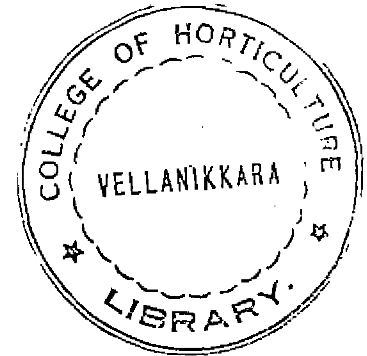
STANDARDISATION OF *IN VITRO* PROPAGATION TECHNIQUE IN BANANA



By

JYOTHI BHASKAR

THESIS

submitted in partial fulfilment of the requirement for the degree of

MASTER OF SCIENCE IN HORTICULTURE

Faculty of Agriculture
Kerala Agricultural University

Department of Pomology & Floriculture

COLLEGE OF HORTICULTURE

Vellanikkara, Trichur

1991

Dedicated to my family

DECLARATION

I hereby declare that this thesis entitled **"Standardisation of *in vitro* propagation technique in banana"** is a bonafide record of research work done by me during the course of research and that the 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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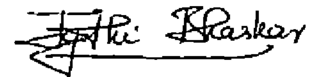

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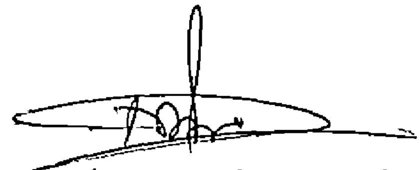
Certified that this thesis entitled "**Standardisation of *in vitro* propagation technique in banana**" is a record of research work done independently by **Mrs.JYOTHI BHASKAR** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.



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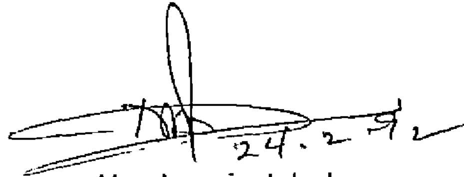

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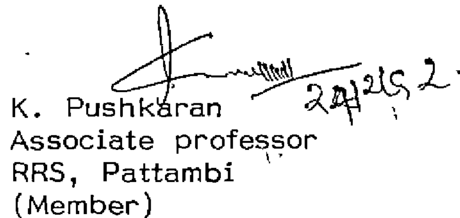

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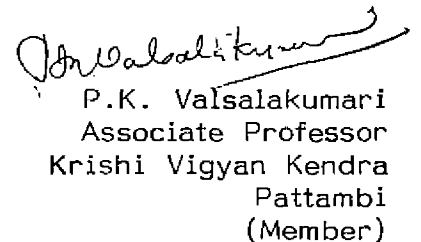

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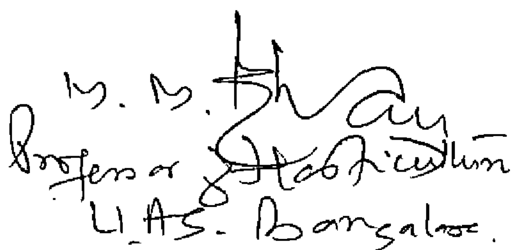

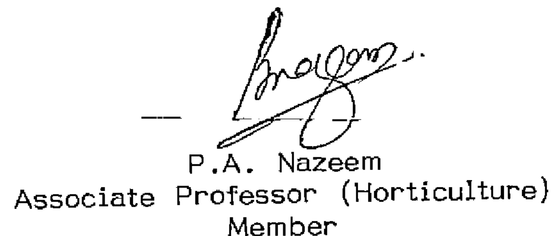

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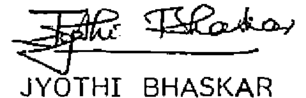

JYOTHI BHASKAR

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ABBREVIATIONS

AC	Activated charcoal
BA	Benzyladenine
BPM	Basal proliferation medium
CH	Casein hydrolysate
cv	Cultivar
CW	Coconut water
2,4-D	2,4-Dichlorophenoxyacetic acid
EDTA	Ethylene diamine tetra acetate
GA	Gibberellic acid
HCl	Hydrochloric acid
IAA	Indole-3-acetic acid
IBA	Indole-3-butyric acid
2ip	2-isopentenyl adenine
KC	Knudson's C (1975) medium
MS	Murashige and Skoog's (1962) medium
NAA	1 -Naphthalene acetic acid
NaOCl	Sodium hypochlorite
NaOH	Sodium hydroxide
PCPA	Pentachlorophenoxyacetic acid
ppm	parts per million : mg/l
PVP	Poly vinyl pyrrollidene
2,4,5-T	2,4,5-Trichlorophenoxyacetic acid
v/v	volume in volume
w/v	weight in volume

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INTRODUCTION

INTRODUCTION

Banana (*Musa* spp.) is one of the most important tropical fruits of the world. India ranks second in average under this crop (2,77,919 hectares) with an annual production of 47,84,648 tonnes. The major banana growing areas in the country are in the states of Kerala, Tamil Nadu and Maharashtra, together occupying 49.35 per cent of total area, contributing 55.16 per cent to the annual production. The incidence of deadly diseases such as bunchy top virus disease, bacterial wilt, sigatoka leaf spot diseases and infestation of nematodes and other pests are often attributed as the major production constraints. Further more, the varietal situation in India is very complex, each region having preference to certain dessert/cooking varieties. 'Nendran' (AAB) is the most popular dual purpose variety grown in Kerala. While 'Palayankodan' (AAB) is the most common dessert variety grown in homesteads, the Red banana (AAB) is highly popular in Southern districts of Kerala. The polyclonal homestead system of cultivation prevalent in Kerala is unique and is attributable to the low productivity of banana in the state.

Conventional propagation of banana is through suckers. As the production of suckers per plant is rather low, the rate of multiplication is very slow. Since forcing buds under field conditions is a tedious and time consuming procedure, amassing

of a sufficient amount of suitable planting material is slow. This is a problem when a new clone is being generated to replace the older plantings or when a large number of uniform planting materials of a particular variety is needed for conduct of a trial. The prevalence of disease and the need for generating clean planting stock in large quantities has stimulated recently a surge of interest in production of clonal material of both cooking and dessert bananas by the use of aseptic micropropagation techniques.

The major advantages offered by _in vitro_ propagation technique in banana are increased rate of multiplication, clean disease free, uniform propagules, easier transportation, improved crop yield, scope for selection and multiplication of elite ecotypes, and year round availability of planting materials.

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 and especially in a state like Kerala wherein 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 commercial clones of Kerala viz. Nendran (AAB), Palayankodan (AAB) and Red banana (AAA), a study was taken up in the Department of Pomology, College of Horticulture, Vellanikkara during the period 1988-90.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

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. This success was achieved using medium containing inorganic salts, sugar, amino acids, vitamins and some undefined organics such as malt extract and coconut water. The use of undefined substances caused some problems because it was not possible to quantify their contribution.

Vasil *et al*. (1979) reported that the techniques of micropropagation has been standardised 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 (Hu and Wang, 1983).

According to Murashige (1974) there are three possible routes for *in vitro* propagule multiplication; (i) enhanced release of axillary buds (ii) production of adventitious shoots through organogenesis and (iii) somatic embryogenesis.

In the first, route, meristems like shoot tips are cultured which assures genetic uniformity of the progeny to a great extent (Chand and Roy, 1980; Rao and Lee, 1986). This method is being adopted commercially in various crop species for rapid clonal multiplication. The second route, callus mediated somatic organogenesis, is not recommended for clonal propagation, but may be ideal for recovery of useful variant lines. Somatic embryogenesis, the third route is limited to a few species but results in the most rapid mode of plant regeneration (Evans *et al.*, 1981).

The tissue culture techniques, increasingly being used for crop improvement, are the meristem/shoot apex culture and somatic embryogenesis for rapid propagation of selected genotypes; the cell culture for the recovery of useful variants; *in vitro* mutagenesis for obtaining desirable mutants; anther culture for the production of haploid plants and homodiploids; embryo culture and *in vitro* pollination/fertilization for effecting distant crosses; protoplast

culture for facilitating somatic hybridisation and introduction of new genetic material into plant cells and cryopreservation for long term storage of germplasm. Drew *et al.* (1989) demonstrated the transmission of banana bunchy top virus in an apparently symptomless condition in micropropagated bananas.

