BIOCHEMICAL CHANGES IN MICROPROPAGATED BANANA (*Musa* sp.) cv. DWARF CAVENDISH (AAA) DUE TO ARBUSCULAR-MYCORRHIZAL FUNGI (AMF) INOCULATION DURING *EX VITRO* ACCLIMATIZATION

THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF

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in

Horticulture

By

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Thesis submitted in partial fulfillment of the requirements for the award of the degree of

DOCTOR OF PHILOSOPHY

IN

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by

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Place: Varanasi ***(Vartika Srivastava)***

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ABBREVIATION

µg	Microgram
µM	Micromolar
2-iP	2-Isopentenyl adenine
AMF	Arbuscular-mycorrhizal fungi
APX	Ascorbate peroxidase
BAP	6-benzylaminopurine
Ca	Calcium
$CaCl_2.2H_2O$	Calcium chloride
CAT	Catalase
Cl	Chlorine
cm	Centimeter
CO_2	Carbon dioxide
$CoCl_2.6H_2O$	Cobaltus chloride
Cu	Copper
$CuSO_4.5H_2O$	Copper sulphate
Fe	Iron
$FeSO_4.7H_2O$	Iron sulphate
FYM	Farm yard manure
g	Gram
H_2O_2	Hydrogen peroxide
H_2SO_4	Sulfuric acid
H_3BO_3	Boric acid
ha	Hectare
HCl	Hydrochloric acid
$HClO_4$	Perchloric acid
HNO_3	Nitric acid
IAA	Indole-3-acetic acid
IBA	Indole-3-butyric acid
K	Potassium
kg	Kilogram
KH_2PO_4	Potassium di-hydrogen orthophosphate
KI	Potassium iodide
KNO_3	Potassium nitrate
KOH	Potassium hydroxide
M	Molar
Mg	Magnesium
mg	Milligram
$MgSO_4.7H_2O$	Magnesium sulphate
mL	Milliliter
mm	millimeter
mM	Millimolar
Mn	Manganese
$MnSO_4.4H_2O$	Manganese sulphate
MS	Murashige and Skoog
N	Normal

$Na_2EDTA.2H_2O$	Disodium EDTA
$Na_2MoO_4.2H_2O$	Disodium molybdate
NAA	Napthaleneacetic acid
NaOH	Sodium hydroxide
NH_4NO_3	Ammonium nitrate
nm	Nanometre
P	Phosphorus
PBZ	Paclobutrazol
POX	Peroxidase
PPO	Polyphenol oxidase
SOD	Superoxide dismutase
TDZ	Thidiazuron
Zn	Zinc
$ZnSO_4.7H_2O$	Zinc sulphate

PREFACE

Since this thesis is written as the final thesis for doctoral degree in Horticulture, it is primarily aimed to understand the morpho-physiological and biochemical changes in micropropagated banana under control and mycorrhized condition during hardening under water stress and I hope it will be of interest of Horticulturists and Biochemists in elucidating the mechanism of water stress resistance in plants especially banana due to mycorrhizal inoculation. The present study deals with the problems encountered during the hardening phase of the banana plantlets under low input conditions like limited water supply and reduced nutrients without use of fungicide or pesticides. Also this study will be helpful in ascertaining the role of mycorrhiza in morpho-physiological changes under moisture stress as well as to recognize the biochemical changes associated with biotization of micropropagated banana.

The research work was carried out during 2008-09 in the tissue culture lab and 2009-10 in the net-house of the Institute of Agricultural Sciences, Banaras Hindu University, Varanasi under the supervision of Dr. Anand Kumar Singh.

Chapter I provides introduction part, which includes general account of the topic, objectives of the study and its relevance in agriculture.

Chapter II, Review of literature deals with current situation of banana micropropagation, use of mycorrhiza during hardening of plantlets, morphological, physiological and biochemical changes associated with the addition of mycorrhiza under moisture stress. It also explains the research work carried out by different workers in this direction.

Chapter III, Materials and Methods deals with the methodology adopted for carrying out the experimental work, treatment details and method used for statistical analysis.

Chapter IV, Experimental findings deals with the results of research work along with graphs and tables.

Chapter V, Discussion explains the findings in the light of available literature.

Chapter VI, Summary and conclusion provides brief description of findings and the conclusions drawn from the present investigation.

Chapter VII, Bibliography deals with the references consulted during the course of study.

Chapter I

Introduction

Chapter I

INTRODUCTION

Banana (*Musa* sp.) which belongs to the family Musaceae, is the man's oldest and most valued fruit crop. It is the cheapest, plentiful and most nourishing of all fruits. It contains nearly all essential nutrients including minerals and vitamins and has several medicinal properties. It is prized for its nutritive value with high carbohydrates (22.2%), fiber (0.84%) and protein (1.1%) with less fat (0.2%) and water (75.7%) thus considered as rich source of energy (Stover and Simmonds, 1987). Banana is the fourth important global food commodity. It is the major fruit crop of tropics and sub-tropics and makes a vital contribution in economy of the world (Ray, 2001)**.**

The word banana is derived from the Arabic word "*banan*" for finger. Banana, by virtue of its multiple uses, is popularly known as '*Kalpataru*' (a plant with virtues). Apart from fresh consumption as dessert fruit, some types are also used for culinary purposes. The flower bud and also the central core of the pseudostem are made into tasty dishes. Banana leaf is used as 'biological plate' for serving food. Leaf sheaths are used as wrapping material and dry leaves as fuel. Pseudostem, leaves, underground rhizomes are used as cattle feed. Various processed products like banana puree, powder, flour, chips, vinegar, jam, jelly, wine can be prepared from it. Banana fiber can be made into attractive napkins, placemats, carry bags, etc. On-farm consumption is considerable, concerning three quarters of world production.

The dessert banana has a therapeutic value. Ripe banana is easily digestible, highly refreshing, invigorating and nutritionally far superior to the other tropical fruits. Therefore, it can be used as food for three month old babies. It is a suitable fruit for persons with high blood pressure and heart diseases as it contains less sodium, little fat and no cholesterol. Banana is ideal for patients with gout or arthritis as it is free from substances that give rise to uric acid. Banana is included in the special diets of kidney disease sufferers because of low sodium and protein content in the fruit. It is also used in ulcer therapy due to its capacity to neutralize free hydrochloric acid. Abnormally fat people may also use banana in their diet due to low lipid content of the fruit.

The edible banana is believed to have originated in the hot, tropical regions of south-east Asia (Sauer, 1952) stretching from India to New Guinea. Plants of the Musaceae family as a whole are strictly old world plants and predominantly Asian (Simmonds, 1966). India stands second largest in diversity of indigenous bananas in the world. More than 600 types of *Musa* germplasms comprising wild forms and cultivated species are reported to be world over. India has more than 300 types of germplasms but only a few are commercially important.

The world's production of banana is about 97 million tonnes and most of the production is consumed locally. In India, it is the most important fruit crop and is grown in 0.74 million hectares with a total production of 26.99 million tonnes and an average productivity of 36.08 tonnes per hectare (NHB, 2009). Several cultivars of banana are cultivated in the country among which, Dwarf Cavendish and Robusta are predominantly grown because of higher yields, resistance to strong winds and short cropping duration besides a good profit (Singh, 1990). Other cultivars that are considered of commercial importance are Poovan, Rasthali, Lalkela, Safed velchi and Karibale monthan. Banana is severely affected by viral (bunchy top virus, cucumber mosaic virus, banana streak virus), bacterial (moko or bacterial wilt, bacterial soft rot), fungal (black sigatoka, fusarium wilt) diseases besides vulnerable to insects/pests and nematodes. It is a long duration crop of one and a half years and is conventionally propagated through suckers. However, transmission of soil borne diseases through rhizome and viral infection causing bunchy top are major bottlenecks in propagation of banana through conventional method. Besides, this method is slow and season bound. The production of suckers varies in different genotypes ranging from 5-10 per plant per year. Crop productivity and maturity is dependent on the size and age of suckers and uneven maturity extends the duration by 3-4 months. Suckers also carry soil nematodes, disease causing organisms such as bunchy top virus, leaf spot etc., thereby affecting the crop production considerably. Therefore, biotechnological approaches such as cell and tissue culture, protoplast fusion and gene transfer are far reaching to be used as useful tools (Novak *et al*., 1993).

In vitro propagation of banana provides excellent advantages over traditional propagation by way of high multiplication rate, physiological uniformity, year round

availability of disease-free material, and rapid dissemination of new plant materials throughout the world, uniformity of shoots, short harvest interval and faster growth in the early growing stages compared to conventional materials (Vuylsteke, 1989; Arias, 1992). Tissue culture also plays a vital role in the distribution and conservation of germplasm, safe exchange of planting materials and rapid propagation of newly selected hybrid cultivars.

The prevalence of diseases and the need for generating clean planting stock in large quantities have recently stimulated a surge of interest in the production of banana clones raised through aseptic micropropagation techniques. In the recent years, tissue culture propagation of banana through shoot-tip as well as floral apices has been utilized to increase production. The process involves initiation of cultures from sterilized shoot-tips obtained from the parent banana plant, shooting and rooting in the test tube, primary hardening in the laboratory, secondary hardening in the nursery and planting in the field.

In certain plant species, establishment of tissue culture raised plants under glasshouse and later in field is often very poor. This is mainly attributed to the inability of such plants to tolerate different type of stresses such as transplant shock, excessive water loss, pathogen attack, poor photosynthesis etc.

Mycorrhizal inoculation to *in vitro* propagated transplants has been found effective in respect of tolerance to different stresses, improvement in vegetative growth and mineral nutrient status (Gianinazzi *et al.*, 1989). The benefits of mycorrhiza for micropropagated plantlets have been reported in high value crops such as grapes, oil palm, apple, plum, pineapple, avocado, strawberry, raspberry, cherry, pear, *Hortensia* spp. and Rhododendron (Varma and Schuepp, 1995; Fortuna *et al.*, 1996; Lovato *et al.*, 1996; Azcon-Aguilar and Barea, 1997). Banana shows a great ability to establish mycorrhizal symbiosis (Jaizme-Vega *et al.*, 1991, 1998, 2002; Rizzardi, 1990; Declerck *et al.*, 1994; Yano-Melo *et al.*, 1999). However, despite the great number of studies on banana, there are not many references concerning the effect of rhizosphere microbiota on tissue cultured banana root architecture (Jaizme-Vega *et al.*, 1994; Garcia-Perez and Jaizme-Vega, 1997) and their consequences on

plant growth and health. Also, very less work is done regarding the biochemical changes taking place alongwith the physiological adjustments in plant system due to mycorrhizal inoculation under various stresses.

In nature plantlets were frequently exposed to adverse environmental conditions that have a negative effect on plant survival, development and productivity. Drought is considered as most important abiotic factor limiting plant growth and yield. The findings of present investigation with micropropagated banana under water stress during hardening phase would be helpful in ascertaining the role of arbuscular-mycorrhizal fungi in growth and development of banana plantlets. The study of biochemical parameters would be helpful in finding the answers to the morphological changes in plantlets occurring due to mycorrhizal fungi treatments.

Keeping these in view the present investigation entitled "Biochemical changes in micropropagated banana (*Musa* sp.) cv. Dwarf Cavendish (AAA) due to arbuscular-mycorrhizal fungi (AMF) inoculation during *ex vitro* acclimatization" was undertaken with the following objectives:

1. To develop the protocol for micropropagation of banana cv. Dwarf Cavendish.
2. To study the effect of mycorrhizae on growth of the micropropagated banana plantlets during the acclimatization phase under moisture stress.
3. To study the biochemical changes in the AMF treated and untreated plantlets under water stress conditions.

Chapter II

Review of Literature

Chapter II

REVIEW OF LITERATURE

2.1. BANANA TISSUE CULTURE

Banana is conventionally propagated through suckers because being a triploid plant, seed setting and propagation by seed is not possible. The major problem in propagation through conventional method is the transmission of soil born diseases through rhizome and viral infection causing bunchy top. Besides, this method is slow and season bound. Now-a-days, micropropagation of banana is being practiced on commercial scale.

The first report on banana tissue culture came in early 1970's from Taiwan when Ma and Shii (1972) produced *in vitro* adventitious buds from decapitated shoot apex of banana. In 1974, Berg and Bustamante from Honduras, used meristem culture in combination with thermotherapy for the production of virus free banana plants. Since then, various protocols regarding different aspects of banana tissue culture were developed by various workers as a key tool for improving overall banana production. Majority of workers used MS medium with some additional components. Isolated meristems, which were initially creamy and usually begin to turn green within 4 days, were the most commonly used explants for micropropagation of banana. The pace at which growth proceeded and the growth form obtained largely depended on the type of culture medium (liquid or semisolid).

2.1.1. Selection of explant

The most commonly used explant sources to initiate banana cultures are shoot apices obtained from removing leaf sheaths from the pseudostem, suckers, peepers, lateral buds and terminal inflorescence. The terminal floral apex and axillary flower buds marked morphogenetic plasticity in their juvenile stage and can be induced to revert to vegetative growth, producing multiple shoots *in vitro*. Banana meristem culture is now commonly used for the clonal multiplication and maintenance of banana cultivars. Regeneration of meristems proceeds through organogenesis instead of somatic embryogenesis due to which sometimes chimeric plants are obtained

(Hwang *et al*., 1984; Banerjee and Sharma, 1988; Blakesley, 1991; Alvard *et al*., 1993; Sagi *et al*., 1995; Mendes *et al*., 1996, 1999; Jasrai *et al*., 1999; Nandwani *et al*., 2000; Madhulatha *et al*., 2004).

Micropropagation of banana attempted from different parts of the plant has shown the success of shoot-tip culture as the most potential source to reduce the spread of disease. Micropropagation has become popular in many banana growing regions. Doreswamy *et al.* (1983) explored the possibilities of banana propagation through tissue culture. Shoot-tips isolated from the rhizomes were found suitable for plantlet production *in vitro*. Shoot-tips with several older sheathing leaf bases enclosing the axillary buds regenerated in to multiple plantlets. When separated and subcultured, they produced a new crop of multiple shoots. Cronauer and Krikorian (1984) successfully established rapidly multiplying cultures of two dessert and two plantain clones from isolated shoot-tips on modified MS medium supplemented with 5 mgL^{-1} BA. Apices cultured on semi-solid media produced single shoot while those placed in liquid media produced shoot clusters. Hwang *et al.* (1984) successfully established in the field, plantlets originated from adventitious buds of explants from the decapitated shoot apex of a Cavendish banana sucker. These gave rise to mature plants with uniform growth and normal yield. Cronauer and Krikorian (1985) isolated and cultured the terminal floral apices of Dwarf Cavendish banana in the modified MS medium supplemented with BA (5 mgL^{-1}) 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 mgL^{-1}) and activated charcoal (0.025%).

Wong (1986) described *in vitro* multiplication of banana from shoot-tip explants isolated from lateral suckers. He used explants with apical domes and cultured successfully 22 banana cultivars on modified MS medium containing 6-benzylaminopurine (BA) and indole-3-butyric acid (IBA). Shoot-tip explants produced multiple shoot initials in the presence or absence of apical domes, but the survival rates were higher when apical domes were retained. Cultivars varied widely in their multiplication rates in response to cytokinins and BA was found to be more

effective than kinetin. Although kinetin was less effective in this regard, it stimulated vigorous root growth. Rooted plantlets were successfully established in soil.

Gupta (1986) attempted meristem-tip culture for rapid clonal multiplication of mosaic-free planting materials. Heat therapy and meristem tip culturing were used in various cultivars of banana. Suckers were subjected to heat therapy at 38-40°C for 14 days prior to the culture of their meristem tips (1.5-2.0 mm long having 6-8 vertical incisions) on modified MS medium containing 1.0 mgL^{-1} thiamine HCl, 0.5 mgL^{-1} nicotinic acid, 0.5 mgL^{-1} pyridoxine HCl, 25 mgL^{-1} ascorbic acid (filter sterilized), 0.7 mgL^{-1} kinetin. This culture medium alone was effective in preventing the oxidation of phenolic compounds present in explants, and in producing up to 13 rooted plantlets from a single meristem within 10-12 weeks. Plants derived from heat-treated meristems of infected plants were free from the disease, as determined by visual inspection, mechanical inoculation to *Cucumis sativus* and electron microscopy. Micropropagation of *Musa* was achieved through shoot proliferation and somatic embryogenesis using varying explants such as shoot-tip, meristem and flower suspension culture Vuylsteke (1998).

Navarro *et al.* (1997) regenerated banana plants via somatic embryogenesis of diploid (*Musa acuminata* spp. *malaccensis*) and triploid (Grand Nain) bananas from immature zygotic embryos and male flower bud primordia.

Kosky *et al*. (2002) initiated cell suspension of the hybrid cultivar FHIA-18 (AAAB) which was established from sections of embryogenic tissue derived from male flowers. Plant regeneration from embryogenic suspension cultures of *Musa acuminata* cv. Mas (AA) from male inflorescence was done by Jalil *et al.* (2003).

Micropropagation assures the supply of quality planting material on regular basis. Though no genetic improvement is expected from shoot-tip culture, planting materials obtained through shoot-tip culture have proved better over traditional planting materials under proper management conditions. Even under low input conditions, the performance of micropropagated plants show uniformity in flowering and harvest. 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.

2.1.2. Banana explant surface sterilization protocols

Generally the explants for *in vitro* culture initiation are collected from field grown plants, so the plant materials are liable to be contaminated by microorganisms. Therefore, they must be disinfected before being transferred to *in vitro* conditions.

Berg and Bustamante (1974) initiated the *in vitro* cultures without any disinfection. They were the first to realize the potential of meristem culture for the production of disease free banana plantlets. They found that heat treatment of rhizomes or meristem cultures *in vitro* was not sufficient alone to obtain virus free plantlets. However, by heat treatment of both the rhizomes and grown *in vitro* plantlets, they were able to obtain a banana culture that was 75% virus free. They also concluded that the smaller the explant the greater was the likelihood that any viral contaminant might be eliminated.

Gupta (1986) reported the successful use of thermotherapy for the production of mosaic virus free banana and plantain using a technique very similar to that used by Berg and Bustamante (1974). Suckers were subjected to heat therapy at 38-40°C for 14 days prior to the culture of their meristem tips (1.5-2.0mm long having 6-9 vertical incisions) on modified MS medium containing 1.0 mgL^{-1} thiamine HCl, 0.5 mgL^{-1} nicotinic acid, 0.5 mgL^{-1} pyridoxine HCl, 25 mgL^{-1} ascorbic acid (filter sterilized).

Variations in sterilization procedures have been proposed by several researchers. Some reports suggest that sodium hypochlorite is the most effective disinfectant for surface sterilization of banana explants (Sandra and Krikorian, 1984; Mendes *et al*., 1996; Muhammad *et al*., 2004). For the explant disinfected after excision, a shorter time and a lower hypochlorite concentration (0.0525%) have been effective (Vessey and Rivera, 1981; Krikorian and Cronauer, 1984). Some investigators have replaced sodium hypochlorite with low concentrations of mercuric chloride (Banerjee and Sharma, 1988; Habiba *et al*., 2002; Titov *et al*., 2006). Double sterilization technique has also been adopted by several workers, in which first large

sized explant is disinfected, followed by shoot tip excision and finally sterilization with some other surface disinfectant (Silva *et al*., 1998a; Nandwani *et al*., 2000; Madhulatha *et al*., 2004). Sometimes explants are treated with fungicides and antibiotics to minimize the contamination *in vitro* culture (Van den Houwe *et al*., 1998; Nandwani *et al.*, 2000). Ethanol has also been used by a number of research workers for disinfection purposes (Silva *et al.*, 1998a; Rahman *et al.*, 2002; Jalil *et al.*, 2003).

Hamill *et al.* (1993) preferred double sterilization over single sterilization methods for rapid processing of large consignments in banana tissue culture. They recommended treatment with 3.5% sodium hypochlorite with Tween 80 for 15 minutes followed by removing bleached tissues and again sterilization with sodium hypochlorite (3.5%) for 5 minutes.

2.1.3. Culture initiation:

As soon as the shoot tips are isolated after following proper sterilization protocol, explants are cultured *in vitro* for shoot initiation. At this stage, the explant consists of the shoot apical meristem, covered by 3-6 leaf primordia and small portion of rhizomatous tissue. The shoot tip is cultured on the medium intact, wounded or fragmented in to pieces. Decapitatation of shoot tips before culture initiation was done by Ma and Shii (1972) and Hwang *et al.* (1984). A number of vertical cuts into the meristematic dome were applied by Vessey and Rivera (1981) and Jarret *et al.* (1985). The numbers of incisions ranged from 2-10 and were made in such a way that the base of explant was kept intact. A number of investigators (Cronauer and Krikorian, 1984; Jarret *et al.*, 1985) used fragmentation procedure in which shoot apex was cut longitudinally into halves or more pieces and used as individual explants.

Different media compositions were used for culture initiation. Some investigators used same media composition for initiation as well as multiplication, while others used low concentrations of hormones for culture initiation. Most commonly used culture media for banana tissue culture was MS media with some modifications as reported by many workers (Hwang *et al.*, 1984; Cronauer and

Krikorian, 1984; Thomas *et al.*, 1995; Silva *et al.*, 1998a; Nandwani *et al.*, 2000; Roels *et al.*, 2005).

Assani *et al*. (2003) initiated cultures using anthers on MS medium containing vitamins of Morel supplemented with 500 mgL^{-1} casein hydrolysate, 4.4µm BAP and 2.3µm IAA.

When kinetin was used as sole cytokinin, adenine sulphate was also added in the medium as a conducive agent for shoot induction (Hwang *et al.*, 1984; Nandwani *et al.*, 2000).

Some investigators reported only single cytokinin for culture initiation (Cronauer and Krikorian, 1984; Thomas *et al.*, 1995; Silva *et al.*, 1998a; Roels *et al.*, 2005), while others used mixture of cytokinins (Nandwani *et al.*, 2000). A combination of cytokinin and auxin was also used for banana culture initiation by a number of researchers (Hwang *et al.*, 1984; Zaffari *et al*., 2000).

2.1.4. *In vitro* multiplication:

Plant growth regulators are essential media components for the manipulation of growth and development of explants *in vitro*. Their concentrations and ratio in the medium often determine the pattern of development *in vitro.* Cytokinins and auxins are used as growth regulators for banana tissue culture. The most widely used and the most effective cytokinin for this purpose is adenine based cytokinin BAP.

Sandra and Krikorian (1984) recorded 9.1 shoots per explant during *in vitro* multiplication of 'Philippine Lacatan' and 'Grande Naine' on a modified MS medium supplemented with 5 mgL^{-1} 6-benzylaminopurine (BAP). On the other hand Rahaman *et al*. (2002) achieved 4.52 shoots per explant on the same concentration of BAP on MS medium during *in vitro* multiplication of Bari-1, indicating the genotypic response towards cytokinins. Highest shoot length (3.62 cm) was achieved when MS medium was supplemented with 1.5 mgL^{-1} NAA.

Mendes *et al*. (1996) used 4.5 mgL^{-1} BAP in MS medium during the study of the behavior of vegetative apices coming from different rhizomes. They reported that

shoot-tips coming from different rhizomes behaved differently under *in vitro* conditions, some were highly productive and others produced a much smaller number of plants under similar culture conditions.

Micropropagation of banana (*Musa paradisiaca*) through cormlet initiation by *in vitro* culture of apical meristem slices was achieved by Priyono (2001). He reported cormlet production on medium supplemented with 5-20 mgL^{-1} BAP combined either with 10-40 percent sucrose or 5-20 mgL^{-1} ancymidol (ANC). Venkatachalam *et al*. (2006) achieved direct shoot regeneration from leaf sheaths of silk banana (AAB), when cultured on medium containing 22.5 µm BA.

Some researchers have reported that a combination of BAP and auxins enhanced shoot proliferation and shoot length during tissue culture of banana Bhagyalakshmi and Singh (1995) used MS medium with 8.9 M benzyladenine and 0.98 M indolebutyric acid during shoot culture of three cultivars of banana, Cavendish, Bluggoe and Silk. Similarly Okole and Schulz (1996) used MS medium along with 10 µM BAP and 1 µM IAA for shoot multiplication during culture of leaf segments from banana plants as an alternate approach for the production of adventitious shoots and callus. An average of 15 shoot buds were obtained from micro cross sections derived from each explant on a shoot-inducing multiplication medium.

Assani *et al*. (2003) reported production of haploids from anther culture of banana [*Musa balbisiana* (BB)]. The solid culture medium consisted of MS salts, vitamins of Morel (Morel and Wetmore, 1951), 500 mgL^{-1} casein hydrolysate, 73 mM sucrose, 4.4 µM BAP, 2.3 µM IAA and 6 gl^{-1} agarose. Androgenic embryos were regenerated on MS medium supplemented with vitamins of Morel, 88 mM sucrose, 2.2 µM BAP and 2.3 µM IAA.

Hwang *et al*. (1984) added 2 mgL^{-1} kinetin and 2 mgL^{-1} indole-3-aceticacid (IAA) in MS medium during meristem culture of banana and found that the population of buds increased by five times per month.

Wong (1986) compared kinetin and 6-benzylaminopurine (BAP) along with indole-3-butyric acid (IBA) during *in vitro* multiplication of banana (*Musa* spp.) and found that BAP was more effective than kinetin.

Gupta (1986) and Madhulatha *et al*. (2004) used equal concentrations of BAP and kinetin on MS medium for multiplication. Rahman *et al*. (2002) used BAP, kinetin and 2-iP each at 5 mgL^{-1} alone or in combination during shoot multiplication of cv. Sabri and found that MS medium supplemented with 5 mgL^{-1} each of BAP and kinetin produced the highest number of shoots (3.11) per explant.

Arinaitwe *et al*. (2000) used MS modified medium supplemented with various equimolar concentrations (16.8, 20.8, 24.8, 28.8 mM) of BAP, TDZ, ZN, 2-iP and kinetin to determine the suitable concentration ranges of the cytokinins for micropropagation of banana cultivars. The results showed that shoot proliferation was dependent on cytokinin type, its concentration and the banana cultivar.

Growth retardants are used along with cytokinins to increase the number of multiple shoots per explant. Morphological parameters of Grand Naine banana (*Musa* AAA) after *in vitro* multiplication with growth retardants was studied by a number of research workers. Albany *et al*. (2005) reported the benefits of supplementation of MS medium with 1.0 mgL^{-1} thiamine-HCl and 17.7 µM BAP, 9.75 µM ANC or 8.5 µM PBZ. The addition of ANC induced significant differences in plant height during the acclimatization stage. The incorporation of growth retardants (GR) such as ANC or paclobutrazol (PBZ) in liquid culture media during multiplication stage of bananas decreased the excessive growth of stems and leaves.

Okole and Schulz (1996) induced embryogenic callus by placing micro-cross sections on a Gamborg B-5 medium having 10 µM 3,6-dichloro-o-anisic acid (dicamba) and 1 µM IAA. An average of 5-6 plantlets regenerated from each micro-cross section.

During micropropagation of banana, blackening and necrosis of tissues is commonly observed which interfere with plant growth. Martin *et al*. (2007) controlled tissue necrosis of cultivar Grand Naine (AAA), Dwarf Cavendish (AAA), Nendran

(AAB) and Quintal Nendran (AAB) by the addition of 50-100 mgL^{-1} calcium chloride in the MS medium. Titov *et al*. (2006) controlled oxidation of phenolic compounds secreted by floral explants, by washing them in 0.125 percent potassium citrate: citrate solution before culturing.

Physical state of the medium also played an important role in tissue culture of banana. In most of the research laboratories solid medium is used. Role of liquid verses agar-gelled media in mass propagation and *ex vitro* survival of banana was studied by many workers. Bhagyalakshmi and Singh (1995) during shoot-tip culture of Bluggoe and Silk evaluated agar-gelled, agitated liquid and static liquid media for their ability to support shoot multiplication and *ex vitro* survival. Liquid media was found better for shoot multiplication whereas agar-gelled medium supported maximum *ex vitro* survival.

Alvard *et al*. (1993) described an elegant liquid tissue culture system, based on temporary immersion of explants with liquid medium for 20 minutes after every 2 hours. The results indicated that shoots in liquid medium and those in cellulose substrate, proliferated little or not at all. Shoots on gelled medium subjected to partial immersion, and those in aerated medium displayed multiplication rates of 2.2 to 3.1, and the highest multiplication rate was observed in explants subjected to temporary immersion in the liquid medium.

Kosky *et al*. (2002) reported somatic embryogenesis of the banana hybrid cultivar FHIA-18 (AAAB) in liquid medium and scaled-up. The secondary multiplication of somatic embryos in liquid media on rotary shaker and in bioreactors was successfully achieved.

The effect of liquid pulse treatment of growth regulators on *in vitro* propagation of banana (*Musa* sp. AAA) was studied by Madhulatha *et al*. (2004). Optimal shoot proliferation rate was achieved due to the pulse treatment of 6-benzylaminopurine (BA) and kinetin combination (1:1) at the concentration of 50 mgL^{-1} for 60 minutes. Similarly high frequency of root induction was obtained due to pulse treatment with combination of NAA and IBA (1:1) at a concentration of 100 mgL^{-1} each for 60 minutes.

2.1.5. *In vitro* rooting:

In vitro multiplication of banana is normally carried in the presence of high cytokinin levels which inhibit root formation and elongation. Moreover, during *in vitro* multiplication, shoots may lack roots and grow in the form of bunches which cannot be transferred directly to field conditions. Prior to transfer in free living conditions, individual shoots are separated from cluster and grown on root induction media.

Doreswamy *et al*. (1983) first time used IBA during banana tissue culture. Later, Hwang *et al*. (1984) regenerated roots from *in vitro* plants of *Musa sapientum* L. on MS medium to which 1 gl^{-1} activated charcoal was added. After four weeks of incubation, the plants developed numerous roots and were ready for transfer in the field.

Sandra and Krikorian (1984) added IAA, NAA and IBA at the rate of 1 mgL^{-1} along with 0.025 percent activated charcoal to MS medium and were able to achieve encouraging results for *in vitro* rooting of banana plants which were in conformity with the findings of Cronauer and Krikorian (1984); Jalil *et al*. (2003) and Srangsam and Kanchanapoom (2003).

The concentration of cytokinin in the rooting medium should be lower than that of auxin, so that cytokinin:auxin ratio may become low which is favorable for root induction as reported by Gupta (1986) and Wong (1986). However most of the investigators omit cytokinins entirely from the rooting medium. The most commonly incorporated auxins in rooting medium were NAA, IAA and IBA.

Banerjee and Sharma (1988) achieved rooting on semi-solid medium with IBA (0.2 mgL^{-1}) during plant regeneration from long term banana cultures. Nandwani *et al*. (2000) found 1.0 mgL^{-1} IBA suitable in MS medium during mass propagation of Basrai. Further, Habiba *et al*. (2002) and Muhammad *et al*. (2000, 2004) regenerated roots on half strength MS medium having 1 and 2 mgL^{-1} IBA respectively. The use of IBA and NAA in combination during optimization of liquid pulse treatment for

production of *in vitro* rooted plants of cv. Nendran (*Musa* spp. AAA) was reported successfully by Madhulatha *et al*. (2004, 2006).

Napthalene acetic acid (NAA) was another auxin used frequently at lower concentrations for root induction of *in vitro* raised banana plants. Cronauer and Krikorian (1984) found 1 mgL^{-1} NAA to be satisfactory in *Musa textiles*, AAA and AAB bananas respectively. Arinaitwe *et al*. (2000) achieved rooting on MS medium containing 1.2 µM NAA during the study of proliferation rate effects of cytokinin on Kibuzi, Bwara and Ndizwemiti banana cultivars. Rahman *et al*. (2004) used different concentrations of NAA for root induction of *Musa sapientum* and found that 2 mgL^{-1} was better.

Jasrai *et al*. (1999) used 0.1 µM IAA on MS medium during the study of *ex vitro* survival of *in vitro* derived banana plants without green house facilities, while Assani *et al*. (2003) used MS medium containing 1.2 mM NH_4NO_3 for root induction of haploids regenerated from anther culture of banana (*Musa balbisiana* BB).

There are reports that roots can be induced without growth regulators (Albany *et al*., 2005; Silva *et al*., 1998a) but most of the authors agreed with the inclusion of the growth regulators for root induction.

2.2. HARDENING OF MICROPROPAGATED BANANA PLANTS:

The transfer of *in vitro* rooted plantlets directly from aseptic culture conditions to external environment can result in significant losses of plant. The micropropagated plants, after removal from culture room must be allowed to adjust to the outside environment associated with varying light levels, changing temperature, reduced humidity, lower nutrient availability and pathogen attack. Tissue cultured plants are generally poor in cuticle and losses water rapidly upon transfer to natural conditions. Moreover, due to limited space and presence of excess carbon source, their photosynthetic apparatus does not fully develop. As a result of which their energy demands are fulfilled by reserves of starch accumulated during culturing. These reserves fastly deplete and create a crisis of availability of energy during hardening.

During *in vitro* culture, plantlets grow in air-tight cultivation vessel, which provides higher air humidity and lower irradiance than those prevail in conventional culture. The use of closed vessels in order to prevent microbial contamination decreases air turbulence which increases leaf boundary layers and limits the inflow of CO_2 and outflow of gaseous plant products from the vessels. The cultivation media are often supplemented by saccharides as carbon and energy sources. This addition considerably decreases the water potential of the medium and increases the risk of bacterial and fungal contamination. Furthermore, the plantlets are usually supplied with large doses of growth regulators. These conditions result in the formation of plantlets of abnormal morphology, anatomy and physiology (Kozai, 1991; Pospíšilová *et al.*, 1992, 1997; Buddendorf-Joosten and Woltering, 1994; Desjardins, 1995; Kozai and Smith, 1995).

After transfer from the *in vitro* to the *ex vitro* conditions the plantlets have to correct the above-mentioned abnormalities. In the greenhouse, and especially in the field, irradiance is much higher and air humidity is much lower than those prevail in the vessels. Even if the water potential of the substrate is higher than the water potential of media with saccharose, the plantlets may quickly wilt as water loss of their leaves is not restricted. In addition, water supply can be limiting factor because of low hydraulic conductivity of roots and root-stem connections (Fila *et al*., 1998). Many plantlets die during this period. Therefore, after *ex vitro* transplantation plantlets usually need some weeks of acclimatization with gradual lowering in air humidity **(**Preece and Sutter, 1991; Kadleček, 1997; Bolar *et al.*, 1998).

Silva *et al.* (1998b) studied growth and development of *in vitro* raised plants of cv. Pioneira (*Musa* sp. AAAB) during hardening. *In vitro* rooted plantlets were transferred to plastic bags containing organic substrate. Different parameters of growth were recorded in green house, under tree canopy, humid chamber under tree canopy and in field conditions. All treatments showed 100 per cent plantlets growth, except for the direct field planting (39.7 percent).

