IN VITRO PROPAGATION IN TWO COMMERCIAL DIPLOID BANANAS OF KERALA

By

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THESIS

submitted in partial fulfilment of the requirement for the degree of

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DECLARATION

I hereby declare that this thesis entitled "***In vitro* propagation in two commercial diploid bananas of Kerala"** is a bonafide record of research work done by me during the course of research and that this thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title of any other University or Society.

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CERTIFICATE

Certified that this thesis, entitled **"*In vitro* propagation in two commercial diploid bananas of Kerala"** is a record of research work done independently by **Miss. Sapheera. C.P,** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

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Sapheera. C. P

Affectionately Dedicated to My Mother

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ABBREVIATIONS

ABA	-	Abcisic acid
AC	-	Activated charcoal
BA	-	Benzyl adenine
BBTV	-	Banana bunchy top virus
BPM	-	Basal proliferation medium
cv.	-	Cultivar
2,4-D	-	2,4- Dichlorophenoxy acetic acid
HCl	-	Hydrochloric acid
IAA	-	Indole acetic acid
IBA	-	Indole butyric acid
2ip	-	2- isopentenyl adenine
$HgCl_2$	-	Mercuric chloride
MS	-	Murashige and Skoog medium
NAA	-	Naphthalene acetic acid
PCPA	-	Pentachloro phenoxy acetic acid
RWS	-	Refined white sugar
NaOCl	-	Sodium hypochlorite
NaOH	-	Sodium hydroxide
2,4,5- T	-	2,4,5- Trichlorophenoxy acetic acid
UBS	-	Unrefined brown sugar
ULBS	-	Unrefined light brown sugar
UV	-	Ultra violet
v/v	-	volume/volume

INTRODUCTION

1. INTRODUCTION

Banana (*Musa* spp.) is one of the most popular nutritious fruit crops of the world in terms of per capita consumption. Bananas and plantains are major fruit crops in the tropics and subtropics and make a vital contribution to the economies of a number of countries. India is the largest producer of banana accounting for 17.8 per cent of world share followed by Brazil (FAO, 2002). The state of Kerala is blessed with a wide array of banana varieties with specific regional preferences and commercial importance. It occupies an area of 1.06 lakh ha with a total production of 7.69 lakh tons in Kerala (FIB, 2004).

Conventional propagation of banana is through suckers. As the production of suckers per plant is rather low, the rate of multiplication is very low. This is a problem especially when a new clone is being generated to replace older clones or when a large quantity of planting material of a specific pathogen free clone is required for field testing and assessment.

Lack of uniformity in harvesting is yet another problem faced by the banana growers throughout the country. The harvesting period extends even up to four months in several cases. This is mainly due to the use of heterogenous planting material. Farmers suffer great loss due to this non uniformity in bunch maturity. The prevalence of disease problems and the need for generating clean disease free uniform planting stock in large quantities have stimulated a surge of interest in the production of clonal material of both dessert and cooking bananas by the use of aseptic *in vitro* cleaning technique. Maximum yield gains from *in vitro* derived plants range from 20.0 per cent in bananas to 70.0 per cent in plantains.

Micropropagation techniques were developed during the past two decades and are now well established. Tissue culture laboratories supply the regular replanting needs of the large export banana plantations. Shoot tip culture is

simple, easy and applicable to a wide range of *Musa* genotypes. Application of micropropagation has greatly improved *Musa* germplasm handling, clonal propagation and breeding.

The varietal situation in India is very complex, each region having preference to certain dessert/ cooking bananas. The morphological variations in the cultivars of banana, which consist mainly of triploids and diploids, are quite wide and complex, with combinations of different degrees of expression of the parental species. In Kerala, diploid cultivars have got high demand and are getting premium price in domestic market. However scanty tissue culture work has been done in the case of domesticated diploid banana cultivars. Since the diploid group constitute only a small number of cultivars mainly of South Indian origin, it may differ from those of commonly cultivated triploid bananas and may require technology refinement for *in vitro* multiplication. The limited source of planting material is one of the major problems in cultivation of diploid bananas like Njalipoovan, Nivedyakadali, Kunnan etc. and these cultivars have received less attention in the field of tissue culture.

Developing and improving *in vitro* methods for reproducing banana plants is regarded as essential to long term efforts aimed at improving banana culture world wide especially in a state like Kerala where productivity is noted at the lowest due to polyclonal, homestead system of banana cultivation coupled with prevalence of sucker transmitted deadly diseases like bunchy top and bacterial wilt and nematode infestation. Therefore, to develop *in vitro* techniques for rapid multiplication of important diploid clones of Kerala namely, *Musa* (AB) 'Njalipoovan' and *Musa* (AA) 'Nivedyakadali', a study was taken up in the Department of Pomology and Floriculture, College of Horticulture, Vellanikkara during the period 2003-2005 with following objectives

1. Standardisation of explant and explant establishment (stage I)
2. Standardisation of basic proliferation medium and induction of axillary shoots (stage II)
3. Standardisation of *in vitro* rooting (stage III)
4. Acclimatization of plantlets (stage IV)

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Commercial micropropagation industry is considered relevant to agribusiness as tissue culture has the capability to break the plateau in yield improvements reached by green revolution and has several exclusive practical applications leading to improvement in crop productivity, which are not possible in conventional science. However, high cost of production of tissue culture plantlets limits this application.

Clonal propagation of plant species through tissue culture is based on the concept of cell totipotency derived from the cell theory that Schleiden proposed in 1838. The origin of plant tissue culture could be traced to the earliest studies by Haberlandt (1902), who aseptically cultured single cells on a liquid medium supplemented with sugar.

The identification and purification of indole-3- acetic acid (IAA), the first known growth regulator, by Kogl *et al.*, (1934) and then by Thimann (1935), made it possible to control the growth of plants, tissues and cells. Reinert (1958) and Steward and Mapes (1958) were the first to obtain plantlets from callus and cell suspension cultures.

Vasil *et al.*, (1979) reported that the technique of micropropagation has been standardized in about 332 crop species. According to George and Sherrington (1984) studies on *in vitro* culture systems are in progress at least in 1051 crop species of which not less than 94 are fruit crops including bananas. The greatest success in plant tissue culture has been achieved in herbaceous horticultural species.

The review has been confined to the *in vitro* propagation of banana and its cost effective methods, the important aspect of which are dealt under six major heads

1. Explant
2. Media for culture establishment and multiplication
3. Culture conditions
4. *In vitro* rooting
5. Hardening off and planting out
6. Cost effective methods in micropropagation

2.1. EXPLANT

Several workers had reported the use of both vegetative buds and inflorescence buds for *in vitro* culturing of banana (Ma and Shii, 1974; Cronauer and Krikorian, 1985a and b; Fitchet and De Winnaar, 1987; Aravindakshan, 1989; Doreswamy and Shajiram, 1989; Drew *et al.*, 1989; Bhaskar, 1991). Shoot tip explant was found to be the commonly used explant in most of the literature.

2.1.1. Shoot tip explant

Ma and Shii (1972) reported adventitious bud formation in *in vitro* banana shoot apex culture using a combination of semi-solid and liquid media. The applicability of excised shoot tip culture technique to a number of *Musa* clones was assessed by De Guzman *et al.*, (1976), Vessey and Rivera (1981), Banerjee and De Langhe (1985), Vuylsteke and De Langhe (1985), Aravindakshan (1989) and Bhaskar (1991).

Doreswamy *et al.* (1983) explored the possibility of clonal propagation of banana through tissue culture in the cultivar Robusta (AAA). Shoot tip isolated from the sucker was found to be the best explant. Excised shoot tips with the

youngest leaves produced only one plantlet, while shoot tips with several older sheathings leaf bases enclosing the axillary buds regenerated multiple plantlets. Individual shoots, when separated and sub cultured, produced multiple shoots.

According to Krikorian and Cronauer (1984b) one of the major features of shoot apex culture technique was that shoot multiplication could be induced by releasing buds at the leaf bases. Sub culturing could be carried out from the proliferating mass of the shoots formed. Cronauer and Krikorian (1984a and b) established rapidly multiplying cultures of dessert banana clones, Philippine Lacatan and Grand Naine (AAA) and Plantain clones Saba and Pelipita (ABB) on a modified MS medium supplemented with 5.0 mgl^{-1} BA. They added that multiple shoot cultures could be induced by longitudinally splitting a young cultured shoot through the apex and placing each half upright on semisolid medium. Hwang *et al.*, (1984) reported that the plantlets originating from adventitious buds of explants obtained from the decapitated shoot apices of banana suckers established well and gave rise to mature axillary shoots which on transfer to hormone free medium resulted in rapid and extensive root formation.

Damasco and Barba (1985) reported successful shoot tip culture for Saba bananas. They reported that by repeated sub culturing at an interval of 2 months, 2,00,000 plantlets could be obtained from a single explant in 10 months time when culturing in MS medium with BA 10.0 mgl^{-1}. According to Sun (1985) 93 out of 103 banana clones developed adventitious buds in meristem culture on an MS medium supplemented with BA 5.0 mgl^{-1} and IAA 2.0 mgl^{-1}.

Wong (1986) reported *in vitro* multiplication of banana (*Musa* spp.) from shoot tip explants isolated from lateral suckers. Using explants with apical domes, a total of 22 cultivars (genomes AA, AAA, AAAA, AAB, AB, ABB) were successfully cultured on a modified MS medium containing BA and IBA. The medium used was MS containing 0.4mg thiamine HCl, 100.0 mg L-tyrosine, 100.0 mg myo- inositol, 2.0 mg IAA, 2.0 mg kinetin, 160.0 mg adenine sulphate,

30.0 g sucrose and 8.0 g agar per litre. Repetitive dissection of the aggregated adventitious buds was made for increasing bud population. These buds were transferred to the above medium amended with 1.0 gl^{-1} activated charcoal for root and shoot development.

Jarret *et al.* (1985) established shoot tip cultures of two cooking bananas, Saba and Pelipita (AAB) on a modified MS medium supplemented with BA in combination with IAA. Splitting shoots tips along their longitudinal axis and reculturing individual pieces on basal medium supplemented with BA 5.0 mgl^{-1} initiated propagation cultures. Fitchet and De Winnaar (1987) developed a method for rapid multiplication of banana by tissue culture using apical meristems from small suckers of Dwarf Cavendish and Williams banana on MS basal medium supplemented with IAA, NAA, kinetin, activated charcoal and sodium phosphate. Sub culturing was carried out at four weeks intervals. Huang and Chi (1988) also reported the use of banana shoot tip for *in vitro* culturing. Mateille and Foncelle (1988) developed an improved micropropagation method for *Musa* (AAA) Poyo. Apices of sucker buds and lateral buds were cultured in auxin free cytokinin (BA) rich MS medium. Shoot proliferation was achieved by subculturing them in the same medium. Drew *et al.* (1989) established plants *in vitro* from banana bunchy top virus (BBTV) infected plants using vegetative shoot apices.

2.1.2. Response of genotypes

Variation in response of different genotypes was observed in banana tissue culture. Vuylsteke and De Langhe (1985) studied the *in vitro* shoot proliferation potential of a number of banana cultivars. They observed that the genome influenced the rate and type of proliferate growth. The triploids gave higher multiplication rate than diploids. Triploid cultivars with one or two B genomes produced more meristem tips per explant than did AAA triploids.

Bhaskar (1991) viewed that different cultivars of banana, namely Red Banana (AAA), Palayankodan (AAB) and Nendran (AAB) responded differently to a fixed set of treatments.

Das *et al.* (1998) reported best explant survival as well as growth responses by Kanchikela (*Musa* ABB) compared to the genotypes Martaman (*Musa* AAB), Singapuri (*Musa* AAA) and Giant Governor (*Musa* AAA) under investigation. Cultivars Singapuri and Giant Governor both belonging to the Cavendish (*Musa* AAA) group showed moderate response to the micropropagation technique.

2.1.3. Explant preparation

The size of the explant used, though varies depending upon the plant species, influences the success or failure of the culture. In general, the larger the size of the explant, the more will be the survival rate and growth in the culture media (Hussey, 1983). Krikorian and Cronauer (1984a) stated that in meristem culture the smaller the size of the meristem used, the mortality due to sterilization may be more, but, chances of getting virus elimination from the meristem will also be more. In banana, the usual size of shoot bud explant ranges from 10 x 15 mm^2 in size with two to three leaf primordia covering the apical dome (Doreswamy *et al.*, 1983; Cronaeur and Krikorian, 1984a; Jarret *et al.*, 1985; Vuylsteke and De Langhe, 1985; Israeli *et al.*, 1995).

2.1.4. Physical treatments to explants

The technique of splitting banana shoot tips longitudinally through their apex in order to induce multiple shoot formation was first described by De Guzman *et al.* (1980). Injuring the bud apex by giving 7 to 12 vertical incisions was found to induce the production of a cluster of shoots with in one month as reported by Vessey and Rivera (1981). Jarret *et al.* (1985) and Bhaskar(1991) observed dividing the apical dome into two halves and culturing each half separately was

found to be best one in enhancing the release of axillary buds. Wong (1986) reported that survival rate of shoot tip explants was considerably less when the apical dome removed.

2.1.5. Season of explant collection

It has been fairly well documented that tissues taken from field grown plants are not equally amenable to culture conditions throughout the year. Shoot tip collection in banana during November to April resulted in least contamination rate and maximum explant survival (Bhaskar, 1991). Sundararasu (2003) reported that maximum survival percentage of explants was recorded when explants were collected from the field during March - April.

2.1.6. Surface sterilization

The explants collected from field grown plants harbour numerous fungi and bacteria, which when inoculated into a nutrient medium contaminate the entire *in vitro* system. Hence, surface sterilization is resorted prior to inoculation of explants.

Different workers used various sterilizing agents and different concentrations and length of time in banana tissue culture. Rao *et al.* (1982) used mercuric chloride (0.2 %) solution for 10 minutes to 15 minutes to sterilise the inflorescence explants.

The shoot tips were washed with liquid detergent (Cleansol) and surface sterilized with saturated chlorine water for 15 to 20 minutes (Doreswamy *et al.*, 1983). All traces of chlorine water were removed by washing several times with sterilized distilled water. Krikorian and Cronauer (1984a) reported sterilization with one per cent to two per cent commercial bleach (0.0525 % NaOCl) and Tween 20 for five minutes, followed by three to four rinses in sterile water.

Gupta (1986) surface sterilized the banana shoot tips in 10 per cent Chlorox for 10 minutes, followed by three to five washings in sterile water.

According to Fitchet and De Winnaar (1988), 10 per cent Calcium hypochlorite gave the best results. Bhaskar (1991) reported best results with mercuric chloride (0.05 - 0.2 %)

2.1.7. Explant and media blackening

Addition of activated charcoal (AC) prevent phenol oxidation from micro cuttings and explants and to induce rooting of cuttings. The effect of AC attributed to three factors such as darkening of medium (favours rooting) (Proskauer and Berman, 1970); absorption of inhibitory compounds and growth hormones from the medium (Weatherhead *et al.*, 1978). In banana, for *in vitro* rooting, 0.025 per cent w/v AC is commonly used (Krikorian and Cronauer, 1984a).

Blackening, as a result of phenolic oxidation, was reduced by dipping the explant in antioxidants, such as cystein (Jarret *et al.*, 1985; Sandoval, 1985), citric or ascorbic acid (Gupta, 1986) before being transferred to the medium. Alternatively, antioxidants could be included in the medium itself (Banerjee *et al.*, 1986; Vuylsteke, 1989; Vuylsteke *et al.*, 1990; Bhaskar, 1991).

2.2. MEDIA FOR CULTURE ESTABLISHMENT AND MULTIPLICATION

The most widely used medium contains the Murashige and Skoog (1962) (MS) formulation with certain modifications. Smith and Murashige (1970) reported a version with additional phosphate. Several basal medium formulations

have been reported to sustain growth and proliferation of the explants (Cronauer and Krikorian, 1986). An efficient medium for *in vitro* propagation of banana

cultivar William, Grand Naine and Maghraby was developed by Bekheet and Saker (1999). The major constituents of tissue culture medium are mineral salts, carbon and energy sources, vitamins, plant growth regulators and other organic compounds.

2.2.1. Composition of medium

The salt composition of several media has been reviewed by Gamborg *et al.* (1976). Banerjee and De Langhe (1985) omitted copper sulphate and zinc sulphate from MS medium for better response in micropropagation of banana. Several research workers reported desired success in MS medium (Cronauer and Krikorian, 1986; Gupta, 1986; Wong, 1986; Aravindakshan, 1989; Novak *et al.*, 1989. and Bekheet and Saker (1999).

A carbon energy source is inevitable in any tissue culture media. Sucrose is the most widely accepted carbon source. Besides serving as carbohydrate source, sucrose regulates osmolarity of the culture media and also plays a role during morphogenesis (Thorpe, 1978 and Sopory, 1979). Matielle and Foncelle (1988) reported that lower concentration of sucrose 10.0 g l^{-1} is the most favourable for shoot elongation and rooting. In general sucrose 30.0 gl^{-1} is used in banana micropropagation.

Bhaskar (1991) stated that sucrose at 1.5 per cent concentration was found optimum for the production of sufficient roots and normal plantlets. Sundararasu (2003) observed that table sugar 2.0 per cent and 3.0 per cent were found to be an effective alternative to sucrose 3.0 per cent.

2.2.2. Growth regulators

Two principal classes of growth regulators are used in tissue culture studies, namely auxins and cytokinins. Most commonly used auxins are 2,4-D,

IBA, NAA, IAA, 2,4,5-T and PCPA. The most commonly used cytokinins are BA, kinetin and 2 ip. Auxins most frequently used to induce *in vitro* rooting are IAA, NAA and IBA (Murashige, 1974). In banana both IBA (0.5 mgl^{-1} to 2.0 mgl^{-1}) and NAA (0.02 mgl^{-1} to 2.0 mgl^{-1}) have been reported to induce rooting of shoots and embryoids (Banerjee *et al.*, 1987).

No universal ratio of auxin and cytokinin has so far been developed for root and shoot induction. For axillary shoot proliferation, cytokinin has been utilized to overcome the apical dominance of shoots and to enhance the branching of lateral buds from leaf axils (Murashige, 1974). A kind of synergism between two cytokinins namely, kinetin and BA has also been reported in certain cases in *in vitro* axillary bud proliferation (Gupta *et al.*, 1981).

The success or otherwise of an *in vitro* system in *Musa* dependent upon the choice of correct growth regulators and their use in optimum concentration (Krikorian, 1982). Rodriguez *et al.* (1987) stated that high concentration of BA (5.0 mgl^{-1}) is necessary for intense proliferation of shoots. In shoot tip culture of banana, on an average, each explant released 11.00 axillary shoots when the basal proliferating medium (BPM) containing NAA 1.0 $mgl^{-1}$and BA 10.0 mg l^{-1} (Bhaskar, 1991).

An efficient procedure for the *in vitro* production of tetraploid banana plantlets (*Musa* spp. cv. FHIA, AAAB group) was developed by Oliveira and Silveira (2001). They stated that higher multiplication rates were obtained on the MS media supplemented with 4.0 mgl^{-1} BAP. Survivability rate of plantlets in pots was relatively higher in NAA than IBA (Rahman *et al.*, 2002). Robles and Fernandez (2003) reported that, for accelerated micropropagation of banana species (*Musa* spp. cv. Cavendish, AAA group) the optimum concentration for shooting stimulation (from the formation of off shoots to explant) was BAP 6.0 mgl^{-1}+ NAA 1.0 mgl^{-1} .The MS medium with BAP 5.0 mgl^{-1} is better for *in vitro* response including survivability of banana meristem, whereas BAP 4.0 mgl^{-1} +

NAA 1.5 mgl^{-1} should be recommended for higher shoot multiplication (Rahman *et al.*, 2004).

2.2.3.Vitamins

Murashige and Skoog (1962) vitamin mixture consisting of myo-inositol 100 mgl^{-1}, nicotinic acid 0.5 mgl^{-1} and pyridoxine 0.5 mgl^{-1} is used for banana tissue culture. Additions like glycine 0.2 mgl^{-1}, ascorbic acid 100 mgl^{-1} (Banerjee *et al.,* 1986) and thiamine HCl 0.4 mgl^{-1} (Vuylsteke and De Langhe, 1985; Krikorian and Cronauer, 1984b) have also been reported to be favourable. According to Thorpe and Patel (1984) thiamine is the most often added vitamin, followed by nicotinic acid and pyridoxine.

2.2.4. Physical form of the medium

The physical form of the medium, that is whether it is solidified or liquid, plays an important role in *in vitro* growth and differentiation. In banana, shoot multiplication was found to be stimulated by transferring tissue pieces alternatively between liquid and semi-solid medium of same composition at two weeks interval (Debergh *et al.*, 1981; Cronauer and Krikorian, 1985a and b). Huang and Chi (1988) reported the advantage of using gelrite instead of agar in banana tissue culture.

2.2.5. pH of the medium

Plant cells in culture require an acidic pH and an initial pH of 5.5 to 5.8 is optimum (Gamborg and Shyluck, 1981). In bananas, the pH of the culture media was adjusted to 5.8 using potassium hydroxide or sodium hydroxide before autoclaving (Berg and Bustamante, 1974; Cronauer and Krikorian, 1984 a and b; Banerjee and De Langhe, 1985).

2.3. CULTURE CONDITIONS

Light, temperature and humidity conditions provided inside the tissue culture room plays a significant role in the success of any tissue culture technique.

2.3.1. Light

The light intensity, quality and duration are the three major factors affecting the growth of *in vitro* culture (Murashige, 1974). Continuous light of about 30000 lux is reported by Vuylsteke and De Langhe (1985), complete darkness for callus induction was reported by Novak *et al.* (1989).

According to Bhaskar (1991), a light intensity ranging between 80W and 120W for 16 hours followed by 8 hour dark period was found to be giving maximum growth and development of axillary shoots in shoot tip culture and a light intensity of 80W was found to be best for eye bud and floral apex culture. For banana shoot tip culture, a light intensity of 1000 to 30000 lux and a photoperiod of 16 hours are recommended by various workers (Damasco *et al.*, 1984; Krikorian and Cronauer, 1984a and b; Israeli *et al.*,1995).

2.3.2.Temperature

Yeoman (1986) reported that the usual environmental temperature at the original habitat of a particular species should be taken into consideration while regulating the temperature of *in vitro* systems. The optimum temperature for bananas is reported to be 28 ^{0}C (Krikorian and Cronauer, 1984a and b; Israeli *et al.*, 1995).

2.3.3.Humidity

Humidity is rarely a problem except in arid climates, where rapid drying of medium occurs. This can be reduced by the use of tightly closed containers, covering closures such as foam or cotton wool plugs with aluminium foil. In climates with high humidity, dehumidifier in the culture room may be

advantageous (Yeoman, 1986). Israeli *et al.* (1995) reported 60.0 to 70.0 per cent relative humidity for *in vitro* banana cultures.

2.4. ROOTING MEDIA

Although a number of plants root spontaneously in culture, shoot of most species multiplied *in vitro* lack a root system. Quite often NAA (Gupta, 1986; Fitchet, 1989; Vuylsteke, 1989) or IBA (Banerjee *et al.*, 1986; Raut and Lokhande, 1989) is used in the medium. Israeli *et al.* (1995) used MS medium supplemented with IAA 2.0 mgl^{-1} and kinetin 5.0 mgl^{-1}.

2.4.1. Composition of rooting medium

The concentration of inorganic salts in the basal medium influences the *in vitro* rooting regardless of the growth substances present. Several workers had shown that *in vitro* rooting can be successfully achieved by reducing the salt concentration in the media particularly in high salt media like MS and its derivative (Kartha *et al.*, 1974; Lane, 1979; Skirvin and Chu, 1979). But in such cases, it resulted in poor top growth. (Gupta *et al.*, 1981). Doubling the concentration of all the salts in MS medium was found to reduce the number of roots produced in banana (Rodriguez *et al.*, 1987). Half concentration of MS medium was found favourably effect the rooting of banana without affecting the shoot growth unlike in certain species (Wang, 1978; Gupta *et al.*, 1981; Banerjee and De Langhe, 1985; Vuylsteke and De Langhe, 1985).

As a source of energy as well as factor for osmoregulation for optimizing the rooting response, sucrose has already been recognized (Chong and Pua, 1985). Sucrose at 1.5 per cent concentration was found optimum for the production of sufficient roots and normal plantlets. The favourable effect of reducing the sucrose concentration to half on *in vitro* rooting and shoot elongation has been reported by Matielle and Foncelle (1988). When the sucrose

concentration was reduced to half in the rooting medium, there was an increase in the number of roots produced (8.75/shoot) and the number of days taken for root initiation was reduced (Bhaskar, 1991).

2.4.2. Growth regulators

Rooting can be achieved either by subculturing to a medium lacking cytokinin, with or without a rooting hormone or by treating the shoots as conventional cuttings after removal from sterile culture (Yeoman, 1986). Since, auxin is essential for root initiation, majority of stage III media contain auxin as a supplement. The concentration of rooting hormone required is often critical to provide sufficient stimulus to initiate roots while preventing the excessive formation of callus.

All cytokinins inhibit rooting and BA, which is widely used for shoot multiplication, does so particularly strongly, even after transfer to cytokinin free medium. The use of 2ip or kinetin in place of BA in the final stages of multiplication often improves subsequent rooting (Webb and Street, 1977). It seems that optimal hormone concentration of the medium and cytokinin/auxin ratios have to be adjusted for each clone and laboratory procedures. As shoot elongation and rooting were accomplished on the same medium, there is no need for special rooting medium (Israeli *et al.*, 1995).

Bhaskar (1991) concluded that among the auxins (NAA and IBA), NAA 5.0 mg l^{-1} was the best with respect to root initiation in maximum number of cultures by 100 per cent within the shortest period of 6 days. Maximum number of roots (6.75/ shoot) was also obtained by adding 5.0 mg l^{-1} NAA to the basal rooting medium.

According to Rahman *et al.* (2002) MS medium supplemented with 5.0 mg l^{-1} each of BAP + Kinetin was the best for rapid multiplication of shoots of

banana cv. Sabari (AAB) and MS medium supplemented with 4.0 mg l^{-1} NAA was suitable for rooting of microshoots. Molla *et al.* (2004) reported that a good number of healthy roots were produced on half MS supplemented with 0.5 mg l^{-1} IBA.

2.4.3. Triazoles

Triazoles are sterols inhibiting fungicides, which have got both fungicidal and plant growth regulating activity. The plant growth regulating properties of sterol inhibiting fungicides was reviewed by Fletcher (1985). Triademefon, one of the triazole derivative, was found to protect plants from injury due to drought, chilling and ozone (Fletcher and Nath, 1984). The mode of action of triademefon is that it increases stomatal resistance, thereby contributing significantly to the maintenance of the turgor in the treated plants.

Transient increase in abscissic acid is triggered by triademefon. Abscissic has been implicated as an initial trigger in the hardening process of various types of plant stress (Boussiba *et al.,* 1975). The effect of triademefon on higher survival percentage of planted out plantlets was also reported in gladiolus (Hussain, 1995). Bhaskar (1996) reported that addition of triademefon in the media recorded better survival percentage and maximum healthy and lengthy roots at 2.0 mg level. This together with anti transpirant activity helped in the better survival of plants, which were cultured in traidemefon containing media and planted out.

