg/l), except for cv. Mysore which proliferated best at 15 mg/l BA (Wong 1986). Kinetin supplemented media also promoted shoot proliferation from sub-cultured shoots but was not as effective as BA, kinetin, however, enhanced root formation in cultured shoots (Olivia *et al.* 1984). In the experiment with Zeatin (10 μM) as the cytokinin addition, lower proliferation rates were obtain than with BA (10μM). Because of this and its high cost, Zeatin is no longer considered (Vuylsteke and De Langhe 1985).

In the present investigation, different concentrations of cytokinin alone, in combination with cytokinins or auxins were used to see the response of shoot-tip explants obtained from field-grown plants and *in vitro* grown plantlets.

In case of single shoot regeneration two types of cytokinin (BAP, KIN) in different concentrations were used. The explants were cultured in semisolid MS media. Among all the different cytokinin the best results were obtained for six cultivars (Sabari, Mehersagar, Ranginsagar, Jahaji, Agniswar and Binathuri) in BAP. Cultivars varied widely in their response to cytokinins. BAP was found superior to kinetins (Cronauer and Krikorian 1984a, Damasco and Barba 1985, Wong 1986, Zomora *et al.* 1986, Pandey *et al.* 1993). Therefore the multiplication rate under *in vitro* condition is a function of BAP concentration. In banana BA is the preferred cytokinin

and is usually added in a concentration of 0.1-20.0 mg/l (Banerjee and De Langhe 1985). Higher concentrations of the cytokinin BA tend to have an adverse effect on the multiplication rate and morphology of the culture and should therefore be avoided. In the experiment with Zeatin (10 μM) as the cytokinin addition, lower proliferation rates were obtained with BA (10 μM). Because of this and its high cost, Zeatin is not longer considered (Vuylsteke and De Langhe 1985).

In the present investigation cvs. Sabari, Mehersagar, Ranginsagar, Jahaji, Agniswar and Binathuri showed the highest percentage of shoot regeneration, maximum number of shoots per culture, highest mean length of shoot in MS medium supplemented with 5.0 mg/l BAP. Cronauer and Krikorian (1984a,b) were also able to successfully established rapidly multiplying cultures of two dessert and two plantain clones from isolated shoot-tips on a modified MS medium supplemented with 5.0 mg/l BAP. The present findings were different from Cronauer and Krikorian (1984) because they did vertical incisions to the shoot through the apex. This finding was also differed from Vessey and River (1981) because they used meristem with 7 – 12 times vertical incisions. Rabbani *et al.* (1996) reported the effect of BAP and IBA on micropropagation of different banana cultivars viz. Amritsagar, Sabari, Anajee and Mehersagar and observed that BAP at the rate of 5.0 mg/l produced the highest number of shoots in Amritsagar and Mehersagar. In Amritsagar banana (AAA) meristem tip generated highest number of shoots on MS medium supplemented with 30 μM BAP and it was reported by Khanam *et al.* (1996).

Different concentrations and combinations of BAP + KIN were used for single shoot proliferation. At 5.0 mg/l BAP + 5.0 mg/l KIN in MS media was best for single shoot proliferation in Sabari. But in case of Mehersagar, Ranginsagar and Jahaji MS medium + 0.5 mg/l BAP and 0.5 mg/l KIN was best for single shoot proliferation. In case of Agniswar and Binathuri 8 mg/l BAP + 1 mg/l KIN and 3.5 mg/l BAP + 1 mg/l KIN were best for single shoot proliferation. In the treatment where KIN was used alone or the BAP/KN ratio was law, roots formed and the average shoot length increased.

Following the first report of the *in vitro* production of meristem derived banana plants (Ma and Shii 1972, 1974) shoot-tip and meristem culture is now widely used in banana production. Abdulla *et al.* (1997) noticed that meristem tips with 2 pairs of leaf primodia from dessert banana clones GNGOA, SH 3362, Williams Highgate and Basri rapidly proliferated shoots on MS medium containing 20 µM BA. They also observed good tillering in all the genotypes. A BAP concentrations range of 8.9 – 22.2 µM is recommended for *Musa in vitro* propagation (Crouch *et al.* 1998). The use of rates beyond this range induces increased rates of somaclonal variants (Trijillo and Garcia 1996).

Alvarez *et al.* (1982) reported the culture of plantain shoot-tips in MS supplemented with BAP and IAA. Bower (1982) used MS media supplemented with 5 mg/l BAP + 2 mg/l IAA for shoot-tip culture and produced multiple shoots in banana. Sandra *et al.* (1983) claimed that semi-solid medium was most suitable for shoot multiplication. Ying Fang Sun (1985) reported production of *Musa* spp. plantlets successfully through tissue culture method. They used MS salt supplemented with BAP 5 ppm + IAA 1 ppm.

Begum (1983) used different combinations and concentrations of BAP with KIN for shoot multiplication in modified MS medium. She also reported that modified MS solid medium was the best for the shoot multiplication of Sagar. Rajeevan and Pandey (1986) observed that shoot-tip explants induced to form multiple shoots when they were successfully cultured in media with KIN and NAA and with BAP and NAA. In the present experiment BAP + KIN + IAA in different concentrations and combinations with MS media were used for shoot multiplication. At 5.0 mg/l BAP + 2.0 mg/l KIN + 2.0 mg/l IAA; cvs. Mehersagr, Ranginsagar, Sabari and Jahaji showed the beeter results. In cv. Agniswar, best performance was found in MS + 8.0 mg/l BAP + 1.0 mg/l KIN + 1.0 mg/l IAA. In case of Binathuri 3.5 mg/l BAP + 1.0 mg/l KIN + 1.0 mg/l IAA showed the better experiment are closely similar to the findings of Habiba *et al.* (2002) who reported that the best medium for shoot multiplication was MS + 5.0 mg/l BAP + 2.0 mg/l KIN + 2.0 mg/l NAA.

Atique Akbar *et al.* (2003) reported that among the different combinations of BAP, KIN and IAA, the best medium for shoot induction and multiplication was 0.5 mg/l BAP + 0.5 mg/l KIN + 0.5 mg/l IAA.

In the present experiments from *in vitro* grown shoot-tips six banana cultivars were inoculated in MS medium with different combinations and concentrations of BAP, IAA and IBA for the production of multiple shoots. At 4.0 mg/l BAP + 2.0 mg/l IAA + 2.0 mg/l IBA in MS medium was the best for multiple shoot production in case of all cultivars except cvs. Agniswar and Binathuri. Similar experiment was conducted by Habiba *et al.* (2002). They also obtained better result in the some hormonal supplements in MS medium. In Agniswar, 8.0 mg/l BAP + 1.0 mg/l IAA + 1.0 mg/l IBA and in Binathuri, 4.0 mg/l BAP + 1.0 mg/l IAA + 1.0 mg/l IBA were found to the best for the multiple shoot production. This findings is partially agree with the finding of Vidhya (2002) where he observed multiple shoots were produced in MS medium supplemented with 8 mg/l BA + 0.1 mg/l NAA cv. Red banana.

Dore Swamy *et al.* (1982) used apical shoots and axiliary bud for the production of banana plantlets and were successful in regenerating shoots in MS medium supplemented with CW (15% V/V) + BA (5 .0 and 10.0 mg/l) + IBA (5.0 mg/l). But in the present study 13% coconut water (CW) + 0.5 mg/l BAP + 0.5 mg/l KIN were used and the successful multiple shoots have been obtained in Mehersagar, Ranginsagar and Jahaji. In Sabari 5.0 mg/l BAP + 5.0 mg/l KIN + 13% coconut water were found to the best for multiple shoot production. In case of Agniswar and Binathuri 8.0 mg/l BAP + 1.0 mg/l KIN + 13% CW and 3.5 mg/l BAP + 0.5 mg/l KIN + 13% CW were showed best performance respectively. Begum (1983) used BAP (0.5 mg/l) + KIN (0.5 mg/l) instead of coconut water for shoot regenration in banana. Roots were also obtained in the same medium.

Hadiuzzaman *et al.* (1997) reported that at specific concentration (5.0 mg/l) of KIN, the cluster of shoots initiating on MS + 2.0 mg/l BAP + 2.0 mg/l IAA instead of giving normal plantlets were transformed in tuber like structures. Sharma *et al.* (1997) observed, KIN supplemented medium showed formation of bulbous structure without indication of distinctive shoot buds. It was also reported that a mass of tiny adventitious buds developed *de novo* on white bulbous tissue. This tissue can not be

called a callus since there was no trace of disorganized cell proliferation and differentiation as present in explanted tissue of banana fruit (Mohan Ram and Steward 1964). Rabbani *et al.* (1996) also illustrated that 2ip have the more tendency of in producing ball like structure. Similar results were also obtained by Habib (1994), who observed that some ball like structures formed from the base of the shoot during shoot multiplication. Another evidence was showed by Arinotwe *et al.* (2000) on bulbous structure formation. They reported that at 16.8 µM equimolar concentration shoot proliferation on TDZ supplemented media was manifested by the appearance of numerous fleshy bulbous structures each of them producing several stunted tiny adventitious buds on their surfaces. In the present study multiplication of shoots in five cultivars of banana were being carried out by repeated subcultures addition of higher concentration of kinetin (5 mg/l) in the multiplication medium. The cluster of shoots from MS + 5 mg/l BAP + 2 mg/l IAA showed the more tendency in the induction of hard meristematic bulbous structure. This appearance of meristematic bulbous structure was a new addition in the research of *in vitro* banana propagation. From this bulbous structure multiple shooting induced by implanting the bulbous explant on the shoot multiplication medium. Similar results obtained by Hadiuzzaman *et al.* (1997). Different author, obtained different results which might be due to the genotypic difference as well as source material.