Jasrai *et al.* (1999) developed protocols for hardening of *in vitro* derived banana plantlets without greenhouse facilities. *In vitro* raised plants were transferred

in polythene bags which were perforated 6 cm from base. The bags containing the plants were placed inside a plastic tray. High humidity was maintained by spraying water after every two hours. On an average 92 percent of the plantlets survived.

2.2.1. Use of arbuscular-mycorrhizal fungi during acclimatization:

Mycorrhizal inoculation to *in vitro* propagated transplants has been found effective in respect of tolerance to different stresses, improvement in vegetative growth and mineral nutrient status (Gianinazzi *et al*., 1989). The benefits of mycorrhiza for micropropagated plantlets have been reported in high value crops such as grapes, oil palm, apple, plum, pineapple, avocado, strawberry, raspberry, cherry, pear, *Hortensia* spp. and Rhododendron (Varma and Schuepp, 1995; Fortuna *et al.,* 1996; Lovato *et al.,* 1996; Azcon-Aguilar and Barea, 1997). Banana shows a great ability to establish mycorrhizal symbiosis (Jaizme-Vega *et al.* 1991, 1998, 2002; Rizzardi, 1990; Declerck *et al.,* 1994; Yano-Melo *et al.,* 1999).

Yao-Qing *et al.* (2004) reported that mycorrhizal colonization of banana plantlets varied from 22.8 to 32.9% when treated with a single fungal species (*G. versiforme*) and inoculation with mixed fungal species (*G. versiforme, G. epigaeum* [*G. versiforme*] and *G. caledonium*).

In a study of root colonization percentage with thirty three banana genotypes belonging to five genomic groups (AAA, AAB, ABB, AABB and AAAB), Panja *et al.* (2007) reported that arbuscular-mycorrhizal fungi (AMF) did not have host specificity but have preferential association with some plants and even with the genotypes of a plant. Root colonization intensity of genotypes varied from 10% to more than 92% but the majority of the genotypes had more than 50%. Results indicated that the banana genotypes had host specific influence on the selection of specific and compatible AM fungal partner for efficient symbiotic association.

2.2.2. Morphological changes in banana plantlets due to AMF inoculation during hardening:

Various workers have demonstrated the use of mycorrhizal fungi during the hardening phase of *in vitro* grown banana plantlets. Lin and Chang (1987) obtained

increased height, diameter of the pseudostem and dry matter weight of banana plantlets inoculated with species of *Glomus*, four months after transplant from *in-vitro* culture to the greenhouse.

Micropropagated plantlets are free from diseases, but they lack arbuscular-mycorrhizal fungi (AMF). AMF are known to increase the vigor of plants by increasing absorption of water and mineral nutrients, especially phosphorus (P). Moreover, AMF can protect host plants from root pathogens and mitigate the effects of extreme variation in temperature, pH and water stress (Dixon and Marx, 1987).

Jaizme-Vega *et al.* (1991) observed that root fresh matter of micropropagated banana plantlets (*Musa acuminata* Colla AAA, subgroup Cavendish) increased simultaneously with increase in root colonization by *G. mosseae* and *G. fasciculatum*.

Declerck *et al.* (1994) reported that *G. mosseae* was more effective than *G. geosporum* in improving growth of micropropagated banana plants. They also observed that promotion of growth varied among banana cultivars. The plants inoculated with *G. macrocarpum* grew more than those inoculated with *G. mosseae* (Declerck *et al.*, 1995).

Berta *et al.* (1995) demonstrated that AMF association altered the branching pattern of roots of *Prunus cerasifera*. However, the inoculum type used in the acclimatization was important.

The beneficial effect of the symbiosis formed with the root system of plants from tissue culture manifests itself in the development of vigorous plants with high photosynthetic and transpiration rates, improved absorption of nutrients and water and increased stress tolerance (Azcon-Aguilar *et al.*, 1997; Jaizme-Vega *et al.*, 1997).

Arias *et al.* (1999) inoculated micropropagated banana plantlets with 4 arbuscular mycorrhizas (*Glomus albidum* MA1, MA2 and MA3 and *G. maculosum* MA4) at the beginning of the adaptation phase and reported high percentage of root infection in all mycorrhizal treatments. They observed that plant growth and nutrient absorption in mycorrhzed treatments was similar and did not exceed that of a basic fertilizer treatment.

Matos *et al.* (2002) conducted a greenhouse experiment to evaluate the inoculation effect of arbuscular-mycorrhizal fungus (*Glomus clarum*) on the development of good quality banana cv. Nanicao seedlings under a low input system. The results showed a significant positive effect of the inoculation on seedling height and number of leaves, 65 days after acclimatization. At 93 days, this positive effect was shown also by the pseudostem diameter. After harvest, the seedlings inoculated with *G. clarum* showed a significant increase in leaf, pseudostem and root dry matter, as well as total phosphorus content and radicular ratio efficiency in comparison to non-inoculated seedlings.

Abo-El-Ez (2003) studied the effects of combining different N and K rates (48, 96 and 144 g/plant per 4 months), in the form of ammonium nitrate and potassium sulfate, respectively, on in vitro derived 2-month-old banana cv. Williams inoculated with the vesicular arbuscular-mycorrhizal fungi, *Glomus mosseae* and *G. fasciculatum* in the greenhouse. He observed that mycorrhizas increased pseudostem length and diameter, leaf number and root diameter.

Micropropagated plants of banana cultivars Dwarf Cavendish and Robusta were inoculated during the secondary hardening stage with arbuscular mycorrhiza (AM) fungi (*Glomus fasciculatum, Gigaspora margarita* and *Acaulospora laevis*) by Mathews *et al.* (2003). After one and a half months AM fungi increased the growth of the cultivars, the positive effect of the fungi being more evident for Dwarf Cavendish plantlets inoculated with *Glomus fasciculatum*. The mycorrhizal fungi heavily colonized the root system of both cultivars. Plantlets of both cultivars inoculated with *Glomus fasciculatum* exhibited higher height (60.7% over control) and leaf area (2.2 times over control) besides increasing the pseudostem girth (39.6% over control) and shoot biomass production.

Yao-Qing *et al.* (2004) reported that mycorrhizal inoculation either singly or in combination, promoted the vegetative growth and significantly increased shoot and root dry matter of banana plantlets, with mycorrhizal dependency varying from 30.8 to 37.8%. Plant height, leaf number and leaf length also slightly increased due to inoculation. Although fibrous root number increased due to inoculation with a single

fungal species, fibrous root length decreased in both mycorrhizal treatments, resulting in decreased total root length.

2.2.3. Physiological changes in root system due to AMF inocultation:

Banana is a monocotyledonous herbaceous species that shows a great ability to establish mycorrhizal symbiosis (Jaizme-Vega *et al.*, 1991, 1998, 2002; Rizzardi, 1990; Declerck *et al.*, 1994; Yano-Melo *et al.*, 1999). However, despite the great number of studies on banana, there are not many references concerning the effect of rhizosphere microbiota on banana root architecture (Jaizme-Vega *et al.*, 1994; Garcia-Perez and Jaizme-Vega, 1997) and their consequences on plant growth and health. Among the environmental factors involved in root development (for example, soil structure, temperature, water, nutrient availability), communities of soil microbiota must be considered. Soil microbiota interactions, especially with those organisms that colonize the rhizosphere (the soil zone influenced by roots through the release of substrates that affect microbial activity), have also been reported to affect plant health and soil quality. Root-associated microbiota help the host plant under limiting conditions caused by abiotic (water, nutrition) and/or biotic (soil-borne pathogens) factors. Mycorrhizal symbiosis significantly improves plant nutrition under low soil fertility. Mycorrhizal hyphae are more efficient than roots alone in nutrient uptake, especially of elements with low mobility in soil such as phosphorus (P). Some studies have also reported changes in phytohormone balance (Drüge and Schönbeck, 1992). Recently, it has been reported that AMF are even able to change root architecture. These changes lead to more efficient nutrient uptake in mycorrhizal plants (Hooker and Atkinson, 1992).

2.2.4. Physiological and biochemical changes due to AMF inoculation under moisture stress conditions:

Water stress is considered as one of the most important environmental factors limiting growth and development of tissue culture derived plants during acclimatization. Among the diverse consequences of a drought effect on plant development restricted nutrient and water acquisition are commonly recognized (Agnew and Warren, 1996). Ninety percent of the earth's land plant species form

symbiotic associations with arbuscular-mycorrhizal fungi (Gadkar *et al*., 2001). Arbuscular-mycorrhizal (AM) symbiosis was reported to improve the water relations in many plants (Auge, 2001).

Sanchez-Diaz and Honrubia, (1994) reported that the effect of mycorrhiza is often more pronounced in plants grown under water-stressed conditions than under well-watered conditions. In other words, arbuscular mycorrhizas are able to alter plant physiology in a way that confers the plant ability to more efficiently grow under stressful conditions and cope with stresses (Miransari *et al*., 2008).

The mechanisms by which AM fungi have enhanced the water relations of host plants included enhanced absorption of water by external hyphae (Ruiz-Lozano and Azcon, 1995), stomatal regulation through hormonal signals (Goicoechea *et al*., 1997), the indirect effect of improved phosphorus nutrition (Fitter, 1988), and greater osmotic adjustment in AM plants (Auge *et al*., 1986; Ruiz-Lozano, 2003).

Menge *et al.* (1978) considered inoculation with AMF as a good strategy for successful transplantation of plant because of improved water and nutrient absorption. Guillemin *et al.* (1992) pointed out that increase in growth rate is not always related to colonization, but may be influenced by other factors, such as extension of the external mycelium and transport of nutrients from the soil to the host.

Declerck *et al.* (1995) found differences in colonization of banana cultivars by AMF isolates. According to Douds *et al.* (1998), the physiological response of a plant might be the resultant of interactions among environment, plant and fungus genotype.

Yano-Melo and Lima-Filho (1999) found that inoculation with AMF increased growth of micropropagated banana plantlets during the acclimatization period might have accelerated rates of photosynthesis and also nutrient transport by mass flow. AMF also improved the growth and the nutritional status of banana plantlets.

Krishna *et al.* (2005) observed that AMF were potential inoculants for averting transplantation shock experienced by micropropagated grape plantlets during acclimatization under glasshouse conditions. They suggested that such an association brought about gamut of changes especially in biochemical status of plants like

enhancement in the levels of chlorophyll, carotenoids, proline, phenol and enzymes like polyphenol oxidase and nitrate reductase, which mitigated adverse effects of transplanting shock and enhancing *ex vitro* survival.

In plants, metabolism of reactive oxygen species (ROS), such as superoxide radicals, hydrogen peroxide (H_2O_2) and hydroxyl radicals is kept in dynamic balance. Under water stress conditions, the balance is broken and antioxidant systems are needed to decrease the damage to tissues (Foyer *et al*., 1994). Antioxidants include superoxide dismutase (SOD), catalase (CAT), peroxidase (POX), ascorbate peroxidase (APOX) and glutathione reductase (GR) (Foyer and Harbinson, 1994). Recently, Porcel *et al.* (2003); Ruiz-Lozano (2003); Roldan *et al.* (2008) and Wu *et al.* (2008) put forward a hypothesis, according to which, AM fungi protected host plants against oxidative damage was due to increments of enzymatic antioxidants. Stress adaptive mechanisms are quite different, with stress degree, time course, material, soil quality status and experimental plots, thus increasing the complexity of the issue in question. The information about the activity of antioxidant enzymes in the AM symbiosis under water stress conditions has focused mainly on the role of SOD. Little attention has been paid to the role of other important antioxidant enzymes such as CAT and POX (Ruiz-Lozano, 2003).

2.3. ROLE OF AMF IN NUTRITION OF PLANTS

In India, mycorrhizal fungi populations have been found consistently under natural growing conditions (Iyer *et al*., 1988; Girija and Nair, 1988). Lin and Chang (1987) showed that the mycorrhization of *in vitro* plant material was advantageous for plant development. It was found that elements with low mobility in the soil, such as P, Cu and Zn, are absorbed in higher amounts by mycorrhizal plants than that by non-mycorrhizal plants (Stribley, 1987).Other authors (Lin and Fox, 1987; Knight, 1988) have studied the nutrition requirements of banana and evaluated how mycorrhizal inoculation can help plants absorb phosphorus and nitrogen. Rizzardi (1990) studied the effect of two vesicular-arbuscular mycorrhizal fungi (VAM), *Glomus mosseae* and *Glomus monosporum*, on micropropagated ‘Grand Nain’ plants, noting a higher

phosphorous content in the fungi treated plants than in either the control group or in the plants treated with fertilizer but no AMF.

Effectiveness of mycorrhizal fungi is related to better nutrition as well as physiological processes in plant system. Decklerk *et al*. (1994) obtained higher growth increments in micropropagated banana plants inoculated with *G. mosseae* and *G. geosporum* which he correlated with the increment in P and K levels in plants as compared to non-inoculated ones.

Jaizme-Vega *et al*. (2002) reported that mycorrhizal symbiosis significantly improves plant nutrition under low fertility soil conditions. Mycorrhizal hyphae are more efficient than roots alone in nutrient uptake.

Mathews *et al.* (2003) reported that the symbiotic association also increased the shoot P concentration. Thus, mycorrhiza formation appears to be the key factor in improving the vigour and growth of micropropagated banana plantlets, which aids in the acclimatization process.

The influence of arbuscular-mycorrhizal (AM) fungi on the mineral nutrition and vegetative growth of micropropagated banana plantlets (cv. Williams) under potted conditions was studied by Yao-Qing *et al.* (2004). They observed that inoculation with single (*G. versiforme*) and mixed fungal species (*G. versiforme, G. epigaeum* [*G. versiforme*] and *G. caledonium*) significantly increased P and K contents, respectively. N, P and K uptake was enhanced in both mycorrhizal treatments, with magnitude of increase ranging from 70 to 120% for P, approximately 80% for K and ranging from 40 to 60% for N. Growth promotion was slightly better when mixed species were used.

Chapter III

Material & Methods

Chapter III

MATERIALS AND METHODS

The present investigation entitled "Biochemical changes in micropropagated banana (*Musa* sp.) cv. Dwarf Cavendish (AAA) due to arbuscular-mycorrhizal fungi (AMF) inoculation during *ex vitro* acclimatization" was carried out in the Tissue Culture Laboratory and greenhouse of the Department of Horticulture, Institute of Agricultural Sciences, BHU, Varanasi during 2008 to 2010. This chapter contains description of materials used and methods adopted during the course of investigation under appropriate heads and sub-heads.

3.1. MATERIALS

3.1.1. Plant material

Present investigation was conducted on banana cultivar Dwarf Cavendish, which is one of the most cultivated varieties of banana worldwide as well as in Varanasi. This variety is immune to banana wilt (Panama wilt) and excels in terms of quality with high yielding potential as compared to various locally available varieties. The suckers for isolating explants were selected from the mother plant with higher bunch weight, long finger length, more number of hands per bunch, early maturing, free from insect, pest and diseases. The healthy, well-grown and high yielding plants of cultivar Dwarf Cavendish growing in the banana block at Vegetable Research Farm, Department of Horticulture, Institute of Agricultural Sciences, Banaras Hindu University, were selected for collecting the explants.

3.1.2. Explant

The explant i.e., shoot-tip with rhizomatous base were excised from suckers of field grown banana plants. The explants of 1.5-2.0 cm (length) and 1.0 cm (width of rhizome) excised from the inner portion of the sucker were selected in order to minimize the possibility of somaclonal variation often occurring during *in vitro* culture especially through callus phase.

3.1.3. Glassware and chemicals

Glassware used during the investigation was of borosilicate quality, procured from Borosil India (Pvt.) limited. For media preparation and inoculation, measuring cylinders (10-1000 mL), conical flasks (100-1000 mL), beakers (250-1000 mL), pipettes (0.1, 1.0, 2.0, 5.0, 10.0, 25.0 mL), petridishes (7.5 cm diameter), micropipettes, millipore filters, plastic wares etc. were used. Sterilized conical flasks and jam bottles were used for culturing the explants. All the chemicals or inorganic salts used in the present investigation were of analytical reagent (AR) grade procured from Loba Chemicals (Pvt.) Ltd., Mumbai. Sucrose and agar-agar powder procured from Sisco Research Laboratories (Pvt.) Ltd. were used. Plants growth regulators were procured from Hi-Media India Limited, Mumbai.

3.1.4. AMF strains used

Following arbuscular-mycorrhizal strains were used as bio-hardening agents for the micropropagated banana plantlets during acclimatization.

T_1 = AMF free soil medium

T_2 = *Acaulospora scrobiculata*

T_3 = *Glomus intraradices*

T_4 = Mixed AMF strain

3.2. METHODS

3.2.1. Sterilization of equipment and glassware

All the glassware *viz.*, pipettes, beakers, flasks, measuring cylinders, test tubes, petridishes, jam bottles etc. were washed with laboratory detergent (labolene) followed by sufficient washing with running tap water to remove the detergent residue and then rinsed with double-distilled water. The petridishes and beakers wrapped in aluminum foil and plastic wares were sterilized in an autoclave at 121^0C at 1.2 $kgcm^{-2}$ pressure for 30 minutes. All culture vessels used during experimentation were dried in hot air oven at 120-130^0C for 7-8 hours.

The UV-light of laminar air-flow chamber was switched on 30-40 minutes before use and the working bench was surface sterilized by thorough cleaning with methylated sprit. UV-light was turned off with switching on the air-flow during the operation. All the forceps, scalpels, scissors etc. were dipped in sprit inside the laminar air-flow chamber and were frequently flame sterilized and cooled down before use.

3.2.2. Preparation of stock solutions and their storage

All the stock solutions of the salts required for preparing the culture media were prepared by dissolving the required amount of chemicals in double-distilled water. Salts were dissolved by adding one compound at a time. Precipitation of salts was avoided by preparing the stock solutions in 20 or 200 fold concentrations. Stock solutions of growth regulators such as auxin and cytokinin were prepared by dissolving in small volume of 0.1 N NaOH or HCl and then making the final volume by adding double-distilled water. All the stock solutions were kept in bottles with lids and stored in a refrigerator at 4^0C.

Four stock solutions were prepared as follows:

Stock I	–	Macronutrients	(20 x concentration)
Stock II	–	Micronutrients	(200 x concentration)
Stock III	–	Fe-EDTA	(200 x concentration)
Stock IV	–	Vitamins	(200 x concentration)

For preparation of stock solutions I, II, and IV appropriate amount of each chemical was taken in volumetric flasks after dissolving them separately in a beaker with double distilled water and then the final volume was made up by adding double distilled water. Stock III was prepared by weighing $FeSO_4.7H_2O$ and sodium salt of $EDTA.2H_2O$ separately in the required quantities, dissolved by slight warming, and then mixed thoroughly, pH was adjusted to 5.5.

Table 3.1: Composition of Murashige and Skoog (MS) medium.

Components		Concentration (mgL^{-1})	Amount in Stock Solution (mgL^{-1})
I.	**Macronutrients**		(20 x)
	NH_4NO_3	1650	33000
	KNO_3	1900	38000
	$CaCl_2.2H_2O$	440	8800
	$MgSO_4.7H_2O$	370	7400
	KH_2PO_4	170	3400
II.	**Micronutrients**		(200 x)
	KI	0.83	166
	H_3BO_3	6.2	1240
	$MnSO_4.4H_2O$	22.3	4460
	$ZnSO_4.7H_2O$	8.6	1720
	$Na_2MoO_4.2H_2O$	0.25	50
	$CuSO_4.5H_2O$	0.025	5
	$CoCl_2.6H_2O$	0.025	5
III.	**Iron-EDTA**		(200 x)
	$FeSO_4.7H_2O$	27.8	5560
	$Na_2EDTA.2H_2O$	37.3	7460
IV.	**Vitamins and Amino acids**		(200 x)
	Myoinositol	100	20000
	Nicotinic acid	0.5	100
	Pyridoxine HCl	0.5	100
	Thiamine HCl	0.5	20
	Glycine	2.0	400

In addition the MS medium contains 3 per cent sucrose and 0.7 per cent agar and the pH was maintained at 5.85.

3.2.3. Culture media

All the tissue culture experiments were performed using Murashige and Skoog (1962) basal medium (Table 3.1). The MS medium was supplemented with different concentrations of growth regulators *viz.*, 6-benzylaminopurine (BAP) and indole-3-acetic acid (IAA) and their combinations as specified.

3.2.4. Preparation of culture media and its sterilization

The stock solutions were mixed in required amount of distilled water and final volume of the medium was made after dissolving sucrose (30 gL^{-1}). The pH of the medium was adjusted to 5.85 with addition of 0.1 N NaOH or 0.1 N HCl drop-wise using a digital pH meter. The media were heated to melt agar (7.0 gL^{-1}) followed by continuous stirring to prevent the agar from settling and charring. The molten medium was then poured into clean dry bottles. These bottles were capped and sterilized in a vertical autoclave at 121°C under a pressure of 1.2 kg cm^{-2} for 30 minutes. The media containing bottles were then stored at room temperature under dust free condition for further use.

3.2.5. Preparation of shoot-tip explant

The suckers were collected from the experimental field. The sword suckers of not more than one month old were collected in the morning hours. The collected suckers were brought in the laboratory and washed with running tap water for 5 minutes. Dirt was removed with the help of a brush. The suckers were then chopped with the help of sharp knife in to a block of 10 cm (length) × 5 cm (width) size and washed with autoclaved double distilled water. These trimmed blocks of sucker containing cylindrical leaf sheaths and rhizomatous base were then treated with 0.1% bavistin® (carbendazim) for 30 minutes followed by 1% solution of Cetrimide or Tween-20 for next 30 minutes, each treatment was followed by repeated rinsing with sterile double distilled water.

3.2.6. Surface sterilization and inoculation of explant

Before starting the work on laminar air-flow cabinet, the glass bottles containing sterilized nutrient media were kept on the working bench of the cabinet under UV-light for 30 minutes for disinfection of working area and the vessels. After

that the tools used for inoculation like scalpels, forceps, spatula etc. were sterilized by flaming with prior dip in methylated spirit inside the laminar. The outer leaf sheaths of pre-treated explants were removed under laminar air flow before exposing to different sterilizing solutions. Surface sterilization of explants was done aseptically on laminar air-flow cabinet by using different sterilizing agents *viz*., mercuric chloride ($HgCl_2$), sodium hypochlorite (NaOCl) and alcohol (ethanol 70% v/v).

Surface sterilized explants were given 4-6 washings with sterile double-distilled water to remove the traces of sterilizing agent(s) immediately after treatment and the outer bleached leaf sheath as well as rhizome were further trimmed. The explants were then laid on the sterilized petriplate for drying and inoculated into the culture bottles. The culture bottles were kept in an incubation room with 25±2°C temperature. The cultures were kept under fluorescent white light for photoperiod of 16/8 hours light and dark cycles.

3.3. EXPERIMENTAL SETUPS

3.3.1. Experiment I : Standardization of surface sterilization treatment for shoot-tip explants isolated from field collected suckers

3.3.1.1. Single sterilization

3.3.1.1.1. The single sterilization treatment of explants with $HgCl_2$ (0.1%) for 3, 4, 5, 6 and 7 minutes followed by rinsing with sterile distilled water and then transferred to initiation medium. The different treatments with mercuric chloride (0.1%) are as follows:

Treatment	$HgCl_2$ (0.1%) duration (min.)
SS_1	0
SS_2	3
SS_3	4
SS_4	5
SS_5	6
SS_6	7

3.3.1.1.2. Another single sterilization treatment in which explants were sterilized with NaOCl (4%) for 5, 10, 15, 20, 25 and 30 minutes followed by rinsing with sterile distilled water and then cultured on initiation medium. The different treatments with sodium hypochlorite (4%) are as follows:

Treatment	NaOCl (4%) duration (min.)
SS_7	5
SS_8	10
SS_9	15
SS_{10}	20
SS_{11}	25
SS_{12}	30

3.3.1.2. Double sterilization

In the double sterilization treatment, explants were first treated with $HgCl_2$ (0.1%) for 3, 4 and 5 minutes followed by rinsing with sterilized double distilled water and the outer leaf sheath along with rhizomatous base were trimmed. These trimmed explants were given a second sterilization treatment of quick dip (2-4 sec.) in ethanol. The same procedure was followed for employing sodium hypochlorite treatment for 5, 10 and 15 minutes. The treatment combinations for double sterilization are given below:

Treatment	$HgCl_2$ (0.1%) duration (min.)	NaOCl (4%) duration (min.)	Ethanol (70%)
DS_1	3	-	Quick dip
DS_2	4	-	Quick dip
DS_3	5	-	Quick dip
DS_4	-	5	Quick dip
DS_5	-	10	Quick dip
DS_6	-	15	Quick dip

The size of explant was 1.5-2.0 cm (length) and 1.0 cm (width of rhizome) before inoculation. All the explants were inoculated in MS media supplemented with

2 mgL^{-1} BAP and 1.0 mgL^{-1} IAA. Data of contaminated cultures was recorded after every week on visual basis and contaminated cultures were discarded. Survival percentage was noted for explants neither contaminated nor showing any signs of necrosis. The experiment was replicated thrice and eight explants per replication were maintained.

Observations recorded:

1. Per cent contamination of explants: The per cent contaminated explants was calculated using formula as given below:

$$\text{Per cent contamination} = \frac{\text{Number of contaminated explants} \times 100}{\text{Total number of explants cultured}}$$

2. Per cent survival of explants: The per cent survival of explants was calculated as per formula given below:

$$\text{Per cent survival} = \frac{\text{Number of explants survived} \times 100}{\text{Total number of explants cultured}}$$

3. Per cent mortality of explants: The formula for calculating the per cent mortality of explants is given below:

$$\text{Per cent mortality} = \frac{\text{Number of explants died} \times 100}{\text{Total number of explants cultured}}$$

3.3.2. Experiment II: Standardization of micropropagation protocol for culture initiation, multiplication and rooting of banana plantlets.

In this experiment, explants were inoculated in MS medium containing different combinations of growth regulators (auxin and cytokinin) during culture initiation, multiplication and rooting.

3.3.2.1. Culture initiation:

The basal medium used for culture initiation contained Murashige and Skoog (1962) basal salts supplemented with 30 gL^{-1} sucrose (3%). Details of MS macro and micro nutrients are given in Table 3.1. Shoot induction media consisted of different combinations of growth regulators as mentioned in Table 3.2.

A number of vertical cuts into the meristematic dome were applied before inoculation. The cultures were incubated at 25±2°C temperature and 16 hours photoperiod under cool fluorescent light. Eight cultures were transferred to each treatment and each treatment was replicated thrice. Data was recorded after every week for contamination and other physical changes of the explants up to four weeks.

Observations recorded:

(i) Per cent shoot induction

(ii) Days to shoot induction

Table 3.2: Composition of MS medium supplemented with different growth regulators for culture initiation from shoot-tips of cv. Dwarf Cavendish.

IAA (mgL^{-1}) / **BAP (mgL^{-1})**	**0**	**1**	**2**	**BAP Mean**
0	MSI_0	MSI_4	MSI_8	
2	MSI_1	MSI_5	MSI_9	
4	MSI_2	MSI_6	MSI_{10}	
6	MSI_3	MSI_7	MSI_{11}	
IAA Mean				

3.3.2.2. *In vitro* multiplication:

Multiplication medium consisted of MS macro and micronutrients supplemented with different growth regulators. The growth regulators were BAP (0, 2, 4, 6, 8 mgL^{-1}), IAA (0, 1, 2 mgL^{-1}). Details of multiplication media used are given in Table 3.3.

Each treatment consisted of six replications and each replication consisted of single explant cultured in a jam bottle. Observations accounting for shoot multiplication were recorded after four weeks of culturing in multiplication media. The effects of different treatments were quantified on the basis of number of shoots per explant. Data on number and length of shoots were recorded while sub-culturing in laminar-flow.

Observations recorded:

(i) Number of shoots per explant

(ii) Shoot length (cm)

Table 3.3: Composition of MS medium supplemented with different growth regulators for *in vitro* multiplication of banana cv. Dwarf Cavendish.

IAA (mgL^{-1}) / **BAP (mgL^{-1})**	**0**	**1**	**2**	**BAP Mean**
0	MSM_0	MSM_5	MSM_{10}	
2	MSM_1	MSM_6	MSM_{11}	
4	MSM_2	MSM_7	MSM_{12}	
6	MSM_3	MSM_8	MSM_{13}	
8	MSM_4	MSM_9	MSM_{14}	
IAA Mean				

3.3.2.3. *In vitro* rooting of banana plantlets:

When shoots were 4-5 cm long, these were detached from clump and transferred to rooting medium, which consisted of MS (full and half) solidified with 7 gL^{-1} agar supplemented with IAA (0, 0.5, 1.0 mgL^{-1}). One shoot was cultured in one glass jar and the data were collected for ten jars in each replication. The treatments were replicated six times. Parameters *viz*., per cent rooting, days to rooting and number of roots per micro-shoot in each treatment was recorded after four weeks.

Observations recorded:

(i) Per cent rooting

(ii) Days to rooting

(iii) Number of roots per micro-shoot

Table 3.4: Composition of MS medium supplemented with different growth regulators for *in vitro* rooting of banana cv. Dwarf Cavendish.

MS Strength	IAA (mgL^{-1})			Media mean
	0.0	**0.5**	**1.0**	
Full	MSR_0	MSR_2	MSR_4	
Half	MSR_1	MSR_3	MSR_5	
IAA Mean				

3.3.3. Experiment III: Study of morphological, physiological and biochemical changes in micropropagated banana plantlets due to AMF inoculation.

3.3.3.1. Inoculation of micropropagated plants with AMF

3.3.3.1.1. Microbial inoculum source

Pure cultures of arbuscular-mycorrhizal fungi (AMF) were procured from TERI, New Delhi. The pure AMF cultures were multiplied on rhodes grass (*Chloris guyana*) as host plant and maintained in plastic pots (5 kg) filled with autoclaved (1.2 kg cm^{-2} for 2 hr.) potting mixture of soil, sand and FYM (2:2:1) under glasshouse conditions. Microbial treatments consisted of approximately 20 g inoculum containing rhizosphere soil, spores besides hyphae, arbuscules and vesicles (AMF) and root segments of rhodes grass. The spore density ranged from 200 - 250 per g soil.

3.3.3.1.2. Microbial treatments

The following mycorrhizal strains were used for the inoculation of micropropagated banana plantlets during hardening.

S.No.	Treatments	Strain
1.	T_1	AMF free soil medium
2.	T_2	*Acaulospora scrobiculata*
3.	T_3	*Glomus intraradices*
4.	T_4	*Mixed AMF strain*

3.3.3.1.3. AMF inoculation procedure

In vitro raised rooted plants of cv. Dwarf Cavendish of 30 days after root initiation were taken for AMF inoculation. The plantlets with equal length (5 cm) and around 4-5 roots per micro-shoot were transferred to plastic pots filled with sterile soil, sand and FYM (1:1:1) along with 20 g of AMF inoculums placed immediately below the roots.

3.3.3.1.4. Growth conditions and irrigation scheduling

The plantlets after inoculation were irrigated immediately with sterile tap water and maintained in a growth chamber with day-night temperatures ranging from 27±1ºC for 30 days. Day length was extended to 16 hr. with cool white fluorescent lights at 630 µmol m^{-2} sec^{-1}. Plantlets were irrigated with sterile tap water daily up to 15 days and later on irrigated at an interval of 5 days. Plantlets were transferred to net-house at 30 days after inoculation. During this period plants were irrigated at an interval of 5 days. Visual observation indicated that 5 days interval of irrigation in net-house was sufficient to create moisture stress as control plantlets started showing mortality. Biochemical analysis was done at 20, 40 and 60 days after inoculation in water stressed control and mycorrhized plantlets. Neither fertilizer nor fungicide or pesticide was applied to the plantlets during the experimental period.

Observations recorded

1. Per cent survival

The per cent survival of plantlets after imposing moisture stress was recorded at 20, 40 and 60 days after inoculation (DAI).

$$\text{Per cent survival} = \frac{\text{Number of plantlets surviving} \times 100}{\text{Total number of plantlets transferred}}$$

2. Per cent root colonization

Plantlets were sampled at 20, 40 and 60 DAI and assessed for root colonization by staining method as suggested by Phillips and Hayman (1970). Washed root segments from the B-region (elongation zone) were collected in polybags. The samples were dropped in 10 per cent KOH solution in a beaker and

were then autoclaved at 15 lbs $inch^{-2}$ for 10 minutes. After that, rootlets were covered with alkaline H_2O_2 at room temperature for 10-20 minutes until the roots were bleached. Thereafter, rootlets were rinsed at least thrice with the complete change of distilled water to remove the H_2O_2, and were soaked in 1 % HCl for 3-4 minutes and poured off. Rootlets were stained with acid-fuchsin-lactic acid solution with 0.1% trypan blue boiled for 10 minutes to enhance the rate of reaction. Excess staining solution was decanted and destaining was done by immersing the root segments in acid-fuchsin-lactic acid (without trypan blue). For assessment of per cent root colonization, root segments (1 cm long) were selected at random from a stained sample. The segments were mounted on microscopic slides and were observed under phase contrast microscope under 100 x magnification. Extent of root colonization was assessed in twenty segments, averaged and expressed as a percentage of root length.

3. Growth parameters

The following parameters were determined at 20, 40 and 60 days after inoculation (DAI) in control and mycorrhized banana plantlets during acclimatization. All treatments were replicated four times and 10 plants per replication were maintained.

3.1. Plant height

Five plants were randomly selected from each replication. The height of plants was measured from the base (soil surface) to the growing tip of pseudostem with meter scale. Average length was expressed in centimeter (cm).

3.2. Number of leaves

Total number of fully opened leaves per plant was counted in control and mycorrhized plantlets at different stages of growth i.e., at 20, 40 and 60 days after inoculation.

3.3. Leaf area

Total leaf area (small, medium and large leaves) per plant was calculated from the tagged plants by tracing the leaf boundary on a square paper sheet and expressed in cm^2 $plant^{-1}$.

3.4. Shoot fresh and dry weight

Fresh weight of shoots was measured just after sampling. For dry matter determination, samples were put in tissue paper bags and kept in a hot air oven at 70°C till they showed no change in weights. Thereafter, the respective dry weights were determined.

3.5. Number of roots

Total number of roots per plant was calculated for control and mycorrhized treatments in five randomly selected plants, averaged and recorded in each replication.

3.6. Root length

Root length per plant in control and mycorrhized plantlets was measured from five randomly selected plants in each replication and average root length was expressed in cm.

3.7. Root fresh and dry weight

Roots were washed with tap water to remove the adhering potting mixture. Thereafter, the excess water on the root surface was removed by gentle swabbing with blotting paper. Fresh weights were noted immediately, while for determination of dry weights, procedure as mentioned in case of shoot dry weight was followed.