Triazole derivatives, which block gibberellins biosynthesis by inhibiting the oxidation steps from kaurene to kaurenal, make substrates available for ABA and carotenoid biosynthesis (Chin, 1982; Fletcher, 1985). Induced elevation of ABA level serves to protect plants from a variety of environmental stresses (Keith and Mc keirse 1986; Singh *et al.,* 1987). Protection may in part be due to

increases in tocopherol and ascorbic acid, which acts as antioxidants (Singh, 1993).

Triazoles protect plants from several unrelated stresses (Fletcher 1985; Fletcher and Hofstra, 1985). A triazole treatment was shown to protect cabbage and bean plants from chilling, drought and ozone injury (Asare-Boamah and Fletcher, 1986). Smith *et al.* (1991) reported that paclobutrazol, another triazole derivative was amongst growth regulators that had the greatest influence in reducing wilting in chrysanthemum cultured *in vitro*. Paclobutrazol brought about increased deposition of epicuticular wax, improved water stress and thickening of roots, each of which may have contributed towards imparting resistance to wilting in micropropagated chrysanthemum at stage IV.

A significant improvement in hardness and survivability of banana plantlets after transplanting from *in vitro* conditions to peat moss and sand medium was achieved by incorporation of triademefon in the stage III culture medium (Murali and Duncan, 1995).

Sundararasu (2003) observed large number of very short thick roots on *in vitro* medium containing triazole above 2.0 mg l^{-1}.

2.5. HARDENING OFF AND PLANTING OUT

Acclimatization is necessary in the case of micropropagated plants because *in vitro* plant material is not adapted to *in vivo* condition (Brainerd and Fuchigami, 1981). The success of acclimatization of micro propagated plants is largely dependent not only upon the post transfer growth condition but also the pre transfer culture condition (Ziv, 1986). Tissue cultured plants are poorly adopted to resist the low relative humidity, higher light levels and more variable temperature prevailing outside (Wainwright, 1988).

Light, temperature and relative humidity are the three major factors to be controlled during acclimatization. Hu and Wang (1983) suggested a period of humidity acclimatization for the newly transferred plantlets. Rajmohan (1985) reported the use of plastic microscope covers for maintaining 90.0 per cent to 100.0 per cent relative humidity and obtained 55.0 per cent to 60.0 per cent survival of *in vitro* produced jack plantlets. In Poyo banana clone 100.0 per cent survival was achieved when plantlets were grown in the glass house (Matielle and Foncelle, 1988).

The optimal size of plantlets at the time of hardening stage is 4.0-5.0cm (from base to the point of contact between petiole of the two youngest leaves) with four or five leaves having a well defined lamina and pseudostem diameter of 4.0-5.0 mm at its base, and with a well developed root system ((Israeli *et al.*, 1995).

Vermicompost containing 20.0-25.0 per cent carbon was a good alternative to soilrite for primary and secondary hardening. This growing medium was also found to be highly cost effective, and resulted in the maximum growth of stems, leaves and roots (Sainedane *et al.*, 2001).

Zhou *et al.* (2003) reported that to increase the survival of transplanted *in vitro* cultured banana plantlets, the best method involved transplanting the plantlets in to polybags.

2.5.1. Potting media

Physical, chemical and biological properties of potting media is also important in the establishment of *in vitro* regenerated plantlets when planted out. Thorough washing of the plantlets to remove the traces of nutrient medium and sterilizing the potting mixture eliminates problems of fungal infection (Anderson, 1980). Jarret *et al.* (1985) reported that more than 90.0 per cent banana plantlets survived after transfer to methyl bromide treated soil. Cronauer and Krikorian (1985a and b) successfully established banana plantlets in pro mix: vermiculite (v/v) potting mixture. Drew *et al.* (1989) successfully established micropropagation plants in a peat, perlite and polystyrene bead (1:1:1) mixture with 40.0 per cent natural light in a Persplex relative humidity by a Penn humidistat controller and Defensor –505 humidifier.

Soil mixture for potting should be well aerated with high water holding capacity. Varoius combinations viz.,soil promix with vermiculite (Krikorian and Cronauer, 1984a), peat and perlite (Drew and smith, 1990), perlite (Novak *et al.*,1990) and 60.0 per cent peat and 40.0 per cent shredded stryrofoam (Israeli *et al.*, 1995) has been reported for acclimatization and further growth of the banana plantlets.

Sundararasu (2003) viewed that *in vitro* plantlets were better hardened in vermiculite medium in terms of weight of plantlets, number of roots, length of roots and length of shoots, kept under mist chamber.

2.5.2. Nutrition

The importance of nutrition of the micropropagation during rooting and hardening has been well documented and shown to be species dependent. Wong (1986) recommended addition of 3.0g of nutricate (14:14:14 NPK) to each pot one week after transplanting to get healthier *in vitro* banana plantlets for planting

in the main field. In Israel, daily fertigation with N: P: K (1:02:2) was given. The nitrogen concentration was 60-100 ppm and micro elements were also used (Israeli *et al.*, 1995)

Sundararasu (2003) observed that spraying of 17:17:17 (N: P: K complex) 1.0 per cent solution at weekly intervals on the banana plantlets resulted in maximum number of leaves per plantlet, length and breath of leaves and height of plantlets.

2.5. COST EFFECTIVE METHODS IN MICROPROPAGATION

The primary application of micropropagation has been to produce high quality planting material. Low cost tissue culture technology is the adoption of practices and use of equipment to reduce the unit cost of micropropagule and plant production. Low cost options should lower the cost of production without compromising the quality of the micropropagules and plants. In low cost technology cost reduction is achieved by improving process efficiency and better utilization of resources. Low cost tissue culture technology will stay a high priority in agriculture, horticulture, forestry and floriculture of many developing countries for the production of suitably priced high quality planting material (Savangikar, 2002).

2.6.1. Culture media

A 90.0 per cent resource cost reduction in tissue culture of banana (*Musa* spp. cv. Grande Naine) was achieved by replacing tissue culture grade sucrose and Gelrite in the medium with locally available commercial sugar and a starch/Gelrite mixture and by using sunlight instead of artificial light (Kodym and Zapata- Arias, 2001).

The composition of culture media used for shoot proliferation and rooting has a tremendous influence on production costs. The replacement of expensive imported vessels with reusable glass jars and lids, alternatives to gelling agents, use of household sucrose, and some medium components can reduce costs of production (Prakash *et al.*, 2002).

Sundararasu (2003) reported that half tablet of vitamin B complex tablet was an effective alternative to vitamin in MS medium in terms of multiple shoot and also in terms of maximum number of roots, length of roots and length of shoots in *in vitro* rooting media.

2.6.2. Liquid medium

In Anthurium, liquid culture was used to proliferate callus (Pierik, 1975) and to propagate shoots (Leffering and Soede, 1979). *Lilium, Begonia, Vitis* and many other plant species have been propagated using liquid culture on commercial scale (Harris and Mason, 1983; Levin *et al.*, 1988).

The advantages of liquid media for enhancing shoot propagation (Hammerschlang, 1982; Harris and Mason, 1983), growth (Snir and Erex, 1980; Skidmore *et al.*, 1988) or somatic embryogenesis (Jones and Petolino, 1988; Gawel and Robacker, 1990) have been reported for several species. The absence of a gelling agent may increase the availability of water and dissolved substances to the explant (Debergh, 1983).

Alvard *et al.* (1993) investigated six different liquid culture methods, for meristem propagation of banana and compared with solid medium culture. Shoots in simple liquid medium and those on cellulose substrate proliferated little or not at all, the highest multiplication rate of more than five shoots was observed in explants subjected to temporary immersion in the medium.

Bhaghyalakshmi and Singh (1995) assessed different types of media, such as agar gelled, agitated liquid and static liquid for their ability to support shoot multiplication and *ex vitro* survival of shoot cultures from meristem explant of three cultivars of banana, Cavendish, Bluggoe and Silk. Liquid media were found better for shoot multiplication and maximum plantlet production was obtained in culture growing in static liquid medium followed by a brief culture on agar gelled medium.

Teng (1997) reported that in micropropagation with rafts, shoot regeneration was significantly improved; the regeneration efficiency was almost three to five times higher than that of liquid culture. Also yellow firm callus clumps with numerous shoot primordia were obtained in raft culture and they were larger than those in liquid culture.

2.6.3. Filter paper

Bhattacharya *et al.* (1994) reported that glass wool cloth strips (2.5 cm x 15 cm), nylon cloth (2.5 cm x 15 cm), filter paper strips (2.5 cm x 15 cm) and polystyrene foam blocks (2 cm x 2 cm x 1 cm) can be used as supporting matrices in the culture of nodal segments and shoot tip culture of Chrysanthemum (*Dendranthema grandiflorum*). The performance of all the support matrices in terms of length of roots and shoots was almost identical with those of MS-agar medium. In polystyrene foam supported explants, roots were fewer but longer shoots were obtained.

Sundararasu (2003) observed that agar 0.7 per cent, the principal gelling agent was effectively substituted with agar 0.6 per cent or agar 0.5 per cent + gelatin 0.3 per cent and filter paper in terms of multiple shoot production.

2.6.4. Use of growth regulator free medium

For the induction of callus, an explant is normally cultured on a medium containing growth regulating substances. However, some excised tissue produce callus, in the absence of exogenous growth regulators (Yeoman and Mc Leod, 1977; Bayer, 1982). Typically, this is a wound reaction where the cells at the cut surface are induced to undergo mitosis leading to callus formation, i.e., a process of wound healing (Kahl, 1983).

On the other hand, some cultures previously induced in the presence of exogenous growth regulators can habituate to grow in the absence of such a substances. Habituation has been frequently reported in various plant tissues cultured *in vitro* (Gautheret, 1959; Meins, 1989). As well tumour tissues and differentiated explants of tumorous plants can grow well on growth regulator free media (Bayer, 1982).

Frances *et al.* (1995) reported that callus cultures were successfully induced on a growth regulator free medium using all explants types of *Erysium scoparium* (hypogeal cotyledon and radicle). These calli were predominantly initiated at cut edges of the explants.

2.6.5. Carbon sources

Bonaobra (1994) reported that the use of different table grade table sugars like refined white table sugar (RWS), unrefined light brown table sugar (ULBS), unrefined brown table sugar (UBS) at the concentration of 40.0g l^{-1} instead of sucrose for the culture of zygotic embryos of coconut (Makapuno) and found that the different table grade table sugars used had the same effect in terms of shoot length and plant height. Similar results were obtained in *Brassica campestris* cv. Kukitachna (Okuna, 1996) and Potato (Sorvari, 1986a).

Kodym and Zapata- Arias (2001) reported that sugars of cane or sugarbeet origin were suitable carbon sources in the micropropagation of *Musa* spp cv. Grand Naine. Sundararasu (2003) reported that table sugar 2.0 per cent and 3.0 per cent were found to be an effective alternative to sucrose 3.0 per cent in terms of multiple shoot production.

2.6.6. Equipments and glassware

The use of wood or plastic box fitted with UV light or fume hood has been reported to be used as a substitute for expensive laminar air flow (Kumar and Seeni, 1994).

The electronic balance used to weigh out minute quantities of chemicals can be replaced by a simple common balance, whose cost is much less. By way of diluting stock solutions any desired level of chemicals can be added to the culture medium without difficulty (Raju, 1993). The pH meter can be substituted by pH indicating paper. Instead of the refrigerator, ice placed thermocol boxes will be sufficient (Kumar and Seeni, 1994).

Expensive borosilicate glassware which cost over Rs. 2.50 per square centimeter of culture surface can be replaced by any clear, colourless bottles, which cost much lesser than one paise per square centimeter (Raju, 1993).

According to Raju and Kavitha (1996) almost all the items of sophisticated equipments could be replaced by simpler and cheaper alternatives, which will in no way compromise the quality of the plants produced.

2.6.7. Economics of *in vitro* plantlet production

Shah and Dalal (1980) reported that they could produce more than 200,000 liquorice plants from a single axillary bud in a year. After accounting for 50.0 per cent field mortality, it was possible to have a field of 100,000 liquorice

plants within eight months. The cost of producing one tissue culture transplant was worked out to be Rs 0.12 whereas the unit cost of a sucker readily for planting was Rs 0.15.

Rajmohan (1985) reported that by *in vitro* axillary bud proliferation technique in jack, on an average 65.38 numbers of plantlets could be produced per year from a single explant. The unit cost of producing one jack plantlet including one month hardening was found to be Rs 9.09, whereas the cost of a jack grafts is Rs 8.00. Rajeevan and Pandey (1986) could produce 19,200 papaya plantlets from a single explant in a year and the cost of producing one plantlet was estimated to be Rs 0.85.

According to Reghunath (1989) the unit cost of one cardamom plantlet was Rs.4.90 including one month hardening period. Shah (1989) estimated the unit cost of herbs as Rs. 0.73, shrubs Rs 1.15 and trees Rs. 1.53 including two weeks green house stage in a theoretical commercial scale production. The cost of production of one banana plantlet including one month greenhouse stage was found to be Rs. 2.22 (Babylatha, 1993).

Robinson (1996) viewed that it was possible to produce 2000 *in vitro* banana plantlets from one original explant with in one year. In conventional methods of *in vitro* culture of banana, the cost of per plantlet was Rs.11.90 whereas in the cost effective method of *in vitro* shoot tip culture of banana was Rs. 6.83 thereby reducing the cost by Rs. 5.07 per plantlet (Sundararasu, 2003).

MATERIALS AND METHODS

3. MATERIALS AND METHODS

The studies on *in vitro* propagation of two commercial diploid bananas of Kerala namely, *Musa* (AB) 'Njalipoovan' and *Musa* (AA) 'Nivedyakadali', were carried out at the Plant Tissue Culture Laboratory attached to the Department of Pomology and Floriculture, College of Horticulture, Vellanikkara during 2003 - 2005. The details regarding the experimental materials, methodology of experiments and analytical techniques adopted are presented stepwise in this chapter.

In vitro propagation through enhanced release of axillary buds was used for the present study (Murashige, 1974).

3.1. CHEMICALS

The major and minor elements required for the preparation of media were of analytical grade and were procured from British Drug House and Sisco Research Laboratory, and plant growth regulators were obtained from Sigma, USA.

3.2. GLASSWARE

Borosilicate glassware of borosil brand was used for the study. They were cleaned by initially soaking in potassium dichromate solution in sulphuric acid for 12 hours followed by thorough washing with jets of tap water in order to completely remove all traces of dichromate solution. They were then soaked in detergent solution (cleansol) 0.1 per cent overnight, thoroughly washed with tap water and rinsed twice with double distilled water. The glasswares were then dried in a hot air oven at 100^{0} C for 24 hours and stored in cupboards away from dust and contaminants until used.

3. 3. CULTURE MEDIUM

3.3.1. Medium composition

The most widely accepted MS medium (Murashige and Skoog, 1962) with appropriate modifications was used in the present study .The nutrient medium included inorganic salts, vitamins, growth regulators, organic supplements and a carbon source. The composition of the basal medium is given in Appendix 1.

3.3.2. Preparation of the medium

Standard procedures (Gamborg and Shyluk, 1981) were followed for the preparation of the media. Stock solutions of major and minor nutrients, vitamins, Fe-EDTA and growth regulators were prepared first, by dissolving the required quantity of chemicals in double glass distilled water and stored under refrigerated conditions in amber coloured bottle. The stock solution of nutrients was prepared fresh in every four weeks and that of vitamins, amino acids and phytohormones were prepared fresh in every week. Specific quantities of the stock solution of chemicals and phytohormones were pipetted out into a 1000 ml beaker. Sucrose and inositol were added fresh and dissolved. Then the volume was made up to 1000 ml by adding double glass distilled water. The pH of the solution was adjusted using pH meter by adding 0.1N HCl or 0.1N NaOH. Agar (0.7 %) was weighed out, added to the medium and melted by keeping the solution in a water bath maintained at 90 ^{0} C to 95 ^{0}C. The medium (15 ml) was then poured hot to the oven sterilised culture vessels, which were previously rinsed twice with the double glass distilled water. The container with the medium were then tightly closed with non-absorbent cotton wool plugs and were autoclaved at 121 ^{0}C and 15 psi pressure (1.06 kg cm^{-2}) for twenty minutes. After sterilisation, the culture vessels with medium were allowed to cool to room temperature and stored in the culture room until used.

3.4. TRANSFER AREA AND ASEPTIC MANIPULATIONS

All the aseptic manipulations such as surface sterilization of explants, preparation and inoculation of explants and subsequent subculturing were carried out under the hood of a clean 'Thermadyne' laminar airflow cabinet. The working table of the laminar airflow cabinet was first surface sterilized by wiping with absolute alcohol and then by putting on the UV light for 30 minutes. The petridishes as well as the instruments used for the inoculation were first steam sterilized in an autoclave at 15 psi at 121 ^{0}C for 30 minutes and then flame sterilized before each inoculation. Hands were also scrubbed with alcohol before each inoculation.

3.5. CULTURE ROOM

The cultures were incubated at 27 ^{0}C in an air conditioned culture room with a 16 hour photoperiod of light intensity 2000 lux supplied by cool day light fluorescent tubes.

3.6. SELECTION OF VARIETIES

The explants for the study were collected from two popular diploid banana cultivars of Kerala, namely, *Musa* (AB) 'Njalipoovan' and *Musa* (AA) 'Nivedyakadali'.

3.7. SOURCE OF EXPLANTS

The explants for *in vitro* culture were collected from field grown plants of Njalipoovan and Nivedyakadali maintained at Banana Research Station, Kannara.

The apical buds (shoot tips) collected from sword suckers (four month old suckers having narrow sword shaped leaf blade), peeper suckers (very young

suckers having scale like leaf) and eye buds (small buds seen on the sides of mother rhizome) were used for the present study.

3.8. COLLECTION AND PREPARATION OF EXPLANTS FOR CULTURING

Healthy sword suckers, peeper suckers and eye buds were collected from the field. Sword suckers, after separation from the mother rhizome from which the bunch has already been harvested, were de topped and were reduced to a size, which measured about 5.0 cm in length retaining a small portion of the rhizome tissue. Peeper suckers were removed from mother plant using a crowbar without injuring the central bud, retaining the small portion of the rhizome tissue. The explants after collection were immediately taken to laboratory, where they were first washed thoroughly in tap water to remove all the dirt and soil particles adhering to them. To reduce media and explant discolouration, shoot tip explants were kept in running water for 30 minutes before inoculation. Explants were then treated with emisan 0.1 per cent for 20 minutes and again washed with sterile distilled water. Further sterilization procedures were carried out under perfect aseptic conditions in a 'Thermadyne' laminar airflow cabinet.

3.9. INOCULATION OF EXPLANTS

The explants after surface sterilisation were rinsed with sterile distilled water for four times. To dry these explants, they were cautiously transferred to sterile filter paper placed over sterile petridish.

Sterilised explants were further reduced to a size of 0.5 to 1.0 cm by trimming down the cut surface of the rhizomatous tissues and leaf bases, using sterilised scalpel and forceps. The explants were then inoculated in suitable culture medium.

3.10. PHYSIOLOGICAL PRECONDITIONING OF THE EXPLANT AND EXPLANT ESTABLISHMENT (STAGE 1)

3.10.1. Standardisation of surface sterilization

The peeper sucker explants from cv. Nivedyakadali were subjected to surface sterilisation using different chemicals given below

1. Alcohol 70.0 per cent for 5 and 10 minutes
2. Alcohol 95.0 per cent for 15 seconds + mercuric chloride (0.1 %) for 1 minute
3. Alcohol 70.0 per cent for 1 minute + mercuric chloride (0.1 %) for 10 minutes
4. Alcohol 70.0 per cent for 1 minute + mercuric chloride (0.1 %) for 10 minutes + antibiotic cefotaxime (0.01 %) solution for 15 minutes.
5. Alcohol 70.0 per cent for 1 minute + mercuric chloride (0.1 %) for 10 minute + Inoculating in antibiotic media cefotaxime (0.01 %)
6. Mercuric chloride (0.1 %) for 10 minutes + antibiotic cefotaxime (0.01 %) for 15 minutes
7. 4×4 combination of emisan and mercuric chloride - emisan (0.1 and 0.2 %) for 10 and 20 minutes + mercuric chloride (0.1 and 0.2 %) for 5 and 10 minutes
8. 3×2 combinations of mercuric chloride (0.1, 0.2 and 0.5 %) for 5 and 10 minutes
9. 2×3 combinations of emisan (0.1 and 0.3 %) for 5, 10 and 15 minutes
10. 3×3 combinations of sodium hypochlorite (0.1, 0.2 and 0.5 %) for 5, 10 and 15 minutes

The treatment found to be the best were tried with other three different explants of two cultivars under study and their performance was compared.

Observations on per cent of culture survival (as exhibited by the retention of the green colour) and per cent of cultures contaminated were recorded after four weeks of culturing.

3.10.2. Effect of physical injury treatments on explant establishment

In order to overcome the apical dominance of shoot tips and to obtain a higher rate of axillary bud release in culture, the shoot tip explants were subjected to different types of physical injury treatments as follows

T1 Apical dome intact
T2 Apical dome cut longitudinally
T3 Apical dome with (+) cut
T4 Half portion of the apical dome
T5 Quarter portion of the dome

Observations on per cent of culture survival (devoid of microbial contamination), per cent of cultures exhibiting growth and visual rating on the growth response were made on ten explants per treatment after four weeks of culturing.

3.10.3. Effect of various pretreatments on media and explant discolouration

A trial was conducted to reduce media and explant discolouration of banana during *in vitro* culture establishment, the details of which are given below

1. Adding ascorbic acid (50.0 mg l^{-1}) to the media
2. Rinsing with ascorbic acid solution (0.5 %)
3. Keeping in refrigerator over night (18 hours)
4. Keeping in running tap water half an hour before inoculation (control)

Observations on percentage of cultures showing culture survival and percentage of cultures without media and explant discolouration were made on ten explants per treatment after four weeks of culturing.

3.10.4. Seasonal influence on the *in vitro* establishment of explants

Under culture conditions, the establishment of explants collected from field grown plants varied considerably according to the season of their collection. An experiment was therefore conducted to standardise the best season of explant collection in which the culture establishment was more and contamination rate was minimum. Ten shoot tips from sword suckers of Nivedyakadali were collected for this purpose during July 2004 to June 2005 and cultured in MS semisolid medium containing NAA 0.5 mg l^{-1} and BA 3.0 mg l^{-1}. The percentage of cultures established was recorded after four weeks of culturing.

Observations on percentage of culture survival (devoid of microbial contamination) and per cent of cultures exhibiting growth were made on ten explants per treatment after four weeks of culturing.

3.10.5. Standardisation of establishment media

In order to study the morphogenetic response of diploid banana explants in culture, the most widely accepted MS medium (Murashige and Skoog, 1962) was tried using sword sucker explants of diploid cultivars. The basal media was modified with the following treatments:

Media	**Combinations**
Full MS	5.0 x 4.0 combinations of BA (1.0, 2.0, 3.0, 4.0 and 5.0 mg l^{-1}) and NAA (0.5, 1.0, 1.5 and 2.0 mg l^{-1})
Full MS	BA 5.0 mg l^{-1}
Full MS	BA 2.0 mg l^{-1} and NAA 0.05 mg l^{-1}

Full MS	Without growth regulators
Half MS	5.0 x 4.0 combinations of BA (1.0, 2.0, 3.0, 4.0 and 5.0 mg l^{-1}) and NAA (0.5,1.0,1.5 and 2.0 mg l^{-1})
Half MS	BA 5.0 mg l^{-1}
Half MS	Without growth regulators

Observations on the number of days taken for culture establishment and percentage of cultures established were made after four weeks of culturing.

3.10.6. Response of varieties

To find out whether the three explants behave differently or not, a separate experiment was conducted. In this study, three types of explants of two diploid banana cultivars namely *Musa* (AB) 'Njalipoovan' and *Musa* (AA) 'Nivedyakadali' were utilized. These explants were grown on MS semisolid medium supplemented with NAA 0.5 mg l^{-1} and BA 3.0 mg l^{-1} for a period of four weeks.

Observations were made on the number of days taken for culture establishment and per cent of culture survival for eight explants per treatment.

3.11. INDUCTION OF AXILLARY SHOOTS AND RAPID SHOOT MULTIPLICATION (STAGE II)

3.11.1. Standardisation of basic proliferation medium (BPM)

Explants used for induction of axillary shoots were 1.0 to 1.5 cm long shoots from establishment culture.

To study the effect of various concentration of MS medium on multiple shoot production of *in vitro* culture of diploid banana, all the major and minor

elements in MS medium were reduced to lower strength to find a low cost alternative medium. The details of the trial utilizing growth regulating substances viz., cytokinins and auxins were presented below.

1. 4 x 4 combination of BA (4.0, 6.0, 8.0 and 10.0 mg $l^{-1)}$ and NAA (0, 0.5, 1.0 and 1.5 mg l^{-1}) in full MS.
2. 4 x 4 combination of kinetin (4.0, 6.0, 8.0 and 10.0 mg $l^{-1)}$ and NAA (0, 0.5, 1.0 and 1.5 mg l^{-1}) in full MS.
3. 2.0 x 5.0 combination of MS (half and quarter strength) and BA (1.0, 2.0, 3.0, 4.0 and 5.0 mg l^{-1}).
4. Full MS and BA (1.0, 2.0, 3.0 and 5.0 mg l^{-1})

The best multiplication media found in this experiment was tried with three different explants and their effect on multiplication was compared.

There were four cultures per treatment. Observations on number of multiple shoot formation and per cent of cultures developing shoots were recorded at weekly intervals for four weeks.

3.11.2. Effect of different carbon sources on *in vitro* multiple shoot production of diploid banana

Sucrose, the main source of carbon was substituted with various substances to find a low cost alternative to sucrose. There were four cultures per treatment. Observations on number of multiple shoots formed were recorded after four weeks of culturing. The different substitutes used and their levels were as follows

1. Table sugar (1.5, 2.0 and 3.0 %)
2. Glucose (1.5, 2.0 and 3.0 %)
3. Sucrose (1.5, 2.0 and 3.0 %)

3.11.3. Effect of different gelling agents and supporting materials on *in vitro* multiple shoot production of diploid banana

Agar, the principal component of gelling agent was substituted with various levels of different gelling substances and supporting materials as given below.

1. Agar (0.6 %)
2. Gelatin (0.3 %) +Agar (0.5 %)
3. Gelatin (0.7 %)
4. Filter paper (Wattman No.1)
5. White paper (Ordinary)
6. Brown paper (Ordinary)
7. Agar (0.7 %)

Observations on the number of multiple shoots formed were recorded on four explants per treatment after four weeks of culturing.

3.2.4. Effect of different vitamin concentrations on *in vitro* multiple shoots production of diploid banana

In order to reduce the cost of vitamins, the strength of vitamins (Nicotinic acid, Pyridoxine HCl and Thiamine HCl) in MS medium was reduced and adding of vitamin B complex tablets in MS medium was tried.

1. Half tablet of vitamin B complex 2.0 mg l^{-1} (Omega -B Complex)
2. No vitamins in MS
3. Quarter strength of vitamins in MS
4. Half strength of vitamins in MS
5. Full strength of vitamins in MS (Control)

Observations on number of multiple shoot formation were recorded on four explants after four weeks of culturing.