In the present investigation it was conducted on production of multiple shoots by six cultivars (Sabari, Mehersagar, Ranginsagar, Jahaji, Agniswar and Binathuri) by the application of increased subculture cycle. In this experiment it was observed that multiple shoot formation rate varied with the cultivars and also increase gradually with increase in the number of subculture cycle. It was also noticed that maximum number of multiple shoots were produced in the 8^{th} sub-culture and then the multiple shoots production rate declined eventually with the increases in the number of subcultures. Similar results was also observed by Jambhale *et al.* (2001). Banerjee and De Longhe (1985) noticed the number of subculturing seems to have an influence on the proliferation rate. They also observed that most of the cultivars revealed highest proliferation between the 3^{rd} and 6^{th} subculture. In order to minimise the occurrence of somaclonal variation in banana micropropagation, a maximum of six subcultures has been suggested (Reuveni and Israeli 1990,

Muhammad *et al.* 2000). There is no evidence that growth regulators routinely used in tissue culture directly affect the rate of variation, but it has been found that the rate of somaclonal variation is positively related to the generation number. For 'Williams' (AAA) *in vitro* plants obtain after one and five subculture cycles, dwarfism and leaf-off-types counted for 3.7% and 0.7% respectively and increased to 6.1% and 1.9% respectively after five *in vitro* cycles (Reuveni and Israeli 1990). It is therefore recommended that the number of subculture cycles should be limited to ten or that the number of plants produced from a primary explant should be limited to no more than 1000 (Smith 1988).

The central role of auxin is not only to increase the percentage of shoots with roots but also to increase the number of roots per plantlets (Hartmann and Kester 1975). However, some studies indicate that root induction is inhibited by auxin in a wide range of concentrations in media (Masrgerata and Inger 1981), and that the root promoting effects of auxin are based on some circumstances evidence (Hart and Carlson 1967).

Rooting in micropropagation of *Musa* has been induced both in auxin supplemented and auxin free media. For example, NAA and IAA have been used as root-inducing hormones *in vitro* (Bulakrishnamurthy and Rangnasamy 1988, Kshanika and Niranjali 1997). On the contrary, Subramanya and Schwandes (1984), Cronauer-Mitra and Krikorian (1987), Novak (1992) and Bart *et al.* (1993) observed rooting in all *Musa* shoots cultured *in vitro* on auxin-free medium. Auxin have been reported to shorten the time required far the regeneration of roots as well as increase in the number of roots per culture in *Musa* shoots (Hiratsuka *et al.* 1989).

Cronauer and Krikorian (1984) found that NAA, IAA and IBA are all equally effective in inducing root formation and the addition of CW to the rooting medium reduces or antagonizes the auxins activity there by explaining Berge and Bustamante (1974) slow rooting response. Presumably, this is due to the cytokinins naturally present in CW (Steward and Krikorian 1971). Omitting CW from the culture medium or the addition of small amounts of AC (approx. 0.025 per cent) greatly enhanced root formation.

The root formation was observed as early as 5 – 7 days after culturing on root induction medium which is in conformity with Cronauer and Krikorian (1984a) and Bhaskar *et al.* (1993). Satisfactory rooting was observed in two weeks of culture on half MS semi-solid medium supplemented with either IAA or IBA at 1 mg/l. The small amount of activated charcoal (i.e. 0.025 per cent) greatly enhanced root formation (Cronauer and Krikorian 1984a). Khan *et al.* (2001) were cultured the differentiated shoot-tip explants in half strength MS supplemented with IBA (1 mg/l IBA for rooting). Using auxins NAA, IBA or IAA at 1 mg/l with low level of activated charcoal (0.025%) Cronauer and Krikorian (1984) could obtain rooted plantlets. Roots generally appeared within 5 – 7 days.

For some genotypes (*Musa* spp. ABB and BB group) that produce compact proliferating masses of buds, activated charcoal (0.1 – 0.25%) is added to the regeneration/rooting medium to enhance shoot elongation and rooting (Strosse *et al.* 2004).

Before transplanting from the artificial heteromorphic environment of the test tube to automorphic free living existence and into their ultimate location, these regenerated shoots need root initiation. Each of the three auxins IAA, IBA, NAA promoted abundant root formation (Cronauer and Krikorian 1983). The root initiation was noticed invariably in all the treatments. However, the response to different treatments differed markedly with regard to time taken to initiate roots, number of roots produced and length of root.

Early workers reported a number of ways of root formation. Using auxins NAA, IBA, IAA of 1 mg/l with low levels of activated charcoal (0.25-2.5 gm^{-1}) Cronauer and Krikorian (1984) obtained rooted plantlets. Addition of activated charcoal increased the average root length but not root number. Wong (1986) used indole butyric acid (IBA) for induction of root.

Lameria *et al.* (1990) used half-strength MS medium with IBA for root formation. Novak *et al.* (1986) and De Langhe *et al.* (1985) also used ½MS and IBA. Banerjee *et al.* (1985) found that regenerated shoots were rooted subsequently in MS solid medium with half strength of Macro salts + 02 mg/l IBA in banana and plantain. Begum (1983) found roots in plantain in solid ½MS with Thiamin 1 mg^{-1} + 1 mg^{-1}

IBA. Ghose (1993) observed good rooting within a month treated with IBA. Hauque (1986) used MMS2+2 mg^{-1} IBA for root induction. Berg and Bustamante (1974) and De Guzman (1975) used 1 mg^{-1} NAA for rooting in desert banana. Balakrishnamurthy and Sree Rangasamy (1988) used the auxins IAA, IBA and NAA separately each at does of 1.0 and 2.5 mg/l and supplemented with coconut water or without coconut water. They also used activated charcoal at tow concentrations viz. 0.025 and 0.25 percent. A large number of roots were produced in NAA at 2.5 mg/l. With IBA the shoots produced thicker roots. It was found that NAA, IAA and IBA are all equally effective in inducing root formation and the addition of CW to the rooting medium reduces or antagonizes the auxin's activity thereby explaining Berg and Bustamante (1974).

In the present study different experiments were conducted with MS basal medium supplemented with different type of auxins. In all the treatments with auxins, root initiation was observed within 6-8 days. Among different auxins used in this study IBA was found to be the best for root induction. The highest percentage of shoots induced roots was observed in half strength MS media supplemented with the auxin IBA at 1.0 mg/l and 1.5 mg/l concentration. The highest number and growth of roots were recorded in 1.0 mg/l IBA in case of all three varieties. Each cultivar also showed the highest percentage (100%) of root induction in 1.0 mg/l NAA and 2.0 mg/l IAA. In the present study activated charcoal were also used, average root length was higher in MS media supplemented with IBA.

When a plantlet produced sufficient number of roots, the plantlet needed to be transplanted into polythene bag and finally to the condition through gradual acclimatization. At the primary stage of this investigation to transplant *in vitro* grown plantlets was a difficult task. Rooted plantlets in 3-5 cm tall with 5-7 well-developed leaves were brought out of the culture vessels. Their roots were washed thoroughly by rapid running tap water to remove the sticky agar and to prevent unwanted fungal or bacterial growth in the roots. These plantlets that had been transferred on to the polythene bags soil or small potted soil.

Cronauer and Krikorian (1984) reported that rooted shoots had been potted in a soil vermiculite (1:1) mixture in small plastic pots; placed in a misting bed for 7-10 days

and then transferred to normal greenhouse conditions. Hamilton (1965) reported that small pots were placed in a humidity chamber containing dampened vermiculite. After 18 days plantlets were transferred to pots containing a mixture of 1:2:1 of sand soil and vermiculite and were placed in shade.

In the present investigation for the first 8-10 days of transfer, potted plants were maintained under a polythene tent for providing the conditions of high humidity and sufficient light. The polythene cover was being removed periodically and progressively whenever leaves appeared water soaked. After 5-6 weeks when the transplanted plants grew up to 6-8 inches long those were transferred to the field where they grew under conditions of natural environment. Being the delicate nature of *in vitro* regenerated plantlets, special arrangement such as controlled greenhouse condition use of soil free potting like vermiculite, peat plugs and application of fungicides are to be needed for easy and successful acclimatization of plantlets.

In the field condition more than 80% of the transplanted plants survived in this way and resumed growth. *In vitro* grown plantlets of Sabari, Mehersagar, Ranginsagar, Jahaji, Agniswar and Binathuri were showing very good performance in the field condition. From the foregoing discussion it is evident that shoot-tip culture techniques may provide an important and useful tool for the production of healthy plants for *Musa* spp. Since the rate of growth of these *in vitro* grown plantlets was competing with the conventional suckers it can be concluded that there is a bright application to this present piece of work to commercial exploitation.

2.5.2. **Field Evaluation**

Micropropagation of banana using *in vitro* culture had been developed by several workers (De Guzman *et al.* 1980, Vessey and Rivera 1981, Damasco and Barba 1984, Cronauer and Krikorian 1984, Hwang *et al.* 1984 and Novak *et al.* 1986), which has the unique advantage of rapid multiplication, uniformity of planting material, availability of more number of plants in short time, disease free plants and also possibility of non-seasonal production of plants over other propagation methods, when compared with bananas propagated using conventional planting material (i.e., Suckers), micropropagated banana plants were capable of performing equally or better (Hwang *et al.* 1984, Smith and Drew 1990). In general, micropropagated

banana plants established more quickly, grew more vigorously, were taller, had a shorter and more uniform production period, and produced higher yields than conventional propagules (Daniells 1988, Drew and Smith 1990, Hwang *et al.* 1984, Israeli *et al.* 1988, Robinson *et al.* 1993, Zamora *et al.* 1989). Hwang *et al.* (1984) found that plantlets derived from adventitious buds coming from the decapitated shoot apex established well under field conditions. Gupta (1986) observed that meristem-derived plants grew faster and had shorter harvesting period from 3 months to 1.5 months due to uniform growth. In the Philippines, De Guzman *et al.* (1980) reported that micropropagated plants of 'Lakatan' were found to have similar characteristics in terms of vegetative growth, flowering, suckering and fruiting under field conditions. Damasco and Barba (1984) also found that 'Saba' plants derived from *in vitro* grew normally under field conditions. Moreover, Zamora *et al.* (1989) found that micropropagated plants of 'Lakatan', 'Bangulan' and 'Saba' grew faster had higher yield and early to sucker and flower. At maturity, these were similar in horticultural characteristics with those coming from suckers.