4. Physiological parameters

4.1. Photosynthetic rate

From each replication, five randomly selected plants were tagged. Two mature leaves were selected on each plant and their photosynthetic rates were measured using Infra Red Gas Analyzer (IRGA, LiCOR 6200). Average photosynthetic rate was expressed in µmol CO_2 m^{-2} s^{-1}.

4.2. Stomatal conductance

Stomatal conductance was measured in mycorrhized and control plantlets with the help of Infrared Gas Analyzer (IRGA, LiCOR 6200). The observations were recorded between 9.00 to 10.30 hrs on first fully expanded leaf from top.

4.3. Relative water content (RWC)

The RWC in the recently matured leaf was determined using the method suggested by Weatherley (1950). Leaves were collected and 8 mm diameter discs were made. Fresh weight of these discs were measured and then floated over distilled water in pertidish for 4-6 hours. These discs were then surface dried by placing them in between 2 sheets of Whatman's No. 1 filter paper and saturated weight of these discs were recorded. After that, the samples were dried in an oven at 70ºC for 24 hr. or till they showed no change in their weight after two consecutive drying. The dry weight of the sample was then recorded. The RWC was then estimated from the following formula:

$$\text{RWC (\%)} = \frac{\text{(Fresh weight - Oven dry weight)} \times 100}{\text{(Turgid weight - Oven dry weight)}}$$

5. Biochemical parameters

The biochemical changes in water stressed control and mycorrhized banana plantlets were determined at 20, 40 and 60 days after inoculation in foliar parts.

5.1. Total phenols

The method proposed by Malik and Singh (1980) was employed for quantification of total phenols.

5.1.1. Method:

Freshly emerged foliar samples (approximately 500 mg) was homogenized in a mortar by adding 80 per cent ethanol. It was then centrifuged at 10,000 rpm for 20 min. and the supernatant was filtered using filter paper Whatman No. 42. The residue was re-extracted (5 times) with 80 per cent ethanol and the supernatant collected were evaporated to dryness. Residues were dissolved in 5 ml of distilled water, from which

about 0.2 mL was taken and total volume was made up to 3 mL with distilled water. To this, fresh Folin-Ciocalteau reagent (0.5 mL) was added. After 3 minutes, 2 mL of 20% Na_2CO_3 (sodium carbonate) solution was added in each tube, mixed thoroughly and placed in a hot water bath (58ºC) exactly for one min. It was then cooled to room temperature and then absorbance (650 nm) was measured against blank.

5.1.2. Preparation of standard curve:

Twenty mg of catechol was dissolved in small volume of distilled water and then volume made upto 100 mL. In five test tubes (10 mL) 0.2, 0.4, 0.6, 0.8 and 1.0 mL aliquot of catechol was taken and volume was made to 3 mL with distilled water followed by addition of 0.5 mL Folin-Ciocalteau reagent. After 3 minutes, 2 mL of 20 per cent Na_2CO_3 was added and mixed thoroughly. A blank was run simultaneously taking 3 mL of distilled water instead of catechol. All the tubes were placed in boiling water bath for exactly one minute, cooled and absorbance was measured at 650 nm using spectrophotometer (Elico's Mini Spec SL-171). The instrument was adjusted to zero optical density (O.D.) using a blank.

5.2. Proline content

Proline content was determined in first fully expanded leaf from top in water stressed plants at 20, 40 and 60 days after inoculation by the method as described by Bates *et al*. (1973).

5.2.1. Reagents:

a) Sulphosalicylic acid (3 per cent)

b) Orthophosphoric acid (6.0 M)

c) Ninhydrin reagent: Ninhydrin reagent was prepared by dissolving 1.25 g of ninhydrin in 30 mL glacial acetic acid. To this 20 mL 6.0 M orthophosphoric acid was added.

5.2.2. Method:

Leaf sample (0.5 g) was homogenized in 5 mL of sulphosalicylic acid (3 per cent). It was centrifuged at 6000 g for 10 minutes and supernatant was saved. Residue

was again extracted twice with 5 mL, 3 per cent aqueous sulphosalicylic acid. All the supernatant fractions were pooled and final volume was made to 15 mL. 2 mL of this extract was taken in the test tube and 2 mL ninhydrin reagent and 2 mL glacial acetic acid were added. The reaction mixture was put in boiling water bath for 30 minutes. After cooling the reaction mixture, 5 mL toluene was added. Then solution mixture was shaken vigorously and toluene fraction was separated by separating funnel. The absorbance of toluene fraction was read at 520 nm with the help of spectrophotometer (Digispec 11OD) against toluene blank. Concentration of proline in the plant samples was estimated by referring to a standard curve of proline.

5.2.3. Standard curve for proline estimation:

Proline (10 mg) was taken and dissolved in 3 per cent aqueous sulphosalicylic acid and then the solution was diluted to 100 mL. Then 0.2, 0.4, 0.6, 0.8 and 1.0 mL aliquots were taken in to different test tubes and the volume was raised to 2 mL by adding 3 per cent aqueous sulphosalicylic acid solution. Colour was developed in the same way as for samples and absorbance was determined with the help of spectrophotometer (Digispec 110D).

5.3. Total chlorophyll

Changes in total chlorophyll content under moisture stress in leaves of normal and mycorrhized plants were analyzed at 20, 40 and 60 days after inoculation using SPAD meter (Minolta). Amount of chlorophyll is expressed in terms of SPAD units.

5.4. Total soluble sugar

Total soluble sugar content in first fully expanded leaf from top was determined in moisture stressed control and mycorrhized plantlets by anthrone method (Dubois *et al*., 1956). Total sugar content was expressed as mg g^{-1} fresh weight.

5.4.1. Preparation of anthrone reagent:

To prepare anthrone reagent, 200 mg anthrone was dissolved in 100 mL of 98 per cent H_2SO_4. This reagent was prepared at the time of use.

5.4.2. Procedure for soluble sugar estimation:

Leaf sample (100 mg) from freshly harvested first leaf from top was homogenized with 5 mL ethanol (80 per cent). The extract of the sample was then centrifuged at 4000 g for 15 minutes. The supernatant was separated and remaining residue was again extracted twice with 5 mL 80 per cent ethanol. Supernatants were collected in same flask and total volume was maintained to 15 mL with 80 per cent ethanol. Residue was used for the extraction and estimation of starch. 0.1 mL of the extract was taken in a test tube and dried in oven at 60^0C. Finally the volume was made to 1.0 mL with distilled water. Then 5 mL anthrone reagent was added to it. The tubes were placed in a boiling water bath for 10 minutes, after which they were allowed to cool immediately in running water. A blank was prepared in the similar way, but by taking 1.0 mL distilled water. The absorbance was measured at 620 nm by a spectrophotometer (Digispec 11OD). The amount of the sugar in the leaf samples was calculated by standard curve.

5.4.3. Preparation of the standard curve:

Glucose (10 mg) was dissolved in 100 mL distilled water. From this stock solution 0.2, 0.4, 0.6, 0.8 and 1.0 mL aliquots were taken in separate test tubes. The final volume of these aliquots was made to 1.0 mL with distilled water and thereafter 5 mL anthrone reagent was added in each test tube. Test tubes were kept in boiling water bath as described above. Tubes were then cooled and the intensity of the colour was read at 620 nm. The standard curve was prepared by plotting the absorbance value on Y-axis against the concentration of the sugar in solution on X-axis.

5.5. Starch content

Starch content was determined in the first fully expanded leaf from top at 20, 40 and 60 days after inoculation in plants by anthrone method (Dubois *et al*., 1956).

5.5.1. Procedure for soluble sugar estimation:

Starch content was estimated from the residue retained from the samples that was used for soluble sugar estimation. Residue was extracted thrice at 0°C for 20 minutes with 6.5 mL of perchloric acid (52 per cent) and centrifuged at 10,000 g for

10 minutes. Every time supernatant was taken in a volumetric flask and final volume was made to 20 mL. 0.1 mL of the extract was taken in a test tube and final volume was made to 1.0 mL with distilled water. Then 5 mL anthrone reagent was added to each test tube. The tubes were then placed in a boiling water bath for 10 minutes after which they were allowed to cool in the running water. A blank was prepared in a similar way, but by taking 0.1 mL perchloric acid (52 per cent) and 0.9 mL distilled water and treating in the same way as the samples. The colour was measured at 620 nm by spectrophotometer (Digispec 110D). The amount of the starch in the leaf samples was calculated by standard curve as described in sugar estimation and expressed in terms of glucose.

5.6. Enzymatic activity

5.6.1. Catalase activity

Catalase activity was assayed spectrophotometrically in first fully expanded leaf from top in moisture stressed plants according to the protocol of Aebi *et al.* (1983) at 20, 40 and 60 days after inoculation.

5.6.1.1. Reagents:

(A) 0.1 M Phosphate buffer pH-6.4

(B) 1.0 per cent H_2O_2

5.6.1.2. Procedure:

One hundred mg fresh leaves of control and mycorrhized plants were taken and homogenized in 5 ml of 0.1M phosphate buffer (pH 6.4) in a chilled pestle and mortar. The crude extract was centrifuged at 10,000 g for 20 minutes at 4ºC. The enzyme extract was stored at low temperature until completion of enzyme assay. The activity of enzyme was assayed by taking 2.6 mL, 0.1M phosphate buffer (pH 6.4), 0.1 mL enzyme extract and 0.1mL, 1.0 per cent H_2O_2. The reaction mixture was mixed rapidly at room temperature. A blank was prepared similarly in which 0.1M phosphate buffer (pH 6.4) was added in reaction mixture instead of enzyme extract. Changes in absorbance at 230 nm at an interval of 15 second for 2 minutes were

noted. The enzyme activity was expressed as enzyme units per g fresh weight according to the formula given below:

$$\text{Enzyme units/mg} = \frac{\delta A_{230/min} \times 1000}{43.6 \times \text{mg protein/mL reaction mixture}}$$

5.6.2. Peroxidase activity

Peroxidase activity was assayed in first fully expanded leaf from top in moisture stressed control and mycorrhized plants at various growth stages. The enzyme assay was performed as per the protocol of Kar and Mishra (1976).

5.6.2.1. Reagents:

(A) 0.1M phosphate buffer (pH 6.4)

(B) 50.0 mM pyragallol

(C) 50.0 µM H_2O_2

(D) 5.0 per cent H_2SO_4

5.6.2.2. Procedure:

Enzyme extraction was done by homogenizing 100 mg leaf tissue of control and mycorrhized plants in 5.0 mL, 0.1M phosphate buffer (pH 6.4) in a chilled pestle and mortar. The crude extract was centrifuged at 10,000 g for 20 minutes at 4ºC. Supernatant was stored at 4ºC till the enzyme assay was performed. Reaction mixture was prepared by adding 4.6 mL 0.1M phosphate buffer (pH 6.4), 0.2 mL pyragallol (50 µM), and 0.1 mL 50µM H_2O_2 and 0.1 mL enzyme extract. Mixture was incubated at 25ºC for 5 minutes. Then 0.5 mL 5.0 per cent H_2SO_4 was added to terminate the reaction. Absorbance was measured at 420 nm with the help of spectrophotometer (Elico, SL196). Enzyme units were calculated as:

(Enzyme Units g^{-1} fresh weight) = Change in absorbance g^{-1} fresh weight $minute^{-1}$

5.6.3. Polyphenol oxidase

Polyphenol oxidase activity was assayed in first fully expanded leaf from top in moisture stressed control and mycorrhized plants at 20, 40 and 60 days after

inoculation. The enzyme assay was performed as per the protocol of Kar and Mishra (1976).

5.6.3.1. Reagents:

(A) 0.1M phosphate buffer (pH 6.4)

(B) 50.0 µM pyragallol

(C) 5.0 per cent H_2SO_4

5.6.3.2. Procedure:

Enzyme extraction was done by homogenizing 100 mg leaf tissue in 5.0 mL 0.1M phosphate buffer (pH 6.4) in a chilled pastel and mortar on ice . The crude extract was centrifuged at 10,000 g for 20 minutes at 4ºC. Supernatant was stored at 4ºC till the enzyme assay was performed. This extract was used for estimation of enzymatic activity. Reaction mixture was prepared by adding 4.6 mL 0.1M phosphate buffer (pH 6.4), 0.2 ml pyragallol (50 µM) and 0.1 ml enzyme extract. Mixture was incubated at 25ºC for 5 minutes. Then 0.5 mL 5.0 per cent H_2SO_4 was added to terminate the reaction. Absorbance was measured at 420 nm with the help of spectrophotometer (Elico, SL196). Enzyme units ware calculated as:

(Enzyme Units g^{-1} fresh weight) = Change in absorbance g^{-1} fresh weight $minute^{-1}$

3.3.4. Experiment IV: Leaf nutrient analysis in micropropagated control and mycorrhized banana plantlets.

The leaves of control and mycorrhized banana plantlets were collected after 60 days of acclimatization. Third leaf from top was selected for sampling in four randomly selected plants in a treatment and dried in hot air oven at 70°C for 24 hr. until constant weight was recorded. The dried leaves were then chopped and grinded before being subjected to nutrient (N, P and K) analysis.

3.3.4.1. Nitrogen determination

Total nitrogen content in leaf samples was determined by nitrogen analyzer (Pelican, Model KEL 20L) adopting Kjeldahl method. It required following reagents:

(a) Catalyst mixture ($CuSO_4$ + K_2SO_4) 1:5 ratio

(b) Concentrated sulphuric acid

(c) 40 per cent NaOH

(d) 4 per cent boric acid

(e) 0.1 N HCl

(f) Indicator: Mixed indicator (0.3 g of bromocresol green and 0.2 g methyl red dissolved in 400 mL of 90 per cent ethanol)

3.3.4.1.1. Sample digestion:

Dried leaf sample (100 mg) was taken in a Kjeldahl digestion tube containing 3 g of catalyst mixture and to this 10 mL of concentrated sulphuric acid was added. Tubes were put in the digestion block, fitted with manifolds and scrubber. The temperature was gradually raised to 350ºC. The digestion continued till the solution became colourless. It took nearly 3-3.30 hours for complete digestion of the dried leaf tissues. After completion, samples were brought to room temperature before starting distillation.

3.3.4.1.2. Distillation:

Distillation of digested samples was done by auto distillation system (Pelican Distyl EM). Kjeldahl tubes containing digested plant leaves were fitted in the assembly. Sufficient amount (20-30 mL), 40 per cent NaOH was added to it till the colour of the solution became brown. At the collection end a conical flask containing 24 mL 4 per cent boric acid and 0.5 mL mixed indicator was put. The sample was allowed to steam distilled for 9 minutes.

3.3.4.1.3. Titration procedure:

The boric acid solution of the conical flask was titrated by 0.1 N HCl with the help of micro titration unit. At the end point light brown colour appeared. The amount of nitrogen in the sample was calculated as:

$$\text{N (mg g}^{-1}\text{ dry weight)} = \frac{14 \times \text{Titrant value} \times \text{normality of acid} \times 100}{\text{Sample weight (g)} \times 1000}$$

3.3.4.2. Phosphorus determination

Phosphorus content in dried leaf samples of control and mycorrhized banana plantlets under water stress was determined by method as described by Olsen *et al*. (1954).

3.3.4.2.1. Reagents:

(A) Sulfuric acid concentrated

(B) Perchloric acid (60 per cent)

(C) Ammonium molybdate-ammonium vandate reagent**:** Dissolved 22.5 g $(NH_4)_6MoO_4.4H_2O$ in 400 mL distilled water. Also dissolved 1.25 g ammonium metavandate in 300 mL boiling distilled water. The vandate solution was added to molybdate solution. It was cooled to room temperature and 250 mL of concentrated HNO_3 was added. The solution was mixed thoroughly in a conical flask and final volume was made to 1 litre with distilled water.

3.3.4.2.2. Sample digestion:

The oven dried leaf samples were digested by di-digestion method using concentrated H_2SO_4 and perchloric acid (60 %). 100 mg of leaf samples were taken in kjehldahl flasks containing 2.0 mL of concentrated H_2SO_4. The samples were heated for 1-2 minutes and then 2.0 mL of perchloric acid (60%) was added to the samples. Then the samples were heated directly in the digestion block till it become colourless. Samples were dried and volume was raised upto 100 mL with double glass distilled water. A blank was prepared by taking same amount of H_2SO_4 and perchloric acid without sample and treating in same manner.

3.3.4.2.3. Estimation:

A 30 mL of the digested stock solution was transferred to a 50 mL volumetric flask. 10 mL of vandate-molybdate solution was added to it and finally volume was made upto 50 mL. It was mixed well and the colour intensity was read at 470 nm by setting blank as zero.

Standard curve for phosphorus was prepared by taking 0.50, 0.75, 1.00, 1.25, 1.50, 2.00 and 2.50 mL of 100 mg KH_2PO_4 L^{-1} stock solution. Colour development was done in the same manner as that of the test solution. Phosphorus content was calculated from the standard curve and the formula used is as follows:

$$\text{P content of sample (\%)} = A / 100\ V$$

Where,

A- Phosphorus concentration in µg as read against the sample reading on the graph.

V- Quantity of test solution taken for colour development out of the 100 mL acid digest made from 1 g sample.

3.3.4.3. Potassium determination

Potassium content in leaf was estimated by atomic absorption spectrophotometer (Elico, Model SL-194) after digestion of the samples in tri acid mixture (HNO_3: H_2SO_4: 60% $HClO_4$ in a ratio of (75:30:15)). 50 mg dried plant material was taken in the digestion tube. To this 10 mL tri acid mixture was added.

Tubes were put on digestion heater (Pelican, Model KES-20L) and heated at 200ºC till the solution became colourless. Solution was brought to room temperature and final volume was made to 100 mL with double distilled water. The concentration of various elements in the solution was determined by atomic absorption spectrophotometer using specific hollow cathode lamp of the elements and standards.

3.4. STATISTICAL ANALYSIS

The data recorded from surface sterilization was analyzed with CRD (Completely Randomized Design) with three replications and 8 units were maintained for each replication where as the data taken from the effect of PGRs on different parameters of shooting and rooting were analyzed by Factorial CRD (Completely Randomized Design) with four replications as suggested by Panse and Sukhatme (1967). The per cent data were transformed by angular transformation before the statistical analysis.

In hardening experiment for determining the significance of difference between the treatment means and to draw the valid conclusions, the data obtained by various treatments at different stages of growth were subjected to statistical analysis by adopting the method of Analysis of Variance for completely randomized design. The significance of variance among the treatments was observed by applying 'F' test and critical difference at 5 per cent level of significance to compare the treatment means for all characters. The results are being presented with the help of tables, graphs and plates.

Chapter IV

Experimental Findings

EXPERIMENTAL FINDINGS

The present investigation entitled "Biochemical changes in micropropagated banana (*Musa* sp.) cv. Dwarf Cavendish (AAA) due to arbuscular-mycorrhizal fungi (AMF) inoculation during *ex vitro* acclimatization" was carried out during 2008-09 and 2009-10 in Department of Horticulture, Institute of Agricultural Sciences, BHU, Varanasi.

The whole experiment was divided in two parts i.e., tissue culture experiment and pot experiment (hardening part) in which biochemical analysis was done after imposing water stress of 5 days at various growth stages of plant. The findings of both the experiments are summarized here.

4.1. MICROPROPAGATION PROTOCOL

4.1.1. Sterilization of explants:

Surface sterilization is an obligatory step prior to *in vitro* culture of any plant tissue. It is an inevitable step for establishment of certain species, especially when the explants are derived from field grown perennial plants. In view of above, an experiment was designed to find out the best sterilant and duration of treatment for establishment of maximum aseptic culture.

4.1.1.1. Single sterilization of explants with mercuric chloride

The response of shoot-tip explants to the surface sterilizing agent namely mercuric chloride 0.1 per cent with various time exposures on establishment of contamination free culture is presented in Table 4.1 and Fig. 4.1. The efficiency of sterilizing agents was evaluated in terms of maximum aseptic cultures. When no sterilant was used all the cultures became contaminated. Highest contamination (95.83 per cent) was recorded with 3 minutes treatment of mercuric chloride showing lowest survival (4.16 per cent) of the explants. Lowest frequency of contaminated cultures (0.00 per cent) was recorded when explants were treated with mercuric chloride (0.1 per cent) for 7 minutes but the survival was very low (12.50 per cent) thus this

concentration was found to be toxic for the explants. The contamination of explants significantly decreased with increase in exposure time of sterilant (Plate 1a, b).

The percent survival of explants was recorded highest (54.16 per cent) when explants were treated with mercuric chloride for 5 minutes. The survival of 37.50 per cent was observed with 4 minutes treatment of $HgCl_2$.

Mortality of the explants increased with increase in exposure time of sterilant and ranged from 0.00 to 87.50 per cent. Maximum mortality (87.50 per cent) was observed with 7 minutes treatment of $HgCl_2$ followed by 6 minutes treatment (54.16 per cent). There was no mortality in control, 3 and 4 minutes treatment with $HgCl_2$. Lowest mortality (4.16 per cent) was observed in 5 minutes treatment with $HgCl_2$.

4.1.1.2. Single sterilization of explants with sodium hypochlorite

The time of exposure to sodium hypochlorite (4%) shows significant effects on contamination, survival and mortality of explants (Table 4.2 and Fig 4.2). When shoot-tips were treated with sodium hypochlorite for 5 minutes, maximum contamination (91.66 per cent) was observed while zero per cent contamination was recorded in 25 and 30 minutes treatments followed by 20 minutes treatment (20.83 per cent).

Highest survival (58.33 per cent) was observed with 15 minutes treatment of sodium hypochlorite followed by 10 minutes treatment (25.00 per cent) which was at par (16.66 per cent) with 20 minutes treatment of sodium hypochlorite. There was no survival of explants when treated with sodium hypochlorite for 30 minutes, which was followed by 5 minutes treatment (4.16 per cent) that was at par (8.33 per cent) with 25 minutes treatment (Plate 1c, d).

Regarding mortality, cent percent mortality was observed with 30 minutes treatment of sodium hypochlorite followed by 25 minutes (91.66 per cent).

4.1.1.3. Double sterilization of explants

Table 4.3 and Fig. 4.3 show double sterilization treatment of mercuric chloride and sodium hypochlorite with ethanol. Minimum contamination per cent (0.00) was observed with 5 minutes treatment of $HgCl_2$ + quick dip in ethanol followed by 15 minutes treatment with sodium hypochlorite + quick dip in ethanol (4.16 per cent). Maximum contamination (50.00 per cent) was observed with 5 minutes treatment of sodium hypochlorite + quick dip in ethanol which was at par with 3 minutes $HgCl_2$ treatment + quick dip in ethanol (45.83 per cent).

Maximum survival (91.66 per cent) was observed in 5 minutes $HgCl_2$ treatment + ethanol quick dip (Plate 1e) followed by 4 minutes $HgCl_2$ + ethanol quick dip (75.00 per cent).

Maximum mortality (25.00 per cent) was observed with 15 minutes sodium hypochlorite + ethanol quick dip treatment, while no mortality was recorded in 3 minutes $HgCl_2$ + quick dip in ethanol and 5 minutes sodium hypochlorite + quick dip in ethanol treatments.

4.2. Effect of cytokinin (BAP) and auxin (IAA) on different regeneration parameters of shoot-tip cultures.

4.2.1. Per cent shoot induction

Data regarding shoot induction are presented in Table 4.4 and Fig 4.4. Shoot induction significantly affected when BAP was added singly in the media. Maximum shoot induction (41.66 per cent) was observed with BAP 4 mgL^{-1}. Further increase in concentration of BAP above 4 mgL^{-1} significantly reduced the culture response (33.33 per cent) at BAP 8 mgL^{-1}. Addition of IAA singly showed significant effect on per cent shoot induction over control. Maximum shoot induction (20.83 per cent) was observed at 1 mgL^{-1} IAA which was at par with 2 mgL^{-1} IAA (20.83 per cent).

As far as the main effect of BAP is concerned, the maximum percentage of shoot induction (68.05) was observed at 6 mgL^{-1} BAP. Though there was not any significant difference between 0 mgL^{-1} of BAP (18.05 per cent) and 2 mgL^{-1} BAP (43.05 per cent) for percent shoot induction, yet significant increase was observed at 4

mgL^{-1} BAP (55.55 per cent) and 6 mgL^{-1} BAP (68.05 per cent). For the main effect of IAA, shoot induction increased significantly with addition of IAA in to the media. Maximum percentage of shoot induction (57.28) was observed at 2 mgL^{-1} BAP, which was at par with 1 mgL^{-1} IAA (52.08 per cent).

Regarding the combined effects of BAP and IAA in the induction media, per cent shoot induction increased significantly with increase in BAP and IAA levels (Fig 4.4 and Plate 2). Maximum shoot induction (87.50 per cent) was observed at BAP 6 mgL^{-1} + IAA 2 mgL^{-1} which was at par with BAP 6 mgL^{-1} + IAA 1 mgL^{-1} (83.33 per cent).

4.2.2. Days to shoot induction

It is evident from Table 4.5 and Fig. 4.5 that the addition of BAP and IAA either singly or in combination significantly decreased the time required for shoot induction. When BAP (2-6 mgL^{-1}) was added alone, the minimum time (43.33 days) requirement for shoot induction was observed with 6 mgL^{-1}. The addition of IAA (1-2 mgL^{-1}) also decreased the time required for shoot induction and minimum time (45.33 days) for shoot induction was recorded with 2 mgL^{-1}.

In the case of main effects of BAP, it was discovered that addition of BAP decreased time for shoot induction significantly and minimum time (35.88 days) to shoot induction was obtained with 6 mgL^{-1} BAP which was followed by 4 mgL^{-1} BAP (40.55 days). Regarding the main effects of IAA, addition of IAA (1-2 mgL^{-1}) reduced the time required for shoot induction significantly. The minimum time (37.25 days) for shoot induction for IAA was observed with 2 mgL^{-1} IAA, which was followed by 1 mgL^{-1} IAA (41.66 days).

The interaction between BAP and IAA was found to have significant effect on reduction in time required for shoot induction. The combination of 6 mgL^{-1} BAP + 2 mgL^{-1} IAA took minimum time (31.66 days) to shoot induction which was at par with 6 mgL^{-1} BAP + 1 mgL^{-1} IAA (32.66 days) followed by 4 mgL^{-1} BAP + 2 mgL^{-1} IAA (35.00 days).

When no growth regulator either BAP or IAA was added in MS basal medium, shoot-tips took maximum period (57.00 days) for complete shoot induction.

4.3. Effect of cytokinin (BAP) and auxin (IAA) on shoot multiplication

4.3.1. Number of shoots per explant

Data presented in Table 4.6 and Fig. 4.6 show that number of shoots was significantly increased by addition of BAP singly in to the medium. Maximum numbers of shoots (4.83) was observed with 6 mgL^{-1} BAP followed by 4 mgL^{-1} BAP (3.33). The addition of IAA singly in the MS media did not show any significant increase in shoot number (Plate 3).

The main effect of BAP indicates that the number of shoots increased with increase in concentration of BAP and was found maximum (5.66) at BAP 6 mgL^{-1} followed by 4 mgL^{-1} BAP (3.61). Further increase in BAP in MS medium significantly decreased the number of shoots to 2.83 at BAP 8 mgL^{-1}. Significant increase in shoot number for IAA was observed at 1 mgL^{-1} IAA (3.50) which was found to be at par with 2 mgL^{-1} IAA (3.06).

When both hormones, BAP and IAA were used in combination, a promoting effect on number of shoots per explant was observed up to certain optimal level and thereafter the number of shoots started declining (Plate 4, 5, 6 and 7). The number of shoots per explant was found to be maximum (6.83) at BAP 6 mgL^{-1} + IAA 1 mgL^{-1} followed by (5.33) at BAP 6 mgL^{-1} + IAA 2 mgL^{-1}. The treatment combination of BAP 6 mgL^{-1} + IAA 1 mgL^{-1} was found significantly superior to other combinations of BAP and IAA where number of shoots regenerated was significantly less.

4.3.2. Average shoot length (cm)

It is clear from Table 4.7 and Fig. 4.7 that when BAP was added singly in to the medium at different levels (2-8 mgL^{-1}), the shoot length increased significantly from control (1.91 cm) up to 4 mgL^{-1} BAP (3.73 cm) which was at par with 6 mgL^{-1} (3.60 cm). The further increase in BAP concentration (8 mgL^{-1}), showed decrease in shoot length significantly (2.31 cm). Addition of IAA (1- 2 mgL^{-1}) singly in to the

media exhibited significant increase in shoot length and was found maximum (4.81 cm) at 2 mgL^{-1} IAA.

As far as the main effects of BAP is concerned, shoot length was found to increase gradually and maximum shoot length (5.15 cm) was observed at 4 mgL^{-1} BAP, while at higher concentration, shoot length was found to decrease and minimum shoot length (2.78 cm) was observed at 8 mgL^{-1}. The main effects of IAA showed significant increase in shoot length and was found to be maximum (4.80 cm) at 2 mgL^{-1} which was at par with 1 mgL^{-1} (4.50 cm).

In the case of combination of BAP and IAA, maximum shoot length (5.95 cm) was observed at BAP 4 mgL^{-1} + IAA 2 mgL^{-1} which was found to be at par with combination of BAP 4 mgL^{-1} + IAA 1 mgL^{-1} (5.78 cm). The next highest shoot length (5.43 cm) was observed at BAP 6 mgL^{-1} + IAA 2 mgL^{-1}.

In general addition of IAA in to the medium increased shoot length significantly over control and the shoot length was found to decrease at higher BAP levels (6 and 8 mgL^{-1}) either added alone or in combination with IAA as evident from Plate 4, 5, 6 and 7.

4.4. Effect of MS media strength and IAA concentrations on different parameters of rooting

4.4.1. Per cent rooting

It is evident from Table 4.8 and Plate 8 (a, b) that when IAA was not added to rooting media, half strength MS media showed maximum rooting (65.00 per cent) which was significantly higher than rooting in full strength MS media (15.00 per cent).

For the main effects of media strength, it was observed that half strength of MS medium showed significant increase in rooting (85.00 per cent) over full strength MS media (57.22 per cent). IAA means showed significant increase in per cent rooting at higher levels of IAA (1 mgL^{-1}). Maximum rooting (100 per cent) was observed at 1 mgL^{-1} IAA both in full and half strength of MS media (Plate 8 c, d).

For the combined effect of media strength and IAA concentrations, the maximum rooting (100 per cent) was found to be in MS (full) + 1 mgL^{-1} IAA which was at par with MS (half) + 1 mgL^{-1} IAA (100 per cent) followed by combination of MS (half) + 0.5 mgL^{-1} IAA (Fig. 4.8).

4.4.2. Days to rooting

It is evident from Table 4.9 that main effects of media (full and half strength) showed non-significant difference for days to rooting and minimum days to rooting (10.94) was obtained with half strength MS media. Significant increase in main effects of IAA means was observed for days to rooting. Maximum days to rooting (18.58) was observed at 0 mgL^{-1} IAA, while minimum days to rooting (7.58) was observed at 1 mgL^{-1} IAA.

Significant decrease in number of days to rooting was observed for combined effects of media strength and IAA concentrations (Fig. 4.9). Maximum days to rooting (21.33) was observed in full strength MS media containing devoid of IAA, whereas minimum days for rooting (6.50) was observed with half strength MS media + 1 mgL^{-1} IAA followed by full strength MS media + 1 mgL^{-1} IAA (8.66).

4.4.3. Number of roots per micro-shoot

The data pertaining to number of roots per micro-shoot are presented in Table 4.10 and Fig. 1.10. Full strength MS media showed significantly less number of roots (2.16) as compared to half strength MS media (4.16) as evident from Plate 8 (a, b).

The main effect of MS media strength (full and half) was not found to be significant on number of roots. Maximum number of roots (6.44) was observed in half strength MS media which was at par with full strength MS media (4.83). Main effects of IAA means showed non-significant difference at 0 and 0.5 mgL^{-1} IAA but significant increase in number of roots was observed between 0.0 mgL^{-1} IAA (3.16 roots) and 1.0 mgL^{-1} IAA (8.24 roots).

Regarding the combination of MS media strength and IAA concentrations, maximum number of roots (8.66) was observed at half strength MS media + 1 mgL^{-1}

IAA which was followed by full strength MS media + 1 mgL^{-1} IAA (7.83) as evident from Plate 8 (c, d).

4.5. HARDENING EXPERIMENT

Micropropagated banana plantlets of equal size (5cm length) and approximately 4-5 rootlets per micro-shoot were selected for hardening. The plantlets were irrigated immediately and covered with perforated polybags (Plate 9) and placed in growth chambers for 30 days (Plate 10, 11). After 30 days of acclimatization in growth chamber, plantlets were shifted to net-house (Plate 12) for further acclimatization in the natural climatic conditions. The morphological, physiological and biochemical data were taken at 20, 40 and 60 days after inoculation.

4.5.1. Per cent survival

It is evident from the Table 4.11 and Fig. 4.11 that under moisture stress the per cent survival of banana plantlets at 20 days after inoculation with different AMF isolates showed significant increase over control (87.5 per cent). Cent percent survival was observed in all mycorrhizal treatments.

Per cent survival at 40 and 60 days after inoculation with different AMF species did not show any deviation, while the control treatment exhibited steady decline (75.00 per cent after 40 days and 67.5 per cent after 60 days) under water stress condition.

4.5.2. Per cent root colonization

Per cent colonization after 20, 40 and 60 days of inoculation with different arbuscular-mycorrhizal fungi showed significant increase over control (Table 4.12 and Plate 13).

The percentage of root colonization in control was found to be zero throughout the experimental period (Fig 4.12). At 20 days after inoculation, maximum colonization (51.25 per cent) was observed in mixed AMF strain which was found to be at par with *Glomus intraradices* treatment (47.50 per cent).

At 40 days after inoculation, very high colonization was recorded in the roots of banana plantlets maximum (83.75 per cent) being observed in mixed AMF strain and *Glomus intraradices* which was at par with *Acaulospora scrobiculata* (81.25 per cent).

Maximum root colonization (96.25 per cent) was observed in mixed AMF strain at 60 days after inoculation which was at par with *Glomus intraradices* (93.75 per cent) and *Acaulospora scrobiculata* (87.50 per cent).

4.5.3. Morphological parameters

4.5.3.1. Plant height (cm)

The average plant height in control and mycorrhized plants showed non-significant difference at 20 days after inoculation (Table 4.13). Maximum plant height at 20 days after inoculation was observed in mixed strain of AMF (7.14 cm) while minimum plant height (6.50 cm) was observed in control.

Significant difference in plant height was observed at 40 days after inoculation between mycorrhizal and control treatments (Fig 4.13). Maximum plant height (13.69 cm) was observed in mixed AMF strain followed by *Glomus intraradices* (12.66 cm). The minimum plant height (7.42 cm) was observed in control.