3.12. *IN VITRO* ROOTING OF BANANA (STAGE III)

3.12.1. Standardisation of basal medium

The study on *in vitro* rooting was conducted on full, half and quarter strength MS medium. Shoots (2.0-3.0 cm length) excised from shoot proliferating cultures were utilized as explants for these trials. The different auxins and their levels tried for rooting of diploid banana shoots were the following

MS Medium	Combinations
Full	NAA (0.5, 1.0, 2.0, 3.0 and 5.0 mg l^{-1})
Full	IBA (5.0 and 10.0 mg l^{-1})
Half	NAA (0.5, 1.0, 2.0, 3.0, 5.0, 10.0 and 15.0 mg l^{-1}
Half	IBA (5.0, 10.0 mg l^{-1})
Quarter	NAA (0.5, 1.0, 2.0, and 3.0 mg l^{-1})

Observations on the per cent of cultures showing root initiation, number of days taken for root initiation, number of roots produced per shoot and length of the longest root were recorded on four cultures per treatment after four weeks of culturing.

3.12.2. Effect of different carbon sources on *in vitro* rooting of diploid banana

Sucrose, the main source of carbon was substituted with various substances to find a low cost alternative to sucrose. The different substitutes used and their levels were as follows.

1. Table sugar (1.5, 2.0 and 3.0 %)
2. Glucose (1.5, 2.0 and 3.0 %)
3. Sucrose (1.5, 2.0 and 3.0 %)

Observations on the per cent of cultures showing root initiation, number of days taken for root initiation, number of roots produced per shoot and length of the longest root were recorded on four cultures per treatment after four weeks of culturing.

3.12.3. Effect of different gelling agents and supporting materials on *in vitro* rooting of diploid banana

Agar, the principal component of gelling agent was substituted with various levels of different gelling substances and supporting materials as given below.

1. Agar (0.6 %)
2. Gelatin (0.3 %) +Agar (0.5 %)
3. Gelatin (0.7 %)
4. Filter paper (Wattman No.1)
5. White paper (Ordinary)
6. Brown paper (Ordinary)
7. Agar (0.7 %)

Observations on the percentage of cultures showing root initiation, number of days taken for root initiation, number of roots produced per shoot and length of the longest root were recorded on four cultures per treatment after four weeks of culturing.

3.12.4. Effect of different vitamin concentrations on *in vitro* rooting of diploid banana

In order to reduce the cost of vitamins (Nicotinic acid, Pyridoxine HCl and Thiamine HCl) in MS medium the strength of vitamins was reduced and adding of vitamin B complex tablets in MS medium was tried. The treatments were as follows.

1. Half tablet of vitamin B complex 2.0 mg l^{-1} (Omega -B Complex)
2. No vitamins in MS
3. Quarter strength of vitamins in MS
4. Half strength of vitamins in MS
5. Full strength of vitamins in MS (Control)

Observations on number of days taken for root initiation, number of roots produced per shoot and length of the longest root were recorded on four cultures per treatment after four weeks of culturing.

3.12.5. Effect of different levels of triademefon on *in vitro* rooting of diploid banana

Different levels of triademefon were incorporated into the rooting medium (Stage III). Medium to which no triademefon was added served as the control treatment. One month old shoot cultures were transferred into culture tubes containing the rooting medium. There were four cultures per treatment. The various concentrations of triademefon tried were given below.

Treatments

Triademefon - (0.0, 0.5, 1.0, 1.5, 2.0, 3.0 and 4.0 mg l^{-1})

Observations on number of roots, length of the longest root per plant and length of shoot were recorded after two weeks of culturing.

3.12.6. Effect of culture vessels on *in vitro* rooting of diploid banana

To study the influence of culture vessels on *in vitro* rooting, 2.0 to 3.0 cm long shoots were inoculated in MS medium containing NAA 2.0 mg l^{-1}. The different types of culture vessels used were as follows

1. Test tube with cotton plug
2. Test tube with plastic cap
3. Jam bottle (250 ml)
4. Conical flask (100 ml)

Observations were made on number of roots produced per shoot and length of the longest root for four cultures.

3.13.ACCLIMATIZATION

3.13.1. Standardisation of medium for *ex vitro* rooting of *in vitro* shoots

The *in vitro* shoots (2.0-3.0 cm length) in stage 2 were taken out without injury from the culture tubes using forceps and put in a beaker containing distilled water. Then the micro shoots were shaken thoroughly in distilled water to remove the adhering pieces of semisolid medium and then dipped in high concentration (1000mg l^{-1}) IBA solution for a second. The treated shoots were planted out in small plastic trays containing different sterilized rooting medium .The various rooting medium tried were as follows.

T_1 Vermiculite

T_2 Sand

T_3 Perlite

T_4 Coir pith compost

T_5 Sand + red earth + cow dung (1:1:1)

Observations on survival per cent and rooting per cent were taken after one month.

3.13.2. Standardisation of potting medium for *ex vitro* establishment of banana plantlets.

The *in vitro* rooted plantlets were taken out without injury from the culture vessels using forceps and put in a beaker containing distilled water and shaken thoroughly to remove the adhering pieces of semisolid medium and then dipped in carbendazim 0.1 per cent for 15 minutes and kept under mist chamber. Plantlets were planted out in small plastic containers containing different sterilised rooting medium. The various potting medium tried were as follows:

1. Vermiculite alone
2. Vermiculite+ perlite (1:1)
3. Vermiculite+ coir pith compost (1:1)
4. Vermiculite+ perlite +Sand (1:1:1)
5. Vermiculite+ coir pith compost +sand (1:1:1)
6. Coir pith compost +sand (1:1)
7. Coir pith compost alone
8. Perlite alone
9 Sand alone
10. Sand + red earth + cow dung (1:1:1)-Control

Observations on survival per cent and number of leaves per plantlet were taken after one month.

3.13.3. Influence of potting mixture on growth and vigour of plantlet

After 15 days of hardening the plantlets were transferred to potting mixture. The types of potting mixture in which the plants were potted was found to influence the growth and vigour of plants. To study this, plantlets were potted in various kinds of potting mixtures made up of different ingredients as given below.

1. Sand+ cow dung +goat manure (1:1:1)
2. Sand+ goat manure (1:1)
3. Sand+ cow dung (1:1)
4. Sand+ red earth +cow dung (1:1:1) –Control

After wetting, the potting mixture was autoclaved at 15 psi for 20 minutes to make it free from soil borne pathogens. The plantlets were planted in the potting mixture on the same day of sterilization after cooling.

Observations on number of leaves, height of plant, length and breadth of leaves were made at fortnightly intervals for two months.

3.13.4. Effect of different hardening units on *ex vitro* establishment of diploid banana plantlets

A trial was conducted to study the influence of hardening unit on *ex vitro* establishment of plantlet. The different structures included were

1. Mist chamber
2. Iron frame covered with polythene sheet (2'x 5'x 2')
3. Grouping three to four potted plantlets under a polythene cover (60 x 60 x 60 cm^3)
4. Covering each potted plantlet with tightly fitting polythene cover.

There were 20 plantlets for each treatment and were planted in vermiculite medium. Observations were made for per cent of plantlet survival after one month of planting out.

3.13.5. Effect of containers on plantlet survival

To study the influence of containers on growth of plantlets, they were potted in different types of containers as shown below

1. White disposable plastic cups of 150 ml capacity with drainage holes at bottom
2. Small mud pots of 150 ml capacity with drainage holes at bottom.
3. Black polythene cover of 7 cm diameter and 12 cm length with four punch holes each on two sides
4. White polythene cover of 7 cm diameter and 12 cm length with four punch holes each on two sides.
5. Black plastic protray.

Observations were made on sixteen plantlets, for per cent of plantlet survival, after one month of planting out.

3.13.6. Effect of different nutrient solutions on growth and vigour of diploid banana plantlets

Weekly spraying of five nutrient solutions were tried in established banana plantlets kept under mist chamber. The various starter solutions tried were as follows:

Treatments	**Combinations**
MS	Full and half strength
18:18:18 (N: P: K complex)	(1.0 and 2.0 %)
Vermiwash	Diluted two times

There were eight plantlets per treatment. Observations on number of leaves, height of plant and length and breadth of longest leaf from top of the plant were made at fortnightly intervals for two months.

3.14. STATISTICAL ANALYSIS

Statistical analysis of the data recorded was carried out in completely randomised design following Panse and Sukhatme (1985). Angular as well as square root transformations were carried out, wherever necessary.

RESULTS

4. RESULTS

Results of studies on *in vitro* propagation of two commercial diploid bananas are presented in this chapter.

4.1. PHYSIOLOGICAL PRECONDITIONING OF THE EXPLANT AND EXPLANT ESTABLISHMENT (STAGE 1)

4.1.1. Surface sterilization

The results of surface sterilization of explants from peeper sucker of cv. Nivedyakadali using various sterilants are presented in Table1a and b. Out of the different sterilants tried, combination of mercuric chloride (0.1 %) absolute alcohol (70 %) and antibiotic cefotaxime (0.01 %) gave best result than other sterilants like sodium hypochlorite, absolute alcohol and mercuric chloride and/ or emisan. This treatment resulted in 100 per cent explant survival with no contamination and explant mortality. Treatments involving sterilizing the explant with mercuric chloride (0.1 %) for 10 minutes + antibiotic cefotaxime (0.01 %) solution for 15 minutes, and the treatment namely, treating the explants with absolute alcohol (70 %) for one minute + mercuric chloride (0.1 %) for 10 minutes + inoculating the explants into the media containing antibiotic cefotaxime (0.01 %) were the next best two treatments. These two treatments gave 83.33 per cent survival. Sodium hypochlorite, emisan, absolute alcohol and mercuric chloride either alone or in combination recorded poor explant survival.

The data pertaining to the response of different explants from cv. Njalipoovan and cv. Nivedyakadali are given in Table 1b. No significant difference between the explants collected from sword sucker, peeper sucker and eye bud was observed with respect to explant survival and contamination, when the best sterilization treatment was tried.

Table 1a. Standardisation of surface sterilization of explant

Sl. No.	Treatments	Contamination (%)	Explant death (%)	Survival (%)
1	Alcohol 70 %-5 minutes	100.00	0.00	0.00
2	Alcohol 70 %-10 minutes	100.00	0.00	0.00
3	Alcohol 95 %-15 seconds + $HgCl_2$ 0.1 %-5 minutes	83.33	0.00	16.67
4	Alcohol 70 %-15 seconds + $HgCl_2$ 0.1 %-10 minutes	33.33	0.00	66.67
5	Alcohol 70 %-1 minute + $HgCl_2$ 0.1 %-10 minutes+ cefotaxime 0.01 %15 minutes	0.00	0.00	100.00
6	Alcohol 70 %-1 minute + $HgCl_2$ 0.1 %-10 minutes+ cefotaxime 0.01 %adding to the medium	0.00	16.67	83.33
7	$HgCl_2$ 0.1 %-10 minutes+ cefotaxime 0.01 %15 minutes	16.67	0.00	83.33
8	Emisan 0.1 %10 minutes+ $HgCl_2$ 0.1 %-5minutes	66.67	0.00	33.33
9	Emisan 0.1 %10 minutes+ $HgCl_2$ 0.1 %–10 minutes	100.00	0.00	0.00
10	Emisan 0.1 %10 minutes+ $HgCl_2$ 0.2 %-5minutes	83.33	16.67	0.00
11	Emisan 0.1 %10 minutes+ $HgCl_2$ 0.2 %-10minutes	66.67	0.00	33.33
12	Emisan 0.1 %20 minutes+ $HgCl_2$ 0.1 %– 5 minutes	83.33	0.00	16.67
13	Emisan 0.1 %20 minutes+ $HgCl_2$ 0.1 %-10minutes	100.00	0.00	0.00
14	Emisan 0.1 %20 minutes+ $HgCl_2$ 0.2 %– 5 minutes	100.00	0.00	0.00
15	Emisan 0.1 %20 minutes+ $HgCl_2$ 0.2 %-10minutes	83.33	0.00	16.67
16	Emisan 0.2 %10 minutes+ $HgCl_2$ 0.1 %– 5 minutes	83.33	0.00	16.67
17	Emisan 0.2 %10 minutes+ $HgCl_2$ 0.1 %–10 minutes	83.33	16.67	0.00
18	Emisan 0.2 %10 minutes+ $HgCl_2$ 0.2 %– 5 minutes	83.33	0.00	16.67

(Continued)

Table 1a. Standardisation of surface sterilization of explant

Sl. No.	Treatment	Contamination (%)	Explant death (%)	Survival (%)
19	Emisan 0.2 %10 minutes+ $HgCl_2$ 0.2 %–10 minutes	83.33	16.67	0.00
20	Emisan 0.2 %20 minutes+ $HgCl_2$ 0.1 %– 5 minutes	100.00	0.00	0.00
21	Emisan 0.2 % 20 minutes+ $HgCl_2$ 0.1 %–10 minutes	83.33	0.00	16.67
22	Emisan 0.2 %20 minutes+ $HgCl_2$ 0.2 %– 5 minutes	100.00	0.00	0.00
23	Emisan 0.2 %20 minutes+ $HgCl_2$ 0.2 %–10 minutes	83.33	0.00	16.67
24	$HgCl_2$ 0.1 %–5 minutes	83.33	0.00	16.67
25	$HgCl_2$ 0.1 %–10 minutes	83.33	0.00	16.67
26	$HgCl_2$ 0.2 %–5 minutes	83.33	0.00	16.67
27	$HgCl_2$ 0.2 %–10 minutes	83.33	16.67	0.00
28	$HgCl_2$ 0.5 %–5 minutes	16.67	83.33	0.00
29	$HgCl_2$ 0.5 %–10 minutes	0.00	100.00	0.00
30	Emisan 0.1 %–5 minutes	100.00	0.00	0.00
31	Emisan 0.1 %-10 minutes	100.00	0.00	0.00
32	Emisan 0.1 %-15 minutes	100.00	0.00	0.00
33	Emisan 0.3 %-5 minutes	100 .00	0.00	0.00
34	Emisan 0.3 %-10 minutes	83.33	16.67	0.00
35	Emisan 0.3 %-15 minutes	83.33	16.67	0.00
36	Sodium hypochlorite 0.1 %-5 minutes	100.00	0.00	0.00
37	Sodium hypochlorite 0.1 %-10 minutes	100 .00	0.00	0.00
38	Sodium hypochlorite 0.1 %-15 minutes	83.33	0.00	16.67
39	Sodium hypochlorite 0.2 %-5 minutes	100.00	0.00	0.00
40	Sodium hypochlorite 0.2 %-10 minutes	100.00	0.00	0.00

(Continued.)

Table 1a. Standardisation of surface sterilization of explant

Sl. No.	Treatments	Contamination (%)	Explant death (%)	Survival (%)
41	Sodium hypochlorite 0.2 %-15 minutes	66.67	16.67	16.67
42	Sodium hypochlorite 0.5 %-5 minutes	83.33	16.67	0.00
43	Sodium hypochlorite 0.5 %-10 minutes	100.00	0.00	0.00
44	Sodium hypochlorite 0.5 %-15 minutes	100.00	0.00	0.00

Table 1b. Response of different explants to surface sterilization treatment

Cultivars	Explants	Contamination (%)	Survival (%)	Explant death (%)
Nivedyakadali	Sword sucker	16.67	83.30	0.00
	Peeper sucker	0.00	100.00	0.00
	Eye bud	0.00	83.30	16.70
Njalipoovan	Sword sucker	16.67	83.30	0.00
	Peeper sucker	0.00	100.00	0.00
	Eye bud	0.00	83.30	16.70

4.1.2. Effect of physical injury treatments on explant establishment

The results of the trial conducted by giving five types of physical injury to shoot tip explants of cv.Njalipoovan (Table 2) showed variation in explant survival (40.0-7.5 %). When the percentage of cultures exhibiting growth were compared, apical dome with a longitudinal cut resulted in maximum (90.0 %) survival and satisfactory growth were observed with treatments including half portion of apical dome (80.0 %) (Plate 3) and apical dome intact (77.5 %) .In visual rating of growth response, apical dome with a longitudinal incision was found to be best as the cultures turned green within two weeks and produced two to three buds per culture (Plate 1). In the case of apical dome with '+' cut and half portion of apical dome, only one to two adventitious buds developed and turned green after three weeks. When explant with intact apical dome was used, only a single bud developed within two weeks (Plate 2).

4.1.3. Effect of various pretreatments on media and explant discolouration

Adding ascorbic acid 50.0 mgl^{-1} into the culture medium resulted in maximum percentage of culture without media and explant discolouration (96.0%) and 88.0 per cent culture survival. Rinsing the explants in ascorbic acid solution (0.5 %) just before inoculation resulted in 20.0 per cent culture without media and explant discolouration and 88.0 per cent culture survival (Table 3). Keeping the explants in running water for half an hour resulted the same culture survival (88.0 %); but keeping in refrigerator over night resulted in only 70.0 per cent survival of explants.

4.1.4. Seasonal influence on the *in vitro* establishment of explants

Explant collection during March to April and November to December, was found to result in least contamination rate (10.0 %) and maximum explant

Table 2. Effect of physical injury treatments on explant establishment

Treatments	Culture established (%)	Culture exhibiting growth (%)	Remarks
T1 - Apical dome intact	87.50	77.50	+
T2 - Apical dome cut longitudinally	95.00	90.00	++
T3 - Apical dome with (+) cut	40.00	32.50	+++
T4 - Half portion of the apical dome	97.50	80.00	+++
T5 - Quarter portion of the dome	85.00	30.00	++++

+ Single bud developing and explants turned green within two weeks

++ Two to three buds developing and explants turned green within two weeks

+++ One to two buds developing and explants turned green after three weeks

+++++ Culture developed only one adventitious bud and turned green after three weeks

Plate 1. Culture showing bud formation from an apical dome with a longitudinal cut

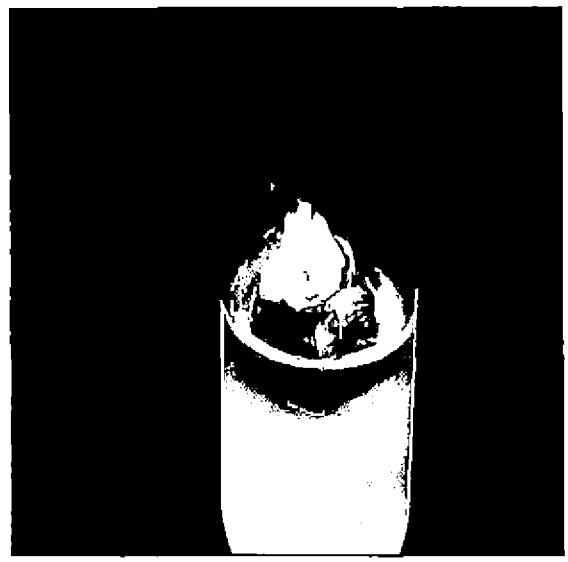


Plate 2. Culture showing bud formation from an intact apical dome

Plate 3. Culture showing bud formation from half portion of apical dome

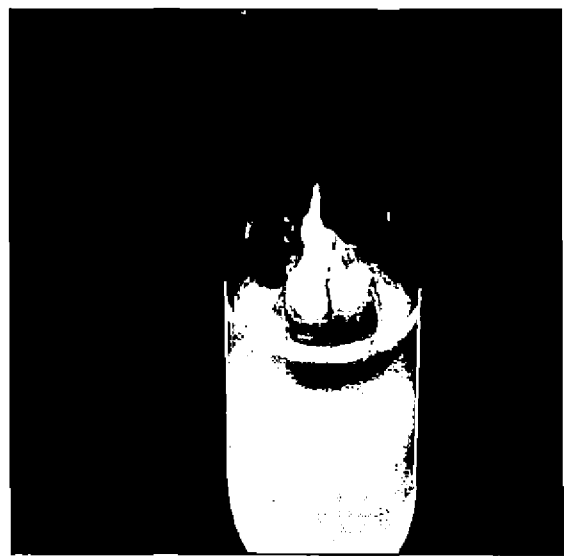


Plate 4. Culture showing bud formation from quarter portion of apical dome

Table 3. Effect of various pretreatments on media and explant discolouration

Sl. No.	Treatments	Survival (%)	Culture without media and explant discolouration (%)
1	Adding ascorbic acid (50 mg l^{-1}) into the media	88.00	96.00
2	Rinsing with ascorbic acid solution (0.5 per cent)	88.00	20.00
3	Keeping in refrigerator overnight	70.00	0.00
4	Keeping in running tap water half an hour	88.00	0.00

Table 4. Seasonal influence on the *in vitro* establishment of explants (cv. Nivedyakadali)

Sl. No.	Month	Contamination (%)	Survival (%)
1	July –August	60.00	40.00
2	September – October	60.00	40.00
3	November – December	10.00	90.00
4	January – February	40.00	60.00
5	March – April	10.00	90.00
6	May –June	40.00	60.00

survival (90.0 %) (Table 4). The period during July to October recorded highest contamination rate (60.0 %) and lowest survival rate (40.0 %).

4.1.5. Standardisation of establishment medium

4.1.5.1. Standardisation of establishment medium for diploid cultivars

Data pertaining to the number of days taken for culture establishment and percentage of cultures established is given in Table 5a and b. It was observed that cv. Njalipoovan took least number of days (8.3) for culture establishment in full MS medium containing BA 3.0 mg l^{-1} and NAA 0.5 mg l^{-1} and this treatment recorded cent per cent culture establishment. The cv. Nivedyakadali also recorded least number of days for culture establishment (10.8) with cent per cent culture establishment in the same medium. The other concentrations of BA and NAA took significantly more number of days for culture establishment. Similarly half MS medium with various combinations of BA and NAA took more number of days for culture establishment.

4.1.5.2. Influence of different explants in the establishment medium

The results of the trial conducted to study the influence of different explants of cv. Njalipoovan and cv. Nivedyakadali; (Table 6), showed variation in the percentage of cultures established and number of days taken for culture establishment. In the case of Njalipoovan, sword sucker explants took lesser number of days for culture establishment (8.00) and 87.50 per cent of cultures got established. Though the peeper sucker explants took 11 days for culture establishment, 93.75 per cent cultures got established. Eye bud explants noted maximum number of days for culture establishment (15.75) with 87.50 per cent of cultures showed establishment. Similar kind of trend was noticed in the case of cv. Nivedyakadali in the number of days taken for establishment. However,

Table 5a. Standardisation of establishment medium for cv. Njalipoovan

Sl. No	Treatments	Number of days taken for establishment	Cultures established (%)
1	Full MS + BA 1.0 mg l^{-1} + NAA 0.5 mg l^{-1}	25.33	66.67
2	Full MS + BA 1.0 mg l^{-1} + NAA 1.0 mg l^{-1}	20.67	100.00
3	Full MS + BA 1.0 mg l^{-1} + NAA 1.5 mg l^{-1}	19.67	100.00
4	Full MS + BA 1.0 mg l^{-1} + NAA 2.0 mg l^{-1}	27.00	66.67
5	Full MS + BA 2.0 mg l^{-1} + NAA 0.5 mg l^{-1}	25.33	83.33
6	Full MS + BA 2.0 mg l^{-1} + NAA 1.0mg l^{-1}	21.67	83.33
7	Full MS + BA 2.0 mg l^{-1} + NAA 1.5 mg l^{-1}	15.67	100.00
8	Full MS + BA 2.0mg l^{-1} + NAA 2.0 mg l^{-1}	20.00	100.00
9	Full MS + BA 3.0 mg l^{-1} + NAA 0.5 mg l^{-1}	8.33	100.00
10	Full MS + BA 3.0 mg l^{-1} + NAA 1.0 mg l^{-1}	11.00	100.00
11	Full MS + BA 3.0 mg l^{-1} + NAA 1.5 mg l^{-1}	15.67	100.00
12	Full MS + BA 3.0 mg l^{-1} + NAA 2.0 mg l^{-1}	19.67	100.00
13	Full MS + BA 4.0 mg l^{-1} + NAA 0.5 mg l^{-1}	22.67	83.33
14	Full MS + BA 4.0 mg l^{-1} + NAA 1.0 mg l^{-1}	25.67	33.33
15	Full MS + BA 4.0 mg l^{-1} + NAA 1.5 mg l^{-1}	25.00	33.33
16	Full MS + BA 4.0 mg l^{-1} + NAA 2.0 mg l^{-1}	23.67	33.33
17	Full MS + BA 5.0 mg l^{-1} + NAA 0.5 mg l^{-1}	22.33	33.33
18	Full MS + BA 5.0 mg l^{-1} + NAA 1.0 mg l^{-1}	22.00	33.33
19	Full MS + BA 5.0 mg l^{-1} + NAA 1.5 mg l^{-1}	30.00	16.67
20	Full MS + BA 5.0 mg l^{-1} + NAA 2.0 mg l^{-1}	29.00	16.67
21	Full MS + BA 5.0 mg l^{-1}	28.67	16.67
22	Full MS + BA 2.0 mg l^{-1} + NAA 0.05 mg l^{-1}	15.67	100.00

(Continued.)