In Israel, Israeli *et al.* (1988) found that with spring planting conventional plants flowered earlier were taller and produced larger bunches than tissue culture (TC) plants. From summer planting, the opposite occurred, with TC plants superior to conventional (CON) plants in all respects. There is evidence that TC plants are larger than CON plants in the first crop cycle, according to work in Israel (Israeli *et al.*, 1986) and Australia (Daniells 1988, Drew and Smith 1990). However, in Costa Rica Arias and Valverde (1987) found that CON plants were larger, and in Taiwan, Hwang *et al.* (1984) found no difference.

In terms of yield potential there is wide spread agreement that TC plants produce larger bunches than CON plants in the first crop cycle. These increases were in the order of 2% (Hwang *et al.* 1984); 5% (Arias and Valverde 1987); 7% (Daniells 1988); 10% (the summer planting of Israeli *et al.* 1988) and 17% (Drew and Smith 1990). Israeli *et al.* (1988) found a TC yield advantage of 12% from a summer planting, but they found a yield increase of 22% in favour of CON, from a spring

planting. In a multiple planting date trial in South Africa, Robinson and Fraser (1992) found no such seasonal interaction with planting materials.

Eckstein and Robinson (1993) demonstrated that TC plants had a higher photosinthetic rate, dry-matter assimilation rate and root growth rate, than CON plants and that this persisted for several months after planting. Further evidence that TC plants are larger than CON plants in the first cycle, was shown by Israeli *et al.* (1986), Daniells (1988) and Drew and Smith (1990).

It is interesting to note that Johns (unpublished data) found that tissue cultured Cavendish bananas of cultivar 'Williams' (AAA group) produced more suckers and were earlier to flower than sucker-derived plans. Daniells (1988) reported taller plants with larger bunches from tissue cultured 'Williams' although bunch emergence was about 3 weeks later. Reuveni *et al.* (1985) found no differences between sucker and tissue culture derived plants of 'William'. These studies indicate some of the difficulties in comparing different form of planting material unless the interactions outlined previously are examined and understood.

Tissue-cultured plants did produce a significantly higher number of sucker up to 8 months after planting, possibly resulting from a carryover effect of BAP in the multiplication medium (Drew and Smith 1990). However, from 8 months to harvest, the rate of sucker production was the same for all plants, and subsequently, conventional planting material produced significantly higher number of suckers. A disadvantage of tissue cultured plants, as the stool matured and development, was their tendency to produce suckers above the soil surface. In subsequent field trials the tissue cultured plants were buried in holes or furrows and back-filled so that the lower leaf bases were covered, and sucker development then proceeded normally.

In 'Cardaba' tissue culture derived planting materials coupled with recommended practices tend to produce sucker earlier ranging from 5-25 days compared to the other treatment. On the other hand no differences were observed among the various treatment in 'Latundan' and 'Lacatan' (Espino *et al.* 1992). Moreover, uniform suckering was also observed in plant coming from tissue culture.

Espino *et al.* (1992) demonstrated that tissue culture planting materials had a highest number of functional leaves at harvest.

The earlier and more vigorous sucker production seen on TC plants in the plant crop was also reported by Daniells (1988), Drew and Smith (1990) and Smith *et al.* (1992). Swennen (1984) found that sucker production was controlled by cytokinin/auxin ratios and that increased suckers development was promoted by gibberellins and cytokinins being synthesized in the root tips. A root system present at planting and a larger overall root system with more root tips, as well as a juvenile rhizome with more axillary buds, could thus explain both the earlier sucker production and higher sucker count on TC plants. The greater number of suckers produced at earlier stages of development could be a reason for the uprooting problems being experienced in the plant crop of TC plants, as also mentioned by Daniells (1988), Drew and Smith (1990) and Fraser (1994). Establishment of TC plants in a 300 mm deep furrow could be an effective solution to this problem.

In field comparisons with CON using the same general management, TC plants had greater uniformity and upto 19% higher production potential, due to more fingers and hands, as well as shorter cycle times (Hwang *et al.* 1984, Drew and Smith 1990, Robinson *et al.* 1993).

The flowering and harvesting in both the tissue cultured cultivars was earlier than in the conventional ones. Rapid growth during the earlier stage offered this advantage to tissue cultured plants. According to Drew and Smith (1990) exposure of these plants to cool fluorescent tube light of 1000 – 3000 lux at 25°C and alternating dark and light phases might influence early fruit bud differentiation. Robinson (1989), Robinson and Anderson (1990) and Espino *et al.* (1992) also reported early flowering in tissue-cultured raised plants.

A vigorous root system, originating from juvenile rhizome tissue (Preece 1992), and the large initial leaf area, enabled TC plants to reach full assimilation potential at an earlier stage of development, with a doubling of both mean functional leaf area and total dry-mass production compared with CON plants, after five months. The faster development of TC plants compared with CON

plants, as also reported by Hwang *et al.* (1984), would have contributed to the shorter crop cycles reported with TC (Drew and Smith 1990, Robinson *et al.* 1993). Increased rooting vigour of TC plants was also reported by Zimmerman and Miller (1991) for apples and by Preece (1992) for rhododendron.

Reports on the yield performance of micropropagated banana plants of the 'Cavendish' subgroup are mixed. Some authors recorded significantly higher yields in micropropagated material (Daniells 1988); Drew and Smith 1990, Robinson *et al.* 1993) and other reported equal (Hwang *et al.* 1984); Zamora *et al.* 1989) or even lower yields than in conventional material. Thus, micropropagation does not seem to confer a consistent yield advantage to *Musa* plants, although *in vitro* plants establish more quickly and have vigorous initial growth. TC plants produce ratoon suckers earlier and with greater vigour than those from CON plants (Eckstein and Robinson 1993). Thus such TC sucker also produce significantly larger ratoon plants than those from CON suckers. The same explanations could also apply, to a lesser extent, in the third cycle. Growth and yield of the ratoon crop was similar with all treatments.

Micropropagation has played a crucial role in rapid development of IITA's plantain breeding program by rapidly supplying large numbers of plants of female fertile plantain cultivars for crossing blocks (Vuylsteke *et al.* 1993, 1993b).

However, the most significant problem that has arisen using *in vitro* produced bananas is the ubiquitous occurrence of off-type plants or somaclonal variants (Israeli *et al.* 1991). Plantings of micropropagated 'Cavendish' bananas had 1% to 50% off-types (Smith 1988), most of which were dwarfs with horticultural trails inferior to those of the original clone (Hwang and Ko 1987, Smith and Drew 1990, Stover 1987). Hwang (1986) found that about 3% of the Cavendish plantlets derived from meristem culture were variants. Some were detectable when the plants were young and other after flowering. Two types of variant were observed at the young stage namely (i) variegation of the leaves and (ii) abnormal leaf shape. In mature plants, four types of variants were recognised differing from ordinary plants in structure, leaf shape, pseudostem colour and bunch characteristics. Similar variations were reported

by Stover (1987) where in 6 – 10% of the plants exhibited abnormal foliage characteristics. Somaclonal variation was also observed in other cultivars such as 'Valery' (12%), 'Willium Hybrid' (3-5%), Umalag (3-5%) and Grand Nain (19%) as reported by Hwang and Ko (1987) in Taiwan and Pool and Irizarry (1985) in Jamaica. On the other hand, Stover (1987) found not a single off-type in 5000 'Saba' plants produced using similar *in vitro* technique in 'Grand Naine'.

Furthermore, the improved phytosanitary status of *in vitro* plants makes them the planting material of choice to reduce the risk of pest and disease introduction in new planting (Hwang *et al.* 1984) or for establishment of field nursery areas (Smith and Drew 1990). These nurseries than can provide clean conventional planting material for production fields. However, for micropropagation to the commercially viable, off-type production must be controlled in the culture laboratory and reliable screening technique to identify rogue dwarf off-types must be developed at either the nursery or laboratory level of minimize the recovery of off-types in the field.

In the present study on the improvement of banana plants were being commercially produced on larger scale through *in vitro* and hence an experiment was conducted to investigate the performance of *in vitro* propagated plantlets and conventional sucker on the growth, yield and yield contributing characters of six cultivars of banana. The vegetative characteristics studied included plant height, base girth of pseudostem, number of functional leaves, sucker production. The reproduction characteristics included in the study were time requirement to attain the flowering stage and maturity of the bunches and yield and fruit characteristics. The study revealed that the TC plants were more vigorous than the suckers as they produced significantly more number of leaves. The TC plantlets produced the taller plant than conventional ones for all the six cultivars of banana. The number of functional leaves of tissue culture derived plants were significantly higher than that of conventional sucker in all of the cultivars. Tissue culture-derived planting material tend to produce sucker earlier compared to the conventional sucker. However, the tissue cultured-derived planting materials had the highest number of sucker present. The TC plants were also earlier in flowering and maturity and shorter in total crop duration than the sucker plants. The superiority of tissue cultured plants of six cultivars over suckers was also

reflected in the bunch characters. Significant differences were recorded for the number of hands and finger/bunch, number of fingers/hand and all these parameters were found to be more in the TC plants then the suckers. The finger girth and length of the finger were significantly more in TC plants. The quality characters like acidity and pulp; pell ratio did not show any significant differences, whereas the TSS was more in TC plants than the sucker plants. The study indicated that tissue-cultured plants of cultivars Sabari, Mehersagar, Ranginsagar, Jahaji, Agniswar and Binathuri are superior to sucker-derived plants in respect to growth, yield and earliness. Based on the results of the present study, it may be recommended that (i) The farmers should be motivated to use tissue culture plantlets; (ii) Tissue culture plantlets should be made available to the farmers for commercial cultivation through Government and different agencies. The present protocol demonstrates a sound commercial feasibility for the cultivation of tissue cultured banana.