Maximum plant height (19.79 cm) at 60 days after inoculation was observed in mixed AMF strain which was at par with *Glomus intraradices* treatment (19.46 cm) while the minimum plant height (8.61 cm) was observed in control (Plate 14).

4.5.3.2. Number of leaves per plant

Non-significant difference in average number of leaves at 20 days after inoculation was found between control and mycorrhizal treatments (Table 4.14 and Fig 4.14).

Significant increase in leaf number of mycorrhized plantlets over control was observed at 40 days after inoculation. Maximum number of leaves (6.76) was observed with mixed AMF strain followed by *Glomus intraradices* (6.32).

At 60 days after inoculation, maximum number of leaves per plant (6.78) was observed with mixed AMF strain which showed non-significant difference with other mycorrhizal treatments. Minimum leaf number (5.56) was observed in control.

4.5.3.3. Leaf area (cm^2 $plant^{-1}$)

It is evident from Table 4.15 and Fig 4.15 that there was non-significant difference in total leaf area at 20 days after inoculation under water stress.

Significant increase in total leaf area per plant was observed at 40 days after inoculation in mycorrhized plantlets over control. Maximum leaf area (285.36 cm^2 $plant^{-1}$) was observed in mixed AMF strain which was at par with *Glomus intraradices* (277.02 cm^2 $plant^{-1}$), while the minimum leaf area (107.63 cm^2 $plant^{-1}$) was recorded in control.

At 60 days after mycorrhizal treatment, maximum leaf area (384.15 cm^2 $plant^{-1}$) was observed in mixed AMF strain followed by *Glomus intraradices* (373.11 cm^2 $plant^{-1}$) which was at par with *Acaulospora scrobiculata* (365.37 cm^2 $plant^{-1}$) treatment. The minimum leaf area (136.11 cm^2 $plant^{-1}$) was observed in control.

4.5.3.4. Shoot fresh weight (g $plant^{-1}$)

For fresh weight of shoot per plant, there was non-significant difference among treatment means at 20 days after inoculation (Table 4.16 and Fig. 4.16).

Maximum fresh weight of shoot (9.67 g $plant^{-1}$) was observed in mixed AMF strain which was at par with *Glomus intraradices* (9.33 g $plant^{-1}$) at 40 days after inoculation. The control plantlets showed significantly less fresh weight of shoot (5.40 g $plant^{-1}$) under water stress.

At 60 days after inoculation, maximum fresh weight of shoot (15.53 g $plant^{-1}$) was observed in mixed AMF strain which was at par with *Glomus intraradices* (14.83 g $plant^{-1}$). Minimum fresh weight of shoot (7.64 g $plant^{-1}$) was observed in control containing no mycorrhizal treatment.

4.5.3.5. Shoot dry weight (g plant^{-1})

At 20 days after inoculation, shoot dry weight among the treatment means showed non-significant difference (Table 4.17). Maximum dry weight of shoot (0.465 g plant^{-1}) was observed in mixed AMF strain, while minimum (0.435 g plant^{-1}) was recorded in control.

At 40 days after inoculation, mycorrhizal treatments showed significantly higher shoot dry weight than control (0.557 g plant^{-1}). Maximum dry weight of shoot (1.87 g plant^{-1}) was observed in mixed AMF strain followed by *Glomus intraradices* (1.72 g plant^{-1}).

Marked increase in dry weight of shoots was observed at 60 days after inoculation (Fig 4.17). Maximum shoot dry weight (3.587 g plant^{-1}) was recorded in mixed AMF strain followed by *Glomus intraradices* (3.357 g plant^{-1}) while the minimum shoot dry weight was observed in control (1.145 g plant^{-1}).

4.5.3.6. Number of roots per plant

Number of roots at 20 days after mycorrhizal treatment showed significant increase over control (Table 4.18 and Fig. 4.18). Maximum number of roots (9.00) at 20 days was observed in mixed AMF strain which was at par with *Glomus intraradices* (8.75).

At 40 days after inoculation, maximum number of roots (15.32) was observed in mixed AMF strain which was at par with *Glomus intraradices* (14.77) treatment. The minimum number of roots (5.40) was observed in control.

At 60 days after inoculation, maximum number of roots (24.75) was observed in mixed AMF strain followed by *Glomus intraradices* (22.22), while the minimum number of roots (8.52) was observed in control (Plate 15).

4.5.3.7. Root length (cm)

Average root length in mycorrhizal treatments was found to be significantly less as compared to the control throughout the experimental period (Table 4.19 and Plate 15).

Maximum root length (6.29 cm) at 20 days after inoculation was observed in control followed by *Acaulospora scrobiculata* (4.66 cm) which was at par with mixed AMF strain (4.63 cm) and *Glomus intraradices* (4.53 cm).

At 40 days after inoculation, maximum root length (9.45 cm) was noted in control followed by *Acaulospora scrobiculata* (7.88 cm) and remained at par with mixed AMF strain and *Glomus intraradices* (Fig 4.19).

Maximum root length (12.61 cm) was observed for control experiment at 60 days after inoculation followed by mixed AMF strain (11.78 cm).

4.5.3.8. Root fresh weight (g plant^{-1})

Data presented in Table 4.20 and Fig. 4.20 show that there was a significant increase in root fresh weight in mycorrhizal treatments over control at all the stages of plant growth.

At 20 days after inoculation, maximum fresh weight of root (1.55 g plant^{-1}) was observed in mixed AMF strain which was at par with both *Glomus intraradices* (1.45 g plant^{-1}) and *Acaulospora scrobiculata* (1.36 g plant^{-1}), while minimum root fresh weight (0.68 g plant^{-1}) was observed in control.

Maximum root fresh weight (5.99 g plant^{-1}) at 40 days after inoculation was recorded in mixed AMF strain followed by *Glomus intraradices* (5.32 g plant^{-1}). Minimum fresh weight of root (1.33 g plant^{-1}) was observed in control.

Significant differences among the treatment means were observed at 60 days after inoculation. Maximum fresh weight of roots (9.88 g plant^{-1}) was observed in mixed AMF strain followed by *Glomus intraradices* (7.85 g plant^{-1}). Minimum fresh weight of roots (1.54 g plant^{-1}) was observed in control.

4.5.3.9. Root dry weight (g plant^{-1})

Significant increase in root dry weight was observed over control (0.056 g plant^{-1}) in mycorrhizal treatments at 20 days after inoculation (Table 4.21 and Fig. 4.21). Maximum root dry weight (0.079 g plant^{-1}) was observed in mixed AMF strain

which was at par with *Glomus intraradices* (0.077 g plant^{-1}) and *Acaulospora scrobiculata* (0.076 g plant^{-1}).

At 40 days after inoculation, maximum root dry weight (1.15 g plant^{-1}) was observed in mixed AMF strain which was at par with *Glomus intraradices* (1.145 g plant^{-1}) and *Acaulospora scrobiculata* (1.137 g plant^{-1}), while minimum root dry weight (0.39 g plant^{-1}) was observed in control.

Root dry weight was found to be significantly different among all treatments at 60 days after inoculation. Maximum dry weight of root (2.55 g plant^{-1}) was observed in mixed AMF strain followed by *Glomus intraradices* (2.367 g plant^{-1}), while minimum dry weight of root (0.657 g plant^{-1}) was recorded in control.

4.5.4. Physiological parameters

4.5.4.1. Photosynthetic rate (µmol m^{-2} s^{-1})

At 20 days after inoculation, photosynthetic rate was found to be maximum (5.21 µmol m^{-2} s^{-1}) in mixed AMF strain which was at par with *Glomus intraradices* (5.17 µmol m^{-2} s^{-1}) whereas minimum photosynthetic rate (5.08 µmol m^{-2} s^{-1}) was observed in control (Fig 4.22).

Significant increase in photosynthetic rate was observed in mycorrhizal treatments over control (10.20 µmol m^{-2} s^{-1}) at 40 days after inoculation. Maximum photosynthetic rate (14.54 µmol m^{-2} s^{-1}) was recorded in mixed AMF strain followed by *Glomus intraradices* (13.58 µmol m^{-2} s^{-1}).

At 60 days after inoculation, maximum photosynthetic rate (18.85 µmol m^{-2} s^{-1}) was observed in mixed AMF strain followed by *Glomus intraradices* (16.57 µmol m^{-2} s^{-1}) whereas minimum photosynthetic rate (13.69 µmol m^{-2} s^{-1}) was observed in control (Table 4.22).

4.5.4.2. Stomatal conductance (mol m^{-2} s^{-1})

Stomatal conductance in mycorrhizal treatments was significantly higher than that of control at 20 days after inoculation (Table 4.23). Maximum stomatal

conductance (0.035 mol m^{-2} s^{-1}) was observed in mixed AMF strains while minimum (0.002 mol m^{-2} s^{-1}) was observed in control.

Stomatal conductance at 40 days after inoculation was found to be maximum (0.090 mol m^{-2} s^{-1}) in mixed AMF strain followed by *Glomus intraradices* (0.052 mol m^{-2} s^{-1}). Minimum stomatal conductance (0.030 mol m^{-2} s^{-1}) was observed in control.

Significant difference in stomatal conductance was observed among all treatments at 60 days after inoculation (Fig. 4.23). Maximum stomatal conductance (0.265 mol m^{-2} s^{-1}) was observed in mixed AMF strain followed by *Acaulospora scrobiculata* (0.137 mol m^{-2} s^{-1}).

4.5.4.3. Relative water content (per cent)

Relative water content in leaves of inoculated plants showed significant increase over control throughout the experimental period (Table 4.24 and Fig. 4.24).

At 20 days, maximum relative water content (88.57 per cent) was observed in mixed AMF strain which was at par with *Glomus intraradices* (88.56 per cent) while minimum relative water content (80.89 per cent) was observed in control.

At 40 days after inoculation, maximum relative water content of 90.93 per cent was observed in mixed AMF strain which was at par with *Glomus intraradices* (90.67 per cent). In control relative water content was found to be 85.14 per cent.

Maximum relative water content (96.30 per cent) was observed in mixed AMF strain at 60 days after inoculation which was at par with *Glomus intraradices* (95.69 per cent). Minimum relative water content (85.23 per cent) was observed in control.

4.5.5. Biochemical analysis

4.5.5.1. Total phenol (mg g^{-1} fresh weight)

Significantly higher phenol content in the leaves of mycorrhized plantlets was observed over control throughout the experimental period (Table 4.25 and Fig. 4.25).

Maximum phenol content (13.40 mg g^{-1} fresh weight) was recorded in mixed AMF strain followed by *Glomus intraradices* (12.49 mg g^{-1} fresh weight) whereas

minimum phenol content (7.25 mg g $^{-1}$ fresh weight) was observed in control at 20 days after inoculation.

At 40 days, phenol content was found to be maximum (53.50 mg g $^{-1}$ fresh weight) in mixed AMF followed by *Acaulospora scrobiculata* (51.48 mg g $^{-1}$ fresh weight). Minimum phenol content (18.50 mg g $^{-1}$ fresh weight) was recorded in control.

Highest total phenol (88.98 mg g $^{-1}$ fresh weight) was recorded in mixed AMF strain followed by *Glomus intraradices* (87.73 mg g $^{-1}$ fresh weight) which was at par with *Acaulospora scrobiculata* (87.25 mg g $^{-1}$ fresh weight). The minimum phenol content (23.36 mg g $^{-1}$ fresh weight) was observed in control.

4.5.5.2. Proline content (mg g^{-1} fresh weight)

Changes in proline content due to moisture stress at various growth stages of micropropagated banana are presented in Table 4.26 and Fig. 4.26. At 20 days after inoculation maximum proline content (0.546 mg g $^{-1}$ fresh weight) was observed in mixed AMF strain followed by *Glomus intraradices* (0.493 mg g $^{-1}$ fresh weight), while minimum proline content (0.079 mg g $^{-1}$ fresh weight) was recorded in control.

At 40 days, maximum proline content (0.546 mg g $^{-1}$ fresh weight) was recorded in mixed AMF strain followed by *Glomus intraradices* (0.493 mg g $^{-1}$ fresh weight). Minimum proline (0.079 mg g $^{-1}$ fresh weight) was recorded in control.

It is evident from Fig. 4.26 that significantly higher proline content (0.721 mg g $^{-1}$ fresh weight) at 60 days after inoculation was recorded in mixed AMF strain followed by *Glomus intraradices* (0.711 mg g $^{-1}$ fresh weight) and lowest proline content (0.186 mg g $^{-1}$ fresh weight) was observed in control.

4.5.5.3. Total chlorophyll (SPAD units)

It is obvious from Table 4.27 and Fig. 4.27 that total chlorophyll content at 20 days after inoculation is significantly higher in mycorrhizal treatments as compared to control and the same pattern was recorded throughout the experimental period. Maximum chlorophyll content (28.83 SPAD units) was recorded in mixed AMF strain which was at par with *Glomus intraradices* (28.51 SPAD units) and *Acaulospora scrobiculata* (28.45 SPAD units).

At 40 days, maximum chlorophyll content (50.67 SPAD units) was observed in *Acaulospora scrobiculata* followed by mixed AMF strain (49.68 SPAD units), while minimum chlorophyll content was observed in control (34.77 SPAD units).

At 60 days after inoculation, maximum chlorophyll content (54.39 SPAD units) was observed in *Acaulospora scrobiculata* followed by mixed AMF strain (52.70 SPAD units). Minimum total chlorophyll content (41.42 SPAD units) was observed in control.

4.5.5.4. Total soluble sugar content (mg g^{-1} fresh weight)

Table 4.28 shows changes in total soluble sugar content in leaves of control and mycorrhized plantlets under water stress. At 20 days after inoculation, though the treatment differences were not significant sugar content was found to be maximum (11.88 mg g^{-1} fresh weight) in mixed AMF strain.

At 40 days, maximum sugar content (34.65 mg g^{-1} fresh weight) was observed in mixed AMF strain followed by *Glomus intraradices* (32.88 mg g^{-1} fresh weight) whereas minimum sugar content (19.34 mg g^{-1} fresh weight) was recorded in control (Fig. 4.28).

Sugar content in leaves of inoculated plants was significantly higher than that of control at 60 days after inoculation. Maximum sugar content (43.21 mg g^{-1} fresh weight) was recorded in mixed AMF strain followed by *Glomus intraradices* (40.06 mg g^{-1} fresh weight). Minimum sugar content (23.61 mg g^{-1} fresh weight) was recorded in control.

4.5.5.5. Starch content (mg g^{-1} fresh weight)

It is discerned from Table 4.29 that starch content in the leaves of control plantlets was significantly higher than that of mycorrhized plantlets at 20 days after inoculation. Maximum starch content (15.57 mg g^{-1} fresh weight) was recorded in control followed by *Acaulospora scrobiculata* (14.76 mg g^{-1} fresh weight). Minimum starch content (14.28 mg g^{-1} fresh weight) was recorded in *Glomus intraradices*.

At 40 days after inoculation, maximum starch content (22.54 mg g^{-1} fresh weight) was recorded in the leaves of plantlets inoculated with mixed AMF strain

followed by *Glomus intraradices* (21.75 mg g $^{-1}$ fresh weight), while minimum (19.94 mg g $^{-1}$ fresh weight) starch was recorded in the leaves of control plantlets.

Maximum starch content (61.99 mg g $^{-1}$ fresh weight) was recorded in mixed AMF strain followed by *Glomus intraradices* (43.88 mg g $^{-1}$ fresh weight) at 60 days after inoculation. Minimum starch content (21.40 mg g $^{-1}$ fresh weight) was recorded in control (Fig. 4.29).

4.5.5.6. Enzymatic activity

4.5.5.6.1. Catalase (Enzyme Units mg protein^{-1})

The activity of catalase in the leaves of control and mycorrhized banana plantlets is presented in Table 4.30 and Fig. 4.30. Significantly higher catalase activity under water stress during acclimatization was recorded in all mycorrhized plants. Maximum catalase activity (3.55 Enzyme Units mg protein^{-1}) at 20 days after inoculation was recorded in mixed AMF strain which was at par with *Glomus intraradices* (3.45 Enzyme Units mg protein^{-1}) and *Acaulospora scrobiculata* (3.36 Enzyme Units mg protein^{-1}). Minimum (2.92 Enzyme Units mg protein^{-1}) catalase activity was recorded in control.

At 40 days, maximum activity of catalase (6.58 Enzyme Units mg protein^{-1}) was recorded in mixed AMF strain which was at par with *Acaulospora scrobiculata* (6.54 Enzyme Units mg protein^{-1}) and *Glomus intraradices* (6.33 Enzyme Units mg protein^{-1}). Minimum catalase activity (4.90 Enzyme Units mg protein^{-1}) was recorded in control.

Maximum activity of catalase (3.68 Enzyme Units mg protein^{-1}) at 60 days after inoculation was recorded *Glomus intraradices* which was at par with *Acaulospora scrobiculata* (3.55 Enzyme Units mg protein^{-1}) and mixed AMF strain (3.53 Enzyme Units mg protein^{-1}), whereas minimum catalase activity (2.27 Enzyme Units mg protein^{-1}) was recorded in control.

4.5.5.6.2. Peroxidase (Enzyme Units g^{-1} fresh weight min^{-1})

It is evident from Table 4.31 and Fig. 4.31 that at 20 days after inoculation, activity of peroxidase in mycorrhized plantlets was significantly higher than that of

control (0.875 Enzyme Units g^{-1} fresh weight min^{-1}). Maximum activity of peroxidase (1.9 Enzyme Units g^{-1} fresh weight min^{-1}) was recorded in mixed AMF strain which was at par with *Glomus intraradices* (1.75 Enzyme Units g^{-1} fresh weight min^{-1}).

At 40 days after inoculation, maximum peroxidase activity (2.02 Enzyme Units g^{-1} fresh weight min^{-1}) was recorded in mixed AMF strain which was at par with *Glomus intraradices* (1.92 Enzyme Units g^{-1} fresh weight min^{-1}) and *Acaulospora scrobiculata* (1.87 Enzyme Units g^{-1} fresh weight min^{-1}). Minimum peroxidase activity (1.5 Enzyme Units g^{-1} fresh weight min^{-1}) was recorded in control.

Activity of peroxidase was found to be maximum (1.77 Enzyme Units g^{-1} fresh weight min^{-1}) in *Acaulospora scrobiculata* at 60 days after inoculation which was at par with *Glomus intraradices* (1.57 Enzyme Units g^{-1} fresh weight min^{-1}) and mixed AMF strain (1.55 Enzyme Units g^{-1} fresh weight min^{-1}). Whereas, minimum peroxidase activity (1.2 Enzyme Units g^{-1} fresh weight min^{-1}) was recorded in control.

4.5.5.6.3. Polyphenol oxidase (Enzyme Units g^{-1} fresh weight min^{-1})

The changes in the activity of polyphenol oxidase enzyme are presented in Table 4.32 and Fig. 4.32. At 20 days after inoculation, maximum polyphenol oxidase activity (2.67 Enzyme Units g^{-1} fresh weight min^{-1}) was recorded in *Glomus intraradices* which was at par with mixed AMF strain (2.57 Enzyme Units g^{-1} fresh weight min^{-1}), while minimum polyphenol oxidase activity (1.17 Enzyme Units g^{-1} fresh weight min^{-1}) was recorded in control.

At 40 days after inoculation, maximum polyphenol oxidase activity (3.80 Enzyme Units g^{-1} fresh weight min^{-1}) was recorded in mixed AMF which was at par with *Glomus intraradices* (3.67 Enzyme Units g^{-1} fresh weight min^{-1}) and *Acaulospora scrobiculata* (3.20 Enzyme Units g^{-1} fresh weight min^{-1}). Minimum activity of polyphenol oxidase (1.73 Enzyme Units g^{-1} fresh weight min^{-1}) was recorded in control.

The activity of polyphenol oxidase was found to be maximum (3.70 Enzyme Units g^{-1} fresh weight min^{-1}) in mixed AMF strain which was at par with *Glomus intraradices* (3.47 Enzyme Units g^{-1} fresh weight min^{-1}). Minimum activity of

polyphenol oxidase enzyme (1.75 Enzyme Units g^{-1} fresh weight min^{-1}) was recorded in control at 60 days after inoculation.

4.6. LEAF NUTRIENT ANALYSIS

The nutrient content (N, P and K) in the leaves of 60 days old fully acclimatized plantlets under nutrient and water stress is presented in Table 4.33 and Plate 16, 17 and 18. The nitrogen content in the leaves of mycorrhized plantlets was significantly higher than the control (0.855mg g^{-1} dry weight). Maximum leaf nitrogen content (2.89 mg g^{-1} dry weight) was observed in mixed AMF strain treated plantlets followed by *Glomus intraradices* (1.84 mg g^{-1} dry weight).

The phosphorus content in mycorrhizal treatments was significantly higher than that of control (0.053 mg g^{-1} dry weight). All mycorrhized plantlets showed at par values (Fig. 4.33). Maximum phosphorus content (0.175 mg g^{-1} dry weight) was observed in mixed AMF strain which was at par with *Glomus intraradices* (0.172 mg g^{-1} dry weight) and *Acaulospora scrobiculata* (0.122 mg g^{-1} dry weight).

Potassium content in the leaves of mycorrhized plantlets was found to be significantly higher than control (0.795 mg g^{-1} dry weight). Maximum value of potassium (1.365 mg g^{-1} dry weight) was recorded in mixed AMF strain which was at par with *Glomus intraradices* (1.295 mg g^{-1} dry weight).

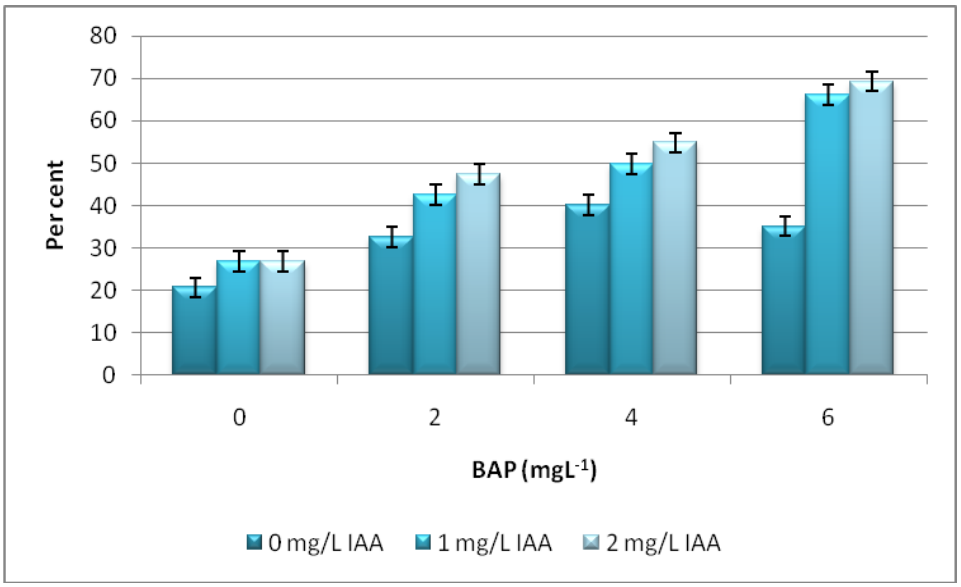


Fig. 4.4: Effect of BAP concentration (0, 2, 4, 6 mgL^{-1}) on percentage shoot induction of explants with 0, 1 and 2 mgL^{-1} IAA.

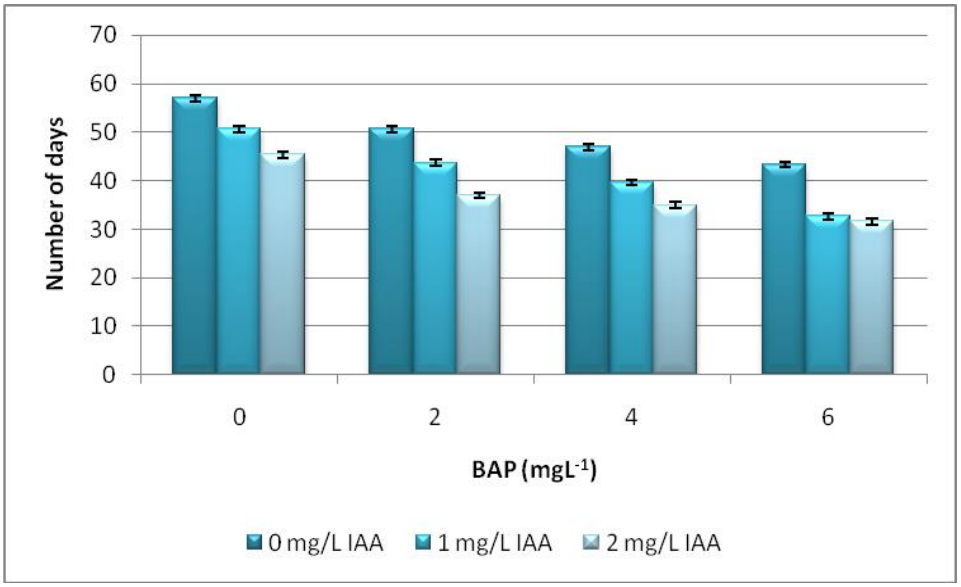


Fig. 4.5: Effect of BAP concentration (0, 2, 4, 6 mgL^{-1}) on days to shoot induction of explants with 0, 1 and 2 mgL^{-1} IAA.

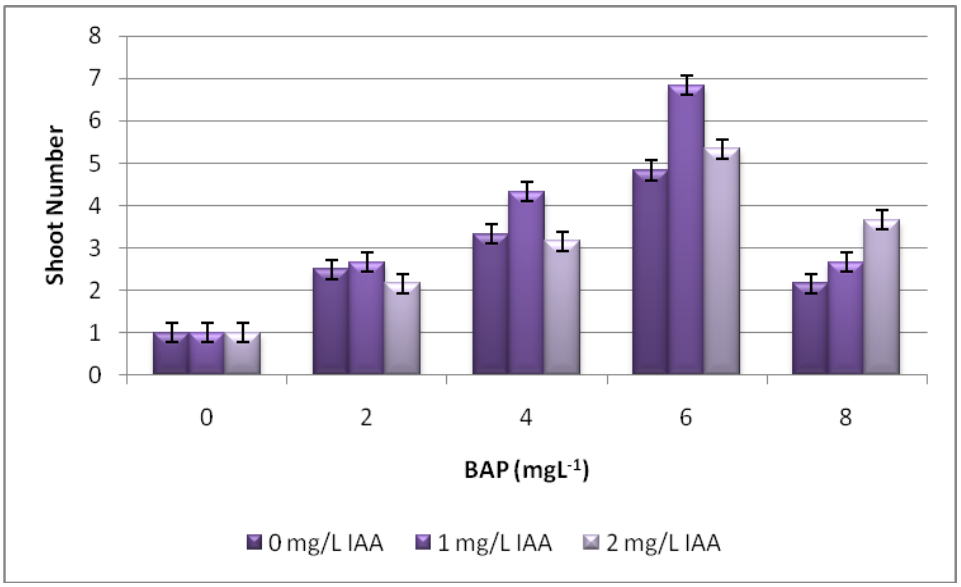


Fig. 4.6: Effect of BAP concentration (0, 2, 4, 6 mgL^{-1}) on number of shoots per explant with 0, 1 and 2 mgL^{-1} IAA.

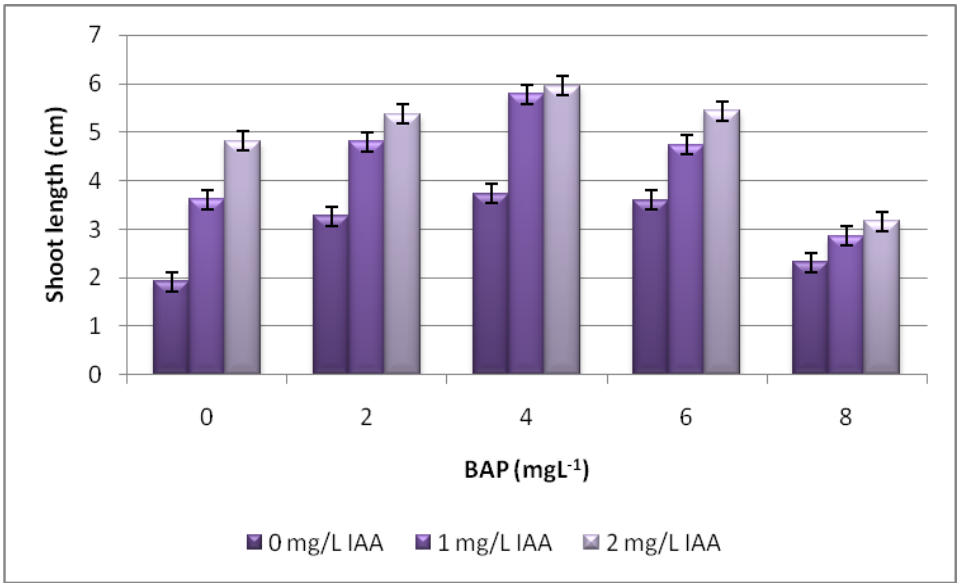


Fig. 4.7: Effect of BAP concentration (0, 2, 4, 6 mgL^{-1}) on average shoot length with 0, 1 and 2 mgL^{-1} IAA.

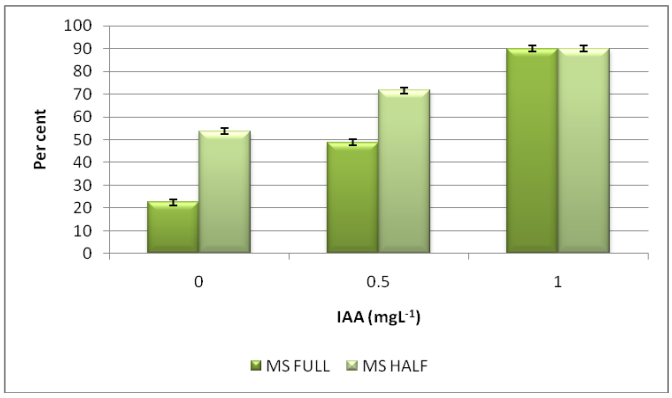


Fig. 4.8: Effect of IAA concentration (0, 0.5, 1 mgL^{-1}) on *in vitro* rooting percentage with full and half strength MS media.

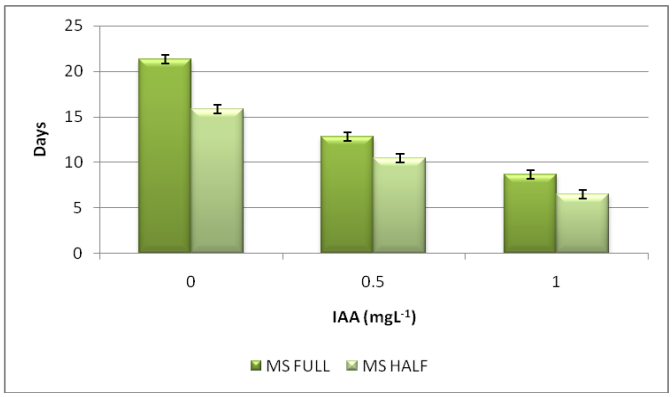


Fig. 4.9: Effect of IAA concentration (0, 0.5, 1 mgL^{-1}) on days to rooting with full and half strength MS media.

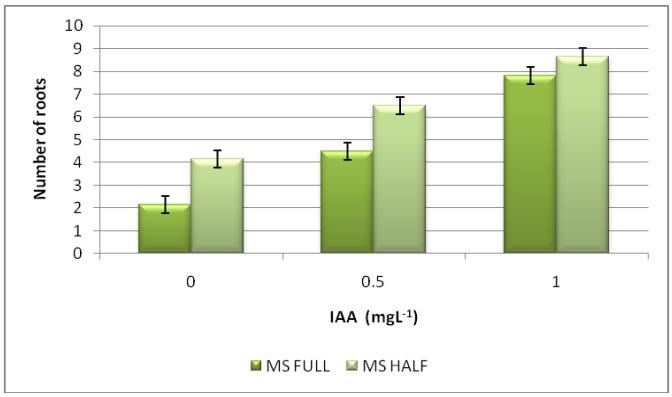


Fig. 4.10: Effect of IAA concentration (0, 0.5, 1 mgL^{-1}) on number of roots per micro-shoot with full and half strength MS media.

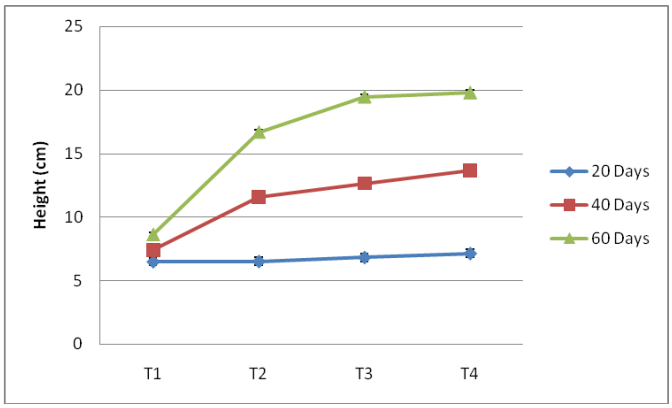


Fig. 4.13: Plant height in control and mycorrhized plantlets under water stress during hardening at 20, 40 and 60 days after inoculation.

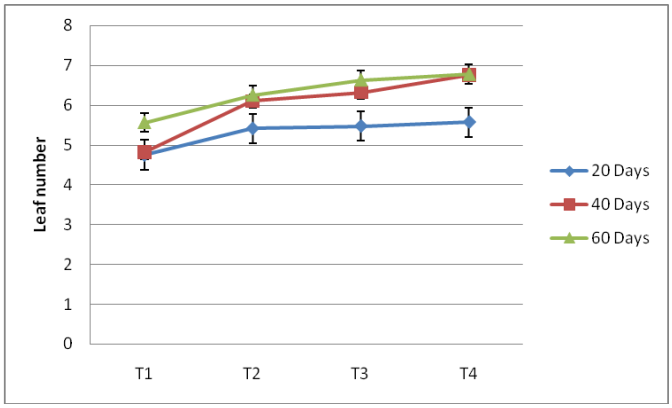


Fig. 4.14: Number of leaves per plant in control and mycorrhized treatments under water stress during hardening at 20, 40 and 60 days after inoculation.