The review has been confined to the *in vitro* propagation of banana, the important aspects of which are dealt under five major head

1. standardization of explant
2. standardization of media
3. standardization of culture conditions
4. *in vitro* rooting of shoots
5. hardening off and planting out of *in vitro* plantlets.

1. Standardization of explant

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

1.1. Apical bud explant

Ma and Shii (1972; 1974) reported adventitious bud formation in vitro in 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) and Aravindakshan (1989).

Berg and Bustamante (1974) were the first to show that virus free plants of Cavendish group (AAA) could be obtained from meristems of lateral buds of virus injected plants, by a combination of heat treatment and aseptic culture. The method involved the use of Knudson's medium (1946) with Berthelots salts, thiamine HCl, coconut water and casein hydrolysate.

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 sheathing leaf bases enclosing the axillary buds regenerated multiple plantlets. Individual shootlets, when separated and subcultured, produced multiple shoots.

Krikorian and Cronauer (1984b) observed that one of the major features of shoot apex culture technique is that shoot multiplication can be induced by releasing buds at the leaf bases. Subculturing could be carried out from the proliferating mass of shoots formed. Cronauer and Krikorian (1984a and b) established rapidly multiplying cultures of dessert banana clones, Phillipine Lacatan and Grande Naine (AAA) and plantain clones Saba and Pelipita (ABB) on a modified MS medium supplemented with 5 mg/1 BA. Multiple shoot cultures could be induced by longitudinally splitting a young cultured shoot through the apex and placing each half upright on semi-solid 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 under field conditions and gave rise to mature plants with uniform growth and normal yield of fruit. The medium used was MS containing 0.4 mg thiamine HCl, 100 mg L - tyrosine, 100 mg myo-inositol, 2 mg IAA, 2 mg kinetin, 160 mg adenine sulphate, 30 g sucrose and 8 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 g/l activated charcoal for root and shoot development.

Banerjee and De Langhe (1985) reported a tissue culture technique for banana for rapid clonal propagation and storage under minimal growth conditions. Excised shoot apices were cultured

on modified MS medium supplemented with IAA 0.18 mg/1 and BA 2.30 mg/1. Established cultures were then successfully stored at 15°C with a light intensity of 1000 lux for 13 to 17 months.

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. Propagation cultures were initiated by spliting shoot tips along their longitudinal axis and reculturing individual pieces on basal medium supplemented with 5 mg/1 BA. Transfer of axillary shoots to hormone free medium resulted in rapid and extensive root formation.

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 proliferative 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.

Damasco and Barba (1985) reported successful shoot tip culture for Saba bananas. They reported that by repeating subculturing at an interval of 2 months, 2,00,000 plantlets could be obtained from a single explant in 10 months time when cultured in MS medium with 10 mg/l BA. According to Sun (1985) 93 out of 103 banana clones developed adventitious buds in meristem culture on a MS medium supplemented with 5 mg/l BA and 2 mg/l IAA.

Gupta (1986) used heat therapy and meristem tip culturing in various cultivars of banana and plantain for rapid clonal propagation of mosaic free plants. Wong (1986) reported, in vitro multiplication of banana (Musa sp.) from shoot tip explants isolated from lateral suckers. Using explants with apical domes, a total of 22 cultivars (genomes AA, AAA, AAAA, AAB, AB and ABB) were successfully cultured on a modified MS medium containing BA and IBA. Shoot tip explants were induced to produce multiple shoot initials in the presence or absence of apical domes, but the survival rates were higher when apical domes were retained. Cultivars varied widely in their response to cytokinins (necessary for multiple shoot production), BA being consistently more effective than kinetin, although kinetin stimulated vigorous root growth.

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. Subculturing were carried out at four weekly intervals. Huang and Chi (1988) also reported the use of banana shoot tips for in vitro culturing. Mateille and Foncelle (1988) developed an improved micropropagation method for Musa AAA cv. 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.

1.2. Floral apices explants

In banana the reinitiation of vegetative growth from floral apex explants was reported by many workers (Rao et al., 1982; Rowe, 1984; Cronauer and Krikorian, 1985a and b; Bakry et al., 1985; Balakrishnamoorthy and Sreerangaswamy, 1988; Doreswamy and Sahijram, 1989; Drew et al., 1989; Aravindakshan, 1989).

Rao et al. (1982) reported the induction of growth in explanted inflorescence axis of Robusta banana. Excised inflorescence axis discs of 5 mm thickness were cultured in MS medium supplemented with auxins and cytokinins at different concentrations and combinations. Although callus was formed and some root formation occurred no other organogenesis could be induced. Bakry et al. (1985) could develop vegetative shoots from inflorescence explants of banana by culturing them in medium containing MS salts and Morel vitamins with or without growth regulators. In plantain AAB sub-group 10-15 plants/explant could be obtained, where as in AAA sub-group and Musa acuminata more than 50 plants could be produced.

Cronauer and Krikorian (1985a) produced banana plants from excised floral apices of Musa acuminata cv. Dwarf Cavendish, in modified MS medium supplemented with BA (5 mg/l) and coconut water (10 per cent v/v). Under these conditions, the determinate floral buds were converted into a multiplying vegetative shoot system. Rooting was obtained by treating plants with NAA (1 mg/l) and activated charcoal (0.025 per cent w/v). Balakrishnamoorthy and Sreerangaswamy (1988) could regenerate plants from inflorescence tip explants in Robusta and Monthan varieties. The inflorescence tip measuring 10 to 15 mm in size was surface sterilized in mercuric chloride 0.1 per cent solution for five minutes and was cultured in MS medium supplemented with BA 2.5 to 5 mg/l. Multiple shoots were produced on repeated subculturing (4 to 12 shoots per explant). Rooting could be induced by culturing individual shoots in media containing 1 mg/l NAA.

Doreswamy and Sahijram (1989) cultured excised floral apices of three banana cultivars viz. 'Chandrabale', 'Rasthali' and 'Robusta' on MS medium supplemented with cytokinins and auxins. The cultures reverted to vegetative state and produced a mass of green leafy shoots which were kept in a state of active growth by repeated subculturing. They had also found that the male flower clusters at different stages of development located on the peduncle subtending and distal to the meristematic zone reverted to vegetative state when cultured in vitro. Drew et al. (1989)

also reported the applicability of floral apex as an explant for multiplying banana plants *in vitro*. For culture initiation MS medium supplemented with 160 g/l adenine sulphate, 2 mg/l IAA, 2 mg/l kinetin was used. Shoot proliferation was achieved when MS medium was upplemented with 5 mg/l BA.

1.3. Selection of explant

The size of the explant used, though varies depending upon the plant species, influences the success or otherwise 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 (1984b) 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 eliminated from the meristem will also be more. In banana, the usual size of shoot bud explant ranges from 0.5 mm to 3 mm in size with two to three leaf primordia covering the apical dome (Doreswamy *et al.*, 1983; Cronauer and Krikorian, 1984a; Vuylsteke and De Langhe, 1985; Jarret *et al.*. 1985).

In banana, the various explants tried varied in their morphogenetic potential. Inflorescence discs (Rao *et al.*, 1982) immature and mature fruit tissue (Mohanram and Steward, 1964), apical bud (Doreswamy *et al.* 1983; Cronauer and Krikorian, 1984a and b; Jarret *et al.* 1985), leaf sheaths with rhizomatous base

excised from meristematic area (Banerjee et al., 1987; Novak et al., 1989), inflorescence apex (Bakry et al. 1985; Balakrishnamoorthy and Sreerangaswamy, 1988) all resulted in varied results. Vuylsteke and De Langhe (1985) reported that no difference could be observed in growth response between shoot tips harvested from eyes, buds, peepers, suckers or the parental pseudostem in banana.