Table 5a.Standardisation of establishment medium for cv. Njalipoovan

Sl.No	Treatments	Number of days taken for establishment	Cultures established (%)
23	Full MS	29.00	16.67
24	Half MS + BA 1.0 mg l^{-1} + NAA 0.5 mg l^{-1}	16.00	100.00
25	Half MS + BA 1.0 mg l^{-1} + NAA 1.0 mg l^{-1}	16.33	100.00
26	Half MS + BA 1.0 mg l^{-1} + NAA 1.5 mg l^{-1}	20.00	83.33
27	Half MS + BA 1.0 mg l^{-1} + NAA 2.0 mg l^{-1}	20.67	83.33
28	Half MS + BA 2.0 mg l^{-1} + NAA 0.5 mg l^{-1}	19.67	83.33
29	Half MS + BA 2.0 mg l^{-1} + NAA 1.0 mg l^{-1}	27.00	16.67
30	Half MS + BA 2.0 mg l^{-1} + NAA 1.5 mg l^{-1}	25.00	83.33
31	Half MS + BA 2.0 mg l^{-1} + NAA 2.0 mg l^{-1}	21.67	100.00
32	Half MS + BA 3.0 mg l^{-1} + NAA 0.5 mg l^{-1}	22.00	100.00
33	Half MS + BA 3.0 mg l^{-1} + NAA 1.0 mg l^{-1}	19.67	100.00
34	Half MS + BA 3.0 mg l^{-1} + NAA 1.5 mg l^{-1}	22.33	100.00
35	Half MS + BA 3.0 mg l^{-1} + NAA 2.0 mg l^{-1}	21.67	100.00
36	Half MS + BA 4.0 mg l^{-1} + NAA 0.5 mg l^{-1}	16.67	100.00
37	Half MS + BA 4.0mg l^{-1} + NAA 1.0 mg l^{-1}	21.33	83.33
38	Half MS + BA 4.0 mg l^{-1} + NAA 1.5 mg l^{-1}	21.67	83.33
39	Half MS + BA 4.0 mg l^{-1} + NAA 2.0 mg l^{-1}	25.33	66.67
40	Half MS + BA 5.0 mg l^{-1} + NAA 0.5 mg l^{-1}	27.33	66.67
41	Half MS + BA 5.0 mg l^{-1} + NAA 1.0 mg l^{-1}	27.67	66.67
42	Half MS + BA 5.0 mg l^{-1} + NAA 1.5 mg l^{-1}	28.00	66.67
43	Half MS + BA 5.0 mg l^{-1} + NAA 2.0 mg l^{-1}	26.00	66.67
44	Half MS + BA 5.0 mg l^{-1}	27.33	66.67
45	Half MS	27.67	66.67
CD (0.05)		2.67	

Table 5b. Standardisation of establishment medium for cv. Nivedyakadali

Sl. No	Treatments	Number of days taken for establishment	Culture established (%)
1	Full MS +BA 1.0 mg l^{-1}+ NAA 0.5 mg l^{-1}	28.00	50.00
2	Full MS + BA 1.0 mg l^{-1}+ NAA 1.0 mg l^{-1}	28.00	50.00
3	Full MS + BA 1.0 mg l^{-1} + NAA 1.5 mg l^{-1}	23.00	50.00
4	Full MS + BA 1.0 mg l^{-1} + NAA 2.0 mg l^{-1}	28.00	33.33
5	Full MS + BA 2.0 mg l^{-1} + NAA 0.5 mg l^{-1}	22.00	83.33
6	Full MS + BA 2.0 mg l^{-1} + NAA 1.0 mg l^{-1}	26.67	83.33
7	Full MS + BA 2.0 mg l^{-1} + NAA 1.5 mg l^{-1}	18.67	100.00
8	Full MS + BA 2.0 mg l^{-1}+ NAA 2.0 mg l^{-1}	20.00	100.00
9	Full MS + BA 3.0 mg l^{-1} + NAA 0.5 mg l^{-1}	10.67	100.00
10	Full MS + BA 3.0 mg l^{-1} + NAA 1.0 mg l^{-1}	15.00	83.33
11	Full MS + BA 3.0mg l^{-1} + NAA 1.5 mg l^{-1}	19.67	33.33
12	Full MS + BA 3.0 mg l^{-1} + NAA 2.0 mg l^{-1}	28.00	33.33
13	Full MS + BA 4.0 mg l^{-1} + NAA 0.5 mg l^{-1}	28.00	50.00
14	Full MS + BA 4.0mg l^{-1} + NAA 1.0 mg l^{-1}	24.33	66.67
15	Full MS + BA 4.0 mg l^{-1}+ NAA 1.5 mg l^{-1}	20.67	66.67
16	Full MS + BA 4.0 mg l^{-1} + NAA 2.0 mg l^{-1}	20.67	100.00
17	Full MS + BA 5.0 mg l^{-1} + NAA 0.5 mg l^{-1}	26.67	50.00
18	Full MS + BA 5.0 mg l^{-1}+ NAA 1.0mg l^{-1}	25.67	50.00
19	Full MS + BA 5.0 mg l^{-1} + NAA 1.5 mg l^{-1}	26.33	50.00
20	Full MS + BA 5.0 mg l^{-1} + NAA 2.0 mg l^{-1}	26.00	50.00
21	Full MS + BA 5.0 mg l^{-1}	27.00	50.00
22	Full MS + BA 2.0 mg l^{-1} + NAA 0.05 mg l^{-1}	15.33	100.00

(Continued..)

Table 5b. Standardisation of establishment medium for cv. Nivedyakadali

Sl.No	Treatments	Number of days taken for establishment	Culture established (%)
23	Full MS	29.00	33.33
24	Half MS + BA 1.0 mg l^{-1} + NAA 0.5 mg l^{-1}	16.00	100.00
25	Half MS + BA 1.0 mg l^{-1} + NAA 1.0 mg l^{-1}	16.00	100.00
26	Half MS + BA 1.0 mg l^{-1} + NAA 1.5 mg l^{-1}	16.00	100.00
27	Half MS + BA 1.0 mg l^{-1} + NAA 2.0 mg l^{-1}	15.00	83.33
28	Half MS + BA 2.0 mg l^{-1} + NAA 0.5 mg l^{-1}	19.00	100.00
29	Half MS + BA 2.0 mg l^{-1} + NAA 1.0mg l^{-1}	27.00	50.00
30	Half MS + BA 2.0 mg l^{-1} + NAA 1.5 mg l^{-1}	27.00	50.00
31	Half MS + BA 2.0 mg l^{-1} + NAA 2.0 mg l^{-1}	26.00	50.00
32	Half MS + BA 3.0 mg l^{-1} + NAA 0.5 mg l^{-1}	23.00	50.00
33	Half MS + BA 3.0 mg l^{-1} + NAA 1.0 mg l^{-1}	28.00	33.33
34	Half MS + BA 3.0 mg l^{-1} + NAA 1.5 mg l^{-1}	25.00	66.67
35	Half MS + BA 3.0 mg l^{-1} + NAA 2.0 mg l^{-1}	25.67	66.67
36	Half MS + BA 4.0 mg l^{-1} + NAA 0.5 mg l^{-1}	16.67	100.00
37	Half MS + BA 4.0 mg l^{-1} + NAA 1.0 mg l^{-1}	20.67	100.00
38	Half MS + BA 4.0 mg l^{-1} + NAA 1.5 mg l^{-1}	22.67	50.00
39	Half MS + BA 4.0 mg l^{-1} + NAA 2.0 mg l^{-1}	22.00	50.00
40	Half MS + BA 5.0 mg l^{-1} + NAA 0.5 mg l^{-1}	28.67	33.33
41	Half MS + BA 5.0 mg l^{-1} + NAA 1.0 mg l^{-1}	28.67	33.33
42	Half MS + BA 5.0 mg l^{-1} + NAA 1.5 mg l^{-1}	29.00	33.33
43	Half MS + BA 5.0 mg l^{-1} + NAA 2.0 mg l^{-1}	28.00	33.33
44	Half MS + BA 5.0 mg l^{-1}	29.33	33.33
45	Half MS	29.67	33.33
CD (0.05)		3.81	

Table 6. Response of varieties and different explants to the establishment medium

Explants	Njalipoovan		Nivedyakadali	
	Number of days taken for establishment	Cultures established (%) *	Number of days taken for establishment	Cultures established (%) *
Sword sucker	8.00	87.50	10.00	81.25
Peeper sucker	11.00	93.75	10.50	93.75
Eye bud	15.75	87.50	12.75	87.50
CD (0.05)	2.24		0.78	

* Mean of 16 observations

maximum culture establishment was obtained in peeper sucker (93.75 %) followed by eye bud (87.50 %).

4.2. INDUCTION OF AXILLARY SHOOT AND RAPID SHOOT MUIPLICATION (STAGE II)

4.2.1. Standardisation of basic proliferation medium (BPM)

The data showing the effect of 46 treatment combinations on the mean number of multiple shoots produced are given in the Table 7a and b. The full MS medium containing BA 5.0 mg l^{-1} alone and BA 8.0 mg l^{-1} + NAA 0.5 mg l^{-1} recorded the maximum number of multiple shoots per culture (7.3) in cv. Njalipooovan and were significantly superior to all other treatments (Plate 5). All the cultures produced shoots in these two treatments (Table 7a).

Data pertaining to the number of multiple shoots per culture and per cent of cultures developing shoots for cv. Nivedyakadali are given in Table 7b. Of the 46 treatment tried, the treatment involving full MS with BA 5.0 mg l^{-1} alone recorded maximum number of shoots per culture (8.3) which was significantly superior to all other treatments (Plate 6).

4.2.2. Influence of different explants in the basic proliferation medium

No significant difference was observed between the three explants collected from cv. Njalipoovan and cv. Nivedyakadali, with respect to the number of multiple shoots produced per culture and percentage of cultures developing shoots (Table7c).

Table 7a. Standardisation of basic proliferation medium (BPM) for cv. Njalipoovan

Sl.No	Treatments	Number of shoots	Cultures developing shoots (%)
1	Full MS+ BA 4.0 mg l^{-1}	1.8	83.33
2	Full MS+ BA 4.0 mg l^{-1} + NAA 0.5 mg l^{-1}	5.3	100.00
3	Full MS+ BA 4.0 mg l^{-1} + NAA 1.0 mg l^{-1}	2.7	100.00
4	Full MS+ BA 4.0 mg l^{-1} + NAA 1.5 mg l^{-1}	2.0	83.33
5	Full MS+ BA 6.0 mg l^{-1}	2.7	100.00
6	Full MS+ BA 6.0 mg l^{-1} + NAA 0.5 mg l^{-1}	5.0	100.00
7	Full MS+ BA 6.0 mg l^{-1}+ NAA 1.0 mg l^{-1}	5.7	100.00
8	Full MS+ BA 6.0 mg l^{-1} + NAA 1.5 mg l^{-1}	5.7	100.00
9	Full MS+ BA 8.0 mg l^{-1}	3.0	100.00
10	Full MS+ BA 8.0 mg l^{-1} + NAA 0.5 mg l^{-1}	7.3	100.00
11	Full MS+ BA 8.0 mg l^{-1} + NAA 1.0 mg l^{-1}	2.5	100.00
12	Full MS+ BA 8.0 mg l^{-1} + NAA 1.5 mg l^{-1}	2.7	100.00
13	Full MS+ BA 10.0 mg l^{-1}	2.2	100.00
14	Full MS+ BA 10.0 mg l^{-1} + NAA 0.5 mg l^{-1}	4.2	100.00
15	Full MS+ BA 10.0 mg l^{-1}+ NAA 1.0 mg l^{-1}	5.8	100.00
16	Full MS+ BA 10.0 mg l^{-1}+ NAA 1.5 mg l^{-1}	2.8	100.00
17	Full MS+ Kinetin 4.0 mg l^{-1}	1.7	83.33
18	Full MS+ Kinetin 4.0 mg l^{-1} +NAA 0.5mg l^{-1}	2.3	83.33
19	Full MS+ Kinetin 4.0 mg l^{-1} +NAA 1.0 mg l^{-1}	1.0	16.67
20	Full MS+ Kinetin 4.0 mg l^{-1} +NAA 1.5 mg l^{-1}	1.0	16.67
21	Full MS+ Kinetin 6.0 mg l^{-1}	1.0	16.67
22	Full MS+ Kinetin 6.0 mg l^{-1} +NAA 0.5 mg l^{-1}	1.0	16.67
23	Full MS+ Kinetin 6.0mg l^{-1} +NAA 1.0 mg l^{-1}	1.2	33.33

(Continued)

Table7a. Standardisation of basic proliferation medium (BPM) for cv. Njalipoovan

Sl.No	Treatments	Number of shoots	Cultures developing shoots (%)
24	Full MS+ Kinetin 6.0 mg l^{-1} +NAA 1.5 mg l^{-1}	1.0	66.67
25	Full MS+ Kinetin 8.0 mg l^{-1}	1.0	16.67
26	Full MS+ Kinetin 8.0 mg l^{-1} +NAA 0.5 mg l^{-1}	1.3	66.67
27	Full MS+ Kinetin 8.0 mg l^{-1} +NAA 1.0 mg l^{-1}	1.3	66.67
28	Full MS+ Kinetin 8.0 mg l^{-1} +NAA 1.5 mg l^{-1}	1.0	16.67
29	Full MS+Kinetin 10.0 mg l^{-1}	1.0	16.67
30	Full MS+Kinetin 10.0 mg l^{-1} +NAA 0.5 mg l^{-1}	1.7	83.33
31	Full MS+Kinetin 10.0 mg l^{-1} +NAA 1.0 mg l^{-1}	2.3	100.00
32	Full MS+Kinetin 10.0 mg l^{-1} +NAA 1.5 mg l^{-1}	3.2	100.00
33	Half MS+ BA 1.0 mg l^{-1}	1.3	66.67
34	Half MS+ BA 2.0 mg l^{-1}	3.0	100.00
35	Half MS+ BA 3.0 mg l^{-1}	3.3	83.33
36	Half MS+ BA 4.0 mg l^{-1}	1.7	66.67
37	Half MS+ BA 5.0 mg l^{-1}	4.8	100.00
38	Quarter MS+ BA 1.0 mg l^{-1}	1.7	66.67
39	Quarter MS+ BA 2.0 mg l^{-1}	2.3	83.33
40	Quarter MS+ BA 3.0 mg l^{-1}	2.0	83.33
41	Quarter MS+ BA 4.0 mg l^{-1}	1.7	83.33
42	Quarter MS+ BA 5.0 mg l^{-1}	2.0	83.33
43	Full MS+ BA 1.0 mg l^{-1}	2.8	100.00
44	Full MS+ BA 2.0 mg l^{-1}	3.7	100.00
45	Full MS+ BA 3.0 mg l^{-1}	3.0	100.00
46	Full MS+ BA 5.0 mg l^{-1}	7.3	100.00
CD (0.05)		1.51	

Table7b. Standardisation of basic proliferation medium (BPM) for cv. Nivedyakadali

Sl.No	Treatments	Number of shoots	Cultures developing shoots (%)
1	Full MS+ BA 4.0 mg l^{-1}	2.8	100.00
2	Full MS+ BA 4.0 mg l^{-1} + NAA 0.5 mg l^{-1}	2.8	100.00
3	Full MS+ BA 4.0 mg l^{-1} + NAA 1.0 mg l^{-1}	1.8	83.33
4	Full MS+ BA 4.0 mg l^{-1} + NAA 1.5 mg l^{-1}	1.0	66.67
5	Full MS+ BA 6.0 mg l^{-1}	1.0	50.00
6	Full MS+ BA 6.0 mg l^{-1} + NAA 0.5 mg l^{-1}	1.0	50.00
7	Full MS+ BA 6.0 mg l^{-1} + NAA 1.0 mg l^{-1}	5.2	100.00
8	Full MS+ BA 6.0 mg l^{-1} + NAA 1.5 mg l^{-1}	4.0	100.00
9	Full MS+ BA 8.0 mg l^{-1}	1.0	50.00
10	Full MS+ BA 8.0 mg l^{-1} + NAA 0.5 mg l^{-1}	2.7	83.33
11	Full MS+ BA 8.0 mg l^{-1} + NAA 1.0 mg l^{-1}	2.8	100.00
12	Full MS+ BA 8.0 mg l^{-1} + NAA 1.5 mg l^{-1}	2.3	100.00
13	Full MS+ BA 10.0 mg l^{-1}	2.5	100.00
14	Full MS+ BA 10.0 mg l^{-1} + NAA 0.5 mg l^{-1}	1.7	100.00
15	Full MS+ BA 10.0 mg l^{-1} + NAA 1.0 mg l^{-1}	2.0	100.00
16	Full MS+ BA 10.0 mg l^{-1} + NAA 1.5 mg l^{-1}	2.0	100.00
17	Full MS+Kinetin 4.0 mg l^{-1}	4.3	100.00
18	Full MS+Kinetin 4.0 mg l^{-1} +NAA 0.5 mg l^{-1}	1.0	16.67
19	Full MS+Kinetin 4.0 mg l^{-1} +NAA 1.0 mg l^{-1}	4.0	100.00
20	Full MS+Kinetin 4.0 mg l^{-1} +NAA 1.5 mg l^{-1}	1.3	83.33
21	Full MS+Kinetin 6.0 mg l^{-1}	4.0	100.00
22	Full MS+Kinetin 6.0 mg l^{-1} +NAA 0.5 mg l^{-1}	3.7	100.00
23	Full MS+Kinetin 6.0 mg l^{-1} +NAA 1.0 mg l^{-1}	3.5	100.00

(Continued)

Table7b. Standardisation of basic proliferation medium (BPM) for cv. Nivedyakadali

Sl.No	Treatments	Number of shoots	Cultures developing shoots (%)
24	Full MS+Kinetin 6.0 mg l^{-1} +NAA 1.5 mg l^{-1}	2.8	100.00
25	Full MS+Kinetin 8.0 mg l^{-1}	1.5	100.00
26	Full MS+Kinetin 8.0 mg l^{-1} +NAA 0.5 mg l^{-1}	2.7	100.00
27	Full MS+Kinetin 8.0 mg l^{-1} +NAA 1.0 mg l^{-1}	4.0	100.00
28	Full MS+Kinetin 8.0 mg l^{-1} +NAA 1.5 mg l^{-1}	1.0	16.67
29	Full MS+Kinetin 10.0 mg l^{-1}	3.5	100.00
30	Full MS+Kinetin 10.0 mg l^{-1} +NAA 0.5 mg l^{-1}	1.0	16.67
31	Full MS+Kinetin 10.0 mg l^{-1} +NAA 1.0 mg l^{-1}	1.5	83.33
32	Full MS+Kinetin 10.0 mg l^{-1} +NAA 1.5 mg l^{-1}	2.5	100.00
33	Half MS+ BA 1.0 mg l^{-1}	1.3	66.67
34	Half MS+ BA 2.0 mg l^{-1}	2.3	83.33
35	Half MS+ BA 3.0 mg l^{-1}	2.7	83.33
36	Half MS+ BA 4.0 mg l^{-1}	3.0	100.00
37	Half MS+ BA 5.0 mg l^{-1}	3.8	100.00
38	Quarter MS+ BA 1.0 mg l^{-1}	1.0	33.33
39	Quarter MS+ BA 2.0 mg l^{-1}	1.3	66.67
40	Quarter MS+ BA 3.0 mg l^{-1}	1.2	66.67
41	Quarter MS+ BA 4.0 mg l^{-1}	2.8	100.00
42	Quarter MS+ BA 5.0 mg l^{-1}	1.0	33.33
43	Full MS+ BA 1.0 mg l^{-1}	1.0	66.67
44	Full MS+ BA 2.0 mg l^{-1}	1.3	83.33
45	Full MS+ BA 3.0 mg l^{-1}	1.0	83.33
46	Full MS+ BA 5.0 mg l^{-1}	8.3	100.00
CD (0.05)		1.33	

Plate 5. *In vitro* multiple shoot production of diploid banana cv. Njalipoovan in full MS + BA 5.0 mg l^{-1}

Plate 6. *In vitro* multiple shoot production of diploid banana cv. Nivedyakadali in full MS + BA 5.0 mg l^{-1}

Table 7c. Response of variteis and explants on BPM

Explants	Njalipoovan		Nivedyakadali	
	Number of shoots	Cultures showing shoot development (%)*	Number of shoots	Cultures showing shoot development (%)*
Sword sucker	7.3	100.00	7.2	100.00
Peeper sucker	7.2	100.00	7.4	100.00
Eye bud	6.7	100.00	7.3	100.00
CD (0.05)	NS		NS	

*Mean of 16 observations

Table 8a. Effect of different carbon sources on *in vitro* multiple shoot production of diploid banana cv. Njalipoovan

Sl.No	Treatments	Number of shoots	Length of longest shoots (cm)	Cultures developing shoots (%)
1	Table sugar 1.5%	2.4	2.6	100.00
2	Table sugar 2.0%	4.1	3.8	100.00
3	Table sugar 3.0%	2.3	2.0	100.00
4	Glucose 1.5%	2.6	3.4	100.00
5	Glucose 2.0%	3.2	4.4	100.00
6	Glucose 3.0%	1.6	3.0	100.00
7	Sucrose 1.5%	2.9	1.7	100.00
8	Sucrose 2.0%	3.1	3.0	100.00
9	Sucrose 3.0%(control)	4.1	3.1	100.00
CD (0.05)		1.14	1.03	

4.2.3. Effect of different carbon sources on *in vitro* multiple shoot production of diploid banana

Data pertaining to the number of multiple shoots produced, length of multiple shoots and per cent of cultures developing shoots by cv. Njalipoovan with various alternatives to carbon sources are given in Table 8a and Figure 1. Number of shoots produced was higher (4.1) in the treatment involving sucrose 3.0 per cent and table sugar 2.0 per cent (Plate 7) which was on par with glucose 2.0 per cent (3.2) and sucrose 2.0 per cent (3.1). Maximum length of shoot was observed by glucose 2.0 per cent (4.4cm) which was on par with table sugar 2.0 per cent (3.8cm) and glucose 1.5 per cent (3.4cm).

In diploid banana cv. Nivedyakadali, the number of multiple shoot produced (4.9) and length of shoot per culture (4.6cm) was maximum in medium containing table sugar 2.0 per cent (Table 8b, Figure 2 and Plate 8). With respect to the number of shoots, sucrose 3.0 per cent (4.6) and sucrose 2.0 per cent (3.6) were on par with table sugar 2.0 per cent. Length of shoots produced per culture was on par in sucrose 3.0 per cent and glucose 2.0 per cent (3.6cm).

4.2.4. Effect of different gelling agents and supporting materials on *in vitro* multiple shoot production

Data on number of shoots produced and survival per cent recorded by cv. Njalipoovan and cv. Nivedyakadali in the media containing various gelling agents and supporting materials are given in Table 9. In cv. Njalipoovan maximum number of multiple shoots (4.1) was produced in the medium containing agar 0.7 per cent. For cv.Nivedyakadali also the same treatment produced maximum number of multiple shoots (4.6). Cent per cent cultures developed shoots in this treatment. Explants in gelatin 0.7 per cent did not survive since the medium did not solidify. Other treatments viz ; ordinary paper, brown paper and agar 0.6 per cent were the next best treatments.

Table 8b. Effect of different carbon sources on *in vitro* multiple shoot production of diploid banana cv. Nivedyakadali

Sl.No	Treatments	Number of shoots	Length of longest shoots (cm)	Cultures developing shoots (%)
1	Table sugar 1.5%	2.1	2.0	100.00
2	Table sugar 2.0%	4.9	4.6	100.00
3	Table sugar 3.0%	3.0	2.5	100.00
4	Glucose 1.5%	2.6	1.8	100.00
5	Glucose 2.0%	2.6	3.6	100.00
6	Glucose 3.0%	3.3	2.9	100.00
7	Sucrose 1.5%	2.6	1.8	100.00
8	Sucrose 2.0%	3.6	2.9	100.00
9	Sucrose 3.0%(control)	4.6	3.6	100.00
CD (0.05)		1.27	0.97	

Figure.1. Effect of different carbon sources on *in vitro* multiple shoot production of cv. Njalipoovan

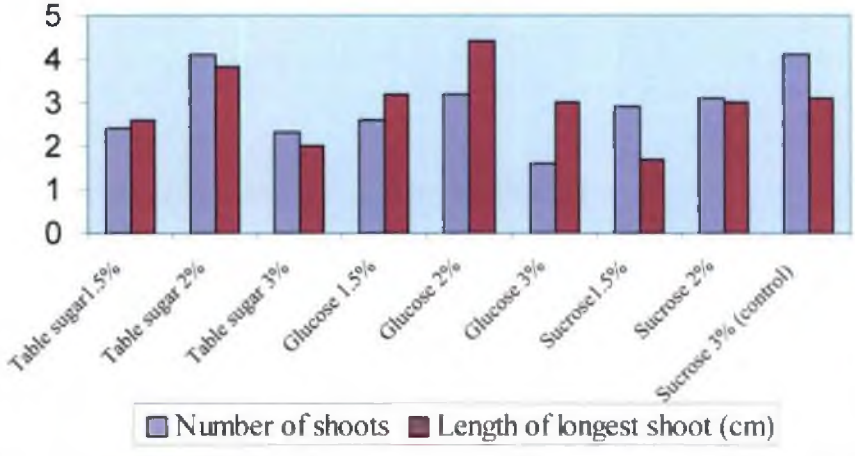


Figure.2. Effect of different carbon sources on *in vitro* multiple shoot production of cv. Nivedyakadali

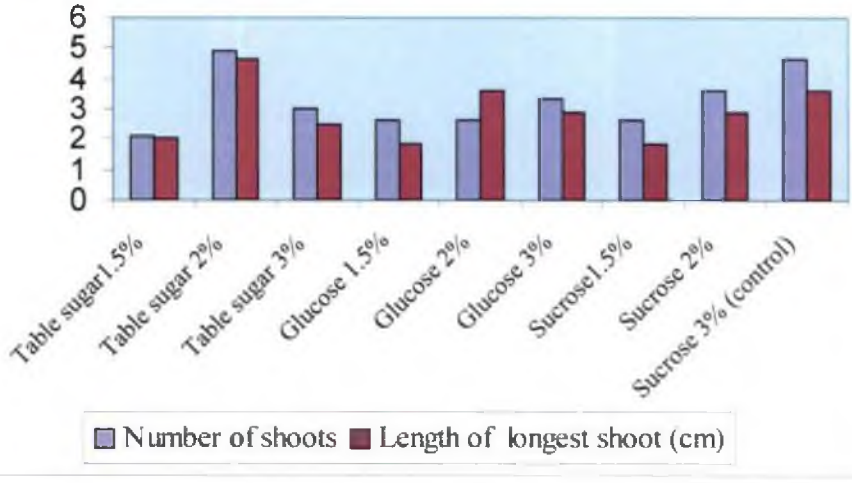


Table 9. Effect of different gelling agents and supporting materials on *in vitro* multiple shoot production of diploid banana

Sl. No.	Treatments	Njalipoovan			Nivedyakadali		
		Number of shoots	Contamination (%)	Cultures developing shoots (%)	Number of shoots	Contamination (%)	Cultures developing shoots (%)
1	Agar 0.6%	2.6	0.00	100.00	3.0	0.00	100.00
2	Gelatin 0.3 % + Agar 0.5%	1.6	0.00	100.00	2.5	0.00	100.00
3	Gelatin 0.7%	0.0	0.00	0.00	0.0	0.00	0.00
4	Filter paper	3.0	12.50	87.50	3.5	6.25	93.75
5	Brown paper	2.7	18.75	81.25	2.9	12.50	87.50
6	Ordinary Paper	3.1	31.25	68.75	3.1	25.00	75.00
7	Agar 0.7%	4.1	0.00	100.00	4.6	0.00	100.00
CD (0.05)		0.78			1.06		

Plate 7. *In vitro* multiple shoot production of diploid banana cv. Njalipoovan in full MS + table sugar (2.0 %)

Plate 8. *In vitro* multiple shoot production of diploid banana cv. Nivedyakadali in full MS + table sugar (2.0 %)

4.2.5. Effect of different vitamin concentrations on *in vitro* multiple shoot production of diploid banana

Data on number of shoots produced per culture by cv. Njalipoovan and cv. Nivedyakadali at various strength of vitamin concentration is given in Table 10. Half strength of vitamins in full MS observed maximum number of multiple shoots (5.3) in cv. Njalipoovan, which was on par with half tablet of vitamin B complex tablet in medium (4.4). In the case of cv. Nivedyakadali, half tablet of vitamin B complex in medium resulted in maximum number of shoots (4.8) which was on par with control treatment involving full strength of vitamins in MS (4.6) and half strength of vitamins in MS medium (Plate 9 and Plate 10).

4.3. *IN VITRO* ROOTING OF BANANA

4.3.1. Standardisation of *in vitro* rooting medium

Data pertaining to the number of days taken for root initiation, per cent of cultures showing root initiation, number of roots and length of longest root produced in different media combinations are given in Table 11a and b. For cv. Njalipoovan the treatment involving full MS + NAA 2.0 mg l^{-1} took significantly least number of days (6.7) with cent per cent root initiation. The number of roots per shoot was maximum (8.0) in half MS +NAA 10.0 mg l^{-1} whereas, longest root was found to be in full MS + NAA 0.5 mg l^{-1} (Table 11 a and Plate 11).

In the case of cv. Nivedyakadali, full MS medium containing NAA 1.0 mg l^{-1} took least number of days for root initiation (6.0) and cent per cent cultures showed root initiation followed by full MS medium + NAA 0.5 mg $l^{-1}$and full MS +NAA 2.0 mg l^{-1}. The number of roots per shoot (5.5) was found to be maximum in Full MS + NAA 0.5 mg l^{-1} to 1.0 mg l^{-1}. Half MS and quarter MS also gave satisfactory results. Longest root (9.5 cm) was observed in the treatment half MS +NAA 0.5 mg l^{-1}(Table 11 b and Plate 12).