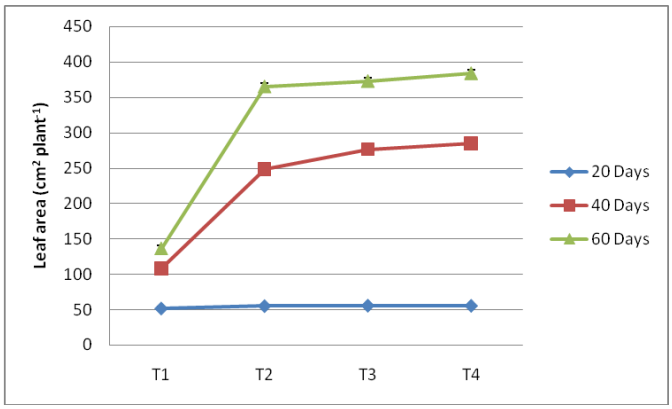


Fig. 4.15: Leaf area per plant in control and mycorrhized treatments under water stress during hardening at 20, 40 and 60 days after inoculation.

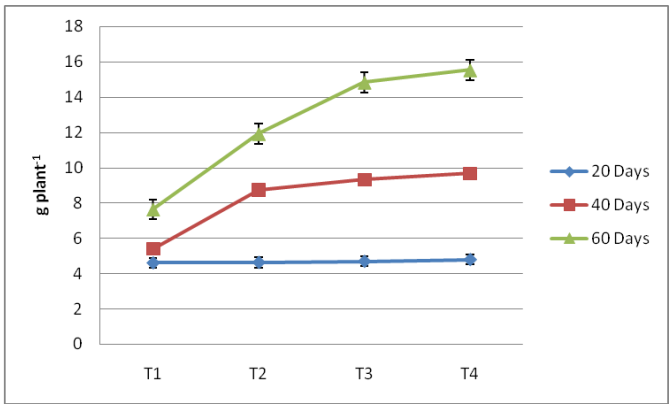


Fig. 4.16: Shoot fresh weight per plant in control and mycorrhized treatments under water stress during hardening at 20, 40 and 60 days after inoculation.

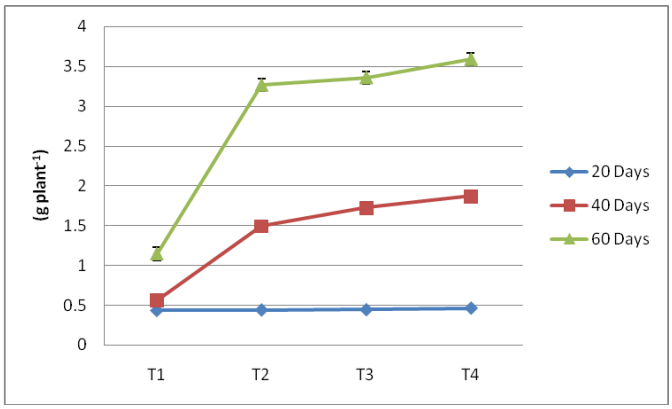


Fig. 4.17: Shoot dry weight per plant in control and mycorrhized treatments under water stress during hardening at 20, 40 and 60 days after inoculation.

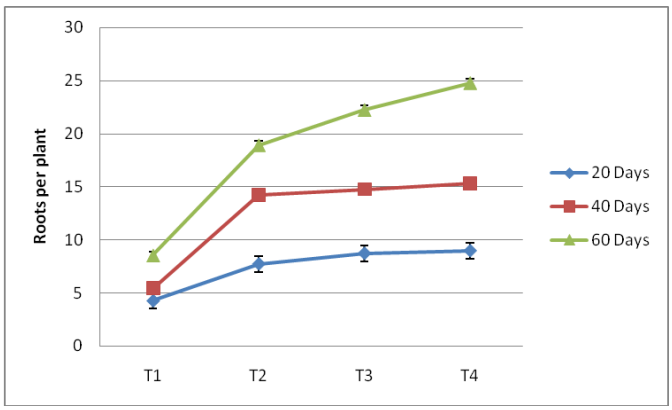


Fig. 4.18: Number of roots per plant in control and mycorrhized treatments under water stress during hardening at 20, 40 and 60 days after inoculation.

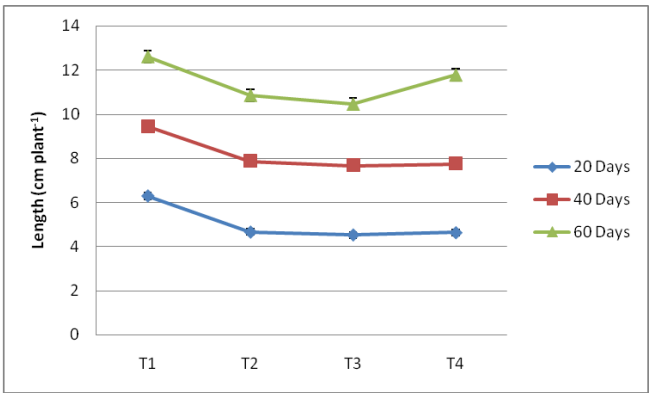


Fig. 4.19: Average root length per plant in control and mycorrhized treatments under water stress during hardening at 20, 40 and 60 days after inoculation.

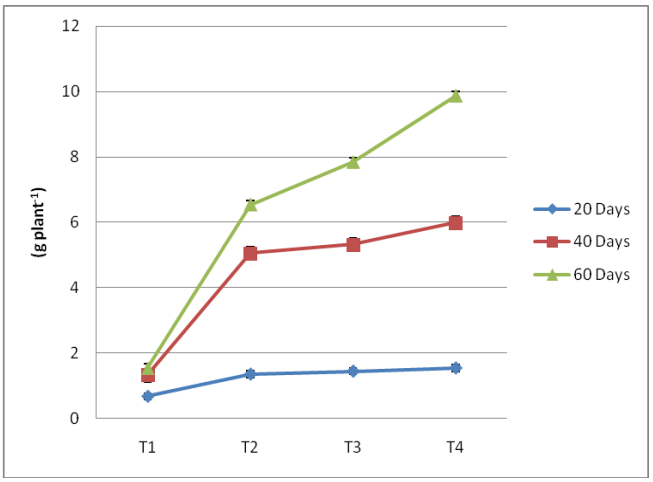


Fig. 4.20: Root fresh weight per plant in control and mycorrhized treatments under water stress during hardening at 20, 40 and 60 days after inoculation.

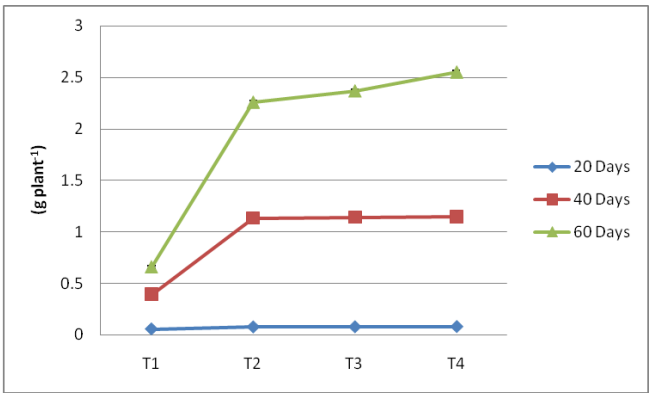


Fig. 4.21: Root dry weight per plant in control and mycorrhized treatments under water stress during hardening at 20, 40 and 60 days after inoculation.

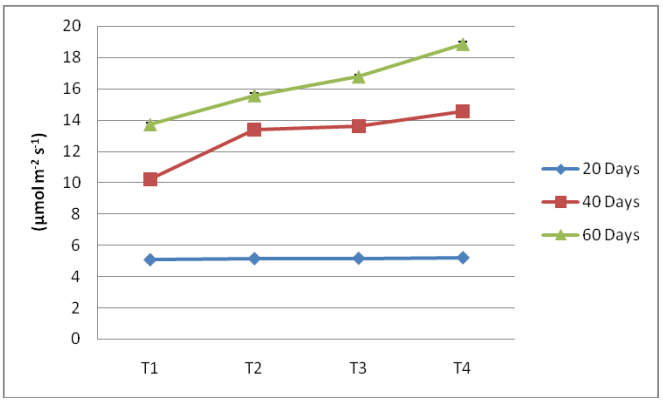


Fig. 4.22: Effect of mycorrhization on photosynthetic rate in micropropagated banana plantlets under water stress during hardening at 20, 40 and 60 days after inoculation.

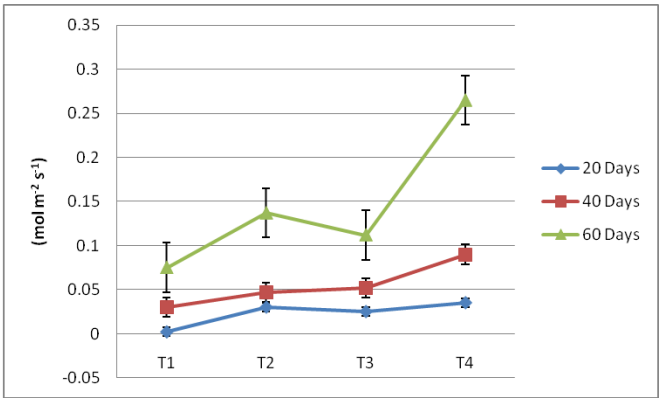


Fig. 4.23: Effect of mycorrhization on stomatal conductance in leaves of micropropagated banana plantlets under water stress during hardening at 20, 40 and 60 days after inoculation.

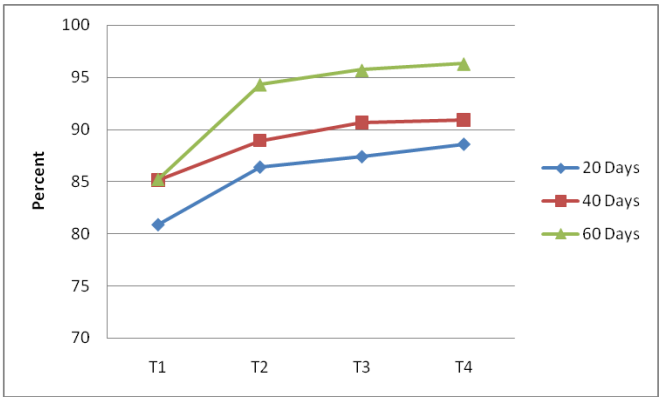


Fig. 4.24: Effect of mycorrhization on relative water content in leaves of micropropagated banana plantlets under water stress during hardening at 20, 40 and 60 days after inoculation.

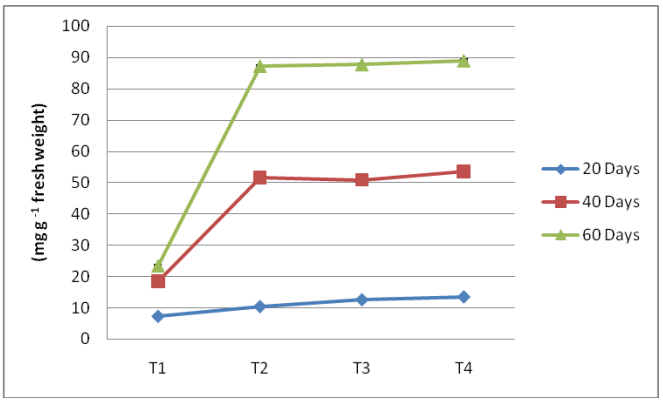


Fig. 4.25: Effect of mycorrhization on total phenol content in leaves of micropropagated banana plantlets under water stress during hardening at 20, 40 and 60 days after inoculation.

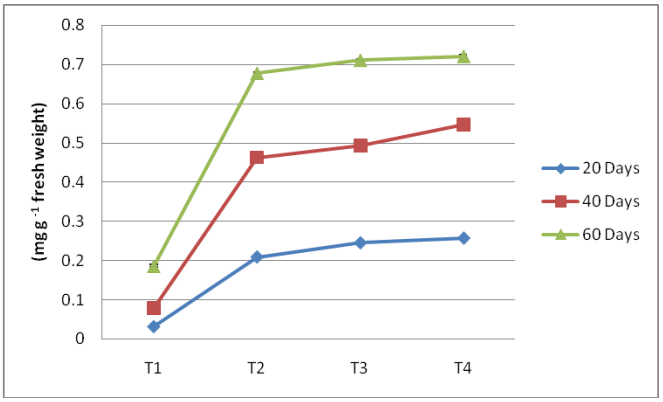


Fig. 4.26: Effect of mycorrhization on proline content in leaves of micropropagated banana plantlets under water stress during hardening at 20, 40 and 60 days after inoculation.

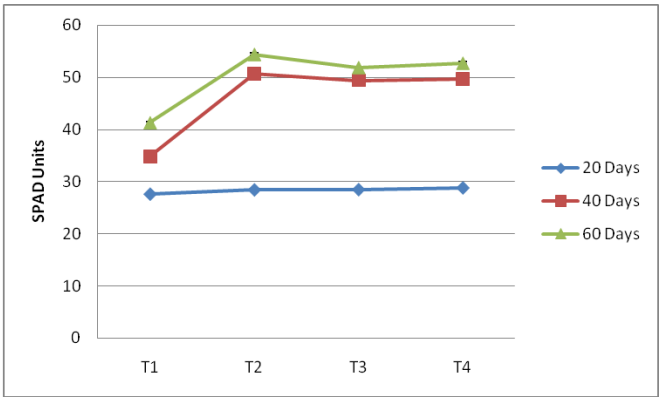


Fig. 4.27: Effect of mycorrhization on total chlorophyll in leaves of micropropagated banana plantlets under water stress during hardening at 20, 40 and 60 days after inoculation.

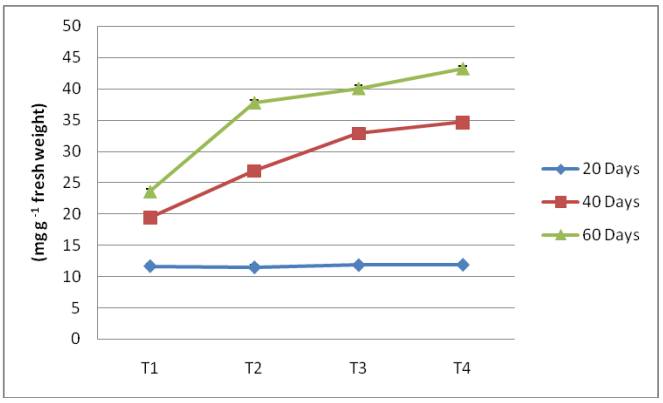


Fig. 4.28: Effect of mycorrhization on total soluble sugar content in leaves of micropropagated banana plantlets under water stress during hardening at 20, 40 and 60 days after inoculation.

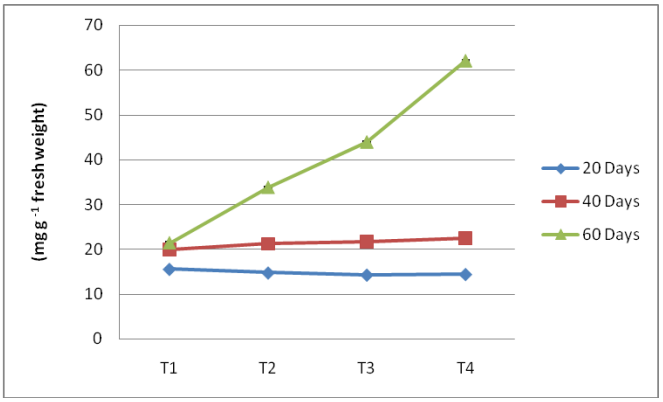


Fig. 4.29: Effect of mycorrhization on starch content in leaves of micropropagated banana plantlets under water stress during hardening at 20, 40 and 60 days after inoculation.

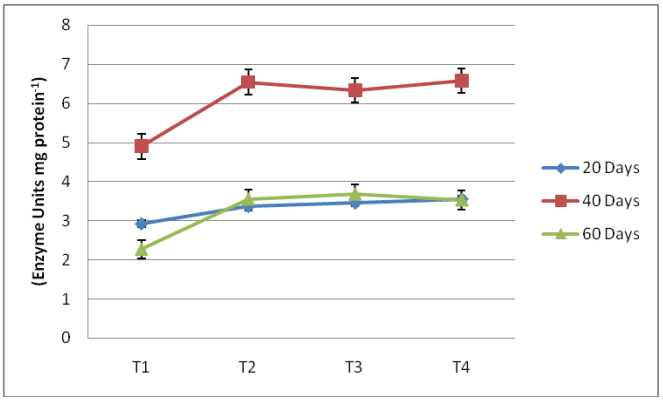


Fig. 4.30: Changes in catalase activity in control and mycorrhized micropropagated banana plantlets under water stress during hardening at 20, 40 and 60 days after inoculation.

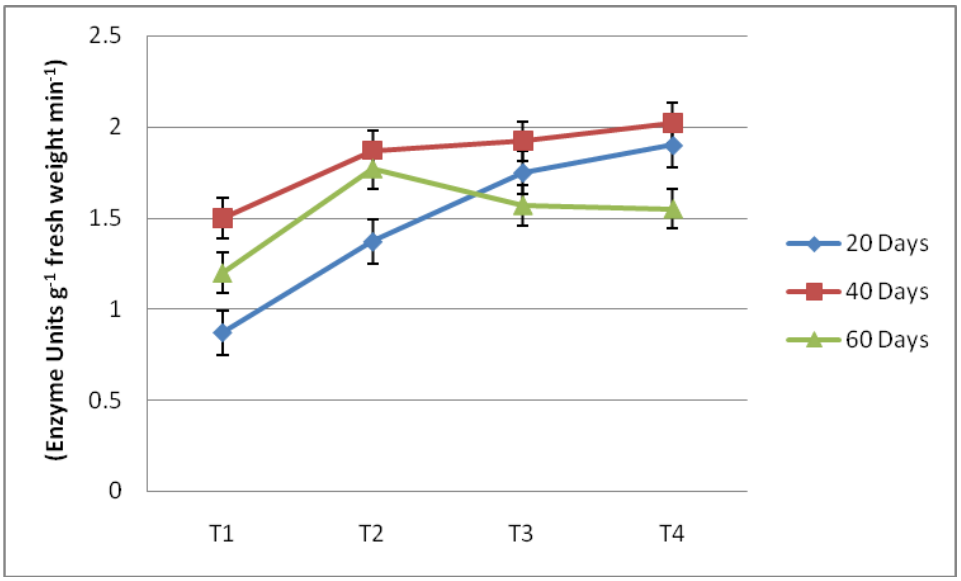


Fig. 4.31: Changes in peroxidase activity in control and mycorrhized micropropagated banana plantlets under water stress during hardening at 20, 40 and 60 days after inoculation.

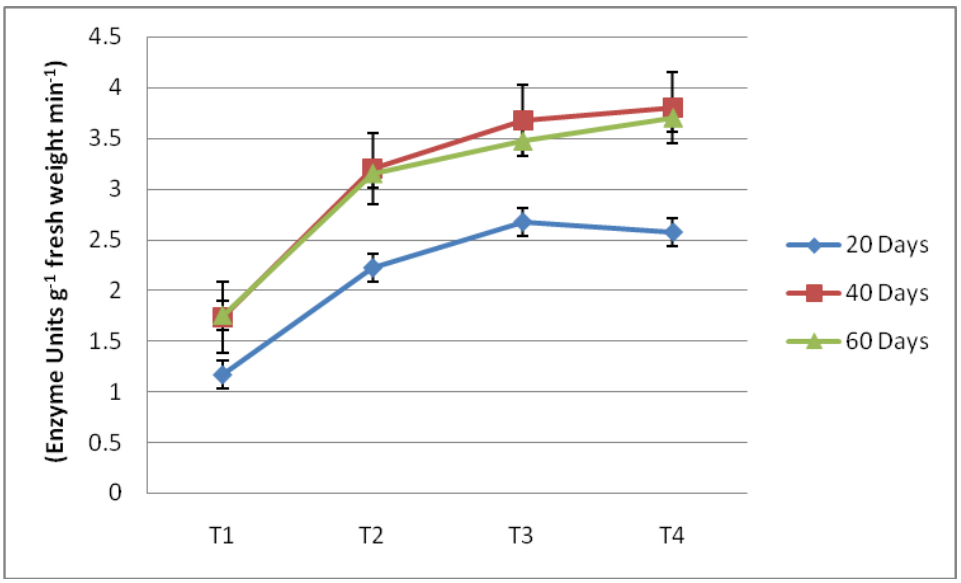
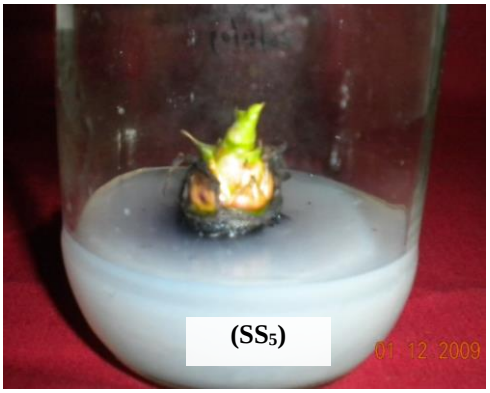
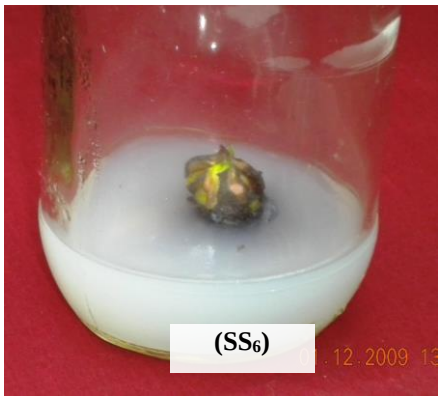


Fig. 4.32: Changes in polyphenol oxidase activity in control and mycorrhized micropropagated banana plantlets under water stress during hardening at 20, 40 and 60 days after inoculation.

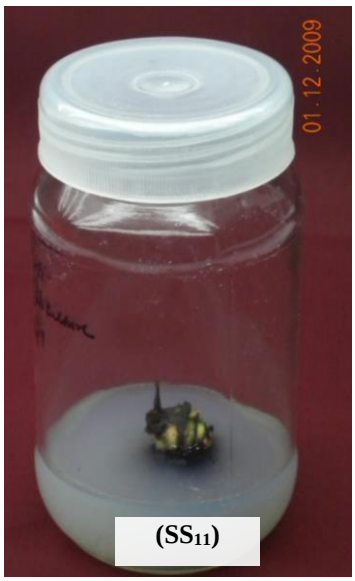
PLATE 1: STERILIZATION TREATMENTS FOR SHOOT-TIP EXPLANTS ISOLATED FROM FIELD COLLECTED SUCKERS OF BANANA cv. DWARF CAVENDISH.



a. Single sterilization with $HgCl_2$ (0.1%) for 6 minutes.

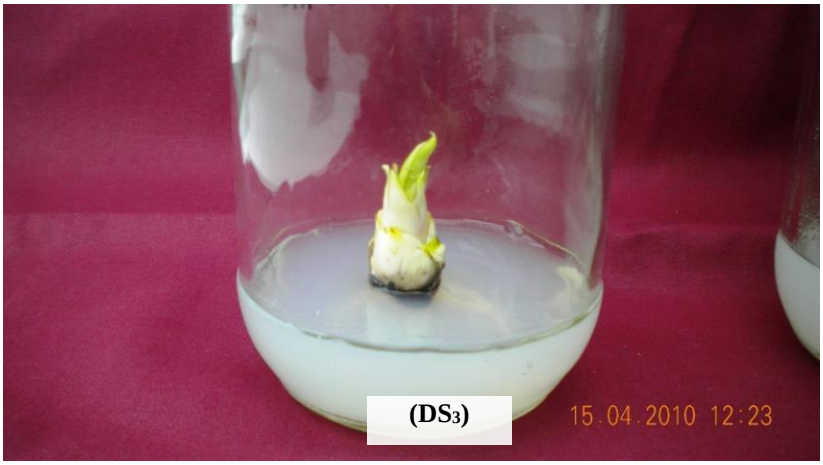


b. Single sterilization with $HgCl_2$ (0.1%) for 7 minutes.



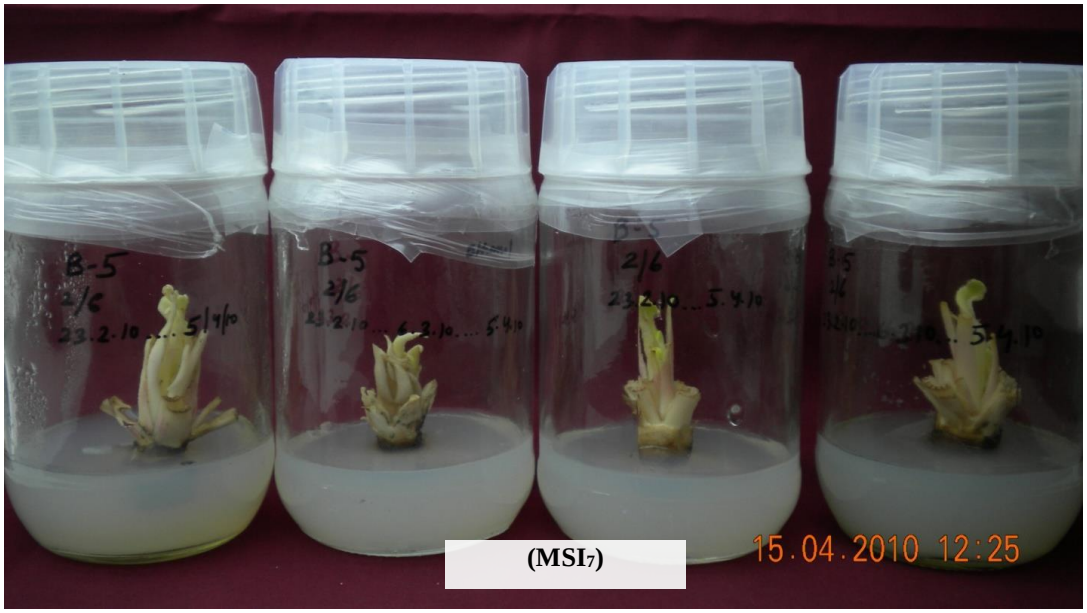
c. Single sterilization with NaOCl (4%) for 25 minutes.

d. Single sterilization with NaOCl (4%) for 30 minutes.



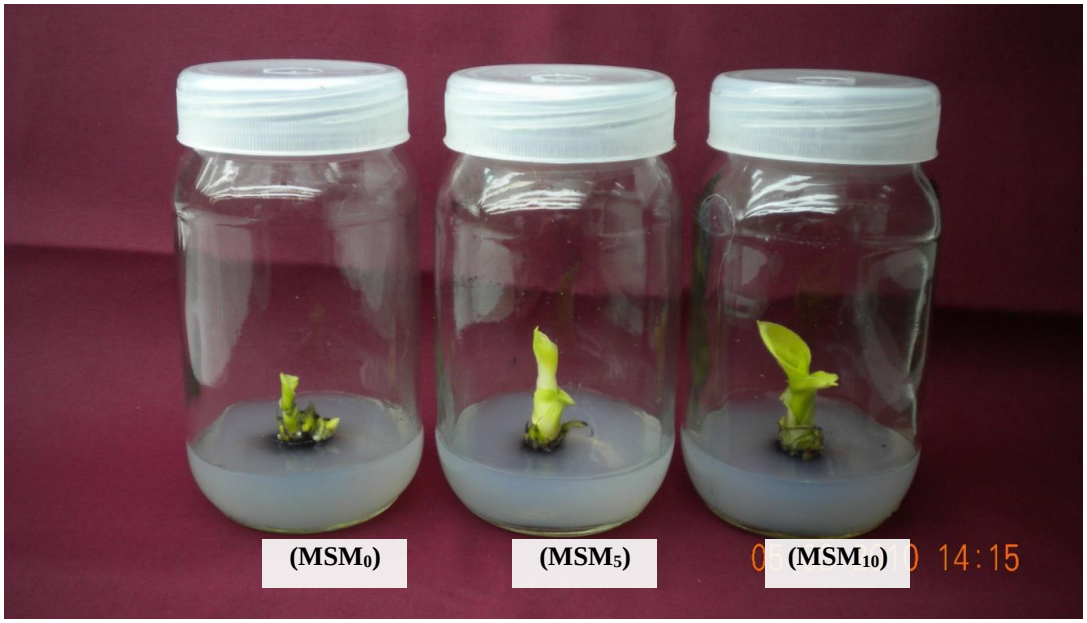
e. Double sterilization with $HgCl_2$ (0.1%) for 5 minutes + Quick dip in Ethanol.

PLATE 2 : DOUBLE STERILIZATION (DS_3) FOLLOWED BY INOCULATION IN SHOOT INDUCTION MEDIUM SUPPLEMENTED WITH BAP 6 mgL^{-1} + IAA 1 mgL^{-1}.



(MSI_7)

PLATE 3 : SHOOT MULTIPLICATION IN MS MEDIA SUPPLEMENTED WITH DIFFERENT LEVELS OF IAA (0, 1 and 2 mgL^{-1})



(MSM_0) **(MSM_5)** **(MSM_{10})**

PLATE 4 : SHOOT MULTIPLICATION AT 4 mgL^{-1} BAP AND DIFFERENT LEVELS OF IAA (0, 1, 2 mgL^{-1})

a. 4 mgL^{-1} BAP + 0 mgL^{-1} IAA.

b. 4 mgL^{-1} BAP + 1 mgL^{-1} IAA.

c. 4 mgL^{-1} BAP + 2 mgL^{-1} IAA.

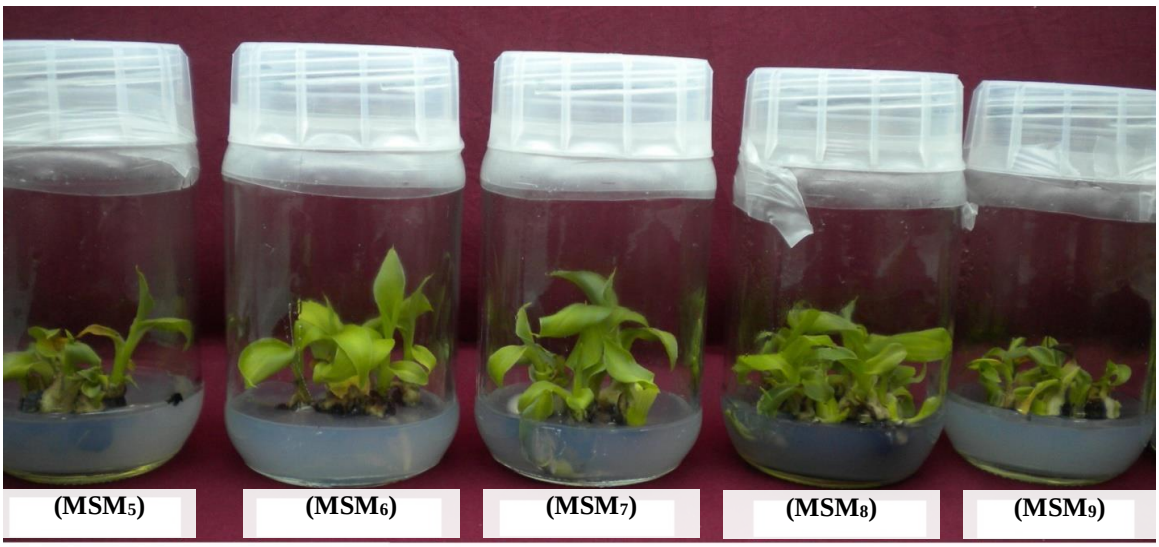
PLATE 5 : SHOOT MULTIPLICATION AT 6 mgL^{-1} BAP AND DIFFERENT LEVELS OF IAA (0, 1, 2 mgL^{-1})

a. 6 mgL^{-1} BAP + 0 mgL^{-1} IAA.

b. 6 mgL^{-1} BAP + 1 mgL^{-1} IAA.

c. 6 mgL^{-1} BAP + 2 mgL^{-1} IAA.

Plate 6 : SHOOT MULTIPLICATION AT DIFFERENT COMBINATIONS OF GROWTH REGULATORS



(MSM$_5$) (MSM$_6$) (MSM$_7$) (MSM$_8$) (MSM$_9$)

PLATE 7 : SHOOT MULTIPLICATION AT 8 mgL^{-1} BAP AND DIFFERENT LEVELS OF IAA (0, 1, 2 mgL^{-1})

a. 8 mgL^{-1} BAP + 0 mgL^{-1} IAA.

b. 8 mgL^{-1} BAP + 1 mgL^{-1} IAA.

c. 8 mgL^{-1} BAP + 2 mgL^{-1} IAA (MSM_{14})

PLATE 8 : *IN VITRO* ROOTING IN BANANA PLANTLETS

a. *In vitro* rooting of banana plantlets in full strength MS media + 0 mgL^{-1} IAA (MSR_0).

b. *In vitro* rooting of banana plantlets in half strength MS media + 0 mgL^{-1} IAA (MSR_1).

c. *In vitro* rooting of banana plantlets in full strength MS media + 1 mgl^{-1} IAA (MSR_4)

d. *In vitro* rooting of banana plantlets in half strength MS media + 1 mgl^{-1} IAA (MSR_5)

PLATE 9 : MICROPROPAGATED BANANA PLANTLETS COVERED WITH PERFORATED POLYBAGS AFTER PLANTING IN PLASTIC POTS.

PLATE 10 : MICROPROPAGATED BANANA PLANTLETS TRANSFERRED TO GROWTH CHAMBER AFTER PLANTING IN POTS.