In in vitro systems it was reported that the juvenile tissues respond better than older tissues (Murashige, 1974; Rajmohan, 1985). Due to the decreased morphogenetic ability of mature material, it has not been possible to apply the technique developed for juvenile material to mature plants. Mante and Tepper (1983) suggested that the lateral shoots produced by an isolated apex could be related to the age of the explant.

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

Different workers used various sterilants at different concentrations and length of time in banana tissue culture. The shoot tips were washed with a liquid detergent (Teepol) 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 (1984b) advised sterilization with 1 to 2 per cent commercial bleach (0.0525 per cent 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. Rao et al., (1982) used mercuric chloride (0.2 per cent) solution for 10 to 15 minutes to sterilize the inflorescence explants. According to Fitchet and De Winnaar (1988) 10 per cent calcium hypochlorite give the best result.

It has been fairly well documented that tissues taken from field grown plants are not equally amenable to culture conditions throughout the year. Litz and Conover (1978) reported that in papaya the time of the year that cultures were established and the cultural conditions in the field were crucial for success of this procedure. According to Reghunath (1989) the most ideal period for collecting shoot bud explants of cardamom from the field was from January to May during which period the rate of culture contamination was less.

2. Standardization of media

One of the most important factors governing the growth and morphogenesis of plant tissue culture is the composition of the medium. The earliest and widely used media were those of White (1943) and Heller (1953). However, since 1962, Murashige

and Skoog's (1962) medium has gained general acceptability among plant tissue culturists. The major constituents of tissue culture medium are mineral salts, carbon and energy source, vitamins, plant growth regulators and other organic compounds.

The salt composition of several media has been reviewed by Gamborg et al. (1976). The Murashige and Skoog (1962) medium is the most widely used one for banana tissue culture. It is high in nitrates, potassium and ammonia. Although MS medium was specifically developed for tobacco tissue culture, it has been successfully used for culturing a variety of plants. However, Banerjee and De Langhe (1985) omitted copper sulphate and zinc sulphate from MS medium for better response. Several research workers reported desired success in Murashige and Skoog (1962) medium (Cronauer and Krikorian, 1986; Gupta, 1986; Wong, 1986; Novak et al., 1989; Aravindakshan, 1989).

A carbon energy source is inevitable in any tissue culture media. Sucrose is the most widely accepted carbon source. The growth of the culture is not only affected by the type of carbon source used but also by the concentration of a particular sugar used in the medium. Thorpe (1978) showed that in tobacco, at least part of the sucrose requirement is osmotic. Mateille and Foncelle (1988) reported that lower concentration of sucrose (10 g/l) is most favourable for shoot elongation and rooting.

The success or otherwise of an *in vitro* system is much dependent upon the choice of correct growth regulators and their use in optimum concentrations (Krikorian, 1982). Skoog and Miller (1957) obtained in tobacco cultures different types of organogenesis by varying the levels of auxins and cytokinins. When the level of cytokinin is high relative to auxin, shoots are induced and when it is low roots are induced. At intermediate concentrations, the tissue grows as unorganised callus.

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 2ip. Auxins most frequently used to induce *in vitro* rooting are IAA, NAA and IBA (Murashige, 1974). In banana both IBA (0.5 mg to 2 mg/l) and NAA (0.02 mg to 2 mg/l) have been reported to induce rooting of shoots and embryoids (Krikorian and Cronauer, 1984a; Banerjee *et al*., 1987).

No universal ratio of auxin and cytokinin has so far been developed for root and shoot induction. Hu and Wang (1983) described the young shoot apex as an active site for auxin biosynthesis. 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). Rodriguez *et al*. (1987) stated that high concentration of BA (5 x 10^{-6} g/ml) is necessary for intense proliferation of shoots.

Murashige and Skoog's (1962) vitamin mixture consisting of myo-inositol (100 mg/l), nicotinic acid (0.5 mg/l) and pyridoxine (0.5 mg/l) is used for banana tissue culture. Additions like glycine (0.2 mg/l), ascorbic acid (100 mg/l) (Banerjee *et al*., 1986) and thiamine HCl 0.4 mg/l (Vuylsteke and De Langhe, 1985; Krikorian and Cronauer, 1984a) are 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.

Favourable effects of coconut water in *in vitro* cultures have been reported by many workers (Van Overbeek *et al*., 1941; Bapat and Rao, 1977; Kunisaki, 1980). The discovery of Pollard *et al*. (1961) that myo-inositol was present in coconut water and had growth promoting activity, led to the inclusion of inositol in plant tissue culture media. A number of cell division factors are present in coconut water (Shantz and Steward, 1952; Letham, 1974; Vanstaden and Drewes, 1974).

To the media activated charcoal (AC) is added to prevent phenol exudation from micro cuttings and explants and to induce rooting of cuttings. The effects of AC may be 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, in vitro cultures for rooting 0.025 per cent w/v AC is commonly used (Krikorian and Cronauer, 1984a).

3. Culture conditions

Light, temperature and humidity conditions provided inside the culture room plays a significant role in the success of any tissue culture technique. The light intensity, quality and duration are the three major factors affecting the growth of in vitro culture (Murashige, 1974, 1977). Murashige (1977) considered the optimum day light period to be 16 hours for a wide range of plants. For banana shoot tip culture, a light intensity of 1000 to 3000 lux and a photoperiod of 16 hours are recommended by various workers (Krikorian and Cronauer, 1984b; Damasco et al., 1984), while continuous light of about 3000 lux is reported by Vuylsteke and De Langhe (1985); complete darkness for callus induction was reported by Novak et al. (1989).

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 in in vitro systems. Most tropical species require a higher temperature

(27 ± 2°C). The optimum temperature for bananas is reported to be 28 ± 2°C (Krikorian and Cronauer, 1984a).

Humidity is rarely a problem except in arid climates, where rapid drying of the 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. In some urban environments, it may be necessary to filter the air entering the culture room (Yeoman, 1986).

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 general, most success in organogenesis is achieved with explants on agar based solid medium [Cronauer and Krikorian (1984a and b); Hwang *et al.* (1984); Banerjee and De Langhe (1985)]. In bananas, shoot multiplication was reported to be stimulated by transferring tissue pieces alternatively between liquid and semi-solid media of same composition at two weeks interval (Cronauer and Krikorian, 1985b). Huang and Chi (1988) reported the advantage of using gelrite instead of agar in banana tissue culture. They found that the medium solidified with gelrite eliminated tissue and medium discoloration.

Plant cells in culture require an acidic pH and an initial pH of 5.5 to 5.8 is optimum (Gamborg and Shyluk, 1981). They

also reported that pH changes during the growing cycle of a cell suspension culture. Though initially there will be a decrease to below pH 5.0 subsequently the pH increases and may reach 6.0 or even higher. In banana, the pH of the culture media were adjusted to 5.8 using potassium hydroxide or sodium hydroxide before autoclaving (Berg and Bustamante, 1974; Cronauer and .rikorian, 1984a and b; Banerjee and De Langhe (1985)

4. *In vitro* rooting of shoot

Although a number of plants root spontaneously in culture, shoots of most species multiplied *in vitro* lack a root system. Rooting can be achieved either by subculturing to medium lacking cytokinin, with or without a rooting hormone or by treating the shoots as conventional cuttings after removal from sterile culture (Yeoman, 1986). 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). Since auxin is essential for root initiation, majority of stage III media contains 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 (Yeoman, 1986).

According to Hu and Wang (1983) three phases are involved in rhizogenesis namely, induction, initiation and elongation. Among the auxins NAA has been the most effective one for induction of rooting (Ancora *et al.*, 1981). The root elongation phase has been found to be very sensitive to auxin concentration. High concentrations of auxins inhibited root elongation (Thimann, 1977).

Several researchers have shown that *in vitro* rooting can successfully be achieved by reducing salt concentrations in the media, particularly in MS. Abundant rooting was observed when the salt concentration in the medium was reduced to one half, one-third or one-fourth of the standard strength (Lane, 1979; Skirvin and Chu, 1979). But in such cases, poor top growth resulted sometimes (Gupta *et al.*, 1981). In banana, abundant rooting was observed, when the concentration of the macrosalts of the MS medium was reduced to half (Banerjee and De Langhe, 1985; Vuylsteke and De Langhe, 1985). 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).