2.6. SUMMARY

In this part of the present investigation micropropagation of six important banana cultivars viz., Sabari, Mehersagar, Ranginsagar, Jahaji, Agniswar and Binathuri and subsequent field evaluation of microclones for commercial exploitation, were studied.

For micropropagation, shoot-tips of one-month-old suckers were surface sterilized with 0.1% $HgCl_2$ for 15 min followed by 3-4 times washing with sterilized distilled H_2O. For the establishment of primary culture the shoot-tips (5×8 mm with 2-3 pairs of leaf primodia together with 3 mm of rhizomatous base) of six banana cultivars were cultured onto agar geled MS medium supplemented with different concentrations and combinations of BAP and KIN. The cultures were inoculated at 25±1°C under cool florescent white light (2500-3000 Lux). *In vitro* culture response during initial establishment was found to influenced with genotypes and culture media formulations. Among the various growth regulator formulations, 5 mg/l BAP were found to be most effective for cvs. Sabari, Mehersagar, Jahaji, and Binathuri. Whereas, BAP 8 mg/l was found most effective formulation for the primary establishment of cv. Agniswar.

For rapid shoot multiplication, the primary cultures were transferred onto agar geled MS medium supplemented with different combinations and concentrations of BAP, KIN, IAA, IBA and coconut water (CW). The primary cultures of all banana cultivars were induced multiple shoots proliferation in most of the culture media formulations. However, both genotypes and media formulations showed marked effect on the multiple shoots proliferation. Among the various media formulations 0.5 mg/l BAP + 0.5 KIN mg/l + 13% coconut water (CW) for cvs. Mehersagar, Ranginsagar and Jahaji, for Sabari 5mg/l BAP + 5 mg/l KIN+ 13% coconut water (CW), for Agniswar 8 mg/l BAP + 1 mg/l KIN and for Binathuri 3.5 mg/l BAP + 0.5 mg/l KIN + 13% coconut water (CW) were most effective media formulation for inducing multiple shoots proliferation. Among the six banana cultivars cv. Sabari (AAB genome) was the most responsive genotypes whereas Binathuri (ABBB) showed the lowest response to micropropagation.

Bulbous structure was noticed to develop during repeated subculture in culture medium containing high concentration KIN (5 mg/l) that showed prolific multiple shoots proliferation. *In vitro* shoot multiplication was also affected with number of subcultures. Shoot multiplication rate was optimum upto 7th subculture; after then, multiplication rate gradually declined with passage of subculture for all banana cultivars.

The micropropagated clones of all banana cultivars after being properly acclimated were cultivated in the field with sucker-derived plants as control. Micropropagated plants of all cultivars showed significantly superior performances for yield (bunch wt/plant) and yield contributing characters over sucker-derived plants.

Chapter III

SOMATIC EMBRYO INDUCTION, PLANT REGENERATION AND EVALUATION OF SOMACLONAL VARIATION

3.1. INTRODUCTION

Somatic embryogenesis techniques in the genus *Musa* have two main goals: The development of a new, high performance micropropagation technique and a cell regeneration system useful for genetic transformation and somatic hybridization. Due to sterility of most important banana and plantain varieties, genetic improvement through conventional breeding is seriously hampered (Swennen *et al.* 2001, Tenkouano *et al.* 2004). Due to these problems, new strategies consisting of tissue culture and molecular biology techniques to complement breeding programs have been undertaken (Murfett and Clarke, 1987). Therfore development of new techniques is increasingly urgent. Cronauer and Krikorian (1988) first time were successfully induced somatic embryos from diploid zygotic embryos of banana. They develop a protocol for inducing somatic embryogenesis in banana that has been used for different *Musa* spp. by different workers. There are numbers of protocols have been developed for inducing somatic embryogenesis. Each procedure relies on different types of explants such as zygotic embryos (Cronauer and Krikorian 1988; Escalant and Teisson 1989; Marroquin *et al.*, 1993), rhizome slices and leaf sheath (Novak *et al.* 1989), proliferous meristems (scalps) (Dhed'a *et al.*, 1991, Schoofs 1997) and immature male/female flowers (Ma 1991, Escalant *et al.* 1994, Grapin *et al.* 1996; Cote *et al.*, 1996 and Grapin *et al.* 1998).

Professor Ma (Ma 1991) at the National University of Taiwan, developed cell suspension procedure using male flower bud as explant for the induction of somatic embryogenesis in banana. The procedure developed by Ma has been widely applicable for the development of somatic embryogenesis in different banana genotypes. Ma's methods has also been used with immature female flowers for those cultivars that do not produce male flowers (Grapin *et al.*, 2000) The first steps, up to the formation of the embryogenic callus, are described in Escalant *et al.* (1994). Descriptions of the initiation, maintenance and regeneration phases of

cellular suspensions are available in Grapin *et al.* (1996) Cote *et al.* (1996) and Strosse *et al.* (2003).

The scalp methods used at Katholicke Universitcit at Leuven relies on highly proliferating cultures initiated from shoot-tips (*in vitro* plants). This methods was first described by Dhed'a (1992) and optimized by Schoofs (1997).

Dhed'a *et al.* (1992) first used meristematic tissue from adventitious buds in the cultivar Bluggoe (ABB). Alternatively, Escalant *et al.* (1994), Cote *et al.* (1996) and Grapin *et al.* (1998) employed male and female flowers in cultivars of the groups AA, AAA, AAB and AAAB to form embryogenic tissue which was later used to establish cell suspension cultures.

The development of bipolar structure of somatic embryos on semi-solid cultures has been demonstrated-histologically in *Musa* spp. (Escalant and Teisson, 1989; Novak *et al.*, 1989). Dhed'a *et al.* (1991) reported the origin of proembryogenic structures and the subsequest process of somatic embryogenesis in suspension cultures.

In bananas and plantain there are major procedures for the development of embryogenic cell suspensions. Each method has its own limitations, rendering the establishment of *Musa* embryogenic cell suspensions still far from routine (Schoofs *et al.*, 1999). Since most edible banana cultivars rarely set seeds, zygotic embryos are of limited value as starting material. Reports on the efficiency of embryogenesis induction in rhizome and leaf tissue are scarce. The scalp-methodology relies on proliferating meristem cultures as explants. In contrast, the starting material for the widely used male-flower technology can be collected directly from the flowering banana plants. In addition this method can not be applied to the highly preferred False Horn and Harton –plantains, non-plantin AAB (Subgroup Maia Maoli, Pacific region) and ABBB (Klue teparod / Binathuri, Asia) which do not produce male flowers. This can be overcome by the use of female flowers as alternative explants, but results in loss of the bunch and is therefore, difficult for large scale use.

Few studies have been published on the incidence of off-types among banana plants produced through somatic embryogenesis. In "Grande Naine", a number of plant

derived from 4-month-old embryogenic cell suspensions were observed to be true-to-type and to have agronomic characteristics (Cote *et al.*, 2000a). Similar findings were obtained with "IRFA 903" plants derived from 7-month-old cell suspensions (Cote *et al.*, 2000b). These data suggest that somatic embryogenesis can be used for genetic transformation given that a proportion of variants can be tolerated for that purpose.

Variation may occur when plants arise from adventitious, meristem differentiated from the explant or from callus, plant cells grown in unorganized (callus, cells and protoplasts) cultures under extensive genetic changes. Genetic variability has been observed in many species during tissue culture. This phenomenon is usually termed somaclonal variation (Larkin and Scowcroft 1981, Meins 1983, Swartz 1991, Karp 1991). Many studies on *Musa* have also demonstrated that some *in vitro* propagated materials differ from the source materials from which they are derived. After the development of the micropropagation technique for bananas using meristem culture (Ma and Shii 1972, Hwang *et al.* 1984), somaclonal variation was commonly found among tissue culture plants (Reuveni and Israeli 1990, Israeli *et al.* 1991, Vuylsteke *et al.* 1991, Daniells and Smith 1993). Israeli *et al.* (1995) gave a comprehensive review on the somaclonal variation in *Musa*. Twenty nine cases of somaclonal variation were reported in various types of bananas and plantain with a range of incidence from 0 to 69%. Daniells and Smith (1993) reported various off-types among several banana cultivars in Australia. Rates of somaclonal variation in banana derived from shoot-tip culure vary from 0 to 70% according to genotype (Israeli *et al.* 1995, Smith 1988, Vuylsteke *et al.* 1991). Somaclonal variation was also observed in other cultivars such as 'Valery' (12%), 'Williums hybrid' (3-5%), 'Umalag' (3-5%) and 'Grande Naine' (19%) as reported by Hwang and Ko (1987) in Taiwan and Pool and Irizarry (1985) in Jamaica. On the other hand, Stover (1987) found not a single off-type in 5000 'Saba' plants produced using similar *in vitro* technique in 'Grand Naine'.