PLATE 11 : CONTROL AND MYCORRHIZED TREATMENTS AT 30 DAYS AFTER INOCULATION.

a. Control (T_1)

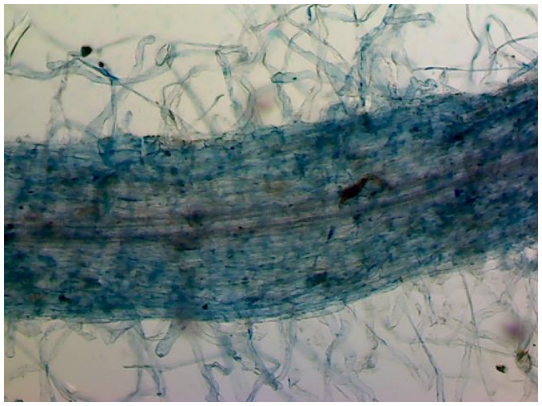
b. *Acaulospora scrobiculata* treated plantlets (T_2)

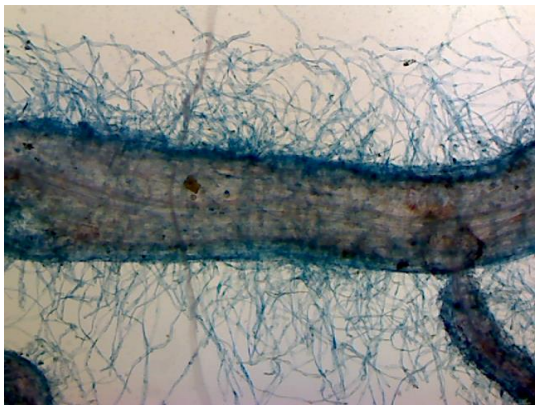
c. *Glomus intraradices* treated plantlets (T_3)

d. Mixed AMF treated plantlets (T_4)

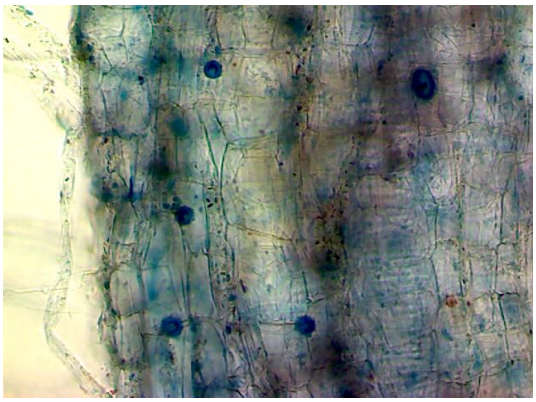
PLATE 12: MICROPROPAGATED BANANA PLANTLETS TRANSFERRED TO NET-HOUSE AFTER 30 DAYS OF ACCLIMATIZATION

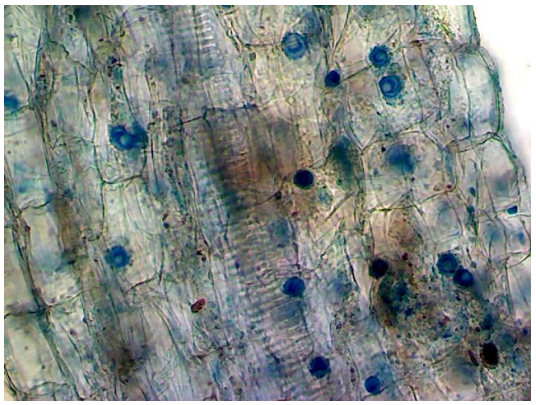
PLATE 13 : BANANA ROOT SECTION COVERED WITH MYCORRHIZAL NETWORK



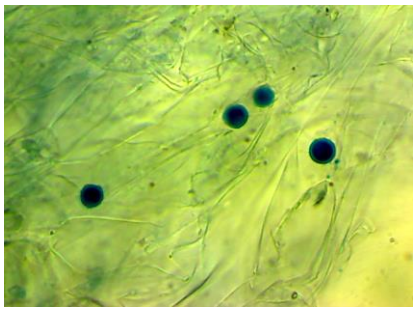


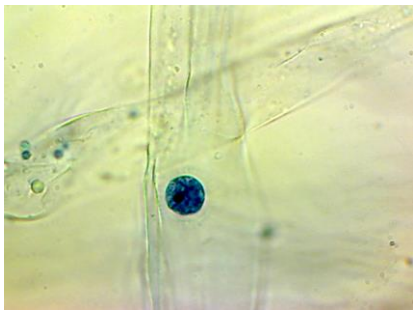
MYCORRHIZAL SPORES IN ROOT CELLS

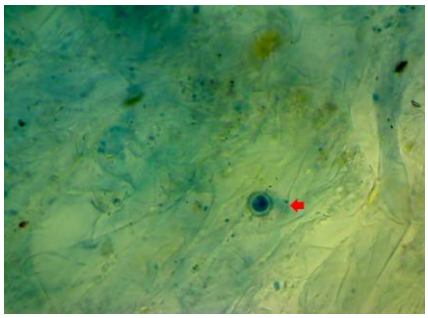




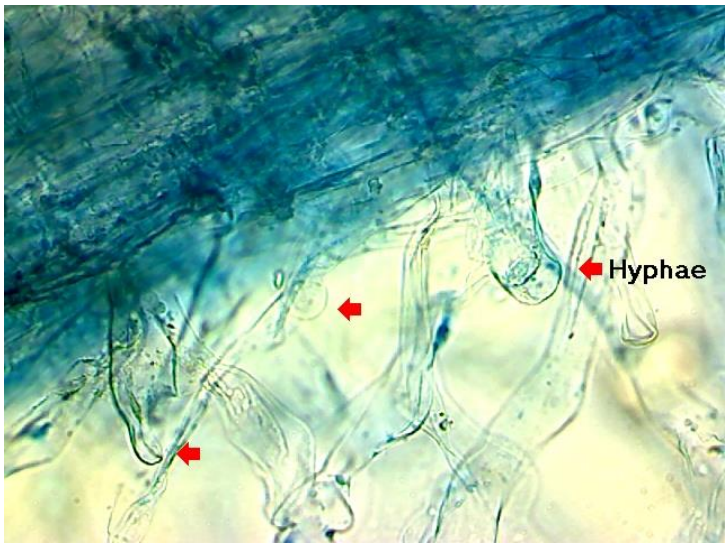
SINGLE SPORE

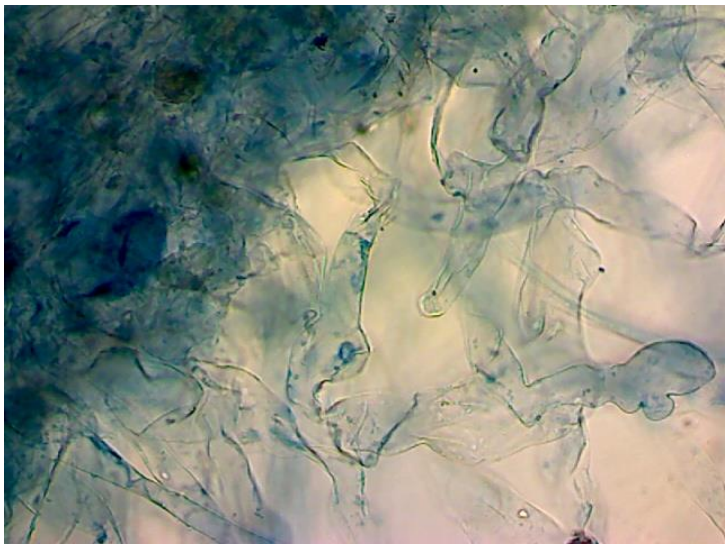






MYCORRHIZAL HYPHAE EXTENDING FROM ROOT OF BANANA PLANT





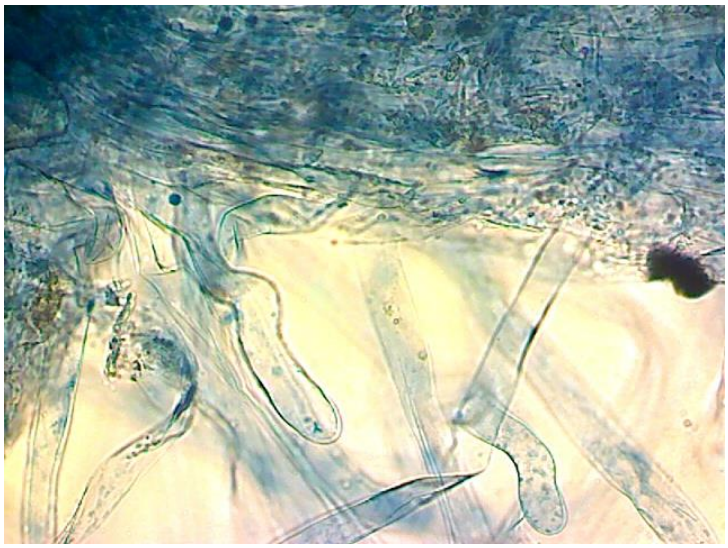


PLATE 14 : BANANA PLANTLETS 60 DAYS AFTER INOCULATION

a. Control (T_1)

b. *Acaulospora scrobiculata* (T_2)

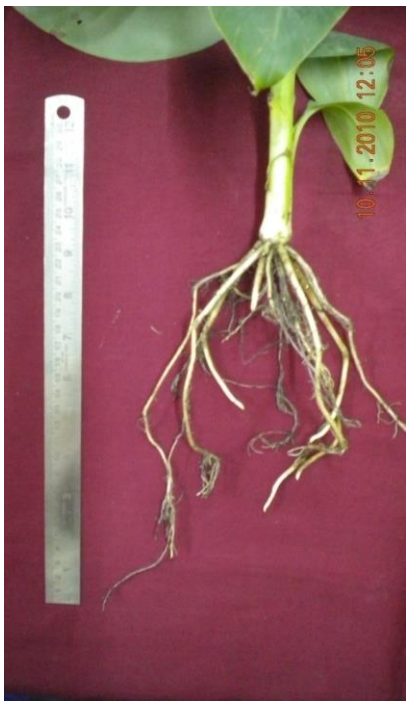
c. *Glomus intraradices* (T_3)

d. Mixed AMF strain (T_4)

PLATE 15 : ROOT MORPHOLOGY OF CONTROL AND MYCORRHIZED BANANA PLANTLETS AT 60 DAYS AFTER PLANTING

a. Control (T_1)

b. *Acaulospora scrobiculata* (T_2)



c. *Glomus intraradices* (T_3)

d. Mixed AMF strain (T_4)

PLATE 16 : CONTROL AND MYCORRHIZED BANANA PLANTLETS IN NET-HOUSE

a. **Control**

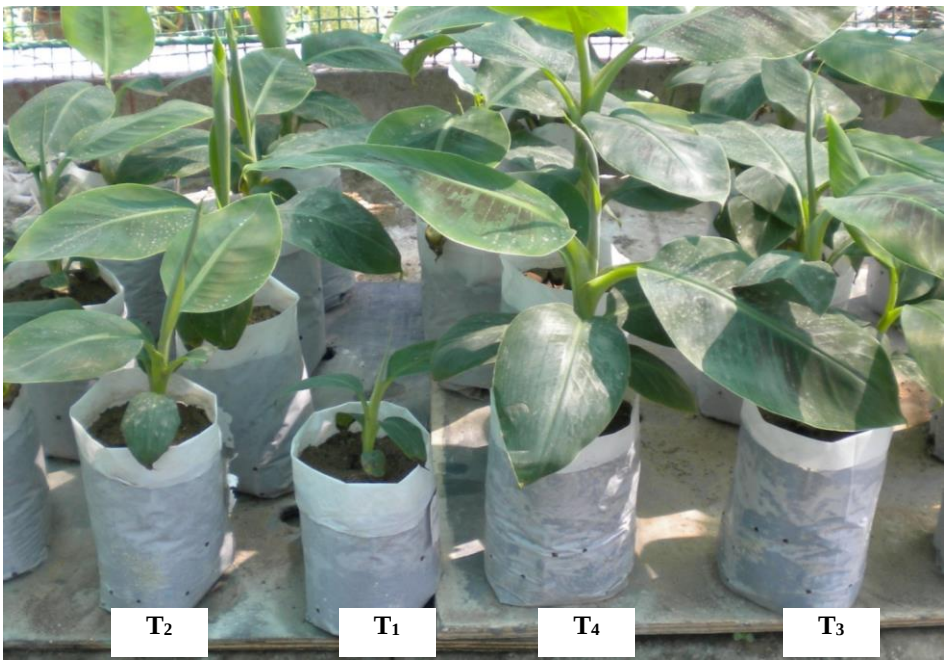
b. ***Acaulospora scrobiculata*** **(T_2)**

c. ***Glomus intraradices*** **(T_3)**

d. **Mixed AMF strain (T_4)**

PLATE 17 : MYCORRHIZED PLANTLETS OF BANANA cv. DWARF CAVENDISH

PLATE 18 : GROWTH OF CONTROL COMPARED TO THE MYCORRHIZED PLANTLETS AFTER 60 DAYS OF INOCULATION.



T_1 = Control, T_2 = ***Acaulospora scrobiculata***, T_3 = ***Glomus intraradices***, T_4 = **Mixed AMF strain**

Chapter V

Discussion

Chapter V

DISCUSSION

Investigation entitled "Biochemical changes in micropropagated banana (*Musa* sp.) cv. Dwarf Cavendish (AAA) due to arbuscular-mycorrhizal fungi (AMF) inoculation during *ex vitro* acclimatization" was carried out during 2008-10 to find out the changes in morpho-physiological and biochemical processes of micropropagated banana plants under normal and mycorrhized condition to identify and correlate the parameters associated with developmental changes during acclimatization under moisture stress.

Experiments were carried out in two parts. During 2008-09 the protocol for micropropagation of banana was standardized and cultures were maintained for at least 5 subculture cycles. In the year 2010, the plantlets were transferred in plastic pots containing sand: soil: FYM (1:1:1) in month of September. The maintained cultures of arbuscular mycorrhizae were applied to each plantlet at the base and mixed in the potting mixture at the rate of 20 g per pot. All the plantlets were immediately irrigated and covered with perforated transparent polybags. Irrigation was provided daily for initial 15 days and after that plants were irrigated at an interval of 5 days. Morphological, physiological and biochemical observations were taken at 20, 40 and 60 days after inoculation. Fertilizer, pesticide and fungicide were not applied throughout the experimental period in all the treatments up to 60 days.

5.1. MICROPROPAGATION PROTOCOL

5.1.1. Surface sterilization of explants

Surface sterilization for field collected banana suckers becomes very crucial step for micropropagation. The most important step in standardization of successful micropropagation protocol is to check the contamination throughout the process of *in vitro* culture. Since field collected suckers are full of soil borne pathogens, it becomes imperative to sterilize the explants before inoculation in sterile growth medium. Though general sterilization procedure for explants has been outlined by various workers, yet there is still lack of published data on the relative success and efficiency of various decontamination methods using field collected banana suckers. Hence, in

present study, it was felt necessary to standardize the sterilants and their duration of treatment separately to propose most efficient and successful surface sterilization technique for banana micropropagation.

Among the sterilizing agents used, mercuric chloride (0.1%) at different time of exposure (3-7 minutes) showed drastic decrease in per cent contamination from 3-7 minutes (Table 4.1). Survival was recorded to be highest (54.16 per cent) at 5 minutes treatment which decreased drastically upon increasing the exposure time i.e., 6 and 7 minutes. No mortality was observed at 3 and 4 minutes of exposure with mercuric chloride but with further increase in treatment duration, from 5-7 minutes, mortality increased drastically from 4.16 per cent to 87.50 per cent showing the detrimental effects of mercuric chloride over the succulent tissues of shoot-tip explants. Though mortality was zero in 3 and 4 minutes treatment, survival was very low because of high percentage of contamination. Some investigators have used low concentrations of mercuric chloride to initiate aseptic cultures of banana (Banerjee and Sharma, 1988; Habiba *et al*., 2002; Titov *et al*., 2006). Resmi and Nair (2007) also recommended use of mercuric chloride (0.1%) for 5 minutes to disinfect explants before inoculation.

When sodium hypochlorite (4%) was used as sterilizing agent for 5-30 minutes, contamination per cent reduced significantly and reached a minimum of zero at 25 and 30 minutes (Table 4.2). The increased survival was recorded from 5-15 minutes exposure reaching maximum (58.33 per cent) at 15 minutes treatment with sodium hypochlorite and then declining trend in survival was observed from 20-30 minutes treatment indicating the harmful effect of treatment duration on explants. The per cent mortality was recorded to be zero in 5 and 10 minutes treatment, it increased significantly from 15-30 minutes and showed maximum (100 per cent) with 30 minutes treatment. Variations in sterilization procedures have been proposed by several researchers. Some reports suggest that sodium hypochlorite is the most effective disinfectant for surface sterilization of banana explants (Sandra and Krikorian, 1984; Mendes *et al*., 1996; Muhammad *et al*., 2004). Wong (1986) also recommended use of sodium hypochlorite (1%) with Tween 80 for 15 minutes.

The best treatment durations (showing minimum mortality) of mercuric chloride and sodium hypochlorite were selected for treatment with ethanol for 2-4 seconds (quick dip) in double sterilization step to compare the effectiveness of sterilants together. Contamination per cent decreased with increase in treatment duration of both sterilants. Minimum contamination (0.00 per cent) was observed in 5 minute mercuric chloride + quick dip treatment with ethanol. Maximum survival (91.66 per cent) was again observed with 5 minute mercuric chloride + quick dip in ethanol. The mortality of explants increased with increase in time of exposure of mercuric chloride as well as sodium hypochlorite (Table 4.3). Double sterilization technique has also been adopted by several workers, in which first large sized explant is disinfected, followed by shoot-tip excision and finally sterilization with some other surface disinfectant (Silva *et al*., 1998a; Nandwani *et al*., 2000; Madhulatha *et al*., 2004). Ethanol has also been used by a number of research workers for disinfection purposes (Silva *et al.,* 1998a; Rahman *et al.,* 2002; Jalil *et al.,* 2003). Hamill *et al.* (1993) also preferred double sterilization over single sterilization methods and recommended treatment with 3.5 per cent sodium hypochlorite with Tween 80 for 15 minutes followed by removing bleached tissues and again sterilization with sodium hypochlorite (3.5 per cent) for 5 minutes.

5.1.2. Effect of cytokinin (BAP) and auxin (IAA) on different regeneration parameters from shoot-tip explants

5.1.2.1. Per cent shoot induction

Explants i.e., shoot-tips were cultured on MS medium supplemented with different concentrations of BAP and IAA added singly as well as in combination to determine their most appropriate combinations. When shoot-tips were cultured on MS medium without plant growth regulators, only 12.50 per cent explants responded towards *in vitro* shoot formation (Table 4.4).

Addition of BAP (2-4 mgL^{-1}) singly in the media significantly increased shoot induction. Further increase in BAP concentration in MS media reduced shoot induction per cent at 6 mgL^{-1} which showed above optimal level. Addition of IAA

singly at 1 mgL^{-1} significantly increased shoot initiation per cent over control but at 2 mgL^{-1} there was no increase in per cent shoot initiation.

Regarding the combined effects of BAP and IAA, maximum shoot initiation (87.50 per cent) was recorded with 6 mgL^{-1} BAP + 2 mgL^{-1} IAA which was at par with 6 mgL^{-1} BAP + 1 mgL^{-1} IAA (83.33 per cent).

The per cent shoot initiation was found to increase with increase in concentration of BAP and IAA in the medium. This may be due to the combined effects of cytokinin and auxin which increased shoot elongation with cell division and enlargement. BAP alone at higher concentration showed reduced percent shoot induction but when IAA was added to the same concentration of BAP, very high shoot induction percentage was observed which showed the positive effect of IAA on shoot elongation. A combination of cytokinin and auxin was also used for banana culture initiation by a number of researchers (Hwang *et al.*, 1984; Zaffari *et al*., 2000).

5.1.2.2. Days to shoot induction

In the present study, it was found that the addition of BAP and IAA either singly or in combination decreased the time required for shoot induction (Table 4.5). In MS media containing no growth regulator, maximum number of days to shoot induction was observed (57 days).

When BAP was added singly in the media minimum duration of 43.33 days to shoot induction was recorded with 6 mgL^{-1}. Addition of IAA singly in the induction media showed minimum number of days to shoot induction (45.33 days) at 2 mgL^{-1}.

As far as the interaction effect of BAP and IAA is concerned, the early shoot induction (31.66 days) was recorded with 6 mgL^{-1} BAP + 2 mgL^{-1} IAA which was at par with 6 mgL^{-1} BAP + 1 mgL^{-1} IAA (32.66 days). The reduction in shoot induction time with higher BAP and IAA contents in the media may be due to the higher cell multiplication and elongation.

In conclusion, the best combination of shoot induction was found to be 6 mgL^{-1} BAP + 1 mgL^{-1} IAA since a lower concentration of growth regulators is always

beneficial over higher concentrations to avoid morphological abnormalities in the explants.

5.1.3. Effect of cytokinin (BAP) and auxin (IAA) on shoot multiplication

5.1.3.1. Number of shoots per explant

The number of shoots per explant increased when MS medium was fortified with BAP and IAA either singly or in combination. In individual effects of BAP, number of shoots increased significantly up to certain levels and was found to be maximum (4.83) when MS medium was fortified with BAP 6 mgL^{-1} (Table 4.6). Further increase in BAP (8 mgL^{-1}) significantly reduced the proliferation rate. This may be due to the inhibitory effects of higher levels of cytokinin in the media. Addition of auxin singly in to the multiplication medium had no additional effect on proliferation. The results are in corroboration with the findings of Sandra and Krikorian (1984); Bhagyalakshmi and Singh (1995); Mandes *et al*. (1996); Rahaman *et al*. (2002).

When BAP and IAA were used in combination, maximum number of shoots per explant (6.83) was recorded with BAP 6 mgL^{-1} + IAA 1 mgL^{-1} in the multiplication medium. The number of shoots was further decreased when higher concentrations of BAP and IAA were added together in the medium (Table 4.6). Similar proliferation behavior with BAP in banana was observed by Vuylsteke (1998) and Arinaitwe *et al.* (2000).

Shoot multiplication with the addition of cytokinin is noticed with a concomitant suppression of shoot length. Also, a moderate concentration of cytokinin increased the shoot proliferation rate, but very high concentrations decreased multiplication and depressed shoot elongation. However, with the addition of auxin in the media, shoot elongation was noticed. The number of shoots per explant increased when MS medium was fortified with BAP and IAA either singly or in combination. In conclusion, it was observed that BAP significantly increased shoot proliferation up to 6 mgL^{-1} either added singly or in combination with IAA. IAA did not increase shoot proliferation significantly when added singly in the media but showed significant effects when used in combination with BAP. Combination of BAP (6 mgL^{-1}) + IAA

(1 mgL^{-1}) was found to be optimum for maximum shoot proliferation. Sandra and Krikorian (1984) recorded 9.1 shoots per explant during *in vitro* multiplication of 'Philippine Lacatan' and 'Grande Naine' on a modified MS medium supplemented with 5 mgL^{-1} 6-benzylaminopurine (BAP). On the other hand Rahman *et al*. (2002) achieved 4.52 shoots per explant on the same concentration of BAP on MS medium during *in vitro* multiplication of Bari-1, indicating the genotypic response towards cytokinins. Highest shoot length (3.62 cm) was achieved when MS medium was supplemented with 1.5 mgL^{-1} NAA.

5.1.3.2. Average shoot length

During *in vitro* multiplication, shoot length varied according to different media composition. It was observed that IAA and interaction of IAA and BAP affected the plant height significantly (Table 4.7).

On the plain MS medium, the average shoot length was 1.91 cm. The length of shoots significantly increased with increasing concentration of BAP up to certain levels and decreased at higher levels of BAP. Maximum shoot length (3.73 cm) was observed in MS medium supplemented with 4 mgL^{-1} BAP. The length of shoots also increased with increase in concentrations of IAA and the maximum shoot length (4.81 cm) was observed with 2 mgL^{-1} IAA. This may be due to the reason that cytokinin promotes shoot proliferation by inducing cell division and enlargement where as the logic for increased shoot length by addition of auxin is that it stimulates cell elongation.

Interaction effects of BAP and IAA resulted in maximum shoot length (5.95 cm) in MS medium fortified with 4 mgL^{-1} BAP + 2 mgL^{-1} IAA which was at par with 4 mgL^{-1} BAP + 1 mgL^{-1} IAA. This may be due to the combined effects of BAP and IAA in shoot elongation. Higher levels of BAP (6-8 mgL^{-1}) showed lower shoot length either alone or in combination with IAA. Some researchers have also reported that a combination of BAP and auxins enhanced shoot proliferation and shoot length during tissue culture of banana Bhagyalakshmi and Singh (1995); Okole and Schulz (1996).

It was observed that at higher concentration of BAP, number of shoots were more with relatively less shoot length. The reason may be the nutritional competition between the shoots and suppression of apical dominance at higher cytokinin levels. Similar negative correlation in shoot number and length was observed by Madhulatha *et al.* (2004) in cv. Nendran. Presence of auxins in the medium affected the cell enlargement and root initiation which might be the reason for increase in plant height. These results are in accordance with the findings of Jarret *et al*. (1985) and Zaffari *et al*. (2000) who achieved more plant height when banana shoots were cultured on medium containing auxins.

5.1.4. Effect of MS media strength and IAA concentrations on different parameters of rooting

During *in vitro* multiplication, plants of banana were very small and were not capable of surviving when transferred to soil. Therefore, it was better to grow rooted plants before *ex vitro* transfer. During *in vitro* multiplication, it was observed that when cytokinin:auxin was low, root formation occurs. Shoots were in cluster when multiplied so they were separated from clusters before being transferred to rooting medium.

5.1.4.1. Per cent rooting

Significantly higher rooting per cent was observed in half strength MS media as compared to full strength MS media either alone or in combination with different levels of IAA (0.5-1 mgL^{-1}). The rooting per cent was recorded minimum (15 per cent) when shoots were cultured in full strength MS media while 65 per cent rooting was observed at half strength MS media. There are reports that roots can be induced without growth regulators (Albany *et al*., 2005; Silva *et al*., 1998a). IAA showed significant increase in per cent rooting with increasing concentrations regardless of media strength. Similar findings of increased rooting percentage with addition of a weak auxin like NAA was reported by many workers Cronauer and Krikorian (1984); Wong (1986); Jasrai *et al*. (1999) and Rahman *et al*. (2004).

For the combined effect of media strength and IAA concentrations, the maximum rooting (100 per cent) was observed in full and half strength MS media at 1

mgL^{-1} IAA (Table 4.8). The results for *in vitro* rooting of banana plants were in conformity with the findings of Cronauer and Krikorian (1984); Nandwani *et al*. (2000); Muhammad *et al*. (2000, 2004); Habiba *et al*. (2002); Jalil *et al*. (2003) and Srangsam and Kanchanapoom (2003).

5.1.4.2. Days to rooting

The time required for root initiation was minimum (15.83 days) in half strength MS media as compared to full strength MS media (21.33 days), while addition of IAA in the media significantly reduced the days to rooting.

Regarding the combined effects of media strength and IAA concentration, minimum number of days to rooting (6.50) was observed with half strength MS media + 1 mgL^{-1} IAA. Addition of auxin significantly reduced the number of days to rooting in both full and half strength media (Table 4.9).

5.1.4.3. Number of roots per micro-shoot

The perusal of data presented in Table 4.10 showed that number of roots in full strength MS medium was significantly lower (2.16) as compared to half strength MS media (4.16). When IAA was added in increasing concentrations, number of shoots increased in both full and half strength MS media.

It is evident from analysis of variance presented in Appendix (Table 10), that interaction of media strength and IAA levels was non-significant. Maximum number of roots (8.66) was observed with half strength MS media + 1 mgL^{-1} IAA.

In conclusion, the best combination for rooting of micro-shoots was found to be half strength MS media + 1 mgL^{-1} IAA. The results of the present investigation are in corroboration with the report of Muhammad *et al*. (2000, 2004); Habiba *et al*. (2002); Jalil *et al*. (2003) and Srangsam and Kanchanapoom (2003).

5.2. MORPHOLOGICAL CHANGES DURING HARDENING

5.2.1. Per cent survival

The data presented in Table 4.11 show higher survival per cent in mycorrhizal treatments as compared to control. Since the plantlets were under moisture stress, the

percent survival of control plants decreased gradually from the day of planting to the termination of the experiment due to oxidative stress. Also, since pesticides and fungicides were not applied, most of the plantlets in control died due to fungal attack after transfer to pots, whereas mycorrhizal treatments showed 100 per cent survival throughout the experimental period which indicates their role in disease control. The role of mycorrhiza in protecting host plants from root pathogens and mitigating the effects of extreme variation in temperature, pH and water stress has been well documented by various workers (Dixon and Marx, 1987; Jeffries *et al*., 2003; Avis *et al*., 2008). Survival rates during the *post vitro* phase of micropropagated material can often be reduced due to poor root systems (Hooker *et al.*, 1994). Role of mycorrhizal symbiosis in enhancing the survival of plants by increasing nutrient and water uptake, pathogenic resistance and production of phytohormone have been reported by Jaffries *et al*. (2003). In our study the AM fungi inoculation significantly increased the number of surviving plants under water stress, a result which is in line with the earlier findings of Gaur and Adholeya (1999), Estrada-Luna and Davies (2003), Marin *et al*. (2003) and Krishna *et al*. (2005) in micropropagated *Syngonium*, chile ancho pepper, persimmon and grape plantlets, respectively.

5.2.2. Per cent colonization

Mycorrhizal root infection was absent in control plants, but well established in the mycorrhizal plants (Table 4.12). At the first assessment (20 days after inoculation) mycorrhizal colonization in mixed AMF strain was 51.25 per cent, at 40 days it was 83.75 per cent, and increased to 96.25 per cent at 60 days after inoculation. Variation in colonization intensities in roots of banana plantlets by AMF isolates was reported to vary from 10 per cent to more than 92 per cent by Panja *et al*. (2007).

The colonization per cent in mycorrhized plantlets was found to increase steadily throughout the experimental period reaching maximum at 60 days after inoculation. Maximum root colonization per cent (96.25) was recorded in mixed AMF which was at par with *Glomus intraradices* treatment (93.75 per cent) and *Acaulospora scrobiculata* (87.50 per cent).

The differences in AMF colonization frequency could be attributed to the differences in mycorrhizal dependency among the host plants and the abiotic factors (Yano-Melo and Lima-Filho, 1999). Superiority of mixed AMF culture may be attributed to that of existing compatible AMF communities. Furthermore, when plants are colonized by more than one AMF isolates, preference of host for specific isolates of the community is noted (Johnson *et al*., 1991).

5.2.3. Growth parameters

As the tissue cultured plantlets are very delicate and prone to biotic and abiotic stresses during *ex vitro* acclimatization, use of bioprotectant agents like AMF has been suggested by many workers for improving the growth and survival of tissue cultured plantlets. Most of the studies in banana have been done on the morphological and nutrient parameters and very little work is done on the biochemical changes which took place during the colonization period with different AMF isolates. Most of the growth parameters like increased survival, shoot length, leaf number, leaf area, fresh and dry weight of shoot have been reported in this study. Growth promotion was slightly better when mixed AMF species were used which is in agreement with other studies (Yao-Qing *et al*., 2004). Regarding root morphology, more number of roots, reduced mean root length and higher fresh and dry weight of root were recorded in micropropagated Dwarf Cavendish banana. These results are in agreement with the findings of other workers (Lin and Chang, 1987; Jaizme-Vega *et al*., 2002).

5.2.3.1. Plant height

Higher plant height was recorded in mycorrhized plants at 20 days after inoculation, although the differences were not significant. Significant differences were observed in control plants and inoculated plants at 40 and 60 days. Mixed AMF strain and *Glomus intraradices* both were found to be at par in increasing plant height. Similar results of increased plant height of tissue cultured banana plants during acclimatization phase has been reported by many workers (Lin and Chang, 1987; Declerck *et al*., 1995; Matos *et al*., 2002; Abo-El-Ez, 2003). Mathews *et al.* (2003) obtained 60.7 per cent increase in height of micropropagated Dwarf Cavendish plantlets over control when treated with *Glomus fasciculatum* during secondary hardening stage in green house.

5.2.3.2. Number of leaves per plant

The number of leaves per plant at 20 days after inoculation was found to be statistically at par among control and mycorrhized plants (Table 4.14). At 40 and 60 days after inoculation, leaf number was found to increase significantly in mycorrhized plants which may be due to increased growth rate of the plantlets. Increase in number of leaves in micropropagated banana treated with various strains of mycorrhiza has been reported earlier by many workers (Matos *et al.*, 2002; Abo-El-Ez, 2003; Yao-Qing *et al.*, 2004).

5.2.3.3. Leaf area per plant

Leaf area per plant was found to increase significantly in mycorrhizal treatments only after 40 days of inoculation and continued to increase up to 60 days. Maximum increase in leaf area was recorded in mixed AMF strain at 60 days after inoculation which was about 2.82 times over control. Increase in leaf area is directly related to the increased plant biomass which indicates better nutrition and water relations in plant due to AMF colonization. A similar result of increased biomass in mycorrhized banana plants was observed by Mathews *et al.* (2003) and Yao-Qing *et al.* (2004).

5.2.3.4. Shoot fresh weight

All the micropropagated banana plants used in this experiment were similar in size at the time of inoculation. Fresh weight of the aerial part of the mycorrhizal plants increased in most of the harvests. Differences between the inoculated and control plants were more marked at 40 and 60 days, when the values were significantly higher in mixed AMF strain and *G. intraradices* inoculated plants.

5.2.3.5. Shoot dry weight

Micropropagated banana plantlets inoculated with different mycorrhizal isolates showed increased shoot dry weight over control plants at 20 days after inoculation though the differences were not significant. At 40 and 60 days after inoculation, the mycorrhized plants showed significantly higher dry matter content in the shoots and maximum increase in shoot dry weight was observed with mixed AMF strain (Table 4.17). The increase in shoot dry weight with addition of mycorrhiza has

been reported by many workers (Lin and Chang, 1987; Rodriguez-Romero *et al.*, 2003).

5.2.3.6. Number of roots

The root system of micropropagated *Musa* plants consists of a series of adventitious roots that develop directly from the corm (subterranean structure). These roots branched in first order laterals, from which second order laterals developed. Mycorrhizal banana plants showed a denser root system than the control plants. Adventitious root number increased over time in both mycorrhizal and non-treated plants, although values of mycorrhizal plants were always significantly higher (Table 4.18). The most important effect of AMF infection on the root system of banana plants was an increase of adventitious root branching. This increase, together with the increase in number of adventitious roots, led to a denser root system, as observed in other mycorrhizal systems, though effects may be different (Berta *et al.*, 1993). It is generally assumed that a denser root system has a greater absorbing power than an elongated one and is typical of plants growing in nutrient rich soils (Glinski and Lipiec, 1990). The extensive network of external mycelium, with its absorbing power and explorative functions, in addition to the denser root system, could improve the "growth effect" characteristic of mycorrhizal plants. Moreover, a much branched root system is particularly useful for banana plants, as they are easily uprooted by winds, which are a frequent problem in many cropping areas of the world.

Root architecture and morphology provide a lot of useful information about plant species and their ability to take up nutrients from the soil. Several soil factors, such as nutrients and microorganisms, greatly affect root development. Soil microbiota interactions, especially with those organisms that colonize the rhizosphere, have also been reported to affect plant health and soil quality. Microbiota helps the host plant under limiting conditions provoked by abiotic and biotic factors. Mycorrhizal symbiosis significantly improves plant nutrition under low fertility soil conditions. Mycorrhizal hyphae are more efficient than roots alone in nutrient uptake, since they increase the root absorption area. Recently, it has been reported that AMF are even able to change root architecture (Jaizme-Vega *et al*., 2002).

5.2.3.7. Root length

In the case of adventitious root length, mycorrhized plantlets significantly showed lower root length than non-treated plantlets, throughout the experimental period. The reduction in length of adventitious roots was accompanied by an increase in their number, as observed in another endomycorrhizal system in which a monocotyledon was involved i.e., *Allium porrum + Glomus E3, Ornithogalum umbellatum + G. fasciculatum* (Berta *et al.*, 1990, 1993). In *Populus* sp. and *Prunus cerasifera* lateral roots were longer in mycorrhizal than in the control plants (Hooker and Atkinson, 1992; Berta *et al.*, 1995) whereas in *Vitis vinifera* and *AIIium porrum* lateral roots were shorter in the mycorrhized plants (Schellenbaum *et al.*, 1991; Berta *et al.*, 1993).