Debergh and Maene (1981) pointed out that rooting *in vitro* may represent the most labour intensive part of micropropagation, because of the need to manipulate shoots on an individual basis rather than in clusters used during proliferation stage. Roots formed *in vitro* do not adapt easily to normal conditions without

some delay in growth. Yeoman (1986) suggested that where it is practicable there is considerable advantage in rooting shoots directly into a conventional medium and elimiinating a further costly transfer in sterile culture. The most efficient method of rooting directly is to transfer shoots into sterile blocks, tubes or pots. Mateille and Foncelle (1988) developed an improved micropropagation method for the Poyo banana clone in which shoot elongation and rooting were obtained simultaneously on a hormone free medium containing 10 g/l sucrose.

5. Hardening-off and planting out of plantlets

Acclimatization is necessary in the case of micropropagated plants because in vitro plant material is not adapted for in vitro conditions (Brainerd and Funchigami, 1981). The success in acclimatization of micropropagated plants is largely dependant upon not only the post-transfer growth conditions but also the pre-transfer culture conditions (Ziv, 1986). Tissue cultured plants are very poorly adapted to resist the low relative humidity, higher light levels and more variable temperatures 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 - 100 per cent relative humidity and obtained 55 - 60 per cent survival of _in vitro_ produced jack plantlets.

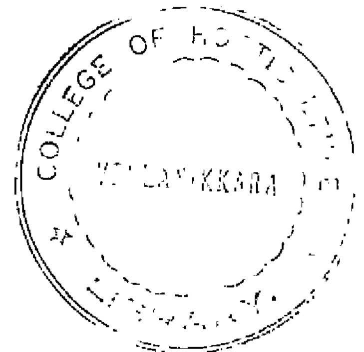
In Poyo banana clone 100 per cent survival was achieved when plantlets were grown in the glass house (Mateille and Foncelle, 1988).

Physical, chemical and biological properties of the potting mixture 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 per cent banana plantlets survived after transfer to methyl bromide treated soil. Cronauer and Krikorian (1985) successfully established banana plantlets in Pro Mix : Vermiculite (v/v) potting mixture. Drew _et al_. (1989) successfully established micropropagated plants in a peat, perlite and polystyrene bead (1:1:1) mixture with 40 per cent natural light in a perspex humidity cabinet, maintained at more than 90 per cent relative humidity by a Penn humidistat controller and 'Defensor-505' humidifier.

Careful field evaluations of micropropagated banana plants are few. Recently certain reports have been published from

plantings and trials (mostly small scale) conducted elsewhere (Hwang _et al_., 1984; Reuveni _et al_., 1986; Ramcharan _et al_., 1987; Pool and Irizarry, 1987; Arias and Valverde, 1987). A shortcoming of these reports is that the exact material used and the methodology adopted is not precisely given (Krikorian, 1989). He also stated that special emphasis should be given to the number of plants evaluated, to stability or lack thereof and to recurrence of plant crop effects in the ratoon. Hwang _et al_. (1984) reported that the plantlets originating from excised decapitated shoot apices of banana suckers, established well under field conditions and gave rise to mature plants with uniform growth and normal fruit yield. Ramcharan _et al_. (1987) observed the performance of tissue cultured plantlets of 2 clones designated Maricongo and Dwarf in U.S. Virgin Island. The clones were found to exhibit considerable variability. By the second harvest, 21 per cent Maricongo had reverted to Tall French types and 38 per cent of Dwarf had reverted to Dwarf French types. But the character of the planting and first ratoon crops were similar. Vuylsteke _et al_. (1988) studied the phenotypic variation among _in vitro_ propagated banana cultivar Agbagba (genome AAB). Observations on the plant crop and successive ratoons revealed five forms of phenotypic variation at a frequency of 6 per cent. Fitchet (1989) stated that tissue culture technique can be used for speeding up evaluation of new cultivars. In a study Robinson (1990a) evaluated the performance of tissue culture

progeny and compared the observations with sucker grown plants. He found that tissue culture plants produced 6 to 7 more leaves before flower emergence and they were taller than suckers at flowering stage due to the production of more leaves per plant and consequently more internodes. Tissue culture plants were found to reach flowering stage two weeks to a month earlier than with suckers. Robinson (1990b) reported that overall yield/annum was 20 per cent higher with tissue culture plants than with sucker grown plants due to the production of larger bunches coupled with shorter cycle time. The increased yield from tissue culture plants was probably related to their greater height, stem circumference and vigour.



MATERIALS AND METHODS

MATERIALS AND METHODS

The studies on the *in vitro* propagation of banana were carried out at the Plant Tissue Culture Laboratory in the Department of Pomology and Floriculture, College of Horticulture, Vellanikkara during 1988-90. The details regarding the experimental material, methodology of experiments and analytical techniques adopted are presented step-wise in the chapter.

In vitro propagation through enhanced release of axillary buds was used for the present study (Murashige, 1974). The different stages involved are illustrated in Fig. 1.

1. Physiological preconditioning of the explant and explant establishment (Stage 1)

1.1. Selection of varieties

The explants for the study were collected from three popular banana cultivars of Kerala namely Nendran (AAB), Palayankodan (AAB) and Red banana (AAA). The morphological description of these varieties are given in Table 1.

1.2. Source of explants and pretreatment

Three different types of explants used for the present study were shoot tip (the main apical bud collected from a five month old sword sucker of banana), eye bud (the main apical bud

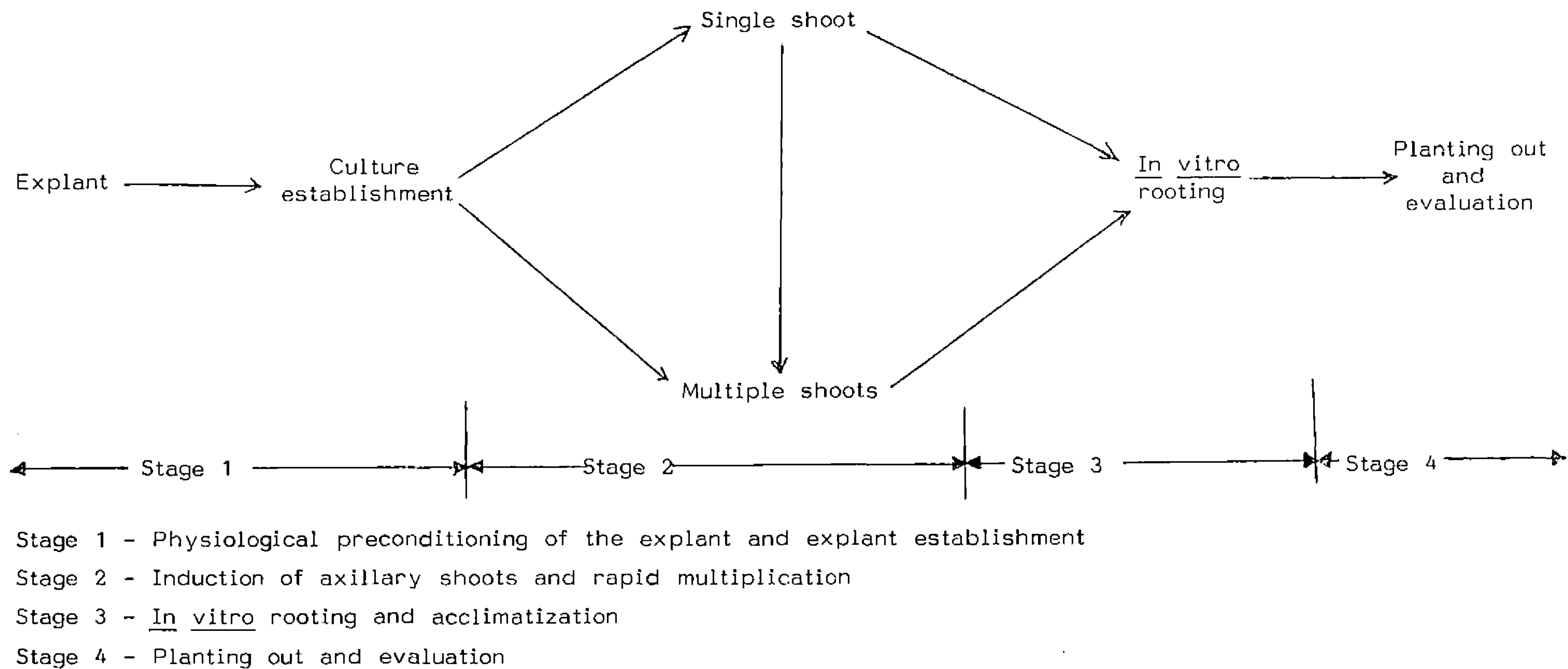


Fig. 1. *In vitro* cloning procedure in banana

Table 1. Morphological features of banana cultivars utilised for *in vitro* culture studies