It is not yet clear, however, whether somatic embryogenesis can be used to mass propagation of banana plants. The available results were obtained in fairly young suspensions (4 to 7 months), but since only a small number of plants can be

regenerated at present such suspensions, this technique cannot be used for multiplication on a large scale.

The present study was intensified to establish protocol for callus induction and subsequent plant regeneration using immature male floral bud of five banana cultivars grown in Bangladesh. Procedures in this chapter describe the establishment of protocol for the initiation and maintenance of callus and plant regeneration from the callus through somatic embryogenesis in five banana cultivars viz. Sabari, Mehersagar, Rangimsagar, Jahaji, and Agniswar.

3.2. OBJECTIVES

I) To induce callus from immature male flowers of five banana cultivars.

II) To induce somatic embryogenesis from male flower derived calli.

III) To regenerate plant from somatic embryos derived from male flower derived calli.

IV) To transplant the somatic embryo derived plant to the field.

V) To study somaclonal variation among the plants derived through somatic embryogenesis.

3.3. MATERIALS AND METHODS

3.3.1. Plant Materials

Male flowers from the following banana cultivars were used as explant source for the induction of callus for somatic embryo development:

1. Sabari
2. Mehersagar
3. Ranginsagar
4. Jahaji
5. Agniswar

Banana cv. Binathuri was excluded in this study due to the absence of male flowers.

3.3.2. Methods

3.3.2.1. ***Explant collection and surface sterilization*** : The initial plant material consisted of male inflorescences of banana were collected from field grown plants. Male flower buds were collected from 8^{th} to 10^{th} position of the inflorescences after 6-10 weeks of flower opening. Collected flowers were brought to laboratory and kept for 24 hours before culture inoculation. Under a steriale condition, the outer overlapping bracts were removed and the remaining part (2-3cm in length) containing male flowers were isolated and were used as explants. The explants were surface sterilized in 70% ethyl alcohol for 1 min.

3.3.2.2. ***Culture inoculation and incubation*** : Surface sterilized explants of male flowers were inoculated onto semisolid MS medium supplemented with different callus inducing growth regulators including 30 g/l sucrose and 1 mg/l biotin. The culture medium was supplemented with different concentrations of 2.4-D, IAA and NAA for callus induction. The culture dishes (9 cm petridishe/250 ml conical flask) were sealed with parafilm to avoid evaporation and to reduced contamination. The culture dishes were inoculated in total darkness at 25±2°C for 4-7 months. The cultures were checked periodically to observe morphogenic change happened to occur during incubation.

3.3.2.3. ***Callus maintenance and somatic embryo induction*:** After callus induction from the explant, the calli were transferred into the fresh medium for further proliferation and maintenance. The embryogenic callus was selected for plant regeneration.

3.3.2.4. ***Plant regeneration*:** For plants regeneration the selected calli bearing somatic embryos were subcultured onto semisolid MS basal salts with Morel's vitamins (Morel and Wetmore 1951), 3% sucrose and 2% gelrite supplemented with BAP and IAA. Fully grown plantlets with good shoot and root system were then transplanted into polybags contaiag potting mix (Manure, sand and garden soil: 1: 1: 1) and placed in a mist condition for 5 weeks.

3.3.2.5. ***Plantlet transplantation and field evaluation*** **:** After three months when the transplanted plants in poly-bag grew up to 12-16 inches long (**Figure A, Plate 11**) were transferred to field and they were grown it to maturity. Fifty plants for each of somatic embryo derived plants (SEDPs) and shoot-tip culture derived plants (STCDPs) were planted in to side-by-side separate block for each of the cultivars. The plants were transplanted in May 2004 in 5 rows, 10 plant/row to plant distance was 2 m and raw to distance was 2.5 m (**Figure B, Plate 11**). During the transplantation of plantlets and post transplantation, special care was taken.

3.3.2.6. ***Data recording for in vitro culture*** **:** Data were collected using the following parameters and the methods of data collection are given below

i) Callus induction frequency

Explants were cultured in 9 cm petridishes / 250 ml conical flask containing media with different concentrations of growth regulators for callus induction. After days of culture frequency of callus induction was calculated using the following formula.

$$\text{Frequency of callus induction (\%)} = \frac{\text{Number of explants induced callus}}{\text{Number of cultured explant}} \times 100$$

$$\textbf{ii) \% of embryogenic callus} = \frac{\text{Number of induced callus}}{\text{Number of embryogenic callus}} \times 100$$

iii) Percentage of embryogenic calli regenerated to plant

As mention earlier data on different parameters from different treatments of shoot proliferation were recorded after 45 days of culture. The percentages of embryogenic calli induced shoots were calculated using formula.

$$\text{Shoots induced } (\%) = \frac{\text{Number of calli induced shoots}}{\text{Total Number of calli cultured}} \times 100$$

a) Number of shoots/embryogenic calli:

Mean number of adventitious shoots per explants (calli) was calculated using following formula.

$$\overline{X} = \frac{\sum xi}{N}$$

$\overline{X}$ = Average number of shoots.

$\sum$ = Summation.

Xi = Total number of shoots.

N = Number of observation.

Field grown plant derived through somatic embryogenesis were visually rated to find out any somaclonal variation.

3.3.2.7 *Data recording for somaclonal variation* : For the evaluation of somaclonal variations and SEDPs and STCDPs data on both morphological and yield characters were recroded.

Morphologicl characters

All (50 plant) SEDPs and STCDPs assessed for finding the out occurrence of possible variation using the following morphological features at different stages of plant development.

i. Dwarf off-type plant: The plants those were shorter in stem height than normally grown plants were considered as dwarf off-types. Data on no. of dwarf off-type plant were recorded six month after transplantation.

ii. Leaf abnormality: Leaf abnormality parameter include as any change or deviation from corresponding normal one such as, short leaf blade, uncommon pigmentation, serrated margin etc. The no. of plants with abnormal leaf were counted six month after transplantation.

iii. Stem colour abnormality: Stem colour abnormality includes presence or disapperance of colour pigment on stem or petiole edge, was recorded six month after transplatation.

iv. Phylotaxic abnormality: Phylotaxic abnormality wax considered as any deviation from normal alternate leaf arrangement which was recorded at the time of inflorescence imergence.

v. Inflorescence abnormality: A normality in inflorescence was characterized as emergence of inflorescence, stem side, inflorescence with short internode, dense hands and fingers, too many female hands or absence of male flower hands; was recorded 30-day after inflorescence emergence.

Yield and its component characters

Data on bunch wt./plant, no. of hands/bunch and no. of fingers/hand were recorded after fruit hervest.

3.3.2.8. ***Data analysis*** : Standard statistical methods were used calculating for range of variation (standard error). Comparison between two sample means (SEDPs and STCDPs) was done according Student t-test.

3.4. RESULTS

3.4.1. Callus Induction, Somatic Embryogenesis and Plant Regeneration

Male flowers of 5 banana cultivars were cultured onto MS medium supplemented with three different concentrations of 2,4-D in combinations with 1 mg/l IAA, 1 mg/l NAA and 1 mg/l biotin (**Table 3.1.**). The cultures were incubated in total darkness at 25 ± 2°C. Explants were left for 5 to 6 months without subculture and morphogenic differentiation was regularly monitored. Data on various *in vitro* parameters such as % of explants induced callus, callus color, degrees of callus growth, time taken to callus induction, % of callus induced somatic embryos and time taken to globular stage embryo formation were recorded for the assessment of the results, which are given in **Table 3.1.** The results on callus induction, somatic embryogenesis and subsequent plant regeneration are illustrated in (**Figures A-E** of **Plate 9**) and (**Figures A-E** of **Plate 10**).

In general callus induction from male flower explants was taken very long time for all of the cultivars. In all cultivars the male flower explants induced to start callus formation within 90-120 days after inoculation. However, morphogenic differentiation of the cultured explants was found vary with culture media formulations and banana cultivars as well.

Among the three culture media formulations 4 mg/l 2,4-D in combination with 1 mg/l IAA + 1 mg/l NAA and 1 mg/l biotin was found to be the most effective in the induction and growth of callus for all the banana cultivars. Among the 5 cultivars cv. Sabari was found to most responsive. In 4 mg/l 2,4-D, the highest 75% explants induced to develop callus. On the other hand cv. Jahaji was the least responsive and highest 55% explants induced callus formation in the same medium formulation. Considerable variation was also observed in the callus morphology and degree of callus growth. The highest degree callus growth from male flower explants for all banana cultivars was observed in culture medium formulation fortified with 4 mg/l 2,4-D.

The extent of embryogenic callus formation was also found to be differed with culture media formulations and also with banana genotypes. In all the cultivars a certain % of calli underwent morphogenic redifferentiation to develop somatic embryos.

Among the five cultivars the highest 55% calli induced in 4 mg/l 2,4-D become subsequently underwent morphogenic differentiation to develop somatic embryos. Extent of somatic embryos formation was the lowest in cv. Jahaji.

Time taken to somatic embryogenesis was also found to be different depending upon banana genotypes. In cv. Sabari the calli induced from male flower explant took the longest 210-240 days for embryo differentiation. Whereas, the male flower derived calli of cv. Mehersagar took the 150-170 days for somatic embryogenesis. The stages of somatic emrbyo differentiation were clearly distinguishable. All the stages of embryogenesis such as proembryonic cells mass, globular, torpedo and heart shaped embryos were visible en masse on the same callus.

Global embryos of 5 banana cultivars were cultured onto MS medium supplemented with three different concentration of BAP in combination with 2 mg/l IAA (**Table 3.2.**). Culture conditions were total darkness at 27 ± 2°C and the embryos remained in the culture medium for 50 days and no subculture was done. Data on various *in vitro* parameters such as numbers of globular embryos in each culture and numbers of globular embryos after multiplication.