Table 10. Effect of different vitamin concentrations on *in vitro* multiple shoot production of diploid banana

Sl. No.	Treatments	Njalipoovan		Nivedyakadali	
		Number of shoots	Cultures developing shoots (%)	Number of shoots	Cultures developing shoots (%)
1	Half vitamin B complex tablet	4.4	100.00	4.8	100.00
2	No vitamins	0.6	100.00	0.8	100.00
3	Quarter strength of vitamins in full MS	2.6	100.00	1.5	100.00
4	Half strength of vitamin in full MS.	5.3	100.00	3.5	100.00
5	Full strength of vitamins in full MS (control)	4.1	100.00	4.6	100.00
CD (0.05)		0.95		2.19	

Plate 9. *In vitro* multiple shoot production of diploid banana cv. Njalipoovan in full MS medium + half vitamin B complex tablet (2.0 mg l^{-1})

Plate 10. *In vitro* multiple shoot production of diploid banana cv. Nivedyakadali in full MS medium + half vitamin B complex tablet (2.0 mg l^{-1})

Table 11a. Standardisation of basal medium for *in vitro* rooting of cv. Njalipoovan

Sl. No	Treatment	Number of days for root initiation	Number of roots	Length of longest roots (cm)	Cultures showing root initiation (%).
1	Full MS + NAA 0.5 mg l^{-1}	9.2	2.5	12.7	100.00
2	Full MS + NAA1.0 mg l^{-1}	9.3	3.5	6.17	100.00
3	Full MS + NAA 2.0 mg l^{-1}	6.7	3.5	6.5	100.00
4	Full MS + NAA 3.0 mg l^{-1}	8.5	2.3	3.5	25.00
5	Full MS + NAA 5.0 mg l^{-1}	11.0	4.2	8.5	75.00
6	Full MS + NAA 10.0 mg l^{-1}	10.0	4.0	10.5	100.00
7	Full MS + NAA 15.0 mg l^{-1}	8.3	2.3	5.4	100.00
8	Full MS + IBA 5.0 mg l^{-1}	10.0	6.0	9.0	100.00
9	Full MS + IBA 10.0 mg l^{-1}	9.7	4.7	4.5	100.00
10	Half MS + NAA 0.5 mg l^{-1}	11.5	3.5	8.5	100.00
11	Half MS + NAA1.0 mg l^{-1}	8.8	3.5	5.5	100.00
12	Half MS + NAA 2.0 mg l^{-1}	9.7	2.3	7.0	100.00
13	Half MS + NAA 3.0 mg l^{-1}	11.2	1.7	4.2	25.00
14	Half MS + NAA 5.0 mg l^{-1}	11.0	4.5	8.5	50.00
15	Half MS + NAA 10.0 mg l^{-1}	9.0	8.0	5.5	100.00
16	Half MS + NAA 15.0 mg l^{-1}	10.0	5.8	5.0	50.00
17	Half MS + IBA 5.0 mg l^{-1}	9.5	1.7	9.0	50.00
18	Half MS + IBA 10.0 mg l^{-1}	9.2	1.8	3.3	100.00
19	Quarter MS + NAA 0.5 mg l^{-1}	10.7	4.5	6.0	100.00
20	Quarter MS + NAA1.0 mg l^{-1}	15.0	1.7	3.3	25.00
21	Quarter MS + NAA 2.0 mg l^{-1}	10.3	1.8	3.3	25.00
22	Quarter MS + NAA 3.0 mg l^{-1}	10.0	5.0	5.0	100.00
CD (0.05)		0.84	1.14	3.30	

Table 11b. Standardisation of basal medium for *in vitro* rooting cv. Nivedyakadali

Sl.No	Treatments	Number of days for root initiation	Number of roots	Length of longest root (cm)	Cultures showing root initiation (%)
1	Full MS + NAA 0.5 mg l^{-1}	6.2	5.5	5.5	100.00
2	Full MS + NAA1.0 mg l^{-1}	6.0	4.7	8.0	100.00
3	Full MS + NAA 2.0 mg l^{-1}	6.7	4.0	5.7	100.00
4	Full MS + NAA 3.0 mg l^{-1}	9.0	2.3	6.0	75.00
5	Full MS + NAA 5.0 mg l^{-1}	7.0	2.7	7.3	100.00
6	Full MS + NAA 10.0 mg l^{-1}	6.8	1.5	7.8	100.00
7	Full MS + NAA 15.0 mg l^{-1}	8.3	4.0	7.3	75.00
8	Full MS + IBA 5.0mg l^{-1}	7.7	5.2	6.3	25.00
9	Full MS + IBA 10.0 mg l^{-1}	8.3	2.0	6.0	100.00
10	Half MS + NAA 0.5 mg l^{-1}	7.3	4.0	9.5	75.00
11	Half MS + NAA1.0 mg l^{-1}	9.0	4.7	7.5	100.00
12	Half MS + NAA 2.0 mg l^{-1}	7.7	3.0	8.8	75.00
13	Half MS + NAA 3.0 mg l^{-1}	9.7	1.8	4.8	25.00
14	Half MS + NAA 5.0 mg l^{-1}	7.0	1.8	5.5	25.00
15	Half MS + NAA 10.0 mg l^{-1}	10.7	1.8	5.0	50.00
16	Half MS + NAA 15.0 mg l^{-1}	11.2	4.7	5.5	100.00
17	Half MS + IBA 5.0 mg l^{-1}	8.3	3.5	7.0	100.00
18	Half MS + IBA 10.0 mg l^{-1}	8.3	2.5	6.3	50.00
19	Quarter MS + NAA 0.5 mgl^{-1}	11.0	4.5	8.3	50.00
20	Quarter MS + NAA1.0 mg l^{-1}	11.7	2.0	8.0	50.00
21	Quarter MS + NAA 2.0 mg l^{-1}	11.8	2.0	7.3	50.00
22	Quarter MS + NAA 3.0 mg l^{-1}	6.7	3.0	7.3	100.00
CD (0.05)		2.22	1.31	2.50	

Plate 11. *In vitro* rooting of diploid banana cv. Njalipoovan in full MS + NAA 1.0 mg l^{-1}

Plate 12. *In vitro* rooting of diploid banana cv. Nivedyakadali in full MS + NAA 1.0 mg l^{-1}

4.3.2. Effect of different carbon sources on *in vitro* rooting of diploid banana

Number of roots and length of roots produced by cv.Njalipoovan and cv.Nivedyakadali with different carbon sources are given in Table 12a and b.Of the various carbon sources tried with cv. Njalipoovan, number of roots produced was significantly higher (6.3) in the medium containing table sugar 3.0 per cent and table sugar 2.0 per cent (Figure 3 and Plate 13). Length of root was maximum (10.2cm) in the medium containing sucrose 3.0 per cent which was on par with sucrose 2.0 per cent (8.9cm) and table sugar 2.0 per cent (8.1cm).

In the case of cv. Nivedyakadali number of roots produced was higher (7.5) in medium containing table sugar 3.0 per cent. Regarding the length roots produced there was no significant difference between the treatments (Figure 4 and Plate 14).

4.3.3. Effect of different gelling agents and supporting materails on *in vitro* rooting of diploid banana

Data pertaining to the survival per cent, number of roots and length of roots produced by cv. Njalipoovan and cv. Nivedyakadali are given in Table 13a and b. Agar 0.7 per cent recorded maximum number of roots (7.4) and agar 0.6 per cent recorded maximum length of root (12.0 cm) in cv. Njaliopoovan (Figure 5).

Of the various gelling agents and supporting materials tried with cv. Nivedyakadali, agar 0.7 per cent recorded maximum number of roots per culture (4.9). Maximum length of roots (7.4cm) was observed in two treatments viz., agar 0.6 per cent and ordinary paper (Figure 6).

Table 12a. Effect of different carbon sources on *in vitro* rooting of cv. Njalipoovan

SL.NO	Treatments	Number of days taken for root initiation	Number of roots	Length of longest roots(cm)
1	Table sugar 1.5 %	8.3	4.1	6.6
2	Table sugar 2.0 %	8.3	6.3	8.1
3	Table sugar 3.0%	7.5	6.3	6.9
4	Glucose 1.5%	9.5	1.9	2.9
5	Glucose 2.0%	9.5	2.4	6.6
6	Glucose 3.0%	9.3	1.0	1.1
7	Sucrose 1.5%	7.3	1.2	2.8
8	Sucrose 2.0%	6.3	5.3	8.9
9	Sucrose 3.0%(control)	6.3	3.8	10.2
CD (0.05)		2.1	0.51	2.96

Table 12b. Effect of different carbon sources on *in vitro* rooting of cv. Nivedyakadali

Sl.No	Treatments	Number of days taken for root initiation	Number of roots	Length of longest root (cm)
1	Table sugar 1.5%	7.5	2.5	6.5
2	Table sugar 2.0%	8.0	2.6	8.3
3	Table sugar 3.0%	8.3	7.5	10.5
4	Glucose 1.5%	9.8	2.3	15.7
5	Glucose 2.0%	9.8	2.8	3.8
6	Glucose 3.0%	9.3	1.0	0.8
7	Sucrose 1.5%	8.3	1.5	13.9
8	Sucrose 2.0%	8.5	6.5	6.8
9	Sucrose 3.0% (control)	7.0	5.7	6.3
CD (0.05)		2.46	0.52	NS

Figure. 3. Effect of different carbon sources on *in vitro* rooting of cv. Njalipoovan

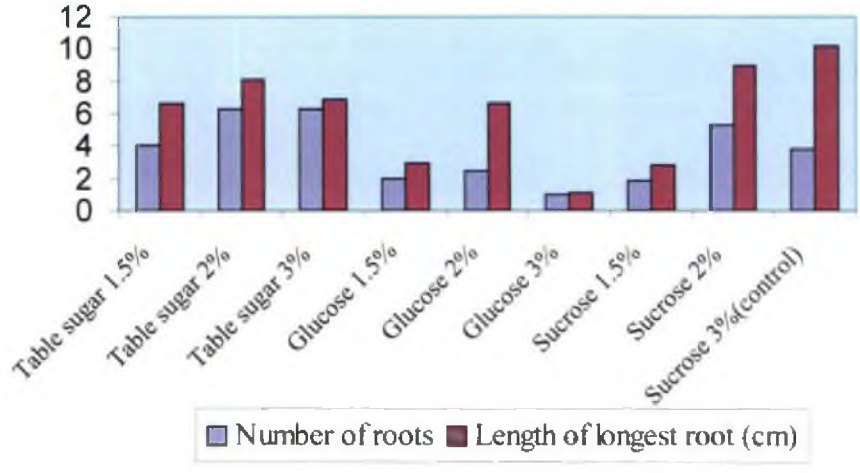


Figure.4. Effect of different carbon sources on *in vitro* rooting of cv. Nivedyakadali

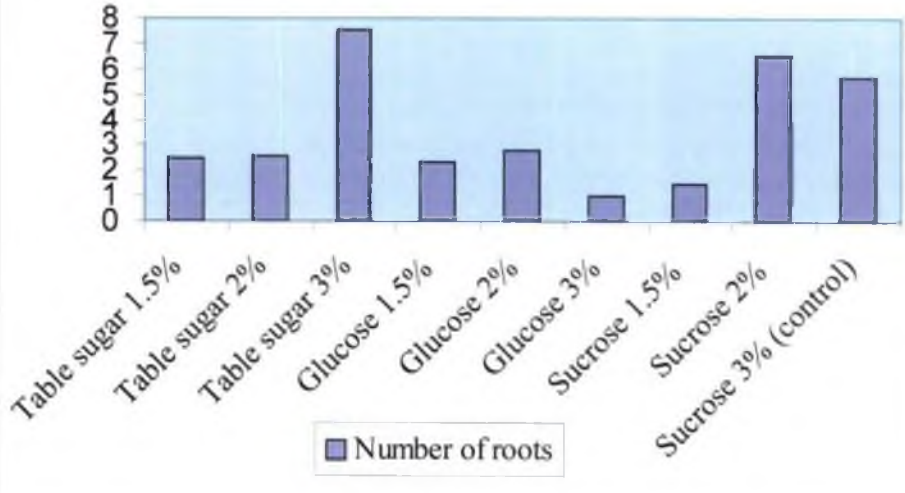


Plate 13. ***In vitro*** **rooting of diploid banana cv. Njalipoovan in full MS + table sugar (3.0 %)**

Plate 14. ***In vitro*** **rooting of diploid banana cv. Nivedyakadali in full MS + table sugar (3.0 %)**

Table 13a. Effect of different gelling agents and supporting matrerials on *in vitro* rooting of cv. Njalipoovan

Sl.No	Treatments	Number of days taken for root initiation	Number of roots	Length of longest root (cm)	Cultures developing roots (%)
1	Agar 0.6%	7.00	5.0	12.0	100.00
2	Gelatin 0.3 % + Agar 0.5%	8.00	3.0	1.5	100.00
3	Gelatin 0.7%	0.00	0.0	0.0	0.00
4	Filter paper	6.8	4.8	3.5	100.00
5	Brown paper	6.8	6.2	1.7	100.00
6	Ordinary Paper	14.0	5.8	9.5	100.00
7	Agar 0.7%	6.8	7.4	7.9	100.00
CD 0.05)		3.37	0.74	1.76	

Table 13b. Effect of different gelling agents and supporting materials on *in vitro* rooting of cv. Nivedyakadali

Sl. No	Treatments	Number of days taken for root initiation	Number of roots	Length of longest root (cm)	Cultures developing roots (%)
1	Agar 0.6%	6.8	3.5	7.4	100.00
2	Gelatin 0.3 %+ Agar 0.5%	8.8	1.8	1.4	100.00
3	Gelatin 0.7%	0.0	0.0	0.0	0.00
4	Filter paper	8.0	1.5	3.5	100.00
5	Brown paper	8.5	2.6	1.6	100.00
6	Ordinary Paper	14.5	3.6	7.4	100.00
7	Agar 0.7%	7.0	4.9	4.5	100.00
CD (0.05)		2.72	1.04	0.38	

Figure.5. Effect of different gelling agents and supporting materials on *in vitro* rooting of cv. Njalipoovan

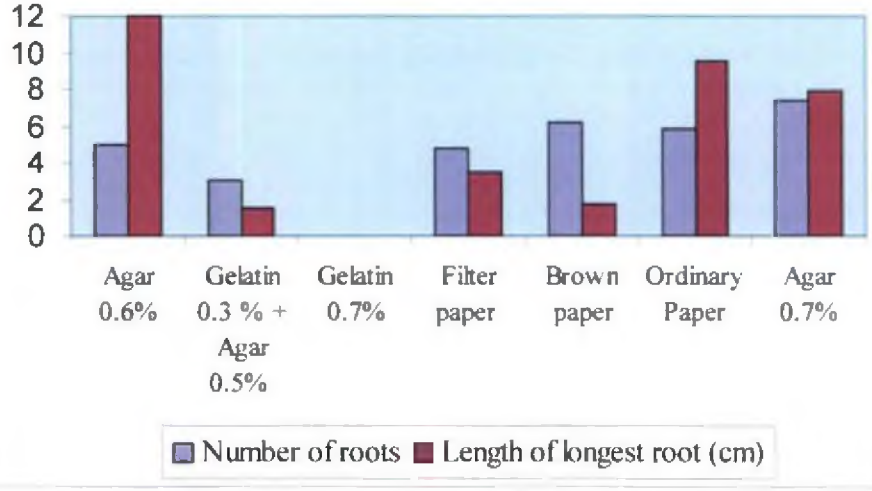
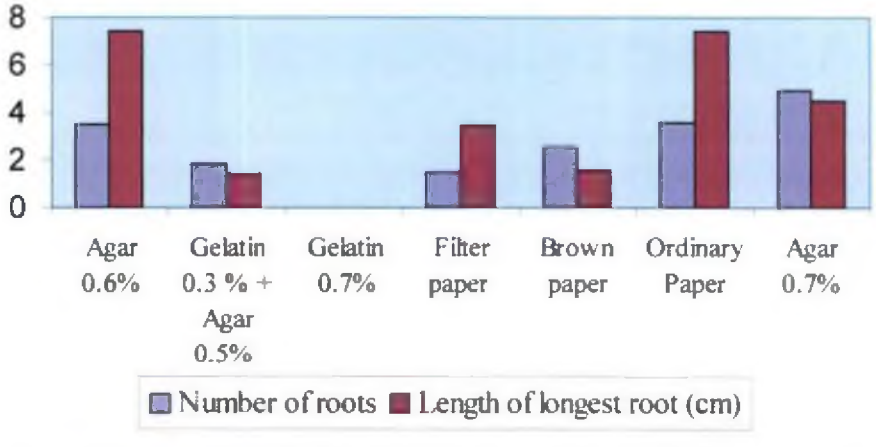


Figure.6. Effect of different gelling agents and supporting materials on *in vitro* rooting of cv. Nivedyakadali



4.3.4. Effect of different vitamin concentrations on *in vitro* rooting

The data related to the number of roots and length of roots produced by the shoots at various strengths of vitamin is given in Table 14a and b. For cv. Njalipoovan, full strength of vitamins in full MS medium resulted in maximum number of roots (6.0) and maximum length of roots (12.6cm) (Figure 7 and Plate 15). The cv. Nivedyakadli recorded maximum number of roots (6.6) in half strength of vitamins in full MS media and length of root was maximum (7.4cm) in full strength vitamin concentration (Figure 8 and Plate 16) , which was on par with half tablet of vitamin B complex (7.3cm).

4.3.5. Effect of different levels of triademefone on *in vitro* rooting

The number of roots, length of roots and length of shoots produced by cv. Nivedyakadali are presented in Table 15. The number of roots (6.8) and length of root (13.7cm) was higher in media containing triademefon 1.0 mg l^{-1}, whereas length of shoot (13.6cm) was maximum in triademefon 2.0 mg l^{-1} treatment (Figure 9).

4.3.6. Effect of culture vessels on *in vitro* rooting of diploid banana cv. Njalipoovan

Data on number of roots per shoot, length of longest root and per cent of cultures devoid of contamination with respect to cv. Njalipoovan are presented in Table 16. No significant influence was noticed among different containers tried, with respect to number of roots per shoot. Test tubes with cotton plug recorded maximum length of root (13.7 cm), which was in par with conical flask and jam bottle. Test tubes with plastic cap produced lowest length of root (7.0 cm) (Figure10). Regarding survival per cent maximum survival (90.0 %) was recorded in test tubes with cotton plug and plastic cap.

Table 14a Effect of different vitamin concentrations on *in vitro* rooting of cv. Njalipoovan

Sl.No	Treatments	Number of days taken for root initiation	Number of roots	Length of longest root (cm)
1	Half vitamin B complex tablet	8.3	4.3	7.9
2	No vitamins	15.5	1.3	2.0
3	Quarter strength of vitamins in fullMS	11.0	1.0	1.8
4	Half strength of vitamin in full MS.	8.3	3.2	8.3
5	Full strength of vitamins in full MS (control)	6.8	6.0	12.6
CD (0.05)		3.16	1.53	0.92

Table 14b. Effect of different vitamin concentrations on *in vitro* rooting of cv. Nivedyakadali

Sl. No	Treatments	Number of days taken for root initiation	Number of roots	Length of longest root(cm)
1	Half vitamin B complex tablet	8.0	3.1	7.3
2	No vitamins	15.5	2.1	2.4
3	Quarter strength of vitamins in full MS	11.5	2.3	3.8
4	Half strength of vitamin in full MS.	8.3	6.6	4.4
5	Full strength of vitamins in full MS (control)	7.0	4.3	7.4
CD (0.05)		3.4	0.99	0.74

Figure.7. Effect of different vitamin concentrations on *in vitro* rooting of cv. Njalipoovan

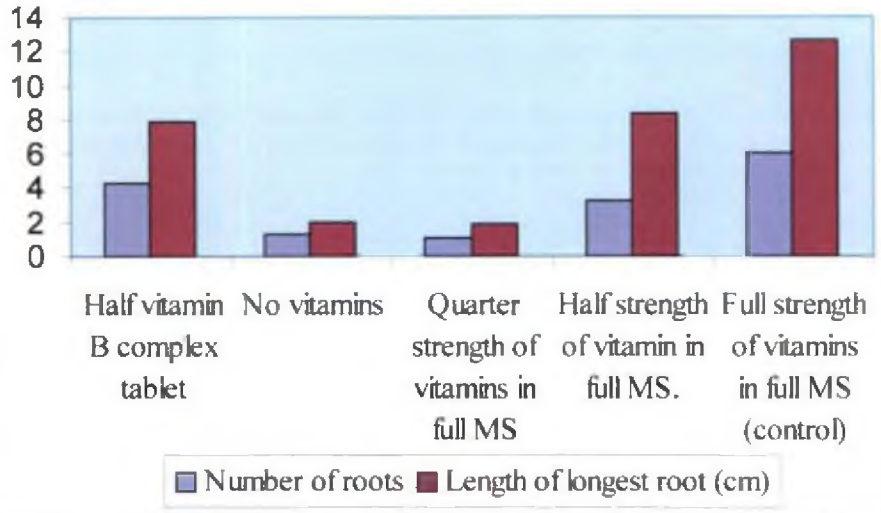


Figure.8. Effect of different vitamin concentrations on *in vitro* rooting of cv. Nivedyakadali

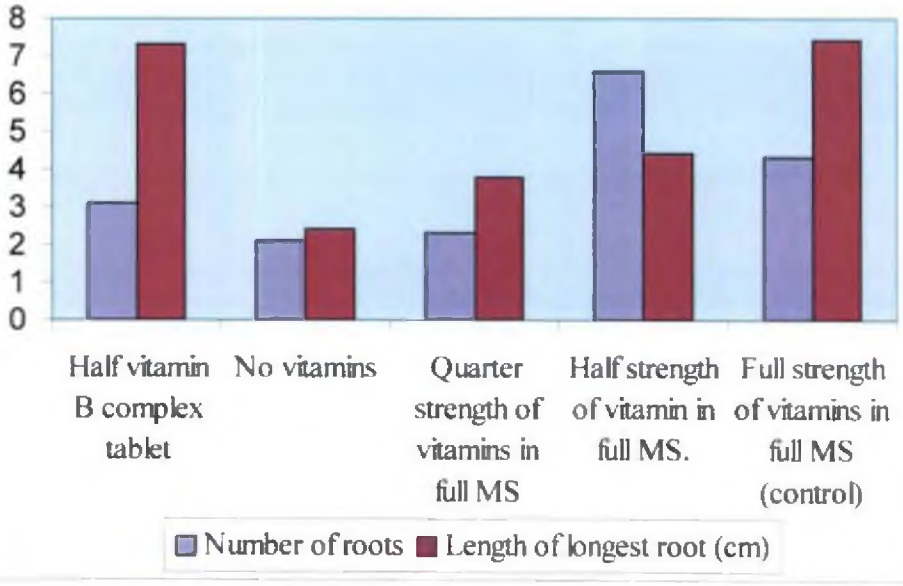


Plate 15. ***In vitro*** **rooting of diploid banana cv. Njalipoovan in full MS + half vitamin B complex tablet (2.0 mg l^{-1})**

Plate 16. ***In vitro*** **rooting of diploid banana cv. Nivedaykadali in full MS + half vitamin B complex tablet (2.0 mg l^{-1})**

Table 15. Effect of different levels of triademefon on *in vitro* rooting of diploid banana cv. Nivedyakadali

Sl.No	Treatments	Number of roots	Length of longest root (cm)	Length of shoot (cm)
1	Triademefon 0.0 mg l^{-1}	4.4	7.3	8.8
2	Triademefon 0.5 mg l^{-1}	3.5	8.9	10.7
3	Triademefon 1.0 mg l^{-1}	6.8	13.7	11.4
4	Triademefon 1.5 mg l^{-1}	3.3	8.8	6.0
5	Triademefon 2.0 mg l^{-1}	4.9	8.6	13.6
6	Triademefon 3.0 mg l^{-1}	3.1	5.8	6.8
7	Triademefon 4.0 mg l^{-1}	3.1	9.1	9.4
CD (0.05)		0.93	3.49	2.21

Figure.9. Effect of different levels of triademefon on *in vitro* rooting of diploid banana cv. Nivedyakadali

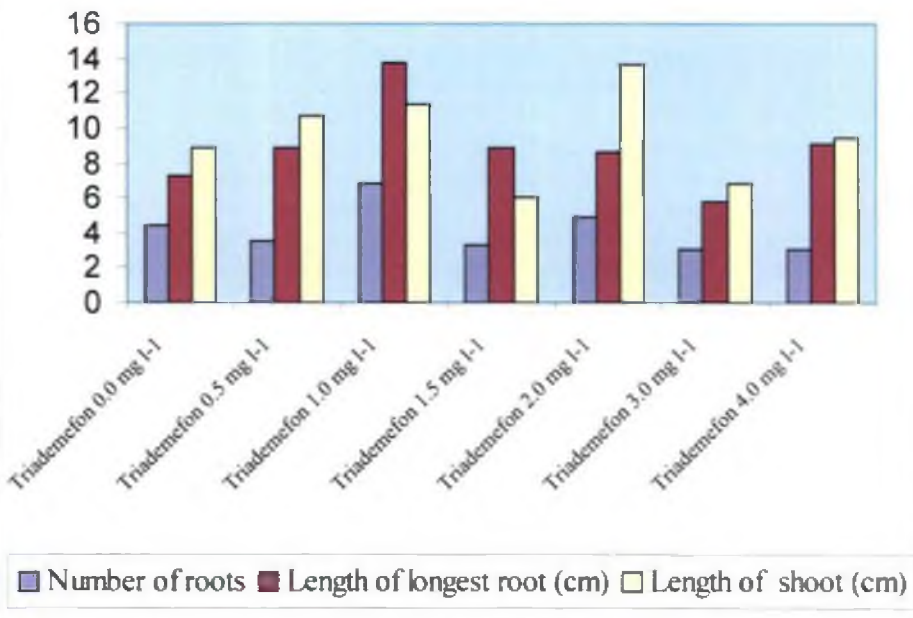


Table 16. Effect of culture vessels on *in vitro* rooting of diploid banana cv. Njalipoovan

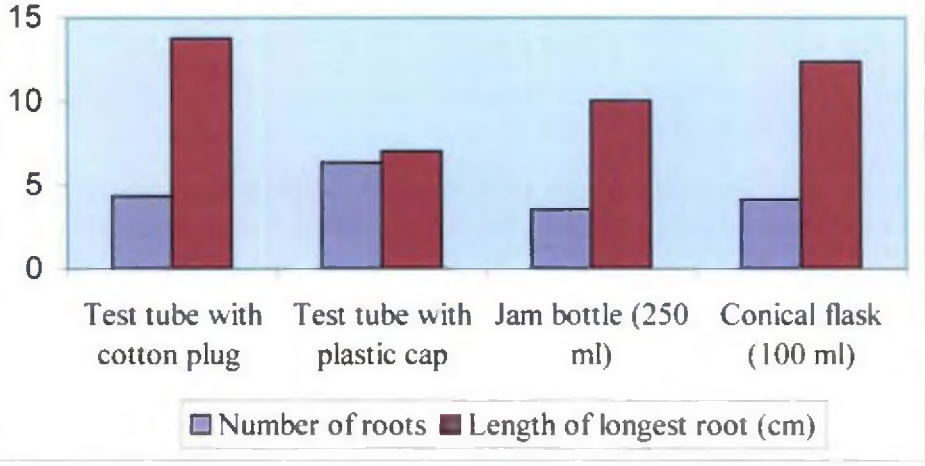
Sl. No	Treatments	Number of roots	Length of longest root (cm)	Survival (%)*
1	Test tubes with cotton plug	4.3	13.7	90.00
2	Test tubes with plastic cap	6.3	7.0	90.00
3	Jam bottles (250 ml)	3.5	10.0	60.00
4	Conical flask (100 ml)	4.1	12.3	80.00
CD (0.05)		NS	4.06	

* Mean of 20 observations

Table 17. Standardisation of medium for *ex vitro* rooting of *in vitro* shoots (cv. Njalipoovan)

Sl. No.	Treatments	Survival (%)	Rooting (%)
1	Vermiculite	20	20
2	Sand	15	15
3	Perlite	10	10
4	Coir pith compost	15	15
5	Sand+ red earth + cowdung (1:1:1)	10	10

Figure.10. Effect of culture vessels on *in vitro* rooting of cv. Njalipoovan



4.4 ACCLIMATIZATION

4.4.1.Standardisation of medium for *ex vitro* rooting of *in vitro* shoots in mist chamber

The experiment conducted for standardisation of *ex vitro* rooting of microshoots from Njalipoovan showed that the treatments had no satisfactory effect on rooting and survival per cent (Table17). The maximum survival and rooting was only 20.0 per cent in vermiculite.