	Red banana	Palayankodan	Nendran
Genomic status	AAA	AAB	AAB
Pseudostem	Greenish purple, with dark brown blotches	Yellow green with brownish black blotches	Yellowing green with reddish tinge and with dark brown blotches towards the base of the petiole
Leaves	Petiole not clasping the pseudostem, margins of petiole spreading	Petiole not clasping the pseudostem, margins of petiole slightly enclosed	Petiole not clasping the pseudostem, margins of petiole enclosed
Peduncle	Short and pubescent	Medium-long and pubescent	Medium-long and glabrous
Inflorescence	Female axis pendulous and male axis slightly ageotropic	Female axis semi-pendulous and male axis positively geotropic	Female axis semi-pendulous and male axis positively geotropic
Bract	Decidious, shoulder high. Outside colour dull purple and crimson inside.	Decidious, shoulder low. Outside colour brownish purple and bright crimson inside.	Persistent, shoulder low. Outside colour brownish purple and bright crimson inside.
Female flowers	Creamy white. Stigma rich yellow.	Cream colour flushed with pink. Stigma colour cream.	Cream colour with pink blotches towards the base. Stigma creamy white.
Male flowers	Creamy white with fertile stamen	Cream flushed with pink with fertile stamen	Creamy white flushed with pink. Stamen fertile.
Bunch	Hands and fingers compact. Fingers almost cylindrical with indistinct apex.	Hands and fingers compact. Fingers slightly angular with prominent apex.	Hands and fingers loose. Fingers angular with distinct apex.
Ripe fruit	Red in colour, do not easily separate from the hand, rind medium thick, pulp colour yellowish cream, soft juicy, taste sweet.	Yellow in colour, firmly attached to the hand, rind thin, pulp colour yellowish cream, taste sweet.	Yellow in colour, firmly attached to the hand, rind thick, pulp yellowish orange, thick, taste sweet.

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collected from the small buds seen on the sides of the mother rhizome of banana) and floral apices (the apical bud collected from the distal end of the inflorescence axis of banana) (Fig.2).

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 shown in Fig. 3. The treatments were,

T_1 apical dome intact

T_2 apical dome cut longitudinally

T_3 apical dome with (+) cut

T_4 half portion of the apical dome

T_5 quarter portion of the apical dome.

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

1.3. Explant establishment

1.3.1. Collection and preparation of explants for culturing

Shoot tip explants were collected from five month old sword suckers grown in the field. Suckers after separation from the mother rhizome (from which the bunch has already been harvested) were detopped and were reduced to a size which measured about

1a, 1b, 1c. Source and excision of eye bud explant.

2a, 2b, 2c. Source and excision of shoot tip explant.

3a, 3b, 3c, 3d, Source and excision of floral apex explant.

4. Culture tube containing the inoculation medium.

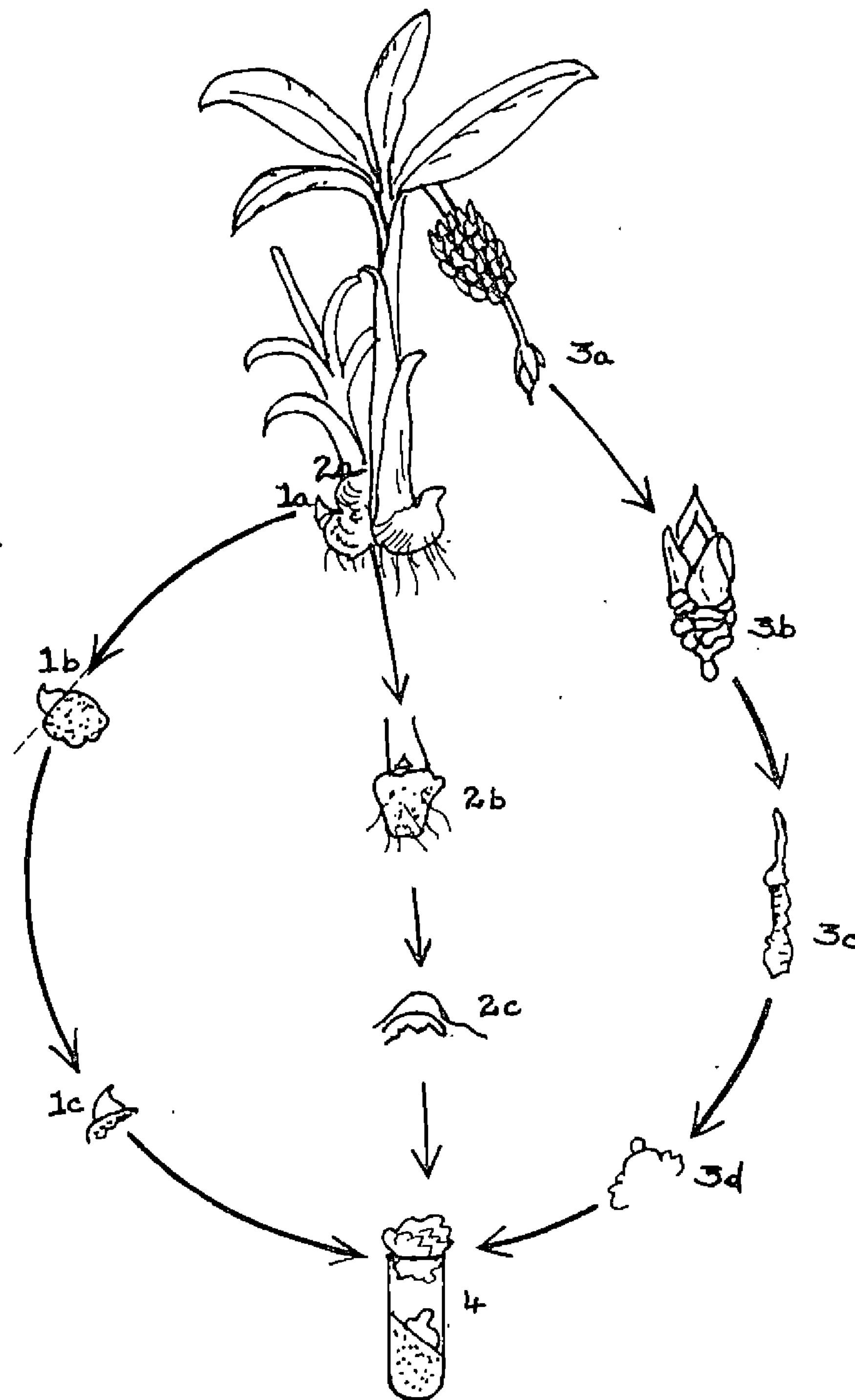


Fig. 2.

A diagrammatic representation of the source of Shoot tip, eyebud and floral apex explant.