Among the three culture media formulations 0.5 mg/l BAP + 2 mg/l IAA was found to be the most effective in the induction and growth of somatic embryos for all the banana cultivars. Among the 5 cultivars cv. Sabari was found to be most responsive in 0.5 mg/l BAP + 2 mg/l IAA. Embryogenic callus exhibited rapid proliferation in the same medium. The highest 65 embryos were recorded in a clump which was obtain from the 10 initial embryos after 50 days of culture. On the other hand cv. Jahaji was the least responsive and highest 45 embryos were recorded in a clump which was also obtained from the 10 initial embryo clump after 50 days of culture.

The extent of embryogenic callus formation was also found to be different with culture media formulations and also with banana genotypes.

Somatic embryos of 5 banana cultivars were cultured onto MS medium supplemented with three different concentrations of BAP in combination with 2 mg/l IAA + Morel vitamins (**Table 3.3.**). The cultures were maintain in an

inoculation room at 25 ± 2°C and 16 h photo-period with 1000 lux. Data on numbers of cultured mature somatic embryos and % of germination were recorded for the assessments of the results which are given in **Table 3.3.** After 45 days of culture germination rates were estimated.

Among the three germinations culture media formulations 0.5 mg/l BAP + 2 mg/l IAA + Morel vitamins was found to be the most effective in the germination of somatic embryos for five cultivars. Among the 5 cultivars cv. Sabari was found to be responsive in 0.5 mg/l BAP + 2 mg/l IAA + Morel vitamins. The highest 70% somatic embryo started to germinate with the emergence of leaves and roots after 45 days of culture. On the other hand, cv. Jahaji was the least responsive and highest 50% embryo induced to germinate in the same media formulation.

3.4.2. Evaluation of Somaclonal Variation

Somatic embryo derived plants (SEDPs) of five banana cultivars (Sabri, Mehersagar, Ranginsagar, Jahaji and Agniswar) were transplanted (fifty plants from each cultivars) into field. The transplanted plants were grown to maturity. Shoot-tip culture derived plants of each cultivar were also planted side-by-side and grown to maturity as a control. Data on morphological characters such as dwarf off-type plants, leaf abnormality, stem colour abnomrlaity and inflorescence abnormality were recorded at different growth stage to evaluate somaclonal variation. Data on yield and its component characters viz., bunch wt./plants, no. of hands/bunch and no. of fingers/hand of both somatic embryo and shoot-tips derived plants were also recorded.

Results on morphological features of somatic embryo derived plant (SEDPs) and shoot-tip culture derived plants (STCDPs) are shown in **Table 3.4.** In general morphological abnormalities were noticed in higher frequency among SEDPs than those of STCDPs. Dwarf off-type plants were frequently seen among SEDPs. Dwarf off-type plants were characterized short stem, weak petiole, short leaves and deformed hands. These plants were found poorly grown and developed small bunches with poorly developed fingers (**Figure C, Plate 11**). Among the five banana cultivars the highest six dwarf off-type plants was recorded for Agniswar. Numbers of dwarf off-type plants out of fifty were four in Sabari, three in Mehershagar, two in Ranginsagar and Jahaji. Dwarf off-type

plants were not noticed among STCDPs of most of the banana cultivars except in Ronginshagar where two out of fifty plants was found dwarf off-type.

Leaf abnormality was characterized as change in the shape of leaf blade such as short, serrated leaf margin, presence of pigmented area etc. Among the SEDPs of five banana cultivars the highest eight plants showed various forms of leaf abnormality in Agniswar which was followed by Jahaji, Sabari, Mehershagar and Ranginsagar. The cultivar Jahaji is dwarf type and developed dark pigmented area at juvenile stage development. Among SEDPs some of the plants the dark pigmented area were persisted even at maturity. Leaf abnormality was observed only among STCDPs of Sabari and Jahaji (Two and one, respectively).

Stem colour abnormality as loss of normal stem pigment or appearance of uncommon pigmented area on stem or petiole edge were also noticed among the SEDPs of all banana cultivars. Among the five cultivars stem colour abnormality was the highest six plants (out of fifty) in Agniswar. The numbers of plants with abnormal stem pigments were four in Sabari and three in Mehershagar, Ranginsagar and Jahahi. Abnormal stem pigmentation was also noticed among the STCDPs of most of the cultivars.

Change in leaf arrangement was also noticed among SEDPs most banana cultivars leaf in banana is grown in regular alternate phylotaxic abnormality was characterized any types of change or deviation from normal sucker derived plants. Number of SEDPs showed change in phyllotexic arrangement was more or less same in Sabari, Mehershagar, Ranginsagar and Agniswar. SEDPs of Jahahi did not any change in leaf arrangement, phyllotaxic abnormality although in lower frequency was observed among STCDPs of Ranginsagar and Agnishwar.

Inflorescence abnormality was common among SEDPs of all banana cultivars. Inflorescence abnormality was characterized as emergence of inflorescence from the side of the stem not from the stem tip, inflorescence with short internode and dense hand, too many female flower hands (**Figure D, Plate 11**) or absence of male flower hands. Numbers of SEDPs with abnormal inflorescence were six (out of fifty plants) in Sabari and Jahahi, five in Agniswar, four in Mehershagar and three in Ranginsagar.

Inflorescence abnormality was less frequent among STCDPs. Only one plant (out of 50) was noticed to bear abnormal inflorescence in Sabari and Jahahi.

Data recorded on average bunch wt. (Twenty five plants), no. of hands/bunch and no. of fingers/hand after the harvest of both SEDPs and STCDPs of five banana cultivars and the results are summarized in **Table 3.5.**

The results presented in **Table 4.2** reveal that yield performances of the plants derived through somatic embryogenesis were in general lower than STCDPs. However, wide range of variations were observed for all three characters among SEDPS for five banana cultivars. Average bunch wt. of SEDPs was significantly lower than those of STCDPs. In Sabari the bunch wt./plant was 15.08 kg for STCDPs where as 12.65 kg/plant for SEDPs. Similarly, bunch wt./plant was 22.44 kg for STCDPs but 20.84 kg for SEDPs. The bunch wt/plant in Ranginsagar and Agniswar were respectively, 25.23 and 18.76 kg for STCDPs where as respectively, 21.35 and 16.38 kg for SEDPs. However, difference observed in bunch wt/plant between STCDPs and SEDPs of Jahaji was non significant. Differences observed for other two yield contributing characters, no. of hands/bunch and no. of fingers/hand between STCDPs and SEDPs were non significant.

Table 3.1 Effect of different concentrations of 2,4-D in MS medium with 1 mg/l IAA+1 mg/lNAA+1 mg/biotin on the induction of embryogenic callus in five cultivars of banana.

Cultivars	Growth regulators (mg/l)	% of immature male flowers induced callus	Callus colour	Degree of callus formation	Time taken to callus induction	% of callus induced to develop somatic proembryos	Time taken to globular stage for embryogenesis
	2 2,4-D	45	W	++	90 – 120	40	210 – 240
Sabari	4 2,4-D	75	W	+++	90 – 120	55	210 – 240
	6 2,4-D	38	LB	+	90 – 120	35	210 – 240
	2 2,4-D	42	LB	++	90 – 120	34	150 – 170
Mehersagar	4 2,4-D	62	W	+++	90 – 120	41	150 – 170
	6 2,4-D	35	LB	+	90 – 120	30	150 – 170
	2 2,4-D	37	LB	+	90 – 120	30	160 – 180
Ranginsagar	4 2,4-D	58	LB	+++	90 – 120	36	160 – 180
	6 2,4-D	31	LB	+	90 – 120	28	160 – 180
	2 2,4-D	38	LB	+	90 – 120	28	160 – 180
Jahaji	4 2,4-D	55	LB	+++	90 – 120	32	160 – 180
	6 2,4-D	30	LB	+	90 – 120	25	160 – 180
	2 2,4-D	50	LB	+++	90 – 120	36	180 – 190
Agniswar	4 2,4-D	65	W	+++	90 – 120	46	180 – 190
	6 2,4-D	41	LB	++	90 – 120	27	180 – 190

W = white; LB = light brown; + = low; ++ = moderate; +++ = massive

Table 3.2 Effect of different concentrations of BAP in MS medium with 2 mg/l IAA on multiplication of globular embryos of five cultivars of Banana. Data were recorded after 40-50 days of culture

Cultivars	Growth regulators (mg/l)	No. of globular embryos in each culture	No. of globular embryos after multiplication
Sabari	0.1 BAP	10	40
	0.5 BAP	10	65
	1.0 BAP	10	45
Mehersagar	0.1 BAP	8	32
	0.5 BAP	8	50
	1.0 BAP	8	35
Ranginsagar	0.1 BAP	6	30
	0.5 BAP	6	48
	1.0 BAP	6	28
Jahaji	0.1 BAP	8	31
	0.5 BAP	8	45
	1.0 BAP	8	25
Agniswar	0.1 BAP	9	40
	0.5 BAP	9	55
	1.0 BAP	9	34

Table 3.3 Effect of different concentration of BAP on somatic embryo germination and plant regeneration from somatic embryos of five cultivars of banana. The isolated embryos were cultured individually onto medium consisted of MS salts + Morel Vitamin and 2 mg/l IAA. Data were recorded 40-45 days after culture

Cultivars	Growth regulators (mg/l)	No. of cultured mature somatic embryos	% of germination
Sabari	0.1 BAP	25	50
	0.5 BAP	25	70
	1.0 BAP	25	60
Mehersagar	0.1 BAP	20	50
	0.5 BAP	20	65
	1.0 BAP	20	60
Ranginsagar	0.1 BAP	20	46
	0.5 BAP	20	60
	1.0 BAP	20	50
Jahaji	0.1 BAP	15	40
	0.5 BAP	15	50
	1.0 BAP	15	45
Agniswar	0.1 BAP	25	48
	0.5 BAP	25	62
	1.0 BAP	25	55