4.4.2. Standardisation of potting medium for *ex vitro* establishment

The number of leaves and survival per cent of plantlets recorded in different types of *ex vitro* rooting medium are given in Table18. Among the different potting media tried, vermiculite + coir pith compost (1:1) resulted in cent percent survival of Njalipoovan plantlets followed by vermiculite alone (80.0 %). Sand+ vermiculite + coir pith compost (1:1:1) medium showed best result in terms of number of leaves (4.1).

In the case of cv. Nivedyakadali, cent per cent survival of plantlets resulted in the vermiculite + coir pith compost (1:1) medium followed by 95.0 per cent survival in sand + coir pith compost (1:1) and vermiculite + perlite (1:1) medium. The number of leaves per plantlet was maximum (4.3) for sand + red earth+ cowdung (1:1:1), which was on par with perlite alone (4.0).

4.4.3. Influence of potting mixture on growth and vigour of plantlet

Different types of potting mixture were found to influence the growth and vigour of the diploid banana plantlets. The cv. Njalipoovan recorded maximum number of leaves (5.9), length of leaves (9.8cm), breadth of leaves (3.3cm) and height of plantlets (14.8cm) in 1: 1: 1 (v/v) mixture of sand, cow dung and goat

Table 18. Standardisation of potting media for *ex vitro* establishment of diploid banana plantlets

Sl. No	Treatments	Njalipoovan		Nivedyakadali	
		Plantlet survival (%)	Number of leaves	Plantlet survival (%)	Number of leaves
1	Vermiculite alone	80.00	3.0	70.00	3.0
2	Vermiculite + perlite (1:1)	30.00	2.3	95.00	3.0
3	Vermiculite +coir pith compost (1:1)	100.00	3.1	100.00	3.8
4	Vermiculite + perlite + sand (1:1:1)	30.00	3.5	90.00	3.8
5	Vermiculite + coir pith compost + sand (1:1:1)	60.00	4.1	90.00	2.8
6	Coir pith compost + Sand (1:1)	70.00	3.9	95.00	3.9
7	Coir pith compost	70.00	2.9	90.00	3.6
8	Perlite alone	60.00	3.8	80.00	4.0
9	Sand alone	60.00	3.6	80.00	3.6
10	Sand + red earth + cow dung (1:1:1)	50.00	3.0	80.00	4.3
CD (0.05)			0.87		0.60

manure (Table19a and Figure 11)). Similar results were obtained with cv. Nivedyakadali, observing maximum number of leaves (5.9), length of leaves (10.1 cm), breadth of leaves (3.4 cm) and height of plantlets (13.1 cm) in the same potting mixture (Table19b and Figure 12).

4.4.4. Effect of different hardening units on *ex vitro* establishment of diploid banana plantlets

Table 20 shows the data on survival per cent of plantlets as affected by different hardening units. In the case of cv. Nivedyakadali plantlets, maximum survival per cent (85.50 %) was obtained by keeping the plantlets in mist chamber. Covering the individual plantlets with polythene cover recorded very low survival (20.0 %).

4.4.5. Effect of containers on plantlet survival

Data on survival per cent recorded by cv. Njalipoovan and cv. Nivedyakadali in different containers is given in Table 21. Both cultivars showed cent per cent survival in mud pot, black polythene cover and white polythene cover. Black protray recorded satisfactory survival (87.5 % and 81.25 % respectively) in cv.Njalipoovan and cv. Nivedyakadali. White disposable cup recorded lowest survival per cent (50.0 % and 62.5 % respectively) in cv. Njalipoovan and cv.Nivedyakadali.

4.4.6. Effect of different nutrient solutions on growth and vigour of diploid banana plantlets

Data pertaining to the effect of different nutrient solutions on growth and vigour of cv.Njalipoovan and cv.Nivedyakadali are given in Tables 22a, 22b, 23a and 23b. Spraying of full MS solution recorded maximum height of 14.1 cm, maximum number of leaves (7.0), length of leaves (17.9 cm) and breadth of

Table 19a. Influence of potting mixture on growth and vigour of plantlet cv. Njalipoovan

Treatments	Number of leaves		Length of leaves (cm)		Breadth of leaves (cm)		Height of plant (cm)	
	30 DAP	60 DAP	30 DAP	60 DAP	30 DAP	60 DAP	30 DAP	60 DAP
T1	4.6	5.9	8.3	9.8	2.0	3.3	7.1	14.8
T2	3.2	4.3	7.4	9.5	1.7	2.8	6.8	11.9
T3	3.0	4.3	6.9	8.3	1.7	2.7	6.1	11.7
T4	3.4	5.4	9.5	10.4	2.0	2.7	5.3	12.2
CD (0.05)	0.57	0.05	1.20	0.84	0.43	0.30	1.00	0.71

Table 19b. Influence of potting mixture on growth and vigour of plantlet cv. Nivedyakadali

Treatments	Number of leaves		Length of leaves (cm)		Breadth of leaves (cm)		Height of plant (cm)	
	30 DAP	60 DAP	30 DAP	60 DAP	30 DAP	60 DAP	30 DAP	60 DAP
T1	3.9	5.9	7.8	10.1	2.1	3.	6.3	13.1
T2	2.4	4.4	6.4	8.8	1.8	2.9	5.5	11.8
T3	2.9	5.0	5.9	7.9	1.8	2.8	5.2	9.6
T4	3.19	4.3	7.3	9.3	2.0	3.1	5.9	12.0
CD (0.05)	0.54	0.38	0.63	0.44	0.36	0.26	0.66	0.05

Treatments
T1- Sand + cow dung +goat manure (1:1:1)
T2- Sand + goat manure (1:1)
T3- Sand + cow dung (1:1)
T4- Sand + red soil + cow dung (1:1:1)

Figure. 11. Influence of potting mixture on growth and vigour of plantlets (cv. Njalipoovan)

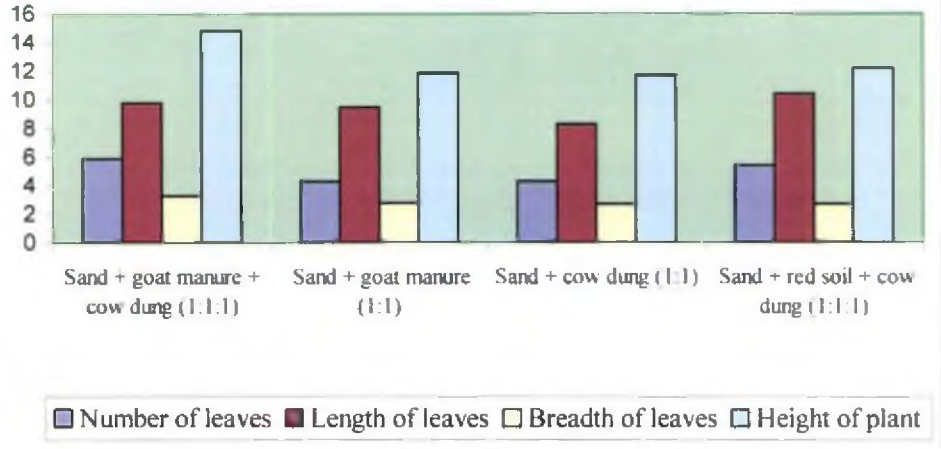


Figure.12. Influence of potting mixture on growth and vigour of plantlets (cv. Nivedyakadali)

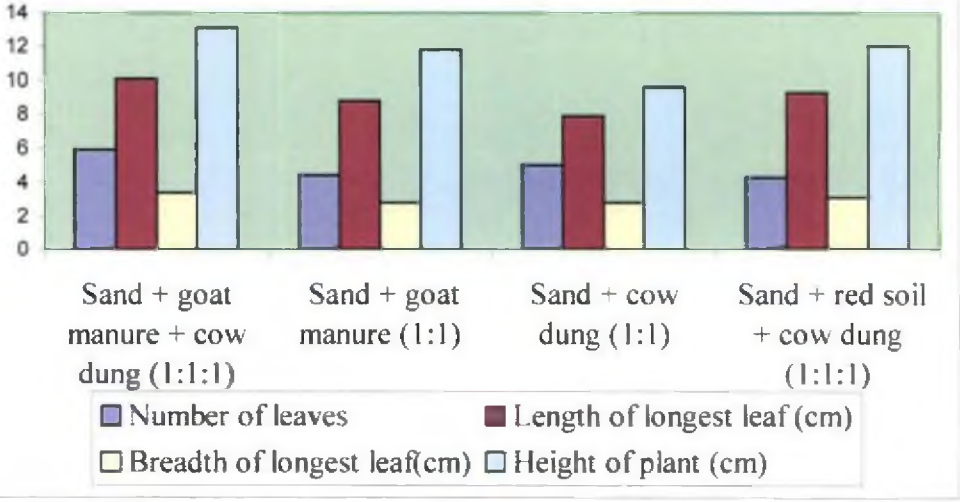


Table 20. Effect of different hardening units on *ex vitro* establishment of diploid banana plantlets (cv. Nivedyakadali)

Sl. No.	Treatments	Plants survived	Survival (%)
1	Mist chamber	18/20	85.00
2	Iron frame covered with polythene sheet	10/20	50.00
3	Covering potted plantlets with polythene cover	6/20	30.00
4	Covering individual plantlets with polythene cover	4/20	20.00

Table 21. Effect of containers on plantlet survival (cv. Njalipoovan)

Sl. No.	Treatments	Njalipoovan (Survival %)	Nivedyakadali (Survival %)
1	Disposable cup	50.00	62.50
2	Mud pot	100.00	100.00
3	Black polythene cover	100.00	100.00
4	White polythene cover	100.00	100.00
5	Plastic protray	87.50	81.25

leaves (5.7cm) in Njalipoovan plantlets after eight weeks of spraying (Figure 13 and Plate 17). The same treatment gave higher values in all these vegetative characters at two weeks, four weeks and six weeks after spraying. Similar results were obtained with cv. Nivedyakadali, observing maximum height of plantlets (17.0cm), number of leaves (6.6), length of leaves (21.6cm) and breadth of leaves (6.2cm) in the same treatment after eight weeks of spraying (Figure 14 and Plate 18).

Table 22a. Effect of different nutrient solutions on height of plantlet and number of leaves (cv. Njalipoovan)

Sl. No.	Treatments	Mean height of plantlet (cm) (Weeks after spray)				Mean number of leaves per plantlet (Weeks after spray)			
		2	4	6	8	2	4	6	8
1	Full MS	4.2	6.1	13.9	14.1	4.3	5.3	5.8	7.0
2	Half MS	3.6	5.1	11.4	12.8	3.8	4.2	4.8	6.6
3	N P K 1 %	3.1	5.1	13.6	13.9	4.1	5.1	5.4	5.8
4	N P K 2 %	2.8	3.6	9.0	9.4	4.1	4.3	5.6	5.9
5	Vermiwash	2.4	2.9	9.2	12.0	3.9	4.9	5.4	6.6
CD (0.05)		0.26				0.16			

Table 22b. Effect of different nutrient solutions on length and breadth of leaves (cv. Njalipoovan)

Sl. No.	Treatments	Mean length of leaves (cm) (Weeks after spray)				Mean breadth of leaves (cm) (Weeks after spray)			
		2	4	6	8	2	4	6	8
1	Full MS	6.1	9.4	16.4	17.9	2.1	3.4	4.9	5.7
2	Half MS	4.7	8.1	14.4	17.6	1.9	3.0	4.8	5.0
3	N P K 1 %	5.6	8.2	14.4	17.0	2.1	2.8	4.5	5.1
4	N P K 2 %	3.9	4.8	10.9	16.7	1.2	1.6	3.5	4.8
5	Vermiwash	3.1	4.4	9.8	14.4	1.1	1.9	3.4	5.0
CD (0.05)		0.26				0.10			

Table 23a. Effect of different nutrient solutions on height of plantlet and number of leaves (cv. Nivedyakadali)

Sl. No.	Treatments	Mean height of plantlet (cm) (Weeks after spray)				Mean number of leaves per plantlet (Weeks after spray)			
		2	4	6	8	2	4	6	8
1	Full MS	3.7	6.4	14.6	17.0	4.2	4.4	5.1	6.6
2	Half MS	3.1	5.5	14.4	15.4	3.9	4.3	4.8	5.5
3	N P K 1 %	3.2	4.2	11.1	16.0	3.5	3.9	5.0	6.5
4	N P K 2 %	2.7	5.5	11.3	15.0	3.3	3.6	5.1	6.3
5	Vermiwash	3.3	5.5	9.4	13.0	3.1	3.8	4.9	6.4
CD (0.05)		0.62				0.20			

Table 23b. Effect of different nutrient solutions on length and breadth of leaves (cv. Njalipoovan)

Sl. No.	Treatments	Mean length of leaves (cm) (Weeks after spray)				Mean breadth of leaves (cm) (Weeks after spray)			
		2	4	6	8	2	4	6	8
1	Full MS	5.8	7.6	15.2	21.4	1.9	2.4	4.6	5.9
2	Half MS	6.2	9.0	15.1	21.6	2.0	3.0	4.4	6.2
3	N P K 1 %	6.1	6.4	10.3	18.7	2.1	2.9	3.8	5.8
4	N P K 2 %	6.1	7.4	10.3	19.8	1.8	2.1	3.8	5.5
5	Vermiwash	4.8	6.9	11.0	16.4	1.4	2.1	3.1	4.9
CD (0.05)		0.628				0.18			

Figure.13. Effect of weekly spraying of different nutrient solutions on diploid banana cv. Njalipoovan

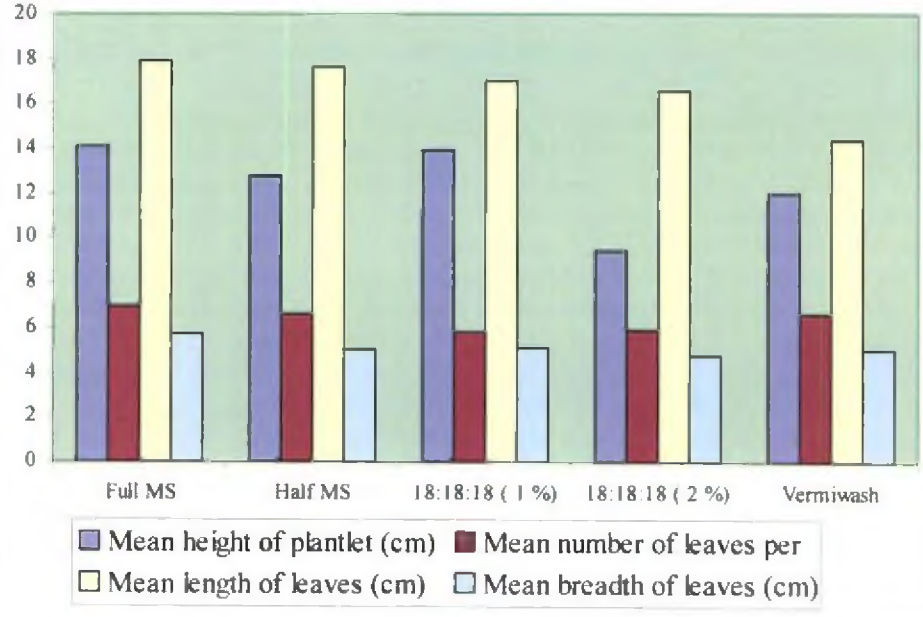


Figure.14. Effect of weekly spraying of different nutrient solutions on diploid banana plantlets cv. Nivedyakadali

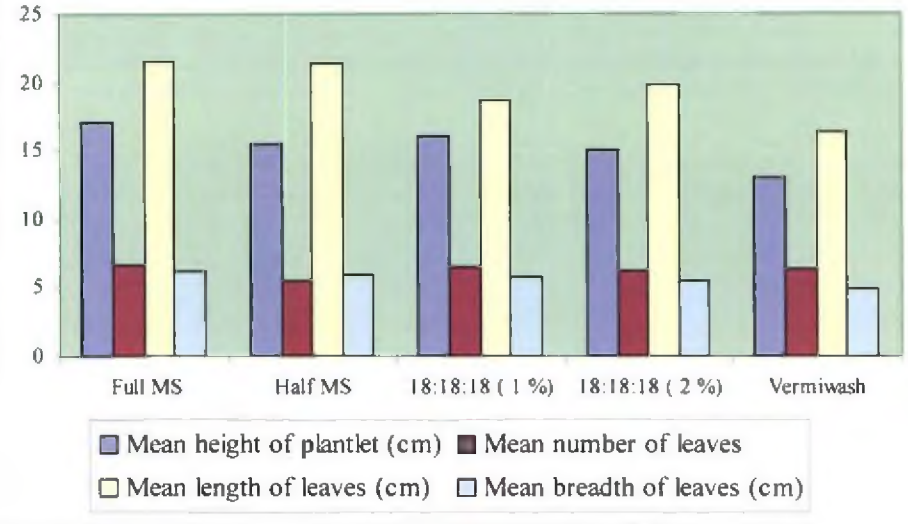


Plate 17.Comparative growth performance of *in vitro* diploid banana plantlets cv. Njalipoovan by weekly spraying of nutrient solutions

Plate 18.Comparative growth performance of *in vitro* diploid banana plantlets cv. Nivedyakadali by weekly spraying of nutrient solutions

DISCUSSION

5. DISCUSSION

All the cultivated bananas are propagated vegetatively either from the suckers, division of the corm or increasingly by *in vitro* propagation. Micropropagation has many advantages, the most important being large scale multiplication of clean, pest and disease free planting materials from selected superior genotypes. Tissue culture techniques can also aid in the cryopreservation of germplasm. Shoot tip culture is simple, easy and applicable to a wide range of *Musa* genotypes.

Successful *in vitro* multiplication of triploid bananas like Nendran, Robusta, Dwarf Cavendish and Red Banana has been developed and commercialized (Cronauer and Krikorian, 1984a; Bakry *et al.*, 1985; Gupta, 1986; Fitchet and De Winnar, 1987; Matielle and Foncelle, 1988; Aravindakshan, 1989; Bhaskar, 1991 and Rahman *et al.*, 2002). However, scanty work has been done in case of domesticated diploid banana cultivars. Since the diploid group constitute only a small number of cultivars mainly of South Indian origin, it may differ from commonly cultivated triploid bananas and may require technology refinement for *in vitro* multiplication.

In view of the above facts, the present investigations were carried out at the Department of Pomology and Floriculture, College of Horticulture, Vellanikkara in order to standardize the *in vitro* propagation in two commercial diploid bananas of Kerala namely, *Musa* (AB) 'Njalipoovan' and *Musa* (AA) 'Nivedyakadali'. The route adopted for *in vitro* propagule multiplication was via enhanced release of axillary buds. The results of the studies are discussed in this chapter.

5.1. PHYSIOLOGICAL PRECONDITIONING OF THE EXPLANT AND EXPLANT ESTABLISHMENT (STAGE 1)

5.1.1. Surface sterilization

As the shoot tip explants emerge from below the ground level they accumulate lot of microbial inoculum along with the adhering dirt and soil. This necessitated a thorough and effective surface sterilization of the explants before culturing. Out of various sterilants tried a combination treatment involving initial rinsing of explants with 70 per cent alcohol for one minute and mercuric chloride treatment 0.1 per cent for 10 minutes followed by dipping the explants in antibiotic cefotaxime 0.01 per cent for 15 minutes was found to give better sterilization than mercuric chloride alone (Table1). This treatment resulted in cent per cent explant survival without any culture contamination and explant death. Though there are reports of using mercuric chloride alone to be more efficient than other sterilants, in the present study it was observed that a combination involving mercuric chloride was more effective. This result was in conformity with the findings of Balachandran (1993) who reported that a combination of mercuric chloride with emisan gave better explant sterilization in Nendran banana.

Different explants collected from sword sucker, peeper sucker and eye bud were used and these three explants behaved similarly with respect to rate of contamination and survival percentage. This may be due to the fact that, the explants used were actually the inner growing point, collected after removing several layers of leaf sheath and base of rhizome.

5.1.2. Effect of physical injury treatments on explant establishment

The establishment stage (stage1) is to activate the dormant buds in the axils of leaf sheath to form branches by suppressing apical dominance in banana. Physical injury treatments like splitting or injuring the bud apex was found to be

favourable in the release of axillary buds. In the present study, culturing the apical dome with a longitudinal incision was found to be the best (Table 2) in enhancing the release of axillary buds with maximum per cent of culture establishment (95.0 %) and maximum per cent of cultures exhibiting growth (90.0 %). The technique of splitting banana shoot tips longitudinally through their apex in order to induce multiple shoot formation was first described by De Guzman *et al.* (1980). Injuring the bud apex by giving 7 to 12 vertical incisions was found to induce the production of a cluster of shoots within one month as reported by Vessey and Rivera (1981). Jarret *et al.* (1985) and Bhaskar (1991) viewed that dividing the apical dome into two halves and culturing each halves separately was found to be best in enhancing the release of axillary buds.

5.1.3. Effect of various pretreatments on media and explant discolouration

In banana, tissue and media discolouration due to oxidation of polyphenols was found to be a serious problem in establishing a good healthy culture. It was found that by the addition of ascorbic acid 50.0 mg l^{-1} into the media 96.0 per cent of cultures were freed from this problem with a survival rate of 88.0 per cent. This result was in agreement with the work of Bhaskar (1991) in Nendran banana.

5.1.4. Seasonal influence on the *in vitro* establishment of explants

In the present study, explants were collected from July 2004 to June2005 to study the seasonal influence on *in vitro* establishment of diploid banana explants. Explant collection during March to April and November to December resulted in least contamination rate (10.0%) and maximum explant survival (90.0 %). This may be due to low rainfall and humidity prevailing in Kerala during this period, which was uncongenial for the growth of microbes. Similar results were also reported by Sundararasu (2003).

5.1.5. Standardisation of establishment medium

Results of the present study indicated that out of 45 different treatments tried using MS medium, the treatment involving NAA 0.5 mg l^{-1} and BA 3.0 mg l^{-1} was found to be the best with respect to the number of days taken for culture establishment and per cent of culture established, for both the cultivars. Reduction in strength of MS medium to half did not support the growth of explants since the salts were not sufficient enough to nourish the explants. The success of an *in vitro* system in *Musa* depend up on choice of correct growth regulators and their use in optimum concentration (Krikorian, 1982). In most of the literature a combination of BA and NAA was found to be best (Jarret *et al.*, 1985; Bhaskar 1991; Babylatha, 1993) for culture establishment.

5.1.6. Influence of different explants on establishment medium

When the performance of sword sucker, peeper sucker and eye bud explants were compared, it was found that sword sucker and peeper sucker behaved similarly and took less time for culture establishment, while the eye bud explants took more time for culture establishment .Sword suckers of Njalipoovan took least time for culture establishment (Table 6). This difference in the performance of different explants of the same plant and between different genotype may be attributed to the difference in the level of endogenous phytohormones, nutrients and metabolites and interaction between various growth factors. Several workers have indicated that the response of banana tissues in culture may vary depending up on the difference in explant and genotype (Cronauer and Krikorian, 1984a and b; Vuylsteke and De Langhe, 1985; Wong, 1986; Bhaskar, 1991).

In the present studies also, it was found that the two diploid banana cultivars (AA and AB) responded differently to a fixed set of treatments. The reason for the observed difference in the behavior of the diploid cultivars can only

be conjectured as due to the difference in the physiological conditions and the level of endogenous phytohormones present as these cultivars belongs to different genomic group.

5.2. INDUCTION OF AXILLARY SHOOT AND RAPID SHOOT MULTIPLICATION (STAGE II)

5.2.1. Standardisation of basic proliferation medium (BPM)

In shoot tip culture of Njalipoovan, the maximum number of multiple shoots (7.3) was recorded in the medium containing NAA 0.5 mg l^{-1} and BA 8.0 mg l^{-1} or BA 5.0 mg l^{-1} alone in full MS whereas in the case of Nivedyakadali BA 5.0 mg l^{-1} in the full MS recorded the highest number of multiple shoots (8.3). In most of the literature BAP alone or in combination with very low concentration of NAA was found to give better results in proliferation media (Bhaskar, 1991; Babylatha, 1993; Rahman *et al.*, 2004).

The favourable effects of axillary bud bursting and multiple shoot production by cytokinins had been demonstrated by Murashige (1974). But at higher levels, cytokinins were proved to have deleterious effect on shoot growth. Auxin added to the medium helps to nullify the suppressive effect of high cytokinin concentration on axillary shoot growth (Lundergan and Janick, 1980). Auxins (NAA and IAA) were found to influence axillary shoot induction and growth of banana shoot tip culture at certain particular combinations (Bhaskar, 1991). In tetraploid banana (*Musa* (AAAB) 'FHIA-01') higher multiplication rate was obtained in the MS medium supplemented with BAP 4.0 mg l^{-1} (Oliveira and Silveira, 2001). During the course of the present study it was clearly understood that the cytokinins added in the medium play a prominent role in the induction of axillary shoots. Out of the two cytokinins tried (BA and kinetin), BA was found to be the most promising one (Table7a and b).

5.2.1.1. Influence of different explants on BPM

The results of the study on the effect of different banana explants on multiple shoot production revealed that, there was no significant difference between the three explants of two cultivars (Table7c). However, Peeper sucker explants of cv. Nivedyakadali recorded more number of multiple shoots (7.4). The slight difference in number of multiple shoots produced between different explants may be attributed to slight variation in the endogenous growth factors.