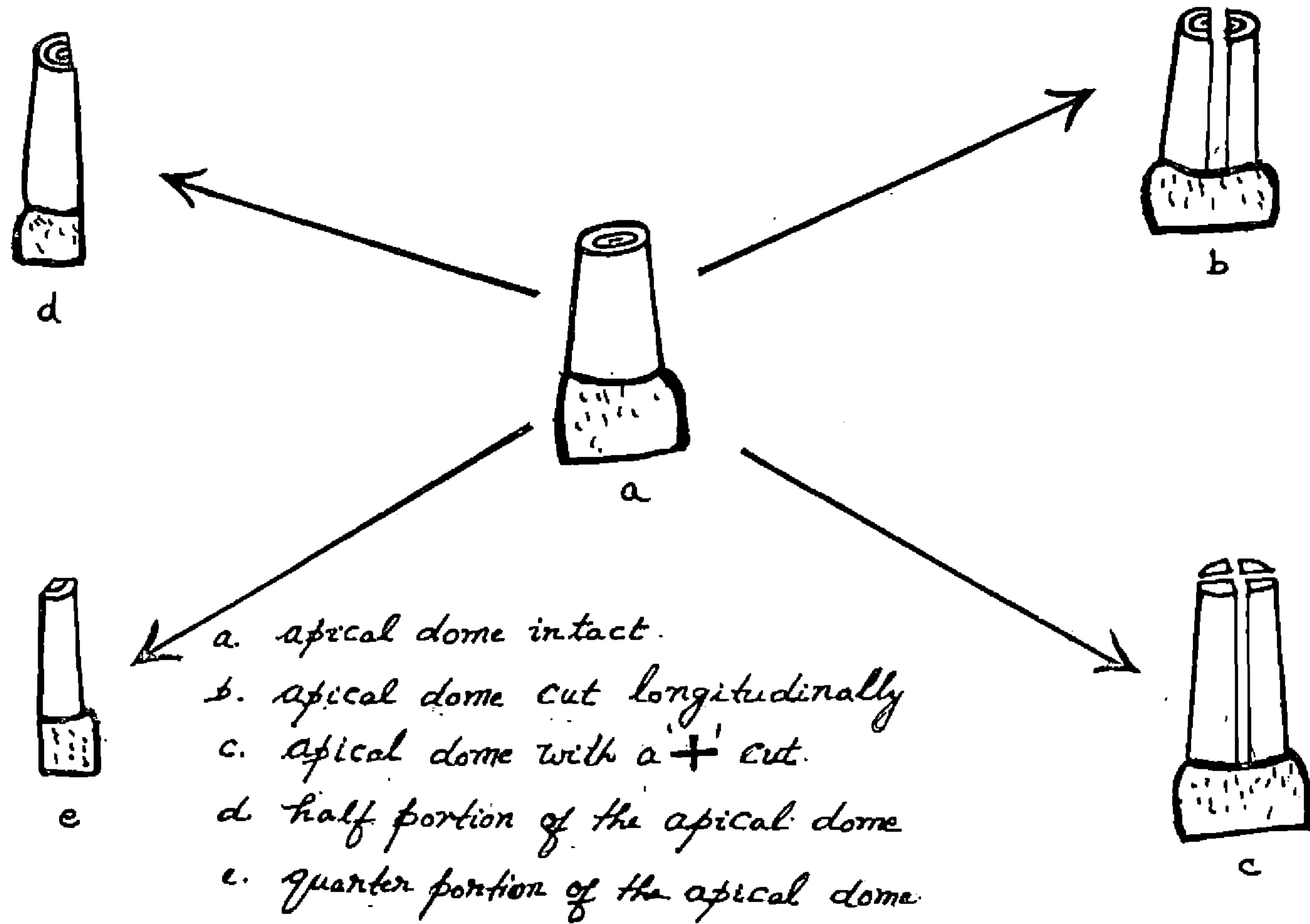


Fig. 3. Types of incisions made on the shoot tip explant of banana.

5 cm in length retaining a small portion (1 cm length) of the rhizome tissue. In the case of eye bud explants, small buds seen on the surface of the mother rhizome were removed using a sharp knife without injuring the central bud also retaining a small portion of the rhizome tissue. For floral apex explants collection, the male buds were collected from the distal end of the peduncle after the completion of the female phase of the bunch. The subtending bracts of the floral apex with their associated hands of female flowers were removed one by one in a stepwise manner until they became too small to be removed by hand. The explants after collection were immediately taken to the laboratory, where they were first washed thoroughly in tap water to remove all the dirt and soil particles adhering to them. After drying them in between folds of tissue paper they were again washed with sterile distilled water. Further sterilization procedures were carried out under perfect aseptic conditions in a 'Thermadyne' laminar air flow cabinet. The explants were then subjected to surface sterilization using different chemicals as given below.

Explant	Sterilant	Concentration (per cent)	Duration
1. Shoot tip	Bleaching powder	10.00	10 min
	Absolute alcohol	95.00	30 s
	Mercuric chloride	0.05	15 min
	Mercuric chloride	0.10	10 min
	Mercuric chloride	0.20	5 min
2. Eye bud	Bleaching powder	10.00	10 min
	Absolute alcohol	95.00	30 s
	Mercuric chloride	0.05	15 min
	Mercuric chloride	0.10	10 min
	Mercuric chloride	0.20	5 min

3. Floral apex	Bleaching powder	10.00	5 min
	Absolute alcohol	95.00	30 s
	+		+
	Mercuric chloride	0.05	10 min
	Mercuric chloride	0.05	15 min
	Mercuric chloride	0.10	10 min
	Mercuric chloride	0.20	3 min

Observations on the percentage of culture survival (as exhibited by the retention of the green colour), percentage of cultures contaminated and percentage of cultures showing explant death were made on twenty explants per treatment after three weeks of culturing.

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

Sterilized shoot tips were further reduced in size by trimming down the cut surface of the rhizomatous tissues and leaf bases, using sterilized scalpel and forceps. In the case of eye buds, two to three outer bud leaves were removed and the extra rhizome tissue was trimmed off retaining a 5 mm base fragment of the underlying corm. Before inoculation, two to three subtending bracts from the floral apices were also removed using sterilized forceps and needles. The explants were then inoculated in suitable culture medium.

1.3.2. Influence of season of explant collection

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 culture establishment was more and contamination rate minimum. Ten shoot tips were collected for this purpose from uniform sized field grown banana plants (cv. Red banana) during each month and cultured in MS (semi-solid) basal proliferation medium containing BA 5.0 ppm. Observations on the percentage of explant establishment, percentage of cultures contaminated and number of axillary shoots initiated per explant were recorded after four weeks of culturing.

To reduce media and explant discolouration of banana different treatments were given to explants, the details of which are given below.

Treatment	Levels
1. Keeping in running tap water before inoculation	½, 1, 1½, 2 h
2. Rinsing in cysteine - HCl solution before inoculation	25, 50, 100 mg/l
3. Rinsing in PVP solution before inoculation	250, 500, 1000 mg/l
4. Adding ascorbic acid into the media	10, 30, 50 mg/l

Observations on the 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.

1.3.3. Basal medium of culture

In order to study the morphogenetic response of banana explants in culture, the most widely accepted MS medium (Murashige and Skoog, 1962) was tried. The composition of the medium is given in Table 2. This medium contained only the basal salts (macro and micro), vitamins and sucrose as reported in the original publication. This was supplemented with cytokinins, auxins and gibberellins at various concentrations as detailed below.