Table 3.4 Somaclonal variant types and percentages of somaclonal varients in somatic embryo derived plants and shoot-tip culture derived plant of five banana cultivars. Morphological evaluation was done on 50 both somatic embryo derived plants (SEDPs) and shoot-tip culture derived plants (STCDPs) for each of the five banana cultivars

Cultivars	Type of variation	Percentage of Variants	
		SEDPs	STCDPs
Sabari	Dwarf off-type plants	4	-
	Leaf abnormality	4	2
	Stem colour abnormality	4	1
	Phylotaxic abnormality	4	-
	Inflorescence abnormality	3	-
Total		22, 44%	3, 6%
Mehersagar	Dwarf off-type plants	3	-
	Leaf abnormality	3	-
	Stem colour abnormality	3	1
	Phylotaxic abnormality	1	-
	Inflorescence abnormality	4	-
Total		14, 28%	1, 100%
Ranginsagar	Dwarf off-type plants	2	1
	Leaf abnormality	3	-
	Stem colour abnormality	3	2
	Phylotaxic abnormality	4	1
	Inflorescence abnormality	3	-
Total		15, 30%	4, 8%
Jahaji	Dwarf off-type plants	2	-
	Leaf abnormality	5	1
	Stem colour abnormality	3	-
	Phylotaxic abnormality	-	-
	Inflorescence abnormality	6	1
Total		16, 32%	2, 4%
Agniswar	Dwarf off-type plants	6	2
	Leaf abnormality	8	1
	Stem colour abnormality	6	3
	Phylotaxic abnormality	4	1
	Inflorescence abnormality	5	-
Total		29, 56%	6, 12%

Table 3.5 Bunch and fruit characters of five banana cultivars of somatic embryo derived plants (SEDPs) and shoot-tip culture derived plants (STCDPs). Data were collected from 25 randomly selected plants for each of the cultivars

Cultivars		Bunch wt./plant (kg) $\bar{X} \pm SE$	No. of hands/bunch	No. of finger /hand $\bar{X} \pm SE$
Sabari	STCDPs	15.08±1.26 b	7.85±0.12 a	5.27±0.36 a
	SEDPs	12.65±6.97 a	7.46±2.73 a	14.65±1.21 a
Mehersagar	STCDPs	22.43±1.32 b	8.66±0.08 a	20.41±0.62 a
	SEDPs	20.84±5.48 a	9.26±2.92 a	20.83±2.08 a
Ranginsagar	STCDPs	25.23±1.22 b	9.34±0.39 a	20.99±0.48 a
	SEDPs	21.35±4.78 a	9.85±3.82 a	19.78±1.55 a
Jahaji	STCDPs	21.96±0.82 a	8.19±0.09 a	20.52±0.96 a
	SEDPs	19.56±4.65 a	8.98±4.65 a	20.46±2.54 a
Agniswar	STCDPs	18.76±0.78 b	7.43±0.06 a	18.77±0.49 a
	SEDPs	16.38±4.18 a	7.56 ± 3.52 a	18.92±1.86 a

Mean value comparison between STCDPs and SEDPs was done using t-test.

Plate 9

Photographs showing induction of callus on male flowers of five banana cultivars.

Figures:

A. Callus formation of Sabari in MS + 4.0 mg/l 2,4-D+ 1.0 mg/l IAA + 1.0 mg/l NAA + 1.0 mg/l biotin 7 months after culture.

B. Callus formation of Mehersagar in MS + 4.0 mg/l 2,4-D+ 1.0 mg/l IAA + 1.0 mg/l NAA + 1.0 mg/l biotin 5 months after culture.

C. Callus formation of Rangiasagar in MS + 4.0 mg/l 2,4-D+ 1.0 mg/l IAA + 1.0 mg/l NAA + 1.0 mg/l biotin 6 months after culture.

D. Callus formation of Jahaji in MS + 4.0 mg/l 2,4-D+ 1.0 mg/l IAA + 1.0 mg/l NAA + 1.0 mg/l biotin 6 months after culture.

E. Callus formation of Agniswar in MS + 4.0 mg/l 2,4-D+ 1.0 mg/l IAA + 1.0 mg/l NAA + 1.0 mg/l biotin 6 months after culture.

Plate 9

Plate 10

Somatic embryos and plant regeneration through the embryo regeneration from male flower derived calli in the culture medium containing MS salts + Morel vitamins +0.5 mg/l BAP +2.0 mg/l IAA. Photographs were taken 45 days after transferring the calli onto regeneration medium.

Figures:

A. Sabari.

B. Mehersagar.

C. Ranginsagar.

D. Jahaji.

E. Agniswar.

Plate 10

Plate 11

Photographs showing field establishment of somatic embryo derived plants of five banana cultivars.

Figures:

A. Potted plants ready for transfer to the field.

B. Established plants in field derived from somatic embryos.

C. Small fruits observed in field due to somaclonal variation in Mehersagar.

D. Abnormal inflorescences observed in field due to somaclonal variation in Agniswar.

Plate 11

3.5. DISCUSSION

Bananas and plantains are monocotyledonous, perenenial herbs, cultivated in nearly 120 countries of the humid and sub humid tropical regions. In the developing world, *Musa* species are one of the major food sources (Erison and Sharrock 1998). Bananas and plantains are, however, prone to many pests and diseases such as fungi, viruses, bacteria, insects and Nematodes (Jones 2000a). There is known chemical or culture control for the pathogen. Hence, varieties attracted by this pathogen must be replaced by resistant varieties (Rowe 1990). Due to sterility of most important banana and plantain varieties, genetic improvement through conventional breeding is seriously hampered (Swennen and Vuylstake 2001, Tenkouno and Swennen 2004). Hence, genetic engineering of bananas and plantains is needed. The choice of candidate tissues for genetic engineering in banana is restricted to meristematic tissue and embryogenic cell suspensions.

The term callus refer to tissue arising from the disorganised proliferated of cells from segments (explants) of plant organs. Callus formed during *in vitro* culture has some similarities to tissue arising *in vitro* injury to plants so called wound callus. However, there often are differences in morphology, cellular structure, growth and metabolism between callus derived through tissue culture and natural wound callus. It has now been well established that any tissue can be changed into callus, if it is cultured on a suitably defined medium under controlled conditions. Callus formation is controlled by the level of plant growth regulators (auxin and cytokinins) in the culture medium concentrations of the plant growth regulators can vary for each plant species and can even depend on the source of explant or individual plant culture conditions (temperature, light etc.) are also important in cultures will be used to study protoplast isolation, cell type, cellular selection, somatic embryogenesis, organogenesis and secondary production.

However, many factors such as genotypes, compositions of the nutrient medium, physical growth factors such as light, temperature, humidity and endogenous supply of growth regulators are important for callus induction (Pierik 1987). For callus culture of dicot plants there are many reports with many species. On the contrary, for monocot plants, it has been believed that callus induction is very difficult, because

they have no secondary growth which occurs through the activity of vascular cambium (Maeda 1980). Since it is sometimes difficult to form callus tissue with monocotyledons embryos, young leaves, seedling or very young flower initials are often resorted to as starting material. A few general rules for the induction of somatic embryos in banana are given below:

I. A high auxin concentration is often required for embryo induction, for further development of the embryos this should be lowered or in some cases it should be completely eliminated from the medium. The auxin 2,4-D is very important for embryogenesis. Cytokinin does not have a vital role in this process.

II. Light generally promotes embryogenesis although it can take place at low irradiance or even in darkness with some species.

III. High temperatures are normally favourable for somatic embryogenesis; some plants (especially anther cultures) require a cold shock to initiate the formation and further development (germination) of the embryos.

The first report of somatic embryogenesis in *Musaceae* was that Cronauer and Krikorian (1983), who obtained somatic embryos from cell suspensions derived from apices cultured *in vitro*. Sterility and polyploidy of banana and plantain the development of new varieties through conventional breeding programs remains difficult. Due to these problems, new strategies consisting of tissue culture and molecular biology techniques to complement breeding programs have been undertaken (Murfett and Clarke 1987).

For several years, different techniques such as somatic embryogeneisis and cellular suspension cultures have made it possible to obtain banana and plantain plants development *in vitro* (Escalant and Teisson 1989, Novak *et al.* 1989, Dhed'a *et al.* 1991, Chou Tou Shii *et al.* 1992).

In bananas and plantains cell suspensions have been obtained from various explants, such as Zygotic embryos (Cronauer and Krikorian 1988, Escalant and Teisson 1989, Marroquin *et al.* 1993) rhizome and leaf sheaths (Novak *et al.* 1989). Proliferous

meristems (Dhed'a *et al.* 1991), immature male flowers (Ma 1991, Grapin *et al.* 1996, Cote *et al.* 1996) and recently immature female flowers (Grapin *et al.* 1998). Each method has its own limitations, rendering the establishment of *Musa* embryogenic cell suspension still far from routine (Schoofs *et al.* 1999). Since most edible banana cultivars rarely set seeds, zygotic embryos are of limited value as starting material. The scalp –methodology relies on proliferating meristem cultures as explants. This involves an extensive material preparation phase preceding induction of embryogenesis. In contrast, the starting material for the widely used male flower technology can be collected directly from flowering banana plants. However, in various types of *Musa* the male part of the axis is short lived, and no male bud, or only a degenerating male bud, is present. The plantain group (AAB) is well known for its important floral morphological heterogeneity (De Langhe 1961, Tezenas *et al.* 1983). Degenerating male bud are also present in other groups: AA (Samba Comoro Islands), non-plantain AAB (subgroup Maia Maoli, Pacific region), and ABBB (Klue Teparod Asia). This can be overcome by the use of female flowers as alternative explants, but results in loss of the bunch and is therefore difficult for large scale use. However, somatic embryogenesis was obtained by culturing young male flowers of different genomic groups of sweet and cooking bananas. The originality of this methods is characterized by its application to a large range of genotypes and by the introduction of temporary immersion system as a very efficient propagation technique (Escalant *et al.* 1994).