5.2.2. Effect of different carbon sources on *in vitro* multiple shoot production

Sucrose, the main carbon source in tissue culture medium can be well substituted by table sugar. In the present study, Njalipoovan produced maximum number of multiple shoots by sucrose 3.0 per cent, which was on par with table sugar 2.0 per cent, sucrose 2.0 per cent and glucose 2.0 per cent. Glucose 2.0 per cent showed maximum length of shoot, which was on par with table sugar 2.0 per cent. Similar trend was noticed in cv. Nivedyakadali, which exhibited maximum number of multiple shoot in medium containing table sugar 2.0 per cent. Result of the present study was in line with the findings of earlier workers who reported the beneficial effects of different carbon sources other than sucrose (Sorvari, 1968; Bonaobara, 1994; Babylatha, 1993; Okuna, 1996). Sundararasu (2003) had also reported that in cost effective *in vitro* multiple shoot production of banana, table sugar 2.0 per cent concentration produced good number of multiple shoots.

5.2.3. Effect of different gelling agents and supporting materials on *in vitro* multiple shoot production

The results generated revealed that both the diploid cultivars responded more or less similarly to the different gelling agents and supporting materials. Agar 0.7 per cent recorded significantly higher number of multiple shoots per

culture. The treatments involving different types of paper supports (filter paper, brown paper and ordinary paper) in liquid media was also satisfactory eventhough inferior to agar 0.7 per cent. The beneficial effects of liquid medium with filter paper and other supporting materials has been pointed out by different workers (Pierik, 1975; Leffering and Soede, 1979; Snir and Erex, 1980; Hammerschlang, 1982; Debergh, 1983; Harris and Mason, 1983; Jones and Petolino, 1988; Levin *et al.*, 1988; Skidmore *et al.,* 1988; Gawel and Robacker, 1990; Priel, 1991; Babylatha, 1993; Bhaghyalakshmi and Singh, 1995 and Teng, 1997). It was also noticed that the contamination rate was comparatively more in liquid media. This might be due to the fact that introduction of the paper into the liquid media might have contributed to more contamination.

5.2.4. Effect of different vitamin concentrations on *in vitro* multiple shoots production

The foregoing discussion on the present study indicate that, Njalipoovan recorded maximum number of multiple shoots in half strength of standard vitamins in full MS, which was on par with half tablet of vitamin B complex (2 mg l^{-1}). In the case of Nivedyakadali, half tablet of vitamin B complex resulted in maximum number of multiple shoots, which was on par with full strength, and half strength of vitamins in full MS media. In quarter strength of vitamins, shoot formation was poor. Dhamankar (1992) reported that molasses could provide most of the nutrients such as vitamins, sugar and inorganic metal ions required for sugarcane callus and shoot formation. Magnaye and Escobido (1996) opined that cost reduction could be achieved by deletion of an expensive organic media compound, myo- inositol in conventional banana propagation. Sundararasu (2003) viewed that the cost of vitamins can be scaled down by 32.22 per cent by the addition of half tablet of vitamin B complex .He also added that reducing the vitamin level by half resulted in moderate amount of multiple shoot production, whereas in the quarter strength of vitamins poor shoot formation occurred.

5.3. *IN VITRO* ROOTING OF DIPLOID BANANA

Stage III involves *de novo* regeneration of adventitious roots from the shoots obtained in stage II. The data on *in vitro* rooting showed that in cv. Njalipoovan, minimum number of days for rooting (6.7) was recorded in full MS+ NAA 2.0 mg l^{-1} and maximum number of root was produced in medium containing half MS+ NAA 10.0 mg^{-1}. In the case of cv. Nivedyakadali, lesser number of days for rooting (6.0) was recorded in the treatment full MS + NAA 1.0 mg l^{-1} with cent per cent of cultures showing root initiation. Maximum number of roots per shoot (5.50) was observed in full MS + NAA 0.5 mg l^{-1}. Regarding the production of longer roots, NAA 0.5 mg l^{-1} gave the best results in the case of both cv. Njalipoovan and cv. Nivedyakadali (Table11a and b). Several researchers have shown that *in vitro* rooting can successfully be achieved by reducing salt concentration in the media particularly in the high salt media like MS and its derivatives (Kartha *et al.*, 1974; Lane 1979; Skirvin and Chu, 1979). Half concentration of MS medium was found to favourably induce the rooting of banana without affecting the shoot growth unlike in certain species (Wang 1978; Gupta *et al.*, 1981 and Sundararasu, 2003). Balachandran (1993) noted that NAA at lower concentration produced long thin roots and NAA at higher concentration produced short thick roots in *in vitro* banana plantlets. The differential behaviour of diploid banana cultivar with respect to *in vitro* rooting may be attributed due to the difference in their physiological conditions and level of endogenous phytohormones, as these cultivars belong to different genomic groups.

5.3.1. Effect of different carbon sources on *in vitro* rooting

A scrutiny of the results revealed that cv. Njalipoovan recorded maximum number of roots in medium with table sugar 3.0 per cent which was on par with table sugar 2.0 per cent. In terms of length of roots same cultivar recorded highest value (10.2cm) in medium with sucrose 3.0 per cent which was on par with sucrose 2.0 per cent and table sugar 2.0 per cent. Least number of days for root

initiation was observed with sucrose 3.0 per cent. In the case of cv. Nivedyakadali, table sugar 3.0 per cent produced maximum number of roots followed by sucrose 3.0 per cent. Least number of days for root initiation was recorded by sucrose 3.0 per cent. The results are in confirmity with the findings of earlier workers (Babylatha, 1993 and Sundararasu, 2003).

5.3.2. Effect of different gelling agents and supporting materials on *in vitro* rooting of diploid banana

In the present study, cv. Njalipoovan produced maximum number of roots in control treatment, agar 0.7 per cent and length of root was maximum in medium containing agar 0.6 per cent. In the case of cv. Nivedyakadali, number of roots produced was maximum in the medium containing agar 0.7 per cent. The other treatments were inferior to agar 0.7 per cent. This finding was in agreement with the works of Bhojwani and Razdan (1983) who reported solidified medium with agar as the best medium for *in vitro* propagation. The desirable success in organogenesis has been achieved with banana explants on agar based medium by several workers (Cronauer and Krikorian, 1984a; Hwang *et al.*, 1984; Banerjee and De Langhe, 1985; Balakrishnamurthy and Sreerangaswamy, 1988; Matielle and Foncelle, 1988)

5.3.3. Effect of different vitamin concentrations on *in vitro* rooting

In this experiment cv. Njalipovan recorded maximum number of roots, length of roots and minimum number of days for root initiation in the medium containing full strength of vitamins in full MS, which served as control treatment. Half tablet of vitamin B complex in full MS and half strength of vitamins in full MS showed satisfactory results in terms of number of roots and length of roots. Whereas cv. Nivedyakadali recorded maximum number of roots in half strength of vitamins and length of root was maximum in full strength vitamin concentration, which was on par with vitamin B complex tablet. Thus it was

observed that vitamin B complex can be substituted to some extent in *in vitro* rooting medium of diploid banana. Sundararasu (2003) in his studies on Nendran banana viewed that the number of roots, length of roots and length of shoots produced by the medium containing full vitamin, half level of vitamin and half tablet of vitamin B complex were on par with each other.

5.3.4. Effect of different levels of triademefon on *in vitro* rooting

The results of the studies using triademefon showed that maximum number of roots and length of roots recorded in triademefon 1.0 mgl^{-1} and maximum length of shoot was in medium containing triademefon 2.0 mgl^{-1}. The beneficial effects of triazole in *in vitro* culture was brought out by several workers (Boussiba *et al.,* 1975; Chin, 1982; Singh, 1983; Fletcher, 1985; Fletcher and Hofstra, 1985; Asare- Boamah and Fletcher, 1986; Keith and Mckeirse, 1986; Singh *et al.,* 1987; Mc Kinless and Andreson, 1991; Smith *et al.* , 1991; Hussain, 1995; Murali and Duncan, 1995 and Bhaskar , 1996).The favourable effects of triazole may be due to the increased stomatal resistance, there by contributing greatly to the maintanence of turgor, transient increase in abscisic acid, a initial trigger in the hardening process of various plant stresses and may be due to increase in tocopherol and abscisic acid which act as antioxidants (Sundararasu,2003).

5.3.5. Effect of culture vessels on *in vitro* rooting of diploid banana

Of the various culture vessels, test tubes with cotton plug recorded maximum length of root with minimum contamination. No significant difference was noticed with respect to number of roots per shoot. Cultures in jam bottle had more contamination though the number of roots and length of roots was satisfactory. The results of the study point out to the fact that if the contamination rate could be reduced by some means, the jam bottles could be effectively substituted to expensive borosil glasswares. Raju (1993) reported that, expensive borosilicate glassware which cost over Rs. 2.50 per square

centimeter of culture surface can be replaced by any clear, colourless bottles, which cost much lesser than one paise per square centimeter.

5.4. ACCLIMATIZATION

5.4.1. Standardisation of medium for *ex vitro* rooting of *in vitro* shoots in mist chamber

Debergh and Maene (1981) pointed out that rooting *in vitro* was the most labour intensive part of micropropagation. In the present investigation also, *in vitro* rooting of individual *in vitro* shoots after separation from the multiple shoot clusters was found to be the most time and labour consuming part. Hence an attempt was made to induce *ex vitro* rooting of *in vitro* banana shoots. No satisfactory results were obtained among the different medium tried with shoots treated IBA 1000 mgl^{-1}. Successful *ex vitro* rooting has been reported in pineapple by direct planting of *in vitro* shoots (Prabha, 1993). The non satisfactory results in *ex vitro* rooting in banana may be attributed to the more succulent nature of the plant and insufficient levels of endogenous rooting hormones.

5.4.2. Standardisation of *ex vitro* rooting media and other conditions for establishment of banana plantlets

The most suitable potting medium for *ex vitro* establishment of cv. Njalipoovan and cv. Nivedyakadali plantlets was found to be the 1:1(v/v) mixture of vermiculite and coir pith compost. According to Reghunath (1989) better performance of vermiculite may be attributed to its ability to maintain optimum moisture status at the same time providing sufficient aeration. Mixing with equal volume of coir pith compost might have helped in giving ample supply of nutrients for the roots of young plantlets besides good aeration. The results of the present study are in agreement with Bhaskar (1991) and Sundararasu (2003).

In an attempt to study the influence of potting mixture on growth and vigour of plantlet, 1:1:1 (v/v) mixture of sand, cow dung and goat manure recorded maximum number of leaves, length of leaves, breadth of leaves and height of plantlets in both the cultivars viz; Njalipoovan (Table 19a) and Nivedyakadali (Table 19b). Babylatha (1993) observed better performance of banana plantlets in 1:1:1(v/v) mixture of soil, sand and vermiculite. The present result is in conformity with the findings of Sundararasu (2003) who reported similar observation in Nendran banana.

The results of the effect of different hardening units on *ex vitro* survival of diploid banana plantlets are presented in Table 20. In this experiment, plantlets were planted out in different hardening units to find out a low cost option. The results of the study revealed that plantlets kept under standard mist chamber gave maximum survival per cent and other hardening units like iron frame covered with polythene sheet, groups of plants kept under polythene cover and individual covering of plantlets with polythene cover resulted in poor survival per cent. The superiority of keeping plantlets in mist chamber over other hardening units in banana has been reported by Sundararasu (2003) The methods of covering the newly transferred plantlets with plastic tent, glass or microscope covers followed by misting for the first two weeks, subsequently removing the cover in a gradual process has been reported by a number of workers for hardening the plantlets (Murashige,1974; Reghunath,1989; Bhaskar,1991 and Babylatha,1993)

Type of container was also found to influence the survival of plantlets. In the present study plantlets in mud pot, black polythene cover and white polythene cover recorded cent per cent survival. Bhaskar (1991) reported different types of containers for the *in vitro* derived banana plantlets.

Application of inorganic nutrients to the hardening plantlets is essential for the growth and vigour of the potted plantlets. A scrutiny of the results revealed that cv. Njalipoovan recorded maximum height of plantlets (14.1cm), number of

leaves (7.0), length of leaves (17.9cm) and breadth of leaves (5.7cm) in the treatment involving weekly spraying of MS inorganic salts at full concentration. Similar results were obtained with cv. Nivedyakadali, observing maximum height of plantlets (17.0cm) number of leaves (6.6), length of leaves (21.6cm) and breadth of leaves (6.2cm) in the same treatment. Application of 5 to10 ml nutrient solution containing MS inorganic salts at half concentration at weekly intervals enhanced the survival and promoted the normal growth of banana plantlets, since the just transferred *in vitro* tissue culture plantlets are highly sensitive and photosynthetically inefficient, weekly foliar spray of starter solution will cope up the initial plant growth (Bhaskar, 1991 and Sundararasu, 2003).

5.5. PROTOCOL FOR *IN VITRO* PROPAGATION OF DIPLOID BANANA

The protocol developed in the present investigation for mass multiplication of diploid bananas viz; cv. Njalipoovan and cv. Nivedyakadali from shoot tip explants are being discussed hereunder. Explants should be collected from field during March to April or November to December. Explants should be surface sterilized with a combination treatment of absolute alcohol 70.0 per cent for 1 minute + mercuric chloride 0.1 per cent for 10 minutes + dipping inn antibiotic cefotaxime 0.01 per cent solution for 15 minutes. Give a longitudinal cut to the apical dome of shoot tip explants for enhancing the release of axillary buds in the culture. To reduce media and explant discolouration due to polyphenol oxidation, ascorbic acid at the rate of 50.0 mgl^{-1} should be added into the medium. For culture establishment, full MS + BA 3.0 mgl^{-1} + NAA 0.5 mgl^{-1} medium should be used. The proliferation media should contain BA 5.0 mgl^{-1}. For cv. Njalipoovan, full MS medium containing BA 8.0 mgl^{-1} and NAA 0.5 mgl^{-1} can also be used in stage II. In multiplication medium, table sugar 2.0 per cent can be used instead of sucrose 3.0 per cent and vitamins can be replaced by half tablet of vitamin B complex. The medium should be solidified with agar 0.7 per cent. In stage III NAA 0.5 mgl^{-1} should be used for longer roots and NAA 1.0 mgl^{-1} for faster rooting. Sucrose 3.0 per cent in rooting medium can be substituted with

table sugar 2.0 per cent or table sugar 3.0 per cent. For cv Nivedyakadali half tablet of vitamin B complex can be used as substitute to standard vitamins in MS medium for *in vitro* rooting. To obtain more number of roots and length of roots triademefon 1.0 mg l^{-1} treatment will be effective. Hardening of plantlets can be done in mud pot, black polythene cover or white polythene cover under mist chamber using 1:1 (v/v) mixture of vermiculite + coir pith compost medium. After one month of hardening, plantlets can be transferred to potting medium containing 1: 1: 1 (v/v) mixture of sand + cow dung + goat manure. To trigger the initial growth of plantlets, foliar spray of nutrient solution containing MS inorganic salts in full concentration at weekly intervals should be adopted to obtain more height of plantlet, number of leaves per plantlet, length and breadth of leaves.

Thus the results of the present study and protocol developed for *in vitro* propagation of two diploid banana cultivars indicate the possibilities for developing shoot tip culture technique as a tool for rapid multiplication of diploid banana cultivars viz ; Njalipoovan and Nivedyakadali, on a commercial scale (Plate 19).

As compared to Nendran and other triploid accuminata types, the number of multiple shoots produced is less in diploid bananas. Hence the possibilities of inducing more number of multiple shoots should be explored. Studies on this line is a topic of future research. More stress also should be given to techniques like somatic embryogenesis, since these techniques are reported to have greater potentialities to enhance the rate of multiplication than axillary bud release method and are useful to generate variants.

Plate 19.Different stages of growth of acclimatised diploid banana plantlets

SUMMARY

SUMMARY

Investigations on *in vitro* propagation in two commercial diploid bananas of Kerala namely *Musa* (AB) 'Njalipoovan' and *Musa* (AA) 'Nivedyakadali' were carried out in the Plant Tissue Culture Laboratory, Department of Pomology and Floriculture, College of Horticulture during the period 2003-2005. The results of the study summerised below.

1. Surface sterilization of explants with a combination treatment of absolute alcohol 70.0 per cent for 1 minute + mercuric chloride 0.1 per cent for 10 minutes + dipping in antibiotic cefotaxime 0.01 per cent solution for 15 minutes recorded cent per cent survival of explant.
2. Shoot tip explants collected from the field during March to April and November to December recorded least contamination rate (10.0 %) and maximum survival (90.0 %).
3. Physical injury of the apical dome of shoot tip explants with a longitudinal cut was found to be best in enhancing the release of axillary buds in the culture.
4. The addition of ascorbic acid into the media at the rate of 50.0 mgl^{-1} reduced media and expalnt discolouration due to polyphenol oxidation.
5. For the better and faster explant establishment and growth of sword sucker and peeper sucker explants, MS medium containing BA 3.0 mgl^{-1} and NAA 0.5 mgl^{-1} was found to be best for Njalipoovan and Nivedyakadali.
6. When the performance of sword sucker, peeper sucker and eyebud explants were compared, sword sucker and peeper sucker explants took comparatively less time. Eye bud explants took more time for culture establishment.
7. In shoot tip culture of Njalipoovan, maximum number of multiple shoots (7.3) was recorded in the medium containing NAA 0.5 mg l^{-1} and BA 8.0 mg l^{-1} or BA 5.0 mg l^{-1} alone in full MS whereas in the case of cv.

Nivedyakadali BA 5.0 mg l^{-1} in full MS recorded the highest number of multiple shoots (8.3).

8. Out of the two cytokinins tried (BA and kinetin), BA was found to be the most efficient one for the induction of axillary shoots in the culture.
9. Sword sucker, peeper sucker and eye bud explants were found to be equally effective in producing maximum number of axillary shoots from a single explant, in both cultivars.
10. In diploid banana cv. Njalipoovan the number of shoots produced was higher (4.1) in the treatment involving sucrose 3.0 per cent and table sugar 2.0 per cent which was on par with glucose 2.0 per cent and sucrose 2.0 per cent. Maximum length of shoot was observed by glucose 2.0 per cent (4.4cm). In cv. Nivedyakadali, the number of multiple shoot produced (4.9) and length of shoot per culture (4.6cm) was maximum in medium containing table sugar 2.0 per cent. With respect to the number of shoots, sucrose 3.0 per cent and sucrose 2.0 per cent were on par with table sugar 2.0 per cent. Length of shoots produced per culture was on par in sucrose 3.0 per cent and glucose 2.0 per cent.
11. In cv. Njalipoovan maximum number of multiple shoots (4.1) was produced in the medium containing agar 0.7 per cent. For cv.Nivedyakadali also the same treatment produced maximum number of multiple shoots (4.6). Explants in gelatin 0.7 per cent did not survive since the medium did not solidify.
12. Half strength of vitamins in full MS observed maximum number of multiple shoots (5.3) in cv. Njalipoovan, which was on par with half tablet of vitamin B complex tablet in medium. In the case of cv. Nivedyakadali, half tablet of vitamin B complex in medium resulted in maximum number of shoots (4.9) which was on par with control treatment involving full strength of vitamins in MS and half strength of vitamins in MS medium.
13. For cv. Njalipoovan the treatment involving full MS + NAA 2.0 mg l^{-1} took significantly least number of days (6.7) for root initiation with cent per cent rooting. The number of roots per shoot was maximum (8.0) in half MS +

NAA 10.0 mg l^{-1} whereas, longest root was found to be in full MS + NAA 0.5 mg l^{-1} . In the case of cv.Nivedyakadali, full MS medium containing NAA 1.0 mg l^{-1} took least number of days for root initiation (6.0) with cent per cent rooting. The number of roots per shoot (5.5) was found to be maximum in NAA 0.5 mg l^{-1} to 1.0 mg l^{-1} in full MS.

14. Of the various carbon sources tried with cv. Njalipoovan, the number of roots produced was significantly higher (6.3) in the medium containing table sugar 3.0 per cent and table sugar 2.0 per cent. Length of root was maximum (10.2cm) in the medium containing sucrose 3.0 per cent which was on par with sucrose 2.0 per cent (8.9cm) and table sugar 2.0 per cent (8.1cm). In the case of cv. Nivedyakadali number of roots produced was higher (7.5) in medium containing table sugar 3.0 per cent. Regarding the length roots produced there was no significant difference between the treatments.
15. Agar 0.7 per cent recorded maximum number of roots in cv. Njaliopoovan. (7.4) and cv. Nivedyakadali (4.9).
16. For cv. Njalipoovan, full strength of vitamins in full MS medium resulted in maximum number of roots (6.0) and maximum length of roots (12.6cm). Nivedyakadli recorded maximum number of roots (6.6) in half strength of vitamins in full MS media and length of root was maximum (7.4cm) in full strength of vitamin concentration, which was on par with half tablet of vitamin B complex.
17. Number of roots (6.8) and length of root (13.7cm) was higher in media containing triademefon 1.0 mg l^{-1}, whereas length of shoot (13.6cm) was maximum in triademefon 2.0 mg l^{-1} treatment.
18. Test tubes with cotton plug recorded maximum length of root which was on par with conical flask and jam bottle. Test tubes with plastic cap produced lowest length of root.
19. Direct transplanting of *in vitro* shoots to *ex vitro* conditions did not result in the survival of the plantlet.

20. The most suitable rooting medium for *ex vitro* establishment of cv. Njalipoovan and cv. Nivedyakadali plantlet was found to be 1:1(v/v) mixture of vermiculite + coir pith compost.
21. Sand + cow dung + goat manure (1: 1: 1) mixture was found to be the best potting mixture for further growth and vigour of *in vitro* derived diploid banana.
22. Plantlets kept under mist chamber recorded the highest survival percentage. Covering the individual plantlets with polythene cover recorded very low survival of the plantlet
23. Mud pot, black polythene cover and white polythene cover was found to be equally effective containers for hardening of plantlet.
24. Weekly spraying of full MS solution recorded maximum height of plantlets, number of leaves, length of leaves and breadth of leaves in both diploid cultivars.

REFERENCES

REFERENCES

Alvard, D., Cote, F. and Tiesson, C. 1993. Comparison of methods of liquid medium culture of banana micropropagation. *Pl. Cell Tiss. Org. Cult.* 32: 55-60

Anderson, W.C. 1980. *Musa* propagation by tissue culture: Principles and techniques. *Proceedings of conference on nursery production of fruit plants: Application and feasibility* (ed. Zimmerman, R.H). United states Department of Agriculture, Newyork, pp.1-14

Aravindakshan, K. 1989. Studies on *in vitro* propagation of diploid banana and hybrids and their parents. Ph. D (Hort.) thesis, Tamilnadu Agricultural University, Coimbatore, 118 p.

Asare-Boamah, N.K. and Fletcher, R.A. 1986. Protection of bean seedlings against heat and chilling injury by triadimefon. *Physiol. Pl.* 67: 353-358

Babylatha, A.K. 1993. *In vitro* propagation studies on banana and papaya. Ph.D (Hort.) thesis, Gujarat Agricultural University, Navasari, 204 p.

Bakry, F., Lavarde – Guignard, F., Rossignol, L. and Demarly, Y. 1985. Development of vegetative shoots from the *in vitro* culture of inflorescence explants of banana plants (*Musa* spp.) *Fruits* 40:459-465

Balachandran, M. 1993. Induction of genetic variability in *Musa sp* var. Nendran by *in vitro* methods. M.Sc (Hort.) thesis, Kerala Agricultural University, Trichur, 113 p.

Balakrishnamurthy, G. and Sreerangaswamy, S.R. 1988. Regeneration of banana plantlets from *in vitro* culture of floral apices. *Curr. Sci.* 57:270-272

Banerjee, N. and De Langhe, E. 1985. A tissue culture technique for rapid clonal propagation and storage under minimal growth conditions of *Musa* (Banana and Plantain). *Pl. Cell Rep.* 4: 351-354

Banerjee, N., Schoofs, J., Dumortier, F.M. and De Langhe, E. 1987. Somatic embryogenesis in *Musa. Proceedings of the third conference of International Association for research on Plantain and other cooking bananas (IARPCB). May 27-31, 1985* (eds. Schoofs. J. and De Kanghe, E.). Abidjan, Ivorycoast, pp.13-14

Banerjee, N., Vuylsteke, D. and De Langhe, E.1986. Meristem tip culture of *Musa*: histomorphological studies of shoot and differentiation. *Plant tissue culture and its applications* (eds. Withers, L.A. and Anderson, P.G.). Butterworths, London, pp.139-147

Bayer, M.H. 1982. Genetic tumours: Physiological aspects of tumour formation in interspecific hybrids. *Molecular Biology of Plant tumours* (eds. Kahl, G. and Schell, J.S.). Academic Press, New York, pp.33-67

Bekheet, S.A. and Saker, M.M. 1999. Rapid mass micropropagation of banana. *Bull. National Research Centre (EGY)*, 24: 221231

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

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

Bhaskar, J. 1991. Standardisation of *in vitro* propagation technique in banana. M.Sc. (Hort.) thesis, Kerala Agricultural University, Trichur, 88 p.

Bhaskar, J. 1996. Micropropagation of *Phalaenopsis*. Ph.D (Hort.) thesis, Kerala Agricultural University, Trichur, 124 p.

Bhattacharya, P., Dey, S. and Bhattacharya, B.C. 1994. Use of low cost gelling agents and support matrices for industrial scale plant tissue culture. *Pl. Cell Tiss. Org. Cult.* 37: 15-23

Bhojwani, S.S. and Razdan, M.K. 1983. *Plant Tissue Culture: Theory and Practice*. Elsveir Science Publishers, Amsterdam, Netherlands, 592 p.

Bonaobra, Z.S. 1994. Effect of table grade sugars on growth and development of Coconut (*Cocos nucifera* L.) embryos *in vitro*. *Proceedings of the 24th International Horticultural Congress, August 21-27,1994* (eds. Dey, S. and Christie, A.). Kyoto International Conference Hall, Kyoto, Japan, pp.165-167

Boussiba, S., Rilcin, A. and Richmond, A.E. 1975. The role of abscissic acid in cross adaptation of tobacco plants. *Pl. Physiol.* 56: 337-339

Brainerd, K.E. and Fuchigami, L.H. 1981. Acclimatisation of aseptically cultured apple plants to low relative humidity. *J. Am. Soc. hort. Sci.* 106: 515-518

Chin, C.K. 1982. Promotion of shoot and root formation in *Asparagus in vitro* by Ancymidol. *HortScience* 17: 590-591

Chong, C. and Pua, E.C. 1985. Carbon nutrition of Ottawa-3 apple root stock during stages of *in vitro* propagation. *J. Hort. Sci.* 60: 285-290

Cronauer, S.S. and Krikorian, A.D. 1984a. Multiplication of *Musa* from excised stem tips. *Ann. Bot.* 53: 321-328

Cronauer, S.S. and Krikorian, A.D. 1984b. Rapid multiplication of bananas and plantains by *in vitro* shoot tip culture. *HortScience* 19:234-235

Cronauer, S.S. and Krikorian, A.D. 1985a. Reinitiation of vegetative growth from aseptically cultured terminal floral apex of banana. *Am. J. Bot.* 72: 1598-1601

Cronauer, S.S. and Krikorian, A.D. 1985b. Aseptic multiplication of banana from excised floral apices. *HortScience* 20:770-771

Cronauer, S.S. and Krikorian, A.D. 1986. Banana (*Musa* spp.) *Biotechnology in Agriculture and Forestry: 1.Trees* (eds. Bajaj, Y.P.S.). Sringer-Verlag Publishers, Berlin, pp.233-252

Damasco, O.P. and Barba, R.C. 1985. *In vitro* culture of Saba banana (*Musa balbisiana cv.* Saba (AAB). International Rice Research Institute (*En.6 ref.*). 41-42

Damasco, O.P., Patena, L.F., Umali, M.C.E. and Babu, R.C. 1984. Tissue culture of fifty Phitoppine banana cultivars. *Philipp. J. Crop Sci.* 9(3): 201-205

Das, A., Paul, A.K. and Choudhari, S. 1998. Banana tissue culture- variation in response of four genotypes. *hort. J.* 11:13-20

Debergh, P.C. 1983. Effects of agar brand and concentration on the tissue culture medium. *Physiol. Pl.* 53: 181-187

Debergh, P.C. and Maene, L.J. 1981. A scheme for commercial propagation of ornamental plants by tissue culture. *Sci. Hort.* 14: 335-345

Debergh, P., Harbaoui, Y. and Lemeur, R. 1981. Mass propagation of globe artichoke (*Cyanara scolymas*): Evaluation of different hypotheses to overcome vitrification with special reference to water potential. *Physiol. Pl.* 53: 181-187

De Guzman, E.V., Decena, A.C. and Ubalde, E.M. 1980. Plantlet regeneration from unirradiated and irradiated banana shoot tip culture *in vitro*. *Philipp. Agricst.* 63: 140-146

De Guzman, E.V., Ubalde, E.M. and Del Rosario, A.G. 1976. Banana and coconut *in vitro* cultures of induced mutation studies. *Proceedings of the Symposium on Improvement of vegetatively propagated plants and tree crops through induced mutations, April 7-8, 1976* (eds. Gamborg, O.L. and Vasil,I.K.). International Atomic Energy Agency, Vienna, pp. 33-54

Dhamankar, V.S. 1992. Molasses, a source of nutrients for *in vitro* sugarcane culture. *Sugarcane* 4: 14-15

Doreswamy, R. and Shajiram, L. 1989. Micropropagation of banana from male floral apices cultured *in vitro*. *Scientia Horticulturae* 40:181-185

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

Drew, R.A. and Smith, M.K. 1990. Field evaluation of tissue-cultured bananas in south-eastern Queensland. *Aust. J. Exp. Agric.* 30: 569-574.