Explant	Treatments
1. Shoot tip	4 x 4 combinations of BA (0.5, 1.0, 2.0, 3.0 ppm) and NAA (0.25, 0.50, 1.00, 2.00 ppm)
2. Eye bud	4 x 2 combinations of BA (0.50, 1.00, 2.00, 3.00 ppm) and IAA (0.50, 1.00 ppm)
3. Floral apex	3 x 3 combinations of BA (0.25, 0.50, 1.00 ppm) and GA (0.50, 1.00, 2.00 ppm)

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

Table 2. Composition of Murashige and Skoog (1962) medium

	Quantity	Volume made up	Volume pipetted
Solution A			
Ammonium nitrate	16.5 g	1 litre	100 ml
Potassium nitrate	19.0 g		
Magnesium sulphate	3.7 g		
Potassium dihydrogen phosphate	1.7 g		
Solution B			
Calcium chloride	4.4 g	500 ml	50 ml
Solution C			
Boric acid	0.62 g	100 ml	1 ml
Manganese sulphate	2.23 g		
Zinc sulphate	0.86 g		
Potassium iodide	0.083 g		
Sodium molybdate	0.025 g		
Solution D			
Ferrous sulphate	2.78 g	500 ml	5 ml
Sodium EDTA	3.73 g		
Solution E			
Cobalt chloride	0.025 g	1 litre	1 ml
Copper sulphate	0.025 g		
Solution F			
Nicotinic acid	50 mg	100 ml	1 ml
Pyridoxine HCl	50 mg		
Thiamine HCl	10 mg		
Glycine HCl	200 mg		
Sucrose	30.00 g		
Inositol	100.00 mg		
Agar	7.00 g		

The chemicals used for preparing the culture medium were of analytical grade from British Drug House (BDH), Sisco Research Laboratories (SRL), Merck or Sigma. Standard procedures (Gamborg and Shyluk, 1981) were followed for the preparation of the media. Stock solutions of major and minor nutrients were prepared first, by dissovling the required quantity of chemicals in double glass distilled water and stored under refrigerated conditions in amber coloured bottles. The stock solution of nutrients were prepared fresh in every four weeks and that of vitamins, aminoacids 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 upto about 1000 ml by adding double glass distilled water. The pH of the solution was adjusted using an electronic pH meter using 0.1 N HCl/NaOH. Agar was added to the medium and final volume made up exactly to one litre. The solution was then melted by keeping in a water bath maintained at a temperature of 90-95°C. The medium (15 ml) was then poured hot to the oven sterilized culture vessels which were previously rinsed twice with double glass distilled water. The container with the medium were then tightly closed with non-absorbant cotton wool plugs. Corning brand test tubes and conical flasks were used.

In order to ensure aseptic condition of the medium, the containers plugged with cotton were autoclaved, for 15-20 min at 15 psi pressure and 121°C temperature. After sterilization, the culture vessels were immediately transferred to an air-conditioned culture room.

1.3.4. Inoculation of explants

All the inoculation operations were carried out under perfect aseptic conditions in a 'Thermadyne' laminar air flow cabinet. The cotton wool plug of the culture vessel was removed and the vessel neck was first flamed over a gas burner kept in the chamber. The sterile explants were quickly transferred to the culture vessels containing suitable culture medium, using sterilized forceps. The neck of the culture vessel was once again flamed and quickly replaced the cotton wool plug. The culture vessels were then transferred to a culture room where they were incubated at a temperature of 27 ± 2°C. Artificial illumination was provided using cool white flourescent lamps. Photoperiod was fixed as 16 h per day and it was automatically regulated by using a diurnal timer.

1.3.5. Response of varieties

In the present study three types of explants of three banana cultivars namely Nendran (AAB), Palayankodan (AAB) and Red banana (AAA) were utilised. To study whether these explants behave

differently or not, a separate experiment was conducted. These explants were grown on MS semi-solid medium supplemented with 0.5 ppm NAA and 3.0 ppm BA for a period of four weeks. Observations on the number of days taken for cultural survival were made on four explants per treatment and on the percentage of culture survival.

2. Induction of axillary shoots and rapid shoot multiplication (Stage 2)

2.1. Standardisation of basic proliferation medium (BPM)

All the trials on stage 2 were carried out on Murashige and Skoog, 1962 (MS) and modified Knudson's C, 1975 (KC) media. The composition of Knudson's C medium is given in Table 3. Explants used for induction of axillary shoots were 30 mm long shoots from establishment culture. The details of the trial on standardisation of basic proliferation medium utilising growth regulating substances viz., cytokinins and auxins are presented below.

Media	Explant	Treatment
1. MS	Shoot tip	1. 4 x 4 combinations of BA (4.0, 6.0, 8.0, 10.0 ppm) and NAA (0, 0.5, 1.0, 1.5 ppm)
	Eye bud	2. 4 x 4 combinations of kinetin (4.0, 6.0, 8.0, 10.0 ppm) and NAA (0, 0.5, 1.0, 1.5 ppm)
	Floral apex	3. 4 x 3 combinations of BA (0.5, 1.0, 2.0, 3.0 ppm) and IAA (0, 0.5, 1.0 ppm)
2. KC		1. 5 levels of BA (2.0, 4.0, 6.0, 8.0, 10.0 ppm)

Table 3. Composition of Knudson's C medium (modified)
(Rowe and Richardson, 1975)

Solution	Constituents	Quantity	Volume made upto stock	Volume pipetted
A	Calcium nitrate	10 g	1000 ml	100 ml
	Ammonium sulphate	5 g		
	Potassium dihydrogen phosphate	2.5 g		
	Magnesium sulphate	2.5 g		
	Berthelots solution			
	Magnese sulphate	1 g		
	Boric acid	25 mg		
B	Potassium iodide	250 mg	500 ml	0.5 ml
	Nickel chloride	25 mg		
	Cobaltous chloride	25 mg		
	Zinc sulphate	50 mg		
	Copper sulphate	25 mg		
	Sulphuric acid	0.5 ml		
	Iron solution			
C	Ferrous sulphate	2.785 g	500 ml	5 ml
	Sodium EDTA	3.725 g		
	Thiamine solution			
D	Thiamine HCl	250 mg	250 ml	1 ml
	Sucrose	40 g		
	Agar	5 g		
	pH	5.6 - 5.8		

Observations on percentage of culture developing shoots and number of shoots produced per culture were made on four explants after four weeks of culturing.

Regular subculturing of the proliferated shoots on to medium containing high concentration of BA was found to increase the number of shoots per culture. A trial was conducted to assess the multiplication rate of shoots, on continuous subculturing at four week interval in ten serial subcultures. Semi-solid MS medium containing BA 5.0 ppm was used for the particular study. Cultures derived from shoot tip explants of three banana cultivar were used for the study. Observations were recorded on four cultures per treatment on the number of shoots produced per culture and the percentage increase in number of shoots over the initial culture.

2.2. Standardisation of medium supplements

Studies were conducted to determine the effect of coconut water on culture survival, culture growth and axillary shoot induction from different explants viz. shoot tip, eye bud and floral apex by adding CW at the rate of 0, 50, 100, 150 and 200 ml per litre of the medium. Semi-solid KC medium containing BA 4.0 ppm was used for the study.

Observations on percentage of culture survival were made on twenty explants and the number of shoots produced per culture

and the visual rating on culture growth were made on five explants after four weeks of culturing.

2.3. Standardisation of physical conditions

After inoculation, the flasks/tubes were properly labelled and incubated under 27 ± 3°C temperature. Artificial illumination was provided using cool white fluorescent lamps. An experiment was conducted to study the effect of light on multiple shoot formation and growth of shoots, the details of which are given below.

Explant	Treatment	Level
1. Shoot tip 2. Eye bud 3. Floral apex	Light	0, 20 W, 40 W, 80 W, 120 W for 16 hours followed by 8 hours darkness.

The photoperiod was provided for 16 h per day and it was automatically regulated by using a diurnal timer. Observations on the number of shoots produced per explant, the length of the longest leaf (cm) and the visual rating of growth response were made on five explants after four weeks of culturing.

3. *In vitro* rooting and acclimatization (Stage 3)

3.1. *In vitro* rooting

3.1.1. Standardisation of basal medium

All the trials on *in vitro* rooting were conducted on KC medium, half and full strength MS medium. Shoots (2 to 3 cm

length) excised from shoot proliferating cultures were utilised as explants for these trials. The different auxins and their levels tried for rooting of banana shoots are the following.