In the present study male flower buds cultured of five cultivars of banana were cultured onto MS medium supplemented with different concentration of 2.4-D, 1 mg/l NAA, IAA and 1 mg/l biotin, showed enlargement of the floral primordia and the development of whitish embryogenic callus, after 2-3 months of inoculation. The embryogenic callus showed several developed embryos on the surface and the smear preparations of the callus tissues showed early stages of embryo development.

Globular embryos could be multiplied further by subculturing onto BAP in combination 2 mg/l IAA in MS medium. At least a certain percentage of somatic embryos induced germination to develop plantlets when the embryos were subcultured onto MS salts with Morel vitamins contained BAP and IAA. The

embroyo derived plant of alls banana cultivars were successfully transplanted to field and grown to maturity.

The methods proposed by Cote *et al.* (1996) and Grapin *et al.* (1996) take between four and seven months just for the formation of calli with embryogenic structures, and a further two months for the establishment of cell suspensions. Furthermore, it is necessary to take into account that the embryogenic response of male flowers cultured on semisolid medium was low. Escalant *et al.* (1994) reported that 0 to 7% of the explants had calli with embryogenic structures in five banana cultivars, including 'Grande Naine'. Daniels *et al.* (2002) reported a success rate of 6.5% with the hybrid FHIA-21 (AAAB). With 'Grande Naine', the mean was 8% (Strosse *et al.* 2003). Khalil *et al.* (2002) obtained better results (58.8%) but with the cultivar 'Brazilian dwarf' (AAB). Moreover, the proportion of embryogenic cell suspensions established from ideal embryogenic calli was between 10% and 30% in 'Grande Naine'.

The levels of germination of somatic embryos obtained after being in a maturation medium were on average 80.5%, a value higher than the 40.6% germination rate for the hybrid FHIA-18 on semisolid culture medium obtained by Barranco (2001) and the 49.3% for the cultivar 'Navolean' (AAB) obtained by Cabrera (2001). Escalant *et al.* (1994) obtained between 60% and 70% germination in different cultivars with a system of temporary immersion of type RITA and Navarro *et al.* (1997) 25% germination in 'Grande Naine'. Daniells *et al.* (2002) obtained high germination rates (82.5%) of somatic embryos for the hybrid FHIA-21 using a maturation medium before germination.

Tissue culture induced genetic variability, popularly known as somaclonal variation, has been observed in many plant species (Larkin and Scowcroft 1981, Meins 1983, Swertz 1991, Karp 1991). Many studies on *Musa* have also demonstrated that somaclonal variation is very common occurrence during *in vitro* culture (Vuylsteke *et al.*, 1991, Daniells and Smith 1992, Smith *et al.* 1993, Cote *et al.* 1993, Hwang and Tung 1995, Reuvini *et al.* 1996, Walther *et al.* 1997, Khayat *et al.* 2004).

Somaclonal variation in the form of different morphological deformities were also observed among the field grown *in vitro* culture derived plants in the present study. This study also reveals that occurrence of different types somaclonal varients were more frequent among somatic embryo derived plant populations of all five banana cultivars than those of shoot-tip culture derived plant populations. Occurrence of dwarf off-types plant was very common in higher frequency. Other morphological characteristics such as, deformed hands, petiole deformation and short leaves were found to be linked with dwarfism. In addition colour modification of leaves, stem or edges of petioles, variations in inflorescence morphology in the forms of fruit size and shape along with bunch morphology were noticed.

In the previous study, somaclonal variations in tissue culture derived banana population have mainly been observed in plant size, inflorescence morphology, limb thickening, high leaf area index, variegated limbs, colour modification on stem and petiole edges etc (Stover 1987, Israeli *et al.* 1991, Marie 1992, Achard and Auboiron 1992, Smith 1988, Vuylsteke *et al.* 1991, Smith *et al.* 1993, Reuveni *et al.* 1996). These results of occurrence of somaclonal variation in banana due to tissue culture are concomitant with the finding of present study.

Introduction of *in vitro* culture allowed production of pathogen free plants and widened the genetic variability of existing banana cultivars, as a result of somaclonal variation (Daniells and Smith 1992, Reuveni *et al.* 1996, Walther *et al.* 1997, Khayat *et al.* 2004). At the same time due to the variation that occurs during the *in vitro* propagation, plants of the same cultivar selected in different locations often differ in same characteristics. In the previous studies somaclonal variations in banana and plantain was evaluated in meristem culture derived micropropagated plant population. In the present study somaclonal variations among shoot-tip culture derived plants are less frequent. Plant regeneration from meristem culture takes longer period than shoot-tip culture. Therefore, the high incidence of off-type plants produced by banana meristem culture may be due to the longer duration in tissue culture and high number of cycles in the multiplication phase of the meristem culture (Khayat *et al.* 2004). Moreover, in the present study the high frequency incidence of somaclonal variations among somatic embryo derived banana plants is also resulted

from prolong incubation period from culture induction to plant regeneration. In induction of embryogenic callus from immature male flower took very long lag period (7-14 months). Somatic embryo induction and plant development from somatic embryo took another 40-45 days. A number of dedifferentiation and redifferentiation processes are involved during plant regeneration through somatic embryogenesis. On the other hand, plant regeneration occurred during intact shoot-tip culture through the activities of pre existing shoot apical meristem that help to maintain "true-to-typeness" among the clones of the source plants.

In the present study, the average yield performance (bunch wt./plant) of somatic embryo derived plants is in general lower than shoot-tip culture derived plant. However, somatic embryo derived plants in all 5 banana cultivars exhibited very wide range of variations in bunch wt. and number of hands/bunch. Even some of the somatic embryo derived somaclonal variation exhibited better yield performances than those of shoot-tip culture derived plants. This results ellucidated that genetic variation of banana cultivars could be widen further through the use of somatic embryo derived plants that could open an opportunity for selection of elite clones. Improvement of banana cultivar by somaclonal variation was first demonstrated by Hwang and Ko (1988) who selected somaclones resistant to *Fusarium oxysporum*. These clones were further improved by somaclonal variation for better field performances (Hwang and Ko 1990, Hwang and Tang 1995, Khayat *et al* 2004) reported significant improvement of yield (bunches/hectare) performance of Grande-Naine through the selection of somaclonal varients.

In spite of the work reported here on somaclonal variation in banana, the genetic aspects of this phenomenon are unknown. The lack of understanding of the genetic evants leading to somaclonal variation puts severe constrains on procedure of tissue cultured banana plants. At the same time it opens an opportunity for selection and improvement of new cultivars with better agronomic characters. Present study shows that use of shoot culture for micropropagation may be minimize the incidence of somaclonal variation for banana cultivars included in this study. Furthermore, the high incidence of somaclonal variations among the plants regenerated through somatic embryogenesis could be used in breeding programme for improvement of banana cultivars.

3.6. SUMMARY

Immature male flowers of cultivars Sabari, Mehersagar, Ranginsagar, Jahaji and Agniswar were surface sterilize by dipping in ethanol (70%) for one min and cultured onto semisolid MS medium supplemented with different concentrations and combinations of 2,4-D, BAP, IAA, NAA and 1mg/l biotin. The cultures were incubated dark at 25±1°C for 4-7 months. The cultures were monitored periodically and after the emergence of somatic embryos, the culture dishes were transferred to light (2000 lux, 16 h/day). Fully developed heart and torpedo shaped embryos were individually transferred to germination medium that consisted of MS salts + Morel vitamins and supplemented with different concentrations of BAP (0.1-1.0 mg/l) with 2 mg/l IAA. Induction of callus from the male flowers and subsequent somatic embryogenesis were found to vary with culture media formulations and also with banana genotypes. Among the different media formulations 4 mg/l 2,4-D + 1 mg/l each of IAA, NAA and biotin was found to the most effective growth regulator formulation for callus induction and growth. Among the five cultivars male flowers of Sabari was found to show the highest response to callus induction and subsequent embryo formation. Somatic embryos when individually transferred to germination media, were continued their root and shoot differentation and eventually developed to miniplant. Somatic embryos of all five-banana cultivars were dedifferentiated into plantlets. However, extent of plantlet regeneration was also found to vary with BAP level and banana genotypes. Among the three BAP level 0.5 mg/l was found most effective in embryo germination. The highest degree of somatic embryo germination (70%) was noticed in cv. Sabari.

The embryo derived plantlets after proper acclimatization were transplanted to field and were grown to maturity along with shoot-tip culture derived plants as control. The embryo derived and shoot-tip culture derived plant were assessed for the occurrence of somaclonal variation using different morphological and yield related characters such as, dwarf off-type plants, leaf abnormality, stem colour,

phyllotaxy, inflorescence abnormality, bunch wt./plant, no. of hands/bunch and no. of fingers/hand. Occurrence of somaclonal variation as manifested from the presence of various abnormalities in morphological characters, was very high among the somatic embryo derived plants than shoot-tip derived plants. The extent of incidence of somaclonal variation among the somatic embryo derived plants was to vary with genotypes. The highest incidence of somaclonal variation was noticed in cv. Agnisawar (58%) followed by cv. Sabari (44%). Somatic embryo derived plants of all banana cultivars at maturity also exhibited wide range of variation in yield (bunch wt./plant) and yield contributing characters.

Chapter IV

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