Drew, R.A., Moisander, J.A. and Smith, M.K. 1989. The transmission of banana bunchy top virus in micropropagated bananas. *Pl. Cell Tiss. Org. Cult.*16: 187-193

FAO. 2002. Food and Agriculture Organisation. Agriculture data. Home page of FAO. [on line] available http:// www.fao.org.[13 aug 2005].

FIB. 2004.. Farm Guide. Farm Information Bureau. Government of Kerala, pp.15-20

Fitchet, M. 1989. Tissue culture speeds up evaluation of new cultivars. *Inf. Bull. Citrus Subtropical Fruit Res. Inst.* Virginia, 197: 1-2

Fitchet, M. and De Winnaar, W. 1987. Rapid multiplication of bananas. *Inf. Bull. Citrus Subtropical Fruit Res. Inst.* Virginia, 176: 11-12

Fitchet, M. and De Winnaar, W. 1988. Effect of sterilants and nutrient media on the establishment of shoot tips of two banana cultivars in culture. *Inf. Bull. Citrus Subtropical Fruit Res. Inst.* Virginia, 187:12-16

Fletcher, R.A. 1985. Plant growth regulating properties of sterol inhibiting fungicides. *Hormonal Regulation of Plant Growth and Development* (ed. Purohit, S.S.). Agro Botanical Publishers, India, pp.103-114

Fletcher, R.A. and Hofstra, G. 1985. Triadimefon a plant multi-protectant. *Pl. Cell Physiol.* 26: 775-780

Fletcher, R.A. and Nath, V. 1984. Triadimefon reduces transpiration and increases yield in water stressed plants. *Physiol. Pl.* 62: 422-426

Frances, J.F.P., Valdas, F. and Martin, R. 1995. Callus induction and culture from explants of *Erysimum scoparium* in a growth regulator free medium. *Pl. Cell Tiss. Org. Cult.* 43: 223-228

Gamborg, O.L. and Shyluck, J.P. 1981. Nutrition, media and characterization of plant cell and tissue culture. *Plant cell tissue culture: Methods and applications in Agriculture* (eds. Thorpe, T.A.). Academic Press, New York, pp. 21-44

Gamborg, O.L., Murashige, T., Thorpe, T.A. and Vasil, I.K. 1976. Plant tissue culture media. *In vitro.* 12: 176-178

*Gautheret, R. 1959. La culture des tissue Vegetaux. *Masson Paris* (French) 71: 97-103

Gawel, N.J. and Robacker, C.D. 1990. Somatic embryogenesis in two *Gossypium hirsutum* genotypes on semi-solid versus liquid proliferation media. *Pl. Cell Tiss. Org. Cult.* 23: 201-204

George, E.F. and Sherrington, P.D. 1984. Plant propagation by tissue culture. *Handbook and Directory of Commercial Laboratories.* Exegetics Ltd., England. 709 p.

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

Gupta, P.K., Mascarenhas, A.F. and Jagannathan, V. 1981. Tissue culture of forest trees-clonal propagation of mature trees of *Eucalyptus cissiodera* Hook. *Pl. Sci. Lett.* 20: 195-201

*Haberlandt, G. 1902. Culturversuche mit isolierten Pflanzenzellen . *Sber. Akad. Wiss. Wien.* (German) 111: 69-92

Hammerschlang, F. 1982. Factors affecting establishment and growth of peach shoots *in vitro*. *HortScience*. 17: 85-86

Harris, R.E. and Mason, E.B.B. 1983. Two machines for *in vitro* propagation of plants in liquid media. *Can. J. Pl. Sci.* 63: 311-316

Hu, C.Y. and Wang, P.J. 1983. Meristem, shoot tip and bud culture. *Handbook of Plant Cell Culture: 1. Techniques for Propagation and Breeding* (eds. Evans, D. A., Sharp, W. R., Ammirato, P. V. and Yamada, Y.). McMillan Publishers, NewYork, pp.177-227

Huang, L.C. and Chi, D.L. 1988. Pivotal roles of Picloram and gelrite in banana callus culture. *Env. Exp. Bot.* 28: 249-258

Hussain, S. 1995. Response of gladiolus to rapid cloning through *in vitro* techniques. M.Sc. (Hort.) thesis, Kerala Agricultural University, Trichur, 122 p.

Hussey, G. 1983. *In vitro propagation of horticultural and agricultural crops.* Plant Biotechnology Press, Cambridge, 138 p.

Hwang, S.C., Chen, C.L., Lin, J.C. and Lin, H.L. 1984. Cultivation of banana using plantlets from meristem culture. *HortScience* 19: 231-223

Israeli, Y., Lahav, E. and Reuveni, O. 1995. *In vitro* culture of bananas. *Bananas and Plantains*. (ed. Gowen S.) Chapman & Hall, London, pp: 147-178

Jarret, R.L., Rodriquez, W. and Fernandez, R. 1985. Evaluation, tissue culture propagation and dissemination of Saba and Pelipita plantains in Costa Rica. *Scientia Horticulturae* 25: 137-147

Jones, A.M. and Petolino J.F. 1988. Effects of support medium on embryo and plant production from cultured anthers of soft red winter wheat. *Pl. Cell. Org. Cult.* 253-261

Kahl, G. 1983. Wound repair and tumour induction in higher plants. *The new frontiers in plant biochemistry* (eds. Akazawa, T. and Imasei, H.). N. Junk Publishers, Hague, pp.193-216

Kartha, K.K., Gamborg, O.L., Constable, F. and Shyluk, J.P. 1974. Regeneration of cassava plants from shoot apical meristems. *Pl. Sci. Lett.* 2: 107-113

Keith, C.N. and Mc Keirse, B.D. 1986. The effect of abcisic acid on the freezing tolerance of callus cultures of *Lotus corniculatus* L. *Pl. Physiol.* 80: 766-769

Kodym, A. and Zapata-Arias, F.J. 2001. Low cost alternatives for the micropropagation of banana. *Plt. Cell Tiss. Org. Cult.* 66: 67-71

*Kogl, F., Smit, A.J.H. and Erxleben, H. 1934. Uber neues auxin (Heteroauxin) aus Hain. *IX Mitt. Zeit. Phys. Chem.* (Latin) 228:90-103

Krikorian, A.D. 1982. Cloning higher plants from aseptically cultured tissues and cells. *Biol. Rev.* 57: 128-151

Krikorian, A.D. and Cronauer, S.S. 1984a. Bananas. *Handbook of plant cell culture: 2.Crop species* (eds. Sharp, W. R., Evans, D. A., Ammirato, P. V. and Yamada, Y.). McMillan, Newyork, pp.327-348

Krikorian, A.D. and Cronauer, S.S. 1984b. Aseptic culture technique for banana and plantain improvement. *Econ. Bot.* 38: 322-331

Kumar, S.K. and Seeni, S. 1994. Fundamentals of *Anthurium* tissue culture. *Proceedings of Symposium on Anthurium-cultivation and propagation through tissue culture, April 15-16, 1993* (eds. Kumar, S.K and Seeni, S.). TBGRI, Palode, pp.5-9

Lane, W.D. 1979. The influence of growth regulators on root and shoot initiation from Flax meristem tips and hypocotyls *in vitro*. *Physiol. Pl.* 45: 260-264

*Leffering, I.L. and Soede, A.C. 1979. Weefselkweek *Anthurium andreanum* onderzoek te boven. *Vakblad Bloemistry* (Dutch) 15: 41-43

Levin, R., Gaba, V., Tal, B., Hinsch, S., De Nola, D. and Vasil, I.K. 1988. Automated plant tissue culture for mass propagation. *Biotechnology* 6: 1035-1040

Lundergan, C.A. and Janick, J. 1980. Regulation of apple shoot proliferation and growth *in vitro*. *Hort. Res.* 20:19-24

Ma, S.S. and Shii, C. T. 1972. *In vitro* formation of adventitious buds in banana shoot apex following decapitation. *J. hort. Soc. China.* 18: 135-142

Ma, S.S. and Shii, C.T. 1974. Growing banana plantlets from adventitious buds. *J. hort. Soc. China.* 20: 6

Magnaye, L.V. and Escobido, E.O. 1996. Commercial micropropagation of popular local banana cultivars. *Philipp. J. Crop. Sci.* 19: 40-41

Matielle, T. and Foncelle, B. 1988. Micropropagation of *Musa* (AAA) cv. 'Poyo' in the Ivory Coast. *Trop. Agric.* 65: 325-328

Mc Kinless, J. and Anderson, P.G. 1991. An anatomical study of rhizome bud formation induced by Paclobutrazol and adventitious root formation in *in vitro* cultures of *Lapageria rosea*. *Ann. Bot.*67: 331-338

Meins, F. 1989. Habituation: Heritable variation in the requirement of cultured plant cells for hormones. *A. Rev. Genet.* 23: 395-408

Molla, M.M.H., Khanam, M.D., Khatun, M.M., Al-Amin, M. and Malek, M.A. 2004. *In vitro* rooting and *ex vitro* plantlet establishment of BARI banana (*Musa* spp.) as influenced by different concentrations of IBA (Indole-3-Butyric acid). *Asian J. Plt. Sci.* 3: 196-199

Murali, T.P. and Duncan, E.J. 1995. The effects of *in vitro* hardening using triazoles on growth and acclimatization of banana. *Scientia Horticulturae* 64: 243-251

Murashige, T. 1974. Plant propagation through tissue culture. *A. Rev. Pl.Physiol.* 25: 135-166

Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassay with tobacco tissue cultures. *Pl. Physiol.* 15: 473-497

Novak, F. J., Afza, R., Vanduren, M., Dallos, P.M., Conger, B.V. and Xiaolang, T. 1989. Somatic embryogenesis and plant regeneration in suspension cultures of dessert (AA and AAA) and Cooking (ABB) bananas (*Musa* spp.). *Biotechnology* 7: 154-159

Novak, F. J., Afza, R., Van Duren, M. and Omar, M.S. 1990. Mutation induction by gamma irradiation of *in vitro* cultured shoot-tips of banana and plantain. *Trop. Agric.* Trinidad, 67:21-28

Okuna, K. 1996. A trial of improvement of micropropagation of *Brassica campestris* cv. Kukitochina by cost stimulation. *J. Soc. High Technol. Agric.* 8 (4): 253-263

Oliveira, R.P. and Silveira, D.G. 2001. BAP concentration and tetraploid banana micropropagation efficiency (AAAB group). *Scientia- Agricola* 58: 73-78

Panse, V.G. and Sukhatme, P.V. 1976. *Statistical Methods for Agricultural Workers.* Indian Council of Agricultural Research, New Delhi, 360 p.

Pierik, R.L.M. 1975. Callus multiplication of *Anthurium andreanum* Lind. in liquid media. *Neth. J. Agric.Sci.* 23 (4): 299-302

Prabha, J. 1993. *In vitro* multiplication and standardisation of hardening techniques in pineapple (*Ananas comosus* (L) Merr.). M.Sc (Hort.) thesis, Kerala Agricultural University, Vellanikkara, Trichur, 142 p.

Prakash, S., Hoque, M.I. And Brinks, T. 2002. Culture media and Containers. *Proceedings of Technical Meeting on Low cost Options for Tissue Culture Technology in Developing Countries, Vienna, 26–30 August 2002.* pp.29-40

Priel, M. 1991. Application of bioreactors in plant propagation. *Micropropagation: Technology and Application* (eds. Debergh, P.C. and Zimmerman, R.H.). Kluwer Academic Publishers, Dordrecht, pp.426-445

Proskauer, K. and Berman, R. 1970. Agar culture medium modified to approximate soil conditions. *Nature* 227: 1161

Rahman, M.M., Rabbani, M.G., Rahman, M.A. and Uddin, M.F. 2002. *In vitro* shoot multiplication and rooting of banana cv. Sabari. *Pakist. J. biol. Sci.* 5 :161-164

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

Rajeevan, M.S. and Pandey, R.M. 1986. Economics of mass propagation of papaya through tissue culture. *Plant tissue culture and its agricultural applications* (eds. Withers, A., Lendsey, P. G. and Alderson, P.G.). Butterworths, London, pp.211-216

Rajmohan, K. 1985. Standardisation of tissue/meristem culture techniques in important horticultural crops. Ph.D (Hort.) thesis, Kerala Agricultural University, Vellanikkara, Trichur,155 p.

Raju, C.R. 1993. Appropriate tissue culture technology for production of planting material at village level. *Proceedings of International Workshop on sustainable farming and the environment, April 23-24, 1993* (eds. Raju,C. R. and Kavitha, R.). Kochi, pp.28-30

Raju, C.R. and Kavitha, R. 1996. Tissue culture technology from COSTFORD in national development. *Proceedings of Eighth Kerala Science Congress, January 27-29,1996* (eds. Raju,C.R. and Kavitha,R.). Kochi, pp.539-541

Rao, N.K.S., Chacko, E.K., Doreswamy, R. and Narayanaswamy, S. 1982. Induction of growth in explanted inflorescence axis of banana. *Curr. Sci.* 51: 666-667

Raut, R.S. and Lokhande, V.E. 1989. Propagation of plantain through meristem culture. *Ann. Plant Physiol.* 3: 256-260.

Reghunath, B.R. 1989. *In vitro* studies on the propagation of cardomom (*Elettaria cardomomum* Maton.). Ph.D. (Hort.) thesis, Kerala Agricultural University, Vellanikkara, Trichur, 155 p.

* Reinert, J. 1958. Morphogenese und thre kontrolle an gewebekulturem aus karotten . *Naturwissen chaften* (German) 45: 344-345

Robinson, J.C. 1996. *Bananas and Plantains.* CABI, Wallingford, U. K, 238p.

Robles, C.H. and Fernandez, C.E. 2003. Accelerated micropropagation in banana species (*Musa* spp.-cavandish). *Agricultura- Tropica- et- Subtropica* 36:69-72

Rodriguez, G.A., Martin, J.R.L. and Endriquez, M.J.R. 1987. *In vitro* propagation of canary island banana (*Musa acuminata* (AAA) var. Dwarf Cavendish): Studies on factors affecting culture retention, preservation and conformity of the plants. *Acta Hort.* 212: 577-583

Saindane, P.V., Baviskar, M.P., Bhalsing, S.R., Teli, N.P., Pathak, S.S., Singh, V.P. and Maheshwari, V.L. 2001. Cost efftective hardening of tissue culture grown banana (*Musa paradisica*) var. Grand Nain plants. *Proceedings of National Symposium on Perspectives in Biotechnology, February 26-27, 1999,* (eds. Redy, S. M. and Rao, D.). Warangal, India, pp.133-138

Sandoval, J.A. 1985. *In vitro* germ plasm conservation of *Musa sp. Asociacion Bananera Nacional (ASBANA) Costa Rico* 9:21-23

Savangikar, V:A. 2002. Role of low cost options in tissue culture. *Proceedings of Technical Meeting on Low cost Options for Tissue Culture Technology in Developing Countries, Vienna, August 26–30, 2002.* pp.11-16

Shah, R.R. 1989. Cost of *in vitro* clonal propagation. *Agricultural Biotechnology* (eds. Sha, R. R. and Kukadia, M. U.) Proceedings of a National Seminar on Agricultural Biotechnology, March 7-8,1989, Navasari, pp.44-52

Shah, R.R. and Dalal, K.C. 1980. *In vitro* multiplication of *Glycyrrhiza. Curr. Sci.* 49: 69-71

Singh, N.K., La Rosa, P.C., Handa, A.K., Hasegawo, P.M. and Bressan, R.A. 1987. Hormonal regulation of protein synthesis associated with salt tolerance in plant cells. *Proc. Nat. Acad. Sci.* USA, 84: 739-743

Singh, V.P. 1993. Uniconazole (s-3307) induced cadmium tolerance in wheat. *J. Pl. Growth Regul.*12: 1-3

Skidmore, D.I., Simon, A.J. and Bedis, S. 1988. *In vitro* culture of shoots of *Pinus caribea* on liquid medium. *Pl. Cell Tiss. Org. Cult.* 14: 129-136

Skirvin, R.M. and Chu, M.C. 1979. *In vitro* propagation of 'For ever yours' rose. *HortScience* 14: 608-610

Smith, R.H. and Murashige, T. 1970. *In vitro* development of the isolated shoot apical meristem of angiosperms. *Am. J. Bot.* 57: 562-568

Smith, E.F., Roberts, A.V. and Moltley, J. 1991. The preparation of *in vitro Chrysanthemum* for transplantation to soil: The effects of eleven growth retardants on wilting. *Pl. Cell Tiss. Org. Cult.* 27: 309-313

Snir, I. And Erex, A. 1980. *In vitro* propagation of Malling Merton apple rootstocks. *HortScience*15: 597-598

Sopory S.K. 1979. Effect of sucrose, hormones and metabolic inhibitors on the development of pollen embryoids in anther cultures of dihaploid *Solanum tuberosum. Can. J. Bot.* 57: 2691- 2694

Sorvari, S. 1986. The effect of starch gelatinized nutrient media in barley anther cultures. *Ann. Agric. Fenn.* 25: 127-133

*Steward, F.C. and Mapes, M.D. 1958. Morphogenes and ihre koutrolle an gewebekulturen aus karotten. *Naturewissenschaften* (German) *45:344-*345.

Sun, Y.F. 1985. Propagation of various *Musa sp.* by tissue culture. *J. agric. Ass. China* 130: 52-57

Sundararasu. N. 2003. Studies on *in vitro* shoot tip culture of banana for the development of cost effective small scale production system. M.Sc. (Hort.) thesis, Kerala Agricultural University, Thrissur. 87 p.

Teng, W.L. 1997. Regeneration of *Anthurium* adventitious shoots using liquid or raft culture. *Pl. Cell Tiss. Org. Cult.* 49: 153-156

Thimann, K.V. 1935. On the growth hormones produced by Rhizopus. *J. Biol. Chem.* 103: 79- 291

Thorpe, T.A. 1978. Physiological and biochemical aspects of organogenesis *in vitro. Frontiers of plant tissue culture* (ed. Thorpe,T. A.).University of Calgary Press, Canada, pp. 49-58

Thorpe, T.A. and Patel, K.R. 1984. Clonal propagation: adventitious buds. *Cell culture and somatic cells genetics: 1. Laboratory procedures and their applications* (ed. Vasil, I.K.). Academic Press, Orlando, pp.49-57

Vasil, I.K., Ahuja, M.R. and Vasil, V. 1979. *Advances in Genetics*. Academic press, Newyork, 127-215 p.

*Vessey, J.C. and Rivera, J.A. 1981. Meristem culture of banana. *Turrialba* 31: 162-163

Vuylsteke, D.R. 1989. Shoot-tip culture for the propagation, conservation and exchange of *Musa* germplasm. *Practical manuals for handling crop germplasm in-vitro, 2.* IBPGR, Rome

Vuylsteke, D. and De Langhe, E. 1985. Feasibility of *in vitro* propagation of bananas and plantains. *Trop. Agric.* 62: 323-328

Vuylsteke, D., Swennen, R. and De Langhe, E. 1990. Tissue culture technology for the improvement of African plantains. *Sigatoka Leaf Spot Diseases of Bananas* (eds. Fullerton, R.A. and Stover, R.H.). INIBAP, Montpellier, pp. 316-337

Wainwright, H. 1988. Overcoming problems in establishing micro-propagates-guidelines for growers. *Prog. Hort.* 2: 67-72

Wang, P. J. 1978. Clonal multiplication of *Cryptomeria japonica* D. *in vitro*. Studies and essays in commemoration of the golden jubilee of Academia sinica, Taipei, pp.559-566

Weatherhead, M.A., Burdon, J. and Hanshaw, G.G. 1978. Some effects of activated charcoal an additive to plant tissue culture media. *Z. Pflanzenphysiol.* 89: 141

Webb, K.J. and Street, H.E. 1977. Morphogenesis *in vitro* of *Pinus* and *Picea*. *Acta Hort.* 78: 259-269

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

Yeoman, M.M. 1986. *Plant cell culture technology.* Blackwell Scientific Publications, Melbourne, 230 p.

Yeoman, M.M. and Mc Leod, A.J. 1977. Tissue (callus) cultures techniques. *Plant tissue and cell culture* (ed. Street, H.). Blackwell Scientific Publications, Oxford, pp. 31-59

Zhou, H.Q., Liu, L.Y. and Xu, X.M. 2003. Techniques for growing in vitro cultured bag plantlets of banana. *South China fruits* 32:30-31

Ziv, M. 1986. *In vitro* hardening and accilimatisation of tissue culture plants. *Plant tissue culture and its agricultural applications* (eds. Withers, L.A. and Alderson, P.G.). Butterworths, London, pp. 187-196

* Originals not seen

Appendix I.

Composition of Murashige and Skoog Medium (1962)

Major elements	mg l^{-1}
$CaCl_2.2H_2O$	440.0
$FeSO_4.H_2O$	27.8
KNO_3	1900.0
KH_2PO_4	170.0
$MgSO_4.7H_2O$	370.0
NH_4NO_3	1650.0
Na_2.EDTA	37.5
Minor elements	
Co $Cl_2.6H_2O$	0.025
$CuSO_4.5H_2O$	0.025
H_3BO_3	6.20
KI	0.83
$MnSO_4$	22.3
$NaMoO_4.2H_2O$	0.25
$ZnSO_4$	8.6
Organic constituents	
G lycine	2.0
Myo- inositol	100.0
Nicotinic acid	0.5
Pyridoxine HCl	0.5
Thiamine HCl	0.1
Sucrose	30.0 g
Agar	7.0 g

IN VITRO PROPAGATION IN TWO COMMERCIAL DIPLOID BANANAS OF KERALA

By

SAPHEERA C. P.

ABSTRACT OF THE THESIS

submitted in partial fulfilment of the requirement for the degree of

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ABSTRACT

The studies on *in vitro* propagation in two commercial diploid bananas of Kerala, namely *Musa* (AB) ' Njalipoovan' and *Musa* (AA) ' Nivedyakadali', were carried out in the Plant Tissue Culture Laboratory, Department of Pomology and Floriculture, College of Horticulture, Vellanikkara. Sword sucker, peeper sucker and eye bud explants were used for the study. The results revealed that surface sterilization of explants with a combination treatment of absolute alcohol 70.0 per cent for 1 minute + mercuric chloride 0.1 per cent for 10 minutes + dipping in antibiotic cefotaxime 0.01 per cent solution for 15 minutes recorded cent per cent survival of explant. Physical injury of the apical dome of shoot tip explants with a longitudinal cut was found to be best in enhancing the release of axillary buds in the culture. Addition of ascorbic acid into the media at the rate of 50.0 mgl^{-1} reduced media and explant discolouration.

For the better and faster explant establishment and growth of sword sucker and peeper sucker explants, MS medium containing BA 3.0 mgl^{-1} and NAA 0.5 mgl^{-1} was found to be best for Njalipoovan and Nivedyakadali. In shoot tip culture of Njalipoovan, maximum number of multiple shoots was recorded in the medium containing NAA 0.5 mgl^{-1} and BA 8.0 mgl^{-1} or BA 5.0 mgl^{-1} alone in full MS. In the case of Nivedyakadali also BA 5.0 mg l^{-1} in the full MS recorded the maximum number of multiple shoots. Out of the two cytokinins tried (BA and Kinetin), BA was found to be more efficient for the induction of axillary shoots in the culture. Sword sucker, peeper sucker and eye bud explants were found to be equally effective with respect to the number of axillary shoots from a single explant, in both cultivars.

Sucrose 3.0 per cent and table sugar 2.0 per cent produced maximum number of multiple shoots in Njalipoovan. In the case of Nivedyakadali too table sugar at 2.0 per cent recorded maximum number of multiple shoots. Half strength of vitamins in full MS observed more number of multiple shoots in cv.

Njalipoovan. In the case of cv. Nivedyakadali, half tablet of vitamin B complex (2.0 mgl^{-1}) in the medium resulted in more number of shoots.

For Njalipoovan the treatment involving full MS + NAA 2.0 mgl^{-1} took significantly least number of days for root initiation with cent per cent rooting. The number of roots per shoot was maximum in half MS + 10.0 mgl^{-1}. In the case of Nivedyakadali, full MS medium containing NAA 1.0 mgl^{-1} took least number of days for root initiation with cent per cent rooting. Of the various carbon sources tried with Njalipoovan, number of roots produced was significantly higher in the medium containing table sugar 3.0 per cent and table sugar 2.0 per cent. In the case of Nivedyakadali number of roots produced was higher in the medium containing table sugar 3.0 per cent. Full strength of vitamins in full MS medium resulted in maximum number of roots and maximum length of roots. Nivedyakadli recorded maximum number of roots in full MS media containing half strength of vitamins whereas length of root was maximum in full strength vitamin concentration. Number of roots and length of root were higher in the media containing triademefon 1.0 mg l^{-1}.

The most suitable rooting medium for *ex vitro* establishment of Njalipoovan and Nivedyakadali plantlets was 1:1(v/v) mixture of vermiculite + coir pith compost. Sand + cow dung + goat manure (1: 1: 1) mixture was the best potting mixture for subsequent growth and vigour of *in vitro* derived diploid banana. Plantlets kept under mist chamber recorded the highest survival percentage. Mud pot, black polythene bag and white polythene bag were found to be equally effective containers for hardening of plantlets. Weekly spraying of full MS solution recorded maximum number of leaves, length of leaves, breadth of leaves and height of plantlets in both the diploid cultivars.