5.2.3.8. Root fresh weight

Significantly higher root fresh weight was recorded throughout the experimental period in mycorrhized plants as compared to control. The increased fresh weight may be due to enhanced number of lateral roots as well as higher diameter of roots. All the mycorrhizal treatments showed at par readings at 20 days after inoculation but later on significant increase in root fresh weight was recorded in mixed AMF strain (Table 4.20). Maximum root fresh weight at 60 days after inoculation was recorded in mixed AMF strain indicating its beneficial effect on shoot growth and development.

5.2.3.9. Root dry weight

Root dry weight increased with increase in colonization intensity. Increased root dry weight in mycorrhized plants was recorded at 20, 40 and 60 days after inoculation but significant difference was observed at 60 days where maximum root dry weight was recorded in mixed AMF strain (Table 4.21). This signifies increased root growth rate and dry matter accumulation due to enhanced photosynthetic efficiency in plants inoculated with mixed AMF strains. Increase in dry weight of roots of mycorrhized banana plants over control has also been reported by Yao-Qing *et al.* (2004).

5.3. PHYSIOLOGICAL CHANGES DURING HARDENING

5.3.1. Photosynthetic rate

The photosynthetic rate was recorded to be very slow during initial phase of *ex vitro* growth of plantlets indicating their gradual shift from heterotrophic to autotrophic nutrition. At 20 days after inoculation, higher photosynthetic rates were recorded in mycorrhizal treatments but the increase was more pronounced at 40 and 60 days after inoculation. Mixed strain of AMF was found to be significantly superior in terms of increased photosynthesis at 60 days after inoculation (Table 4.22). The enhanced photosynthetic rates in inoculated plants suggest that mycorrhizal plants may be able to assimilate more CO_2 and thereby accumulate more biomass. It is plausible that increased number of roots in mycorrhized plants may lead to increased moisture and nutrient availability to the plant leading to enhanced photosynthetic rate. This could be the reason for significantly higher shoot fresh weight, shoot dry weight, root fresh weight and dry weight in plants treated with mixed AMF strain.

Increased photosynthetic rate in mycorrhized plantlets indicates their increased growth rate and earlier acclimatization compared to control. Early establishment and adaptation to the external environment are far reaching in the successful acclimatization and survival of tissue cultured plants. A significant increase in photosynthetic rate of AMF inoculated *Ziziphus nummularia* seedling was observed by Mathur and Vyas (1995). Higher photosynthetic ability in micropropagated mycorrhized grape plantlets at 60 days after acclimatization was also reported by Krishna *et al.* (2005).

5.3.2. Stomatal conductance

In this experiment mycorrhized plants had higher photosynthetic rates and stomatal conductance, which could be associated with the higher CO_2 influx into mesophyll tissues, as indicated by higher stomatal conductance. During acclimatization under *ex vitro* conditions, transpiration gradually decreases because stomatal regulation of water loss becomes more effective and cuticle and epicuticular waxes develop (Grout and Asston, 1977; Wardle *et al*., 1979; Short *et al*., 1984; Fila *et al*., 1998). Similar increase in stomatal conductance in both inoculated and control

plantlet was observed though the increase was much prominent in mycorrhized plants (Table 4.23).

5.3.3. Relative water content

Immediately after transplantation, visible wilting was observed in both control and mycorrhized plantlets. However, the water status of plants became stabilized after some weeks. Relative water content in mycorrhized plantlets was found to be significantly higher than that of control plants after inoculation (Table 4.24). The increased RWC in mycorrhizal plantlets under water stress might be due to an improvement of the water uptake by mycorrhizal root system through extra-radical phase (Ruiz-Lozano and Azcon, 1995). In addition, increased water transport could also be attributed to improvement in P nutrition (Table 4.33). This result is in conformity with the earlier findings of Graham and Syvertsen (1984). In a study of micropropagated mycorrhizal strawberry plantlets, Herandez-Sebastia *et al.* (1999) suggested that the higher concentration of water soluble compounds in plant tissue could be a reason for higher RWC of the whole plantlets.

5.4. BIOCHEMICAL CHANGES DURING HARDENING

5.4.1. Total phenol

During hardening, the tissue cultured plants are not only in stress but also in an array of pathogen spores that cause disease. Phenols and enzymes such as polyphenol oxidase are important components of plant defense mechanism against the diseases. Phenolic compounds occur naturally in plant system and inhibit fungal spore germination and toxin production by pathogens owing to their anti-microbial properties (Vidhyasekaran, 1973). Tang *et al*. (2000) reported that there was a significant increase in the level of phenolic compounds in the bark of poplar plants inoculated with *G. mosseae* showing resistance. The increased levels of total phenols in the present investigation might have increased resistance in inoculated plantlets against diseases, which led to increased plantlet survival under greenhouse conditions (Table 4.25 and 4.11). In present study, highest total phenol at 60 days after inoculation was observed in plantlets treated with mixed AMF strains indicating increased resistance against microbial infections during hardening phase.

5.4.2. Proline

One of the best known responses of plants to salt, drought and other stresses is the accumulation of soluble, low molecular mass solutes such as proline (Paleg *et al*., 1984). Proline content in the leaves of control and inoculated plants showed gradual increase over the period of time but the relative increase in the proline content of mycorrhized plantlets was more pronounced than that of control (Table 4.26). Levels of proline in inoculated plantlets were found to be 2.5 to 3.8 times higher than that of non-inoculated control plantlets at 60 days after inoculation. Higher level of proline in AMF inoculated plantlets is desirable as proline protects the different enzyme systems against dehydration caused by moisture stress (Paleg *et al*., 1984). Furthermore, the higher proline accumulation favours the plants in maintaining the osmotic balance and preventing dehydration of tissue, thereby helping them to grow normal even under stressful conditions.

5.4.3. Total chlorophyll

The mycorrhizal inoculation to micropropagated banana plantlets significantly enhanced total chlorophyll content in leaves. The enhanced chlorophyll level might be responsible for increased photosynthesis in inoculated plantlets (Table 4.27). This can further be attributed to increased Mg and Fe uptake, which are considered essential for chlorophyll biosynthesis. Significantly higher chlorophyll contents in *Acaulospora scrobiculata* treated plants were recorded at 40 and 60 days after inoculation. Similar results of increase in total chlorophyll content in the leaves of micropropagated mycorrhized grape plantlets were reported by Krishna *et al*. (2005).

5.4.4. Total soluble sugar

At 20 days of transplanting, the leaves of control and mycorrhized plants showed non-significant differences for total soluble sugar content. Though higher levels were observed in mixed AMF strain. This may be due to relatively less photosynthetic rates in moisture stressed control and mycorrhized plants.

The leaf soluble sugar content under moisture stress was found to increase at 40 days and 60 days both in control and inoculated plants but the increase was more prominent in the case of mycorrhizal treatments more importantly in mixed AMF

strains treated plants (Table 4.28). The increment in total soluble sugars in leaf of mycorrhized plants under moisture stress may be due to increased photosynthetic activity and more stabilized source sink relationship. Similar observations have been found by Schellenbaum *et al*. (1998); Mathur and Vyas (2000); Sorial (2001) and Wu and Xia (2006). Subramanian *et al*. (1997) provided evidence that the higher foliar concentrations of soluble sugars in mycorrhizal plants after drought, suggest maintenance of greater photosynthetic capacity. Wu *et al*. (2008) observed that during water stress and rewatering, arbuscular mycorrhiza significantly increased the contents of soluble sugars, proteins and the antioxidant enzymes in leaves of *Poncirus trifoliate*, which indicated that AM colonization could improve the osmotic adjustment response of *P. trifoliate*, enhance the defense system and alleviate oxidative damage to membrane lipids and proteins.

5.4.5. Starch

The starch content in the leaves of mycorrhized plants was significantly less than that of control at 20 days after inoculation. It might be due to probable hydrolysis of starch in to sugar and its translocation to the root zone. This may be due to the increased root colonization frequency with mycorrhizal fungi as well as the increased root growth. It seems that reduction in the leaf starch during initial phase of growth may be due to the translocation of reserved food material to the sink (root system and the fungal symbiont) and a relatively less active photosynthetic rate due to moisture stress.

The starch levels were found to increase at 40 and 60 days in mycorrhized plantlets over control (Table 4.29). This increase in the starch levels may be due to increased photosynthetic rate of mycorrhized plantlets which resulted in increased assimilation of photosynthates in the leaves to support greater carbon flow to the root system (form 5-20% greater root carbon demand) needed to support the symbiosis (Smith and Read, 1997). Maintenance of greater photosynthetic capacity during water stress in mycorrhizal plants has also been indicated by higher starch levels in AM than in non-AM plants (Auge *et al*., 1987; Davies *et al*., 1993).

5.4.6. Enzymatic activity

5.4.6.1. Catalase

As the tissue cultured plants were transferred to the *ex vitro* condition, various biotic and abiotic stresses elevated the concentration of reactive oxygen species (ROS) and also the detoxification mechanisms. The increase in catalase activity under moisture stress was observed in both control and mycorrhized plantlets at 20 days after inoculation. Later on, when plantlets were transferred in net-house the activity of catalase increased drastically in mycorrhized as well as in control plantlets in response to various environmental stresses and water stress at 40 days after inoculation. Mycorrhized plantlets showed significantly higher activity of catalase throughout the experimental period. This may be one of the reasons for increased growth and development of mycorrhized banana plants even at higher stressful conditions. As the plantlets acclimatized in greenhouse condition, at 60 days, the relatively lower activity of catalase was noticed in mycorrhized plantlets though it was significantly higher than control (Table 4.30).

Enzymes catalase, peroxidase and polyphenol oxidase tend to scavenge the highly reactive oxygen species which are byproducts of various stresses in plant system. If these species are not detoxified immediately, they cause severe damage to biomembranes and cellular structures (Monk *et al*., 1989; Ushimaro *et al*., 1992; Yan *et al*., 1996). Increased activities of catalase, peroxidase and polyphenol oxidase under stress have been linked with stress resistant mechanisms of the plants (Yordanova *et al*., 2003; Lin *et al*., 2004; Ahsan *et al*., 2007) and higher levels of catalase in inoculated plantlets signifies higher stress tolerance ability of micropropagated plantlets.

5.4.6.2. Peroxidase

Higher activities of peroxidase were recorded in mycorrhizaed plantlets throughout the experimental period which indicate the role of mycorrhiza in stress induced defense mechanism (Table 4.31). It is well known that many stresses, for example high irradiance, drought, high temperature etc., may cause reactive oxygen species production and promote activities of antioxidants. During *ex vitro*

acclimatization of *Doritaenopsis* plantlets, peroxidase activity was not affected by relative humidity but increased at low temperature together with decreased chlorophyll content and photosynthetic efficiency (Jeon *et al*., 2006) similar results were observed in present study with control and mycorrhized plants under water and nutrient stress.

The obtained results suggest that mycorrhizal symbiosis help in increment of enzymatic antioxidant production which in turn help mycorrhized plants to maintain growth under water stress. The increase in antioxidant enzymes resulting from AM inoculation was also obtained by Wu *et al*. (2008) and Roldan *et al*. (2008). Wu *et al*. (2008) attributed the increase in antioxidant activities of AM citrus seedling would partly be due to the lower H_2O_2 concentration, protecting the organism against oxidative damage, in turn enhancing drought tolerance.

5.4.6.3. Polyphenol oxidase

Polyphenol oxidase activity was found higher in mycorrhized plants as compared to control (Table 4.32). Higher level of phenols observed in inoculated plants may be attributed to the increased polyphenol oxidase activity in plants (Mathur and Vyas, 1997; Tang *et al*., 2000). Polyphenol oxidase is an oxidizing enzyme of polyphenols converting them into quinones, a toxin to pathogens. Increased polyphenol oxidase activity in AMF inoculated plants has been observed by Tang *et al*. (2000); Nelson and Achar (2001) and Panwar and Vyas (2002).

The results suggests that activities of antioxidant enzymes *viz*., catalase, peroxidase and polyphenol oxidase increases with the moisture stress both in control and mycorrhized plants but the increase is more pronounced in case of mycorrhized plants. It can also be concluded that the activities of these enzymes also depends on the age of the plant and mycorrhizal colonization frequency as maximum enzymatic activity was recorded after 40 days of inoculation while their activity reduced slightly after 60 days when plants get acclimatized to the external environment.

5.5. LEAF NUTRIENT ANALYSIS

It has often been reported that nutrient uptake by mycorrhizal plants is faster than that by non-mycorrhized roots (Bolan, 1991). In the present study N, P and K

levels in the leaves of mycorrhized plantlets were significantly higher as compared to the non-inoculated control plantlets under water and nutrient stress after 60 days of acclimatization (Table 4.33). Relatively higher biomass accumulation in the mixed AMF treated plantlets may be correlated with the increased levels of N, P and K in the leaf. Similar results of higher P and K levels in banana plant grown in low fertility soil condition was observed by Jaizme-Vega *et al*. (2002); Mathews *et al.* (2003) and Yao-Qing *et al.* (2004).

The increase in plant growth by mycorrhizal association is largely due to increased absorption of nutrients. AMF inoculation was found to increase the fitness of the host plant by increasing uptake of minerals such as P that are relatively immobile in soils (Turk *et al*., 2006). AMF develop intensively inside roots and within the soil by forming an extensive extraradical mycelium which helps the plant in exploiting mineral nutrients and water from the soil. In plants, particularly those with restricted or weak root system, hyphal connections act as a bridge between roots and nutrient sites in soil and facilitate efficient uptake of immobile nutrients by host plants (Azcon-Aguilar and Barea, 1996).

The maximum colonization percentage at 60 days after inoculation may be correlated to the higher levels for N, P and K observed in mixed AMF treated plantlets. Depending on the host plant, colonization by AMF can increase P nutrition. Mycorrhizal structures effectively take up P from lower concentrations in the soil at which normal plant roots fail (Jeffries *et al*., 2003). AMF helps in increasing nutrient uptake by increasing the surface area of absorptive system (roots) of plants and exploring soil by extraradical hyphae beyond the root hair and P-depletion zone. The absorbed P is then converted to polyphosphate granules in external hyphae and passed to the arbuscule for transfer to the host plant (Azcon-Aguilar and Barea, 1996).

Chapter VI

Summary & Conclusion

Chapter VI

SUMMARY AND CONCLUSION

The present investigation entitled "Biochemical changes in micropropagated banana (*Musa* sp.) cv. Dwarf Cavendish (AAA) due to arbuscular-mycorrhizal fungi (AMF) inoculation during *ex vitro* acclimatization" was carried out during 2008-2010 in Department of Horticulture, Institute of Agricultural Sciences, BHU, Varanasi.

The results obtained during the course of investigation are summarized below:

1. MICROPROPAGATION PROTOCOL

i. Single sterilization with either mercuric chloride (0.1%) or sodium hypochlorite (4%) showed very less response towards survival of the explants in culture vessels while, double sterilization of shoot-tips showed encouraging results for maximum culture response.

ii. Lowest contamination (0.00 per cent) was observed with 5 minutes treatment of mercuric chloride (0.1%) followed by quick dip in ethanol.

iii. Maximum survival (91.66 per cent) of shoot-tips was observed in double sterilization with mercuric chloride (0.1%) for 5 minutes followed by quick dip in ethanol.

iv. Per cent mortality was found to be minimum in double sterilization protocol as compared to single sterilization treatments.

v. When explants were inoculated in shoot induction media, maximum shoot initiation (87.50 per cent) was observed at BAP 6 mgL^{-1} + IAA 2 mgL^{-1} which was at par with BAP 6 mgL^{-1} + IAA 1 mgL^{-1} (83.33 per cent).

vi. Minimum time for shoot induction (31.66 days) was observed with BAP 6 mgL^{-1} + IAA 2 mgL^{-1} which was at par with BAP 6 mgL^{-1} + IAA 1 mgL^{-1} (32.66 days).

vii. In multiplication media, number of shoots per explant was found to be maximum (6.83) at BAP 6 mgL^{-1} + IAA 1 mgL^{-1} which was significantly superior to other treatments.

viii. Maximum shoot length (5.95 cm) was observed at BAP 4 mgL^{-1} + IAA 2 mgL^{-1} which was found to be at par with combination of BAP 4 mgL^{-1} + IAA 1 mgL^{-1} (5.78 cm). Addition of auxin in the media significantly increased the shoot length while higher levels of cytokinin (above 4 mgL^{-1}) showed reduction in shoot length in multiplication media.

ix. Maximum rooting per cent (100 per cent) was found in MS (full) + 1 mgL^{-1} IAA as well as in MS (half) + 1 mgL^{-1} IAA (100 per cent).

x. Minimum days for rooting (6.50) was observed with half strength MS media + 1 mgL^{-1} IAA followed by full strength MS media + 1 mgL^{-1} IAA (8.66).

xi. Maximum number of roots (8.66) was observed at half strength MS media + 1 mgL^{-1} IAA.

2. HARDENING EXPERIMENT

i. The survival of mycorrhized micropropagated banana plantlets was significantly higher than the control plantlets and all mycorrhizal treatments showed cent percent survival under moisture stress condition during hardening.

ii. The root colonization percentage in mycorrhized plantlets was found to increase steadily during the experimental period and reached maximum at 60 days after inoculation. The maximum root colonization (96.25 per cent) was recorded in mixed AMF strain treated plantlets which was at par with *Glomus intraradices* treatment (93.75 per cent) and *Acaulospora scrobiculata* (87.50 per cent) treated plantlets.

iii. Mycorrhization improved most of the growth parameters in micropropagated banana during acclimatization under moisture stress.

iv. At 20 days after inoculation, plant height, number of leaves per plant, leaf area, shoots fresh and dry weight were found non-significant, while number of roots, root fresh and dry weight were noted to be significantly higher in mycorrhized plants. However, there was significantly less root length as compared to control.

v. Significant increase in the growth of mycorrhized banana plantlets was recorded at 40 and 60 days after inoculation. Plant height, number of leaves per plant, leaf area per plant, shoot fresh weight, shoot dry weight, number of roots, root fresh and dry weight significantly increased over control. Root length was significantly higher in control as compared to mycorrhized plants. Relatively higher biomass accumulation was observed at 60 days after inoculation in mycorrhized plantlets.

vi. Changes in root morphology of mycorrhized plantlets play an important role in improved growth as number of lateral roots increase surface area for nutrient and water absorption.

vii. Physiological parameters, *viz.*, photosynthetic rate, stomatal conductance and relative water content significantly increased in mycorrhized plants at 20, 40 and 60 days after inoculation which improved overall growth rates of tissue cultured plantlets even under moisture stress.

viii. The enhanced photosynthetic rates in inoculated plants suggest that mycorrhizal plants have ability to assimilate more CO_2 and thereby accumulate more biomass even under stressed condition. This could be the reason for significantly higher shoot fresh weight, dry weight, root fresh weight and dry weight in plants treated with mixed AMF strain.

ix. Biochemical parameters like total phenol, proline, total chlorophyll, and enzymatic activities were found to increase significantly in mycorrhized plantlets under water stress at 20, 40 and 60 days after inoculation.

x. The results suggests that activities of antioxidant enzymes *viz*., catalase and peroxidase increases in moisture stress both in control and mycorrhized plants but the increase is more pronounced in case of mycorrhized plants. It can also be concluded that the activities of these enzymes also depends on the age of the plant and mycorrhizal colonization frequency as maximum enzymatic activity was recorded at 40 days after inoculation while their activity reduced slightly at 60 days after inoculation when plants get acclimatized to the external environment.

xi. All these results suggest that mycorrhizal protection against oxidative stress caused by moisture stress may be one of the most important mechanisms by which the AM symbiosis increases the tolerance of host banana plants to drought.

xii. The N, P and K levels in the leaves of mycorrhized banana plantlets after 60 days of acclimatization under water and nutrient stress were recorded to be significantly higher than the non-treated control plantlets.

xiii. In general mixed AMF strain showed better response towards the transplant shock and water stress through increased colonization, improved plant growth and nutrition.

xiv. The results of the present investigation indicate that AMF inoculation may have certain role in up-regulation of plant defense mechanism through activation of antioxidant enzymatic system.

CONCLUSION

On the basis of the findings of present investigation, it is concluded that for surface sterilization of shoot-tips double sterilization with mercuric chloride (0.1%) for 5 minutes followed by quick dip in ethanol showed best results for minimum contamination and maximum survival of explants. Further, for culture establishment and multiplication 6 mgL^{-1} BAP + 1 mgL^{-1} IAA was found to be the best combination showing maximum shoot induction as well as multiplication. For rooting, half strength MS media + 1 mgL^{-1} IAA was found to be effective for maximum rooting, reduction in days to root induction and number of roots per micro-shoot.

The results with root colonization of micropropagated banana plantlets inoculated with two individual and mixed arbuscular-mycorrhizal strains reveal that AMF are potential inoculants for averting transplant shock experienced by plantlets during acclimatization under net-house conditions. In nutshell, AMF colonization occurred rapidly in about 20 days of inoculation which had a positive impact on photosynthetic rate, stomatal conductance and RWC when plantlets were experiencing desiccation during acclimatization. During nutritional stress, AMF plantlets maintained higher N, P and K levels. This indirectly led to higher chlorophyll levels, accelerated photosynthetic rate and stomatal conductance at the termination of the experiment. Due to these changes mycorrhized plantlets showed better growth responses as compared to control under moisture stress.

This study suggests that changes in levels of total chlorophyll, total soluble sugar, starch, proline, phenol and enzymes effectively overcame transplanting shock along with moisture stress and enhanced *ex vitro* survival under low input conditions. Physiological and biochemical changes brought about by AMF association rendered the plantlets to be more resistant against microbial infection and water stress conditions. Thus, major shortcomings of tissue cultured plants *viz*., poor absorption and transportation and sensitivity to microbial infection could be ameliorated with desirable traits of AMF association. Thus, these microorganisms could be used in future alternative biotechnologies for banana production systems. On the basis of present investigation, it may be concluded that hardening period of banana plantlets could be reduced and made more economic with the use of mycorrhizal fungi during

planting in *ex vitro* conditions. Biopriming with AMFs not only minimizes extra input in terms of water, fertilizer, pesticides and sophisticated structures like mist chambers but also improves resistance of mycorrhized tissue cultured plants to water and nutritional stress which are generally faced by such plants during transport and during growth periods.

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Bibliography

BIBLIOGRAPHY

Abo-El-Ez, A.T., Response of inoculated and un-inoculated Williams banana (*Musa* sp.) *in vitro* plants with endomycorrhizal to mineral nutrients: Effect of different levels of nitrogen and potassium on growth and anatomical structure of mycorrhizal infection in the root system, *Assiut Journal of Agricultural Sciences*, 34(3), 27-47, 2003.

Aebi, H.E., Catalse, In: Methods of enzymatic analysis, Bergmeyer, H.U. (ed.), Verlag Chemie, Weinhern, 273-286, 1983.

Agnew, C. and Warren, A., A framework for tackling drought and land degradation, *J. Arid Environ*., 33, 309-320, 1996.

Ahsan, N., Lee, D. G., Lee, S. H., Kang, K. Y., Bahk, J. D., Choi, M. S., Lee, I. J., Renaut, J. and Lee, B. H., A comparative proteomic analysis of tomato leaves in response to waterlogging stress, *Physiologia Plantarum*, 131(4), 555-570, 2007.

Albany, R.N., Vilchez, J.A., Gracia, L. and Jimenez, E., Comparative study of morphological parameters of Grand Naine bananan (*Musa* AAA) after *in vitro* multiplication with growth retardants, *Plant Cell Tiss. Org. Cult*., 83, 357-361, 2005.

Alvard, O., Cote, F. and Teisson, C., Comparison of methods of liquid medium culture for banana micropropagation, *Plant Cell Tiss. Org. Cult*., 32, 55-60, 1993.

Arias, F., Blanco, F. and Vargas, R., Evaluation of infectivity of arbuscular mycorrhiza in micropropagated banana plants (*Musa* AAA. cv. Valery) during greenhouse and nursery phases, *CORBANA*, 25(52), 173-188, 1999.

Arias, O., Commercial micropropagation of banana, In: Biotechnology Applications for Banana and Plantain Improvement, Inibap, San Jose, Costa Rica, 139-142, 1992.

Arinaitwe, G., Rubaihayo, P.R. and Magambo, M.J.S., Proliferation rate effects of cytokinins on banana (*Musa* spp.) cultivars, *Scientia Horticul*., 86, 13-21, 2000.

Assani, A., Bakry, F., Kerbellec, F. and Haicour, R., Production of haploid plants from anther culture of banana [*Musa balbisiana* (BB)], *Plant Cell Rep*., 21, 511-516, 2003.

Auge, R.M., Schekel, K.A. and Wample, R.L., Leaf water and carbohydrate status of VA mycorrhizal rose exposed to drought stress, *Plant Soil*, 99, 291-302, 1987.

Auge, R.M., Schekel, K.A. and Wample, R.L., Osmotic adjustment in leaves of VA mycorrhizal and nonmycorrhizal rose exposed to drought stress, *Plant Physiol*., 82, 765-770, 1986.

Auge, R.M., Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis, *Mycorrhiza*, 11, 3-42, 2001.

Avis, T., Gravel, V., Antoun, H. and Tweddell, R.J., Multifaceted beneficial effects of rhizosphere microorganisms on plant health and productivity, *Soil Biology and Biochemistry*, 40, 1733-1740, 2008.

Azcon-Aguilar, C. and Barea, J.M., Applying mycorrhiza biotechnology to horticulture: significance and potentials, *Scientia Horticul*., 68, 1-24, 1997.

Azcon-Aguilar, C. and Barea, J.M., Arbuscular mycorrhizas and biological control of soil-borne plant pathogens: an overview of the mechanisms involved, *Mycorrhiza*, 6, 457-464, 1996.

Azcon-Aguilar, C., Cantos, M., Troncoso, A. and Barea, J.M., Benefical effect of arbuscular mycorrhizas on acclimatization of micropropagated cassava plantlets, *Scientia Horticul*., 72, 63-71, 1997.

Banerjee, N. and Sharma, A.K., *In vitro* responses as a reflection of genomic diversity in long term cultures of *Musa, Theor. Appl. Genet*., 76, 733-736, 1988.

Bates, L.S., Waldren, R.D. and Teare, I.D., Rapid determination of free proline for water stress studies, *Plant and Soil*, 39, 205-207, 1973.

Berg, L.A. and Bustamante, M., Heat treatment and meristem culture for the production of virus free bananas, *Phytopathol*., 64, 320-322, 1974.

Berta, G., Fusconi, A. and Trotta, T., VA mycorrhizal infection and the morphology and function of root systems, *Environmental and Experimental Botany*, 33, 159-173, 1993.

Berta, G., Fusconi, A., Trotta, T. and Scannerini, S., Morphogenetic modifications induced by the mycorrhizal fungus *Glomus* strain E_3 in the root system of *Allium porrum* L., *New Phytologist*, 114, 207-215, 1990.

Berta, G., Trotta, A.F., Hooker, J., Munro, R., Atkinson, D., Giovanetti, M., Marini, S., Loreti, F., Tisserant, B., Gianinazzi, P.V. and Gianinazzi, S., The effects of arbuscular mycorrhizal infection on plant growth, root system morphology and soluble protein content in *Prunus cerasifera* L., *Tree Physiol.* 15, 281–293, 1995.

Bhagyalakshmi, and Singh, N.S., Role of liquid versus agar-gelled media in mass propagation and *ex vitro* survival in bananas, *Plant Cell Tiss. Org. Cult*., 41, 71-73, 1995.

Blakesley, D., Uptake and metabolism of 6-benzyladenine in shoot cultures of *Musa* and Rhododendron, *Plant Cell Tiss. Org. Cult*., 25, 69-74, 1991.

Bolan, N.S., A critical review on the role of mycorrhizal fungi in the uptake of phosphorus by plants, *Plant and Soil*, 134, 189-207, 1991.

Bolar, J.P., Norelli, J.L., Aldwinckle, H.S., Hanke, V., An efficient method for rooting and acclimation of micropropagated apple cultivars, *Hort. Science*, 37, 1251-1252, 1998.

Buddendorf-Joosten, J.M.C., Woltering, E.J., Components of the gaseous environment and their effects on plant growth and development *in vitro*, *Plant Growth Regul.*, 15, 1-16, 1994.

Cronauer, S.S. and Krikorian, A.D., Rapid multiplication of bananas and plantains by *in vitro* shoot tip culture, *Hort. Sci*., 19(2), 234-235, 1984.

Cronauer, S.S. and Krikorian, A.D., Reinitiation of vegetative growth from aseptically cultured terminal floral bud apex of banana, *Am. J. Bot.*, 72, 1508–1601, 1985.

Davies, F.T., Potter, J.R. and Linderman, R.G., Drought resistance of mycorrhizal pepper plants independent of leaf P concentration- response in gas exchange and water relations, *Physiol. Plant*., 87, 45-53, 1993.

Declerck, S., Devos, B., Delvaux, B. and Plenchette, C., Growth response of micropropagated banana plants to VAM inoculations, *Fruits*, 49, 103-109, 1994.

Declerck, S., Plenchette, C. and Strullu, D.G., Mycorrhizal dependency of banana (*Musa acuminata*, AAA group) cultivar, *Plant Soil*, 176, 183–187, 1995.

Desjardins, Y., Photosynthesis *in vitro* - on the factors regulating CO_2 assimilation in micropropagation systems, *Acta Hort.*, 393, 45-61, 1995.

Dixon, R.K. and Marx, D.H., Mycorrhizae, In: Bonga, J.M., Durzan, D.J (eds.), Cell and tissue culture in forestry, Forestry Science, Vol. 2 Nijhoff, Dordrecht, 1987.

Doreswamy, R., Srinivasa-Rao, N.K. and Chacko, E.K., Tissue culture propagation of banana. *Scientia Horticul*., 18, 247-252, 1983.

Douds, D.D. Jr., Galvez, L., Becard, G. and Kapulnik, Y., Regulation of arbuscular mycorrhizal development by plant host and fungus species in alfafa, *New Phytol.*, 138, 27-35, 1998.

Drüge, U. and Schönbeck, F., Effect of vesicular-arbuscular mycorrhizal infection on transpiration, photosynthesis and growth of flax (*Linum usitatissimum* L.) in relation to cytokinin levels, *Journal of Plant Physiology*, 141, 40-48, 1992.

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A. and Smith, F., Calorimetric method for determination of sugar and related substances, *Anal. Chem.*, 28, 350-356, 1956.

Estrada-Luna, A.A. and Davies, F.T., Arbuscular mycorrhizal fungi influence water relations, gas exchange, abscisic acid and growth of micropropagated Chile ancho pepper (*Capsicum annum*) plantlets during acclimatization and post-acclimatization, *J. Plant Physiol*., 160(9), 1073-1083, 2003.

Fila, G., Ghashghaie, J., Hoarau, J. and Cornic, G., Photosynthesis, leaf conductance and water relations of *in vitro* cultured grapevine rootstock in relation to acclimatization, *Physiol. Plant*., 102, 411-418, 1998.

Fitter, A.H., Water relations of red clover (*Trifolium pretense* L.) as affected by VA mycorrhizal infection and phosphorus supply before and during drought, *J. Exp. Bot*., 39, 595-603, 1988.

Fortuna, P., Cisternesi, A.S., Moroni, S., Vitagliano, C. and Giovanetti, M., Influence of arbuscular mycorrhizae and phosphate fertilization on shoot apical growth of micropropagated apple and plum rootstocks, *Tree Physiol.*, 16, 757-763, 1996.

Foyer, C.H. and Harbinson, J., Oxygen metabolism and the regulation of photosynthetic electron transport, In: Causes of photooxidative stress and amelioration of defense system in plants, Foyer, C.H. and Mullineausx, P.M. (eds.), CRC Press, Boca Raton, pp. 1-14, 1994.

Foyer, C.H., Descourvieres, P. and Kunert, K.J., Protection against oxygen radicals: an important defense mechanism studied in transgenic plants, *Plant Cell Environ*., 76, 507-523, 1994.

Gadkar, V., David-Schwartz, R., Kunik, T. and Kapulink, Y., Arbuscular mycorrhizal fungi colonization factors involved in host recognition, *Plant Physiol*., 127, 1493-1499, 2001.

Garcia-Perez, J. and Jaizme-Vega, M.C., Influence of infection by mycorrhizal fungus *Glomus intraradices* on plant growth and root development of Grand Naine banana, Abstract in *International Symposium on Banana in the Subtropics*, 10-14 Nov., Puerto de La Cruz, Tenerife, Canary Islands, Spain, p.155, 1997.

Gaur, A. and Adholeya, A., Mycorrhizal effects on the acclimatization, survival, growth and chlorophyll of micropropagated *Syngonium* and *Dracaena* inoculated at weaning and hardening stage, *Mycorrhiza*, 9, 215-219, 1999.

Gianinazzi, V., Gianinazzi, P. and Trouvelot, A., Potentialities and procedures for the use of endomycorrhizas with special emphasis on high value crops, In: J.M. Whipps and B. Lumsden (eds.), *Biotechnology of Fungi for Improving Plant Growth*, Cambridge University Press, Cambridge, UK, pp. 41-54, 1989.

Girija, V.K. and Nair, Y.S., Incidence of VAM in banana varieties, *Indian Journal of Microbiology*, 2(3-4), 294-295, 1988.

Glinski, J. and Lipiec, J., Soil physical conditions and plant roots, CRC Press, Boca Raton, FL, 1990.

Goicoechea, N., Antolin, M.C. and Sanchez-Diaz, M., Gas exchange is related to the hormone balance in mycorrhizal or nitrogen-fixing alfalfa subjected to drought, *Physiol. Plant*., 100, 989-997, 1997.

Graham, J.H. and Syvertsen, J.P., Influence of vesicular-arbuscular mycorrhizae on the hydraulic conductivity of roots of two citrus rootstocks, *New Phytol*., 97, 277-284, 1984.

Grout, B.W.W. and Aston, M.J., Transplanting of cauliflower plants regenerated from meristem culture: Water loss and water transfer related to changes in leaf wax and to xylem regeneration, *Hort. Res*., 17, 1-7, 1977.

Guillemin, J.P., Gianinazzi, S. and Trouvelot, A., Screening of arbuscular endomycorrhizal fungi for establishment of micropropagated pineapple plants, *Agronomie*, 12, 831-836, 1992.

Gupta, P.P., Eradication of mosaic disease and rapid clonal multiplication of banana and plantains through meristem tip culture, *Plant Cell Tiss. Org. Cult.*, 6(1), 33-39, 1986.

Habiba, U., Reza, S., Saha, M.L., Khan, M.R. and Hadiuzzaman, S., Endogenous bacterial contamination during *in vitro* culture of table banana identification and prevention, *Plant Tiss. Cult*., 12(2), 117-124, 2002.

Hamill, S.D., Sharrock, S.L. and Smith, M.K., Comparison of decontamination methods used in initiation of banana tissue cultures from field collected suckers, *Plant Cell Tiss. Org. Cult.*, 33, 343-346, 1993.

Hernandez-Sebastia, C., Piche, Y. and Desjardin, Y., Water relations of whole strawberry plantlets *in vitro* inoculated with *Glomus intraradices* in a tripartite culture system, *Plant Sci*., 143, 81-91, 1999.

Hooker, J.E. and Atkinson, U., Application of computer-aided image analysis to studies of arbuscular endomycorrhizal fungi effects on plant root system morphology and dynamics, *Agronomie, Paris*, 12, 821-824, 1992.

Hooker, J.E., Jaizme-Vega, M.C. and Atkinson, D., Biocontrol of plant pathogens using arbuscular mycorrhizal fungi, In: Gianinazzi, S. and Schüepp, H. (eds.), Sustainable agriculture and natural ecosystems, Birkhäuser Verlag, Basel, Switzerland, pp. 191-200, 1994.

Hooker, J.E., Munro, M. and Atkinson, U., Vesicular-arbuscular mycorrhizal fungi induced alteration in poplar root system morphology, *Plant and Soil*, 145, 207-214, 1992.

Hwang, S.C., Chen, C.L., Lin, J.C. and Lin, H.L., Cultivation of banana using plantlets from meristem culture, *Hort. Sci*., 19, 231-233, 1984.

Iyer, R., Moosa, H. and Kalpana, Y.R., Vesicular-arbuscular mycorrhizal association in banana, *Current Science*, 57(3), 153-155, 1988.

Jaizme-Vega, M.C., Berta, G. and Gianinazzi, S., Effect of *Glomus intraradices* on root system morphology of micropropagated banana plants, Abstract in *Fourth European Symposium on Mycorrhizas*, 11-14 July, Granada Spain, p.152, 1994.

Jaizme-Vega, M.C., Esquivel Delamo, M., Tenoury Dominguezy, P., Rodriguez-Romero, A.S., Efectos de la micorrización sobre el desarrollo de dos cultivares de platanera micropropagada, Info*Musa*, 11(1), 25-28, 2002.

Jaizme-Vega, M.C., Galan-Savco, V. and Cabrera, J., Preliminary results of VAM effects on banana under field conditions, *Fruits*, 46, 19-22, 1991.

Jaizme-Vega, M.C., Sosa Hernandez, B. and Hernandez, J.M., Interaction of arbuscular mycorrhizal fungi and the soil pathogen *Fusarium oxysporum* f. sp. *cubense* on the first stages of 'Grand Naine' banana, *Acta Horticulturae*, 490, 285-295, 1998.

Jaizme-Vega, M.C., Tenoury, P., Pinochet, J. and Jaumot, M., Interactions between the root-knot nematode *Meloidogyne incognita* and *Glomus mosseae* in banana, *Plant Soil*, 196, 27-35, 1997.

Jalil, M., Khalid, N. and Othman, R.Y., Plant regeneration from embryogenic suspension cultures of *Musa acuminata* cv. Mas (AA), *Plant Cell Tiss. Org. Cult*., 75, 209-214, 2003.

Jarret, R.L., Rodriguez, W. and Fernandz, R., Evaluation, tissue culture propagation and dissemination of 'Saba' and 'Palpita' plantations in Costa Rica, *Scientia Horticul*., 25, 137-147, 1985.

Jasrai, Y.T., Kannan, V.R., Remakanthan, A. and George, M.M., *Ex vitro* survival of *in vitro* derived banana plants without greenhouse facilities, *Plant Tiss. Cult*., 9(2), 127-132, 1999.

Jeffries, P., Gianinazzi, S., Perotto, S., Turnau, K.K. and Barea, J.M., The contribution of arbuscular mycorrhizal fungi in sustainable maintenance of plant health and soil fertility, *Biology and Fertility of Soils*, 37, 1-16, 2003.

Jeon, M.W., Ali, M.B., Hahn, E.J., Paek, K.Y., Photosynthetic pigments, morphology and leaf gas exchange during *ex vitro* acclimatization of micro-propagated CAM *Doritaenopsis* plantlets under relative humidity and air temperature, *Environ. Exp. Bot*., 55, 183-194, 2006.

Johnson, N.C., Pfleger, F.L., Crookston, R.K., Simmons, S.R. and Copeland, P.J., Vesicular arbuscular mycorrhizas respond to corn and soybean cropping history, *Agronomy Journal*, 84, 387-390, 1991.

Kadleček, P., Effect of pretreatment by irradiance and exogenous saccharose under *in vitro* conditions on photosynthesis and growth of tobacco (*Nicotiana tabacum* L.) plants during acclimatization after transfer to soil, Diploma Thesis, Charles University, Department of Plant Physiology, Praha, 1997.

Kar, M. and Mishra, D., Catalase, Peroxidase and Polyphenoloxidase activities during rice leaf senescence, *Plant Physiol*., 57, 315-319, 1976.

Knight, S., The phosphorus nutrition of bananas with special emphasis on VA mycorrhizal fungi and the effect of nitrogen, *Banana Newsletter*, 11, 18, 1988.

Kosky, R.G., Silva, M.D.F., Perez, L.P., Gilliard, T., Martinez, F.B., Vega, M.R., Milian, M.C. and Mendoza, E.Q., Somatic embryogenesis of the banana hybrid cultivar FHIA-18 (AAAB) in liquid medium and scaled-up in a bioreactor, *Plant Cell Tiss. Org. Cult*., 68, 21-26, 2002.

Kozai, T., Micropropagation under photoautotrophic conditions, In: Debergh, P.C., Zimmerman, R.H. (eds.), Micropropagation: Technology and Application, Kluwer Academic Publishers, Dordrecht-Boston-London, pp. 447-469, 1991.

Kozai, T. and Smith, M.A.L., Environmental control in plant tissue culture - general introduction and overview, In: Aitken-Christie, J., Kozai, T. and Smith, M.L. (eds.), Automation and Environmental Control in Plant Tissue Culture, Kluwer Academic Publishers, Dordrecht-Boston-London, pp. 301-318, 1995.

Krikorian, A.D. and Cronauer, S.S., Aseptic culture techniques for banana and plantain improvement, *Economic Bot*., 38, 322-331, 1984.

Krishna, H., Singh, S.K., Sharma, R.R., Khawale, R.N., Grover, M. and Patel, V.B., Biochemical changes in micropropagated grape (*Vitis vinifera* L.) plantlets due to arbuscular-mycorrhizal fungi (AMF) inoculation during *ex vitro* acclimatization, *Scientia Horticul.*, 106(4), 554-567, 2005.

Lin, C.Y. and Chang, D.C.N., Effect of three *Glomus* endomycorrhizal fungi on growth of micropropagated banana plantlets, *Trans. Mycol. Soc. Rep. China*, 2(1), 37-45, 1987.

Lin, K.H.R., Weng, C.C., Lo, H.F. and Chen, J.T., Study of the root antioxidative system of tomatoes and eggplants under waterlogged conditions, *Plant Sci.*, 167, 355-365, 2004.

Lin, M.Y. and Fox, R., External and internal P requirements of mycorrhizal and non-mycorrhizal banana plants, *Journal of Plant Nutrition*, 10(9-16), 1341-1348, 1987.

Lovato, P.E., Gianinazzi, P.V., Trouvelot, A. and Gianinazzi, S., The state of the art of mycorrhizas and micropropagation, *Adv. Hortic. Sci.*, 10, 46-52, 1996.

Ma, S.S. and Shii, C.T., *In vitro* formation of adventitious buds in banana shoot apex following decapitation, *J. Chinese Society Hort. Sci*., 18, 135-142, 1972.

Madhulatha, P., Anbalagan, M., Jayachandran, S. and Sakthivel, N., Influence of liquid pulse treatment with growth regulators on *in vitro* propagation of banana, *Plant Cell Tiss. Org. Cult*., 76, 189-191, 2004.

Madhulatha, P., Kirubakaran, S.I. and Sakthivel, N., Effects of carbon sources and auxins on *in vitro* propagation of banana, *Biologia Plantarum*, 50(4), 782-784, 2006.

Malik, C.P. and Singh, M.B., Plants Enzymology and Histo-Enzymology, Kalyani Publishers, New Delhi, p. 286, 1980.

Marin, M., Mari, A., Ibbara, M. and Garcia-Ferriz, L., Inoculation of micropropagated persimmon plantlets, *J. Hort. Sci. Biotechnol*., 78(5), 734-738, 2003.

Martin, K.P., Zhang, C.L., Slater, A. and Madassery, J., Control of shoot necrosis and plant death during micropropagation of banana and plantains (*Musa* sp.), *Plant Cell Tiss. Org. Cult*., 88, 51-59, 2007.

Mathews, D., Hegde, R.V. and Sreenivasa, M.N., Influence of arbuscular mycorrhizae on the vigour and growth of micropropagated banana plantlets during acclimatization, *Karnataka Journal of Agricultural Sciences*, 16(3), 438-442, 2003.

Mathur, N. and Vyas, A., Changes in enzyme of nitrogen metabolism by VA mycorrhizae in *Ziziphus nummularia*, *Journal of Plant Physiology*, 147, 331-333, 1995.

Mathur, N. and Vyas, A., Enzymatic changes in cluster bean by different VAM species, *Agric. Sci. Digest*, 17, 16-18, 1997.

Mathur, N. and Vyas, A., Influence of arbuscular mycorrhizae on biomass production, nutrient uptake and physiological changes in *Ziziphus mauritiana* Lam. under water stress, *J. of Arid Environ*., 45, 191-195, 2000.

Matos, R.M.B., Silva, E.M.R. and Brasil, F.C., Arbuscular mycorrhizal and organic matter on the acclimatization of banana-tree seedlings, cv. Nanicao, *Bragantia*, 61(3), 277-283, 2002.

Mendes, B.M.J., Filippi, S.B. and Demetrio, C.G.B., A statistical approach to study the dynamics of micropropagation rates, using banana (*Musa* sp.) as an example, *Plant Cell Rep*., 18, 967-971, 1999.

Mendes, B.M.J., Mendes, F.J., Neto, A.T., Demetrio, C.G.B. and Pieske, O.R., Efficiency of banana plantlet production by micropropagation, *Pesqui Agropecu Bras*., 31, 863-867, 1996.

Menge, J.A., Davis, R.M., Johnson, E.L. and Zentmyer, G.A., Mycorrhizal fungi increase growth and reduce transplant injury in avocado, *Calif. Agric.*, 32, 6-7, 1978.

Miransari, M., Bahrami, H.A., Rejali, F. and Malakouti, M.J., Using arbuscular mycorrhiza to alleviate the stress of soil compaction on wheat (*Triticum aestivum* L.) growth, *Soil Biol. and Biochem*., 40(5), 1197-1206, 2008.

Monk, L.S., Fagerstedt, K.V. and Crawford, R.M.M., Oxygen toxicity and superoxide dismutase as an antioxidant in physiological stress, *Physiol. Plant*., 76, 456-459, 1989.

Morel, G. and Wetmore, R.H., Fern callus tissue culture, *Am. J. Bot*., 38, 141-143, 1951.

Muhammad, A., Hussain, I., Naqvi, S.M.S. and Rashid, H., Banana plantlet production through tissue culture, *Pak. J. Bot*., 36(3), 617-620, 2004.

Muhammad, A., Hussain, I., Rashid, H. and Quraishi, A., Enhanced *in vitro* multiplication of banana (*Musa* sp.), *Plant Cell Tiss. Org. Cult*., 10(2), 111-117, 2000.

Murashige, T. and Skoog, F., A revised medium for rapid growth and bioassays with tobacco tissue, *Physiol. Plant*., 15, 473-497, 1962.

Nandwani, D., Zehr, U., Zehr, B.E. and Barwale, R.B., Mass propagation and *ex vitro* survival of Banana cv. Basrai through tissue culture, *Garten Bauwissen Chaft*., 65(6), 237-240, 2000.

Navarro, C., Escobedo, R.M. and Mayo, A., *In vitro* plant regeneration for embryogenic cultures of a diploid and a triploid Cavendish banana, *Plant Cell Tiss. Org. Cult*., 51, 17-25, 1997.

Nelson, R. and Achar, P.N., Stimulation of growth and nutrient uptake by VAM fungi in *Brassica oleracea* var. *capitata*, *Biologia plant*., 44(2), 277-281, 2001.

NHB database 2009, http://nhb.gov.in/statistics/area-production-statistics.html.

Novak, F.J., Brunner H., Afza, R., Morpurgo, R., Upadhyay R.K., Van Duren M., Sacchi M., Hawz J.S., Khatri A., Kahl G., Kaemmer D., Ramser J. and Weising K., Improvement of *Musa* through biotechnology and mutation breeding. In: Biotechnology applications for banana and plantain improvement, Proc. of the Workshop. INIBAP, 143-158, 1993.

Okole, B.N. and Schulz, F.A., Micro-cross sections of banana and plantains (*Musa* sp.): morphogenesis and regeneration of callus and shoot buds, *Plant. Sci*., 116, 185-195, 1996.

Olsen, S.R., Cole, C.V., Watanabe, F.S. and Dean, L.A., Estimation of available phosphorus in soils by extraction with sodium bicarbonate, U.S. Dep. of Agric. Circ., 939, 1954.

Paleg, L.G., Stewart, G.R. and Bradbeer, J.W., Proline and glycine betaine influence protein salvation, *Plant Physiol*., 75, 974-989, 1984.

Panja, B.N., Mandal, D., Misra, D. and Saha, J., Diversity of arbuscular mycorrhizal fungi in the rhizosphere of banana genotypes, *Journal of Mycopathological Research*, 45(1), 54-57, 2007.

Panse, V. G. and Sukhatme, P. V., Statistical methods for agricultural workers, Indian Council of Agricultural Research, New Delhi, pp. 359, 1967.

Panwar, J.A. and Vyas, A., AM fungi: a biological approach towards conservation of endangered plants in Thar Desert India, *Curr. Sci*., 82(5), 576-578, 2002.

Phillips, J.M. and Hayman, D.S., Improved procedure for clearing and staining parasitic and vesicular arbuscular mycorrhizal fungi for rapid assessment for infection, *Trans. Br. Mycol. Soc.*, 55, 158-161, 1970.

Porcel, R., Barea, J.M. and Ruiz-Lozano, J.M., Antioxidant activities in mycorrhizal soybean plants under drought stress and their possible relationship to the process of nodule senescence, *New Phytol*., 157, 135-143, 2003.

Pospíšilová, J., Čatský, J., Šesták, Z., Photosynthesis in plants cultivated *in vitro*, In: Handbook of Photosynthesis, Pessarakli, M. (ed.), Marcel Dekker, New York-Basel-Hong Kong, pp. 525-540, 1997.

Pospíšilová, J., Solárová, J., Čatský, J., Photosynthetic responses to stresses during *in vitro* cultivation, *Photosynthetica*, 26, 3-18, 1992.

Preece, J.E. and Sutter, E.G., Acclimatization of micropropagated plants to the greenhouse and field, In: *Micropropagation, Technology and Application*, Debergh, P.C. and Zimmerman, R.H. (eds.), Kluwer Academic Publishers, Dordrecht-Boston-London, pp. 71-93, 1991.

Priyono., Micropropagation of banana (*Musa paradisiaca*) through cormlet initiation by *in vitro* culture of apical meristem slices, *Jurnal ILMU DASAR*, 2(1), 41-48, 2001.

Rahman, M.M., Golam Rabbani, M.D., Rahman, M.A. and Farid Uddin, M.D., *In vitro* shoot multiplication and rooting of banana cv. Sabri, *Pak. J. Biol. Sci*., 5(2), 161-164, 2002.

Rahman, M.Z., Nasiruddin, K.M., Amin, M.A. and Islam, M.N., *In vitro* response and shoot multiplication of banana with BAP and NAA, *Asian J. Plant. Sci*., 3(4), 406-409, 2004.

Ray, P.K., In: *Biotechnology of Horticultural crops*, Vol. I, Parthasarathy, V.A., Bose, T.K., Das, P., Mitra, S.K. and Mohandas, S (eds.), Naya Prokash Pub., pp. 172-205, 2001.

Resmi, L. and Nair, A.S., Plantlet production from male inflorescence tips of *Musa acuminata* cultivars from South India, *Plant Cell Tiss. Org. Cult*., 88, 333-338, 2007.

Rizzardi, V., Effect of inoculation with vesicular-arbuscular mycorrhizal fungi on the growth of micropropagated *Musa acuminata* clone Grand Naine, *Rivista Agricoltura Subtropicale e Tropicale*, 84(3), 473-484, 1990.

Rodriguez-Romero, A.S., Piñero-Guerra, M.S. and Jaizme-Vega, M.C., Effect of the interaction between AMF *Glomus manihotis* and plant growth promoting bacteria (PGPB) from *Bacillus* genus on micropropagated banana plantlets, Abstract in *The Fourth International Conference on Mycorrhizae ICOM4*, August 10-13, Montreal-Canada, 2003.

Roels, S., Escalona, M., Cejas, I., Noceda, C., Rodriguez, R., Canal, M.J., Sandoval, J. and Debergh, P., Optimization of plantain (*Musa* AAB) micropropagation by temporary immersion system, *Plant Cell Tiss. Org. Cult*., 82, 57-66, 2005.

Roldan, A., Diaz-Vivancos, P., Hernandez, J.A., Carrosco, L. and Caravaca, F., Superoxide dismutase and total peroxidase activities in relation to drought recovery performance of mycorrhizal shrub seedlings grown in an amended semiarid soil, *J. of Plant Physiol*., 165(7), 715-722, 2008.

Ruiz-Lozano, J.M. and Azcon, R., Hyphal contribution to water uptake in mycorrhizal plants as affected by the fungal species and water status, *Physiol. Plant*., 95, 472-478, 1995.

Ruiz-Lozano, J.M., Arbuscular mycorrhizal symbiosis and alleviation of osmotic stress, new perspectives for molecular studies, *Mycorrhiza*, 13, 309-317, 2003.

Sagi, L., Panis, B., Remy, S., Schoofs, H., De Smet, K., Swennen, R. and Cammue, B., Genetic transformation of banana via particle bombardment, *Biotechnology*, 13, 481-485, 1995.

Sanchez-Diaz, M. and Honrubia, M., Water relations and alleviation of drought stress in mycorrhizal plants. In: *Impact of arbuscular mycorrhizas on sustainable agriculture and natural ecosystems*, Gianinazzi, S. and Schuepp, H. (eds.), Basel: Birkhauser Verlag, pp. 167-178, 1994.

Sandra, S.C. and Krikorian, A.D., Rapid multiplication of bananas and plantains by *in vitro* shoot tip culture, *Hort Sci*., 19(2), 234-235, 1984.

Sauer, C.O., *Agricultural Origins and Dispersals*, New York, American Geographical Society, 1952.

Schellenbaum, L., Berta, G., Ravolanirina, F., Tisserant, B., Gianinazzi, S. and Fitter, A.H., Influence of endomycorrhizal infection on root morphology in an micropropagated woody plant species (*Vitís vinifera* U.), *Annals of Botany*, 68, 135-141, 1991.

Schellenbaum, L., Muller, J., Boller, T., Wienken, A. and Schuepp, H., Effects of drought on non-mycorrhizal and mycorrhizal maize: Changes in the pools of non-structural carbohydrates, *New Phytol*., 138, 59-66, 1998.

Short, K.C., Wardle, K., Grout, B.W. and Simpkins, I., *In vitro* physiology and acclimatization of aseptically cultured plantlets, In: *Plant Tissue and Cell Culture Application to Crop Improvement*, Novák, F.J., Havel, L. and Doležel, J. (eds.), Inst. Exp. Bot. Czechoslov. Acad. Sci., Prague, pp. 475-486, 1984.

Silva, A.D., Matssumoto, K., Bakry, F. and Souza, R.B.B., Plant regeneration from long term callus culture of AAA-group dessert banana, *Pesq. Agropec. Brasilia*., 33(8), 1291-1296, 1998a.

Silva, S.O., Oliveira, R.P., Matos, A.P., Neto, S.P.S., Prazeres, A.G. and Oliveira, C.A.P., Development of pre-acclimatized banana plantlets obtained from tissue culture, *Acta Hort*., 490, 195-199, 1998b.

Simmonds, N.W., *Bananas*, 2nd Edition, London, Tropical Agriculture Series, Longman, 1966.

Singh, H.P., Country paper report on banana and plantain - India. In: Banana and plantain R & D in Asia and the Pacific INIBAP, 161-185, 1990.

Smith, S.E. and Read, D.J., Mycorrhizal symbiosis, 2nd Edition, Academic Press, Great Britain, 1997.

Sorial, M.E., Growth, phosphorus uptake and water relations of wheat infected with an arbuscular mycorrhizal fungus under water stress, *Ann. of Agric. Sci. Moshtohor*, 39(2), 909-931, 2001.

Srangsam, A. and Kanchanapoom, K., Thidiazuron induced plant regeneration in callus culture of triploid banana (*Musa* sp.) Gros Michel AAA group, *Songklanakrin J. Sci. Technol*., 25(6), 689-695, 2003.

Stover, R.H. and Simmonds, N.W., *Bananas*, 3rd edition, Longman, London, 1987.

Stribley, D.P., Mineral nutrition, In: Ecophysiology of VA mycorrhizal plants, Safir, G.R. (ed.) CRC, Boca Raton, Fla, pp. 59–70, 1987.

Subramanian, K.S., Charest, C., Dwyer, L.M. and Hamilton, R.I., Effects of arbuscular mycorrhizae on leaf water potential, sugar content and P content during drought and recovery of maize, *Can. J. Bot*., 75, 1582-1591, 1997.

Tang, M., Chen, H. and Shang, H.S., Mechanism of vesicular-arbuscular mycorrhizal fungi enhanced the resistance of poplar to canker, *Scientia Silvae Sinica*., 36(2), 87-92, 2000.

Thomas, J.E., Smith, M.K., Kessling, A.F. and Hamill, S.D., Inconsistent transmission of banana bunchy top virus in micropropagated bananas and its implication for germplasm screening, *Aust. J. Agric*., 46, 663-671, 1995.

Titov, S., Salil Kumar, B., Mandal, M.A., Alam, S. and Uddin, S.N., Control of phenolic compound secretion and effect of growth regulators for organ formation from *Musa* sp. Cv. Kanthali floral bud explants, *Amer. J. Bioch. Biotech*., 2(3), 97-104, 2006.

Turk, M. A., Assaf, T.A., Hameed, K.M., Al-Tawaha., Significance of mycorrhizae, *World J. Agric. Sci*., 2, 16-20, 2006.

Ushimaro, T., Shibasaka, M. and Tsuji, H., Development of O_2^- detoxification system during adaptation to air of submerged rice seedlings, *Plant Cell Physiol*., 33, 1065–1071, 1992.

Van den Houwe, I., Guns, J. and Swennen, R., Bacterial contaminations in *Musa* shoot tip cultures, *Acta Horticul*., 490, 485-492, 1998.

Varma, A. and Schuepp, H., Mycorrhization of the commercially important micropropagated plants, *Crit. Rev. Biotechnol.*, 15, 313-328, 1995.

Venkatachalam, L.R., Thimmaraju, R., Sreedhar, R.V. and Bhagyalakshmi., N., Direct shoot and cormlet regeneration from leaf explants of Silk banana (AAB), *In vitro Cell Dev. Biol. Plant*, 42, 262-269, 2006.

Vessey, J.C. and Rivera, J.A., Meristem culture of bananas, *Turriaba*, 31, 162-163, 1981.

Vidhyasekaran, P., Possible role of ortho-dihyroxy phenolics in grapevine anthracnose disease resistance, *Indian J. Exp. Biol*., 11(5), 473-475, 1973.

Vuylsteke, D.R., Shoot tip culture for propagation, conservation and distribution of *Musa* germplasm, Published by IITA, Nigeria, 1998.

Vuylsteke, D.R., Shoot-tip culture for the propagation, conservation and exchange of *Musa* germplasm, International Board for Plant Genetic Resources, Rome, 20-25, 1989.

Wardle, K., Quinlan, A. and Simpkins, I., Abscisic acid and the regulation of water loss in plantlets of *Brassica oleracea* L. var. *botrytis* regenerated through apical meristem culture, *Ann. Bot*., 43, 745-752, 1979.

Weatherley, P.E., Studies in water relation of cotton plants: Field measurement of water deficits in leaves, *New Physiol.*, 49, 81-97, 1950.

Wong, W.C., *In vitro* propagation of banana (*Musa* sp.): initiation, proliferation and development of shoot tip cultures on defined media, *Plant Cell Tiss. Org. Cult*., 6, 159-166, 1986.

Wu, Q.S. and Xia, R.X., Arbuscular mycorrhizal fungi influence growth, osmotic adjustment and photosynthesis of citrus under well-watered and water stress conditions, *J. of Plant Physiol*., 163, 417-425, 2006.

Wu, Q.S., Xia, R.X. and Zou, Y.N., Improved soil structure and citrus growth after inoculation with three arbuscular mycorrhizal fungi under drought stress, *Europ. J. of Soil Biol.*, 44, 122-128, 2008.

Yan, B., Dai, Q. J., Leu, X.Z., Huang, S.B. and Wang, Z.X., Flooding induced membrane damage, lipid oxidation and active oxygen generation in corn leaves, *Plant cell*, 179, 261-268, 1996.

Yano-Melo, A.M. and Lima-Filho, J.M., Effect of arbuscular mycorrhizal fungi on the acclimatization of micropropagated banana plantlets, *Springer-Verlag*, 9, 119-123, 1999.

Yano-Melo, A.M., Orivaldo Jr, J.S., Lima-Filho, J.M., Melo, N.F. and Maia, L.C., Effect of arbuscular mycorrhizal fungi on the acclimatization of micropropagated banana plantlets, *Mycorrhiza*, 9, 119-123, 1999.

Yao-Qing, Zhu-Hong, H. and Yang-Song, Z., Influence of arbuscular mycorrhizal fungi on mineral nutrients uptake and growth of micro-propagated banana plantlets, *Journal of Fruit Science*, 21(5), 425-428, 2004.

Yordanova, R.Y., Alexieva V.S. and Popov L.P., Influence of root oxygen deficiency on photosynthesis and antioxidants in barley plants, *Russ. J. Plant Physiol.*, 50, 163-167, 2003.

Zaffari, G.R., Kerbauy, G.B., Kraus, J.E. and Romano, E.C., Hormonal and histological studies related to *in vitro* banana bud formation, *Plant Cell Tiss. Org. Cult*., 63, 187-192, 2000.

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Appendices

APPENDIX

ANOVA FOR MICROPROPAGATION TABLES

Table 1: Single sterilization with $HgCl_2$ (0.1%)

ANOVA FOR CONTAMINATION PERCENTAGE								
Variation	**df**	**SS**	**Mean SS**	**F cal.**	**SIGNIFICANCE LEVEL**		**SEM**	**$CD_{0.05}$**
					0.05	0.01		
Between treatment	5	17854	3570.8	103.63**	3.10	8.89	3.71	8.08
Within treatment	12	413	34.4					
Total	17	18267						

ANOVA FOR SURVIVAL PERCENTAGE								
Variation	**df**	**SS**	**Mean SS**	**F cal.**	**SIGNIFICANCE LEVEL**		**SEM**	**$CD_{0.05}$**
					0.05	0.01		
Between treatment	5	4939.2	987.8	25.40**	3.10	8.89	3.94	8.59
Within treatment	12	466.5	38.8					
Total	17	5405.7						

ANOVA FOR MORTALITY PERCENTAGE								
Variation	**Df**	**SS**	**Mean SS**	**F cal.**	**SIGNIFICANCE LEVEL**		**SEM**	**$CD_{0.05}$**
					0.05	0.01		
Between treatment	5	13654.2	2730.8	102.21**	3.10	8.89	3.26	7.12
Within treatment	12	320.6	26.7					
Total	17	13974.8						

Table 2: Single sterilization with NaOCl (4%)

ANOVA FOR CONTAMINATION PERCENTAGE								
Variation	**df**	**SS**	**Mean SS**	**F cal.**	**SIGNIFICANCE LEVEL**		**SEM**	**$CD_{0.05}$**
					0.05	0.01		
Between treatment	5	14008.9	2801.7	86.35**	3.10	8.89	3.60	7.84
Within treatment	12	389.3	32.4					
Total	17	14398.2						

ANOVA FOR SURVIVAL PERCENTAGE								
Variation	**df**	**SS**	**Mean SS**	**F cal.**	**SIGNIFICANCE LEVEL**		**SEM**	**$CD_{0.05}$**
					0.05	0.01		
Between treatment	5	2528.5	505.7	7.44*	3.10	8.89	5.21	11.35
Within treatment	12	815.04	67.9					
Total	17	3343.6						

ANOVA FOR MORTALITY PERCENTAGE								
Variation	**df**	**SS**	**Mean SS**	**F cal.**	**SIGNIFICANCE LEVEL**		**SEM**	**$CD_{0.05}$**
					0.05	0.01		
Between treatment	5	24479	4895.9	81.39**	3.10	8.89	4.90	10.68
Within treatment	12	721	60.14					
Total	17	25201						

Table 3: Double sterilization

ANOVA FOR CONTAMINATION PERCENTAGE								
Variation	**df**	**SS**	**Mean SS**	**F cal.**	**SIGNIFICANCE LEVEL**		**SEM**	**$CD_{0.05}$**
					0.05	0.01		
Between treatment	5	5070.5	1014.1	32.17**	3.10	8.89	3.55	7.73
Within treatment	12	378.2	31.5					
Total	17	5448.8						

ANOVA FOR SURVIVAL PERCENTAGE								
Variation	**df**	**SS**	**Mean SS**	**F cal.**	**SIGNIFICANCE LEVEL**		**SEM**	**$CD_{0.05}$**
					0.05	0.01		
Between treatment	5	1902.8	380.5	12.64**	3.10	8.89	3.46	7.55
Within treatment	12	361.1	30					
Total	17	2263.9						

ANOVA FOR MORTALITY PERCENTAGE								
Variation	**Df**	**SS**	**Mean SS**	**F cal.**	**SIGNIFICANCE LEVEL**		**SEM**	**$CD_{0.05}$**
					0.05	0.01		
Between treatment	5	2150.6	430.1	9.03**	3.10	8.89	4.36	9.50
Within treatment	12	571.3	47.6					
Total	17	2721.9						

Table 4: Per cent shoot regeneration

ANOVA								
Sources of Variation	**df**	**SS**	**Mean SS**	**F cal.**	**SIGNIFICANCE LEVEL**		**SEM**	**$CD_{0.05}$**
					0.05	0.01		
Replications	2	65.3	32.6					
BAP	3	4996	1665.3	99.60**	3.04	7.79	8.17	16.95
IAA	2	2069	1034.5	61.87**	3.44	9.61	7.08	14.68
Interactions	6	821	136.8	8.18**	2.54	5.75	2.36	4.89
Error	22	367.8	16.7					
Total	35	8319.3						

Table 5: Number of days to shooting

ANOVA								
Sources of Variation	**df**	**SS**	**Mean SS**	**F cal.**	**SIGNIFICANCE LEVEL**		**SEM**	**$CD_{0.05}$**
					0.05	0.01		
Replications	2	0.722	0.361					
BAP	3	1088.9	362.9	300**	3.04	7.79	2.19	4.56
IAA	2	923.7	461.8	381**	3.44	9.61	1.90	3.95
Interactions	6	31.6	5.268	4.35*	2.54	5.75	0.63	1.31
Error	22	26.61	1.209					
Total	35	2071.6						

Table 6: Number of shoots per explant

ANOVA								
Sources of Variation	**df**	**SS**	**Mean SS**	**F cal.**	**SIGNIFICANCE LEVEL**		**SEM**	**$CD_{0.05}$**
					0.05	0.01		
Replications	5	1.68	0.33					
BAP	4	211	52.91	309**	2.50	5.20	0.82	1.65
IAA	2	8.15	4.07	23**	3.12	7.63	0.64	1.27
Interactions	8	17.4	2.17	12**	2.07	3.77	0.23	0.47
Error	70	11.9	0.17					
Total	89							

Table 7: Average plant height

ANOVA								
Sources of Variation	**df**	**SS**	**Mean SS**	**F cal.**	**SIGNIFICANCE LEVEL**		**SEM**	**$CD_{0.05}$**
					0.05	0.01		
Replications	5	0.115	0.023					
BAP	4	65.72	16.43	135.7**	2.50	5.20	0.69	1.38
IAA	2	62.05	31.02	256.3**	3.12	7.63	0.53	1.07
Interactions	8	8.37	1.04	8.64**	2.07	3.77	0.20	0.40
Error	70	8.47	0.12					
Total	89							

Table 8: Rooting per cent

ANOVA								
Sources of Variation	**df**	**SS**	**Mean SS**	**F cal.**	**SIGNIFICANCE LEVEL**		**SEM**	**$CD_{0.05}$**
					0.05	0.01		
Replications	5	29.47	5.89					
IAA	2	16266	8133.4	1687**	3.26	8.46	5.37	10.91
MEDIA	1	2917.8	2917.8	605**	4.12	12.89	4.39	8.91
Interactions	2	1566.5	783.2	162**	3.26	8.46	1.26	2.57
Error	35	168.6	4.81					
Total	45							

Table 9: Days to rooting

ANOVA								
Sources of Variation	**df**	**SS**	**Mean SS**	**F cal.**	**SIGNIFICANCE LEVEL**		**SEM**	**$CD_{0.05}$**
					0.05	0.01		
Replications	5	3.55	0.71					
IAA	2	742	371	546**	3.26	8.46	2.01	4.09
MEDIA	1	100	100	147**	4.12	12.89	1.64	3.34
Interactions	2	21.16	10.58	15**	3.26	8.46	0.47	0.96
Error	35	23.77	0.67					
Total	45							

Table 10: Number of roots per micro-shoot

ANOVA								
Sources of Variation	**df**	**SS**	**Mean SS**	**F cal.**	**SIGNIFICANCE LEVEL**		**SEM**	**$CD_{0.05}$**
					0.05	0.01		
Replications	5	3.13	0.627					
IAA	2	155.38	77.69	173**	3.26	8.46	1.64	3.32
MEDIA	1	23.36	23.36	52**	4.12	12.89	1.33	2.71
Interactions	2	2.72	1.36	3	3.26	8.46	0.38	0.78
Error	35	15.69	0.44					
Total	45							