Media	Treatment
1. MS (with full concentration of mineral salts)	1. NAA (0.0, 5.0, 10.0, 15.0 ppm) 2. IBA (5.0, 10.0 ppm)
2. MS (with half concentration of mineral salts)	1. NAA (0.0, 5.0, 10.0, 15.0 ppm) 2. IBA (5.0, 10.0 ppm)
3. KC (with full concentration of mineral salts)	1. NAA (0.0, 5.0, 10.0, 15.0 ppm)

Observations on the percentage of cultures showing root initiation, number of roots produced per shoot, the nature of roots and the number of days taken for root initiation were made on four cultures after two weeks of culturing. Treatments found to be best for one variety (Red banana) were repeated for the other two cultivars namely Nendran and Palayankodan in comparative performance trials.

3.1.2. Standardisation of medium supplements

A trial was conducted to observe the effect of various medium supplements on rooting of banana shoots excised from shoot proliferating cultures. The details of various compounds and their levels tested are given below.

Treatment	Level
1. Sucrose	1.5, 3.0, 4.5, 6.0 per cent
2. Activated charcoal powder	0.025, 0.05, 0.1, 0.2 per cent

Observations were made on four explants after two weeks of culturing on percentage of root initiation, the number of roots produced per shoot and the number of days taken for root initiation.

3.2. Acclimatization

3.2.1. Standardisation of hardening treatments

In order to acclimatize the banana shoots produced *in vitro*, initially a trial to standardise the hardening treatments were carried out. Rooted shoots from stage 3 were utilised for conducting this experiment. The rooted plantlets with 3 to 5 leaves and 8 to 10 roots were taken out from the culture vessels with the help of forceps and needles. Then the agar adhering to the roots were completely removed by washing with distilled water. The plantlets were than subjected to various pre-transfer (before planting in the potting mixture) and post-transfer (after planting in the potting mixture) treatments as listed below.

Pre-transfer treatments	Post-transfer treatments
1. Direct planting into the potting mixture	Keeping in the open
2. Keeping in distilled water for 8 h and then for 8 h in 1/10th MS solution under mist (provided using a microscope cover)	Keeping in the culture room
3. Keeping in 1/10th MS solution for 2 days (under mist, provided using a microscope cover)	Covering the plants and pots with plastic cover for four weeks

Observation were made on ten plantlets on the percentage of plantlet survival after one month of planting out.

3.2.2. Influence of potting mixture on plantlet establishment

The type of potting mixture in which the plants are potted are found to effect the growth of plants. To study this, plantlets were potted in various kind of potting mixtures made up of different ingredients as given below.

1. Sand
2. Vermiculite : Sand (1:1 v/v)
3. Red earth : Sand : soil : compost (1:1:1:1)

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

Observation were made on ten plantlets on the percentage of plantlet survival after one month of planting out.

3.2.3. Effect of containers on plantlet survival

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

1. White plastic cups of 250 ml capacity with drainage holes at the bottom.
2. Black plastic pots of 250 ml capacity with drainage holes at the bottom.
3. Tubular polythene bags of 15 cm diameter and 20 cm length with 8 punch holes each on 2 sides.

Observation were made on ten plantlets on the percentage of plantlet survival after one month of planting out.

3.2.4. Use of chemical sterilants for plantlet survival

To ensure better growth of the plantlets the autoclaved potting mixture was treated with various chemical sterilants as listed below.

1. Mercuric chloride 0.2%
2. Bavistin 0.1%
3. Emesan 0.1%

Observations were made on ten plantlets, on the percentage f plantlet survival after one month of planting out.

. Field planting and evaluation (Stage 4)

After 1 month of acclimatization, the plants were planted ut in earthern pots of size 20 cm height and 15 cm diameter ontaining ordinary non-sterile potting mixture (1 sand : 1 soil 1 red earth : 1 dried and powdered cattle manure v/v). A utrient solution containing half concentration of MS mineral salts vas given as additional nourishment once in a week. Plants were ept under partial shade.

Observations were recorded on plant survival, plant height, eaves per plant and length of longest leaf, at three stages ie., fter four weeks under mist, eight weeks after planting out and welve weeks after planting out.

tatistical analysis

The data generated from the various experiments were ubjected to statistical analysis in completely randomised design, vherever necessary as per Panse and Sukhatme (1985).

RESULTS

RESULTS

The results on the *in vitro* propagation of banana conducted at the Plant Tissue Culture Laboratory of the College of Horticulture, Vellanikkara during the period 1988-90 are presented in this chapter.

1. Explant

1.1. Surface sterilization of explants

The results of the surface sterilization of three types of explants viz., shoot tip, eye bud and floral apex using various sterilants are presented in Table 4.

Of the various sterilants tried, mercuric chloride (0.05-0.2 per cent) gave better sterilization of explants than bleaching powder and/or absolute alcohol. An initial rinsing of floral apex explants with 95 per cent absolute alcohol for 30s followed by mercuric chloride treatment (0.05 per cent) for 10 min resulted in least rate of contamination (10 per cent) and maximum percentage of explant survival (85 per cent) and lowest rate of explant death (5 per cent). For shoot tip and eye bud explants, the best sterilant mercuric chloride (0.2 per cent) resulted in very low contamination rate (5 per cent) and maximum percentage of explant survival (80 per cent) eventhough the rate of explant death was high (15 per cent). Bleaching powder was ineffective as a surface sterilant for all the three explants tried, as it resulted in very

Table 4. Standardisation of surface sterilization methods of banana explants

Medium: MS (Semi solid)

Explant	Sterilant	Concentration (per cent)	Duration	Contamination (per cent)	Death of culture due to over concentration (per cent)	Survival (percentage culture alive)
1 Shoot tip	Bleaching powder	10	10 min	100	0	0
	Absolute alcohol	95	30 s	95	0	0
	Mercuric chloride	0.05	15 min	55	5	40
	Mercuric chloride	0.10	10 min	45	10	45
	Mercuric chloride	0.20	5 min	5	15	80
2 Eye bud	Bleaching powder	10	10 min	100	0	0
	Absolute alcohol	95	30 s	100	0	0
	Mercuric chloride	0.05	15 min	60	5	35
	Mercuric chloride	0.10	10 min	50	10	40
	Mercuric chloride	0.20	5 min	5	15	80
3 Floral apex	Bleaching powder	10	5 min	95	0	5
	Absolute alcohol + Mercuric chloride	95 + 0.05	30 s + 10 min	10	5	85
	Mercuric chloride	0.05	15 min	35	10	55
	Mercuric chloride	0.10	10 min	25	15	60
	Mercuric chloride	0.20	3 min	10	20	70

Mean of twenty observations

Culture period - three weeks

high contamination rate and lowest survival percentage eventhough the rate of explant death due to excess sterilant was zero.

1.2. Season of explant collection and culture establishment

Shoot tip collection during November to April period, was found to result in least contamination rate (30-40 per cent) and maximum explant survival (50-60 per cent) (Table 5). The period from May to October recorded greatest contamination rate (50-60 per cent) and lowest survival rate (40-50 per cent). Maximum number of shoots (4.0-4.2/shoot tip) were recorded when shoot tips were collected during July-August and it was minimum during May (2.4/shoot tip) and November (2.6/shoot tip). No significant influence was noted among the months in the induction of axillary shoots, except in the case of two months (May and November).

2. Culture medium

2.1. Culture establishment medium

In order to standardise a suitable culture establishment medium, detailed trails were conducted with different levels of cytokinin (BA), auxins (NAA and IAA) and gibberellin (GA) in MS (semi-solid) medium using excised explants namely shoot tip, eye bud and floral apex explants. All the explants which were not contaminated turned green within a period ranging from 8 to 29 days depending upon the kind and concentration of

Table 5. Influence of season of collection of explants on the establishment of culture and induction of axillary shoot growth in shoot tip culture of banana (cv. Red banana)

Medium: MS + BA 5.0 ppm

Month	Contamination* (per cent)	Survival* (percentage culture alive)	Shoots per** explant
January	40	60	3.2
February	30	70	3.8
March	30	70	3.4
April	40	60	3.2
May	50	50	2.4
June	60	40	3.8
July	60	40	4.0
August	60	40	4.2
September	50	50	4.0
October	50	50	3.6
November	40	60	2.6
December	30	70	4.0
Mean			3.52
CD (5%)			0.98
SEm±			0.49

* Mean of ten observations
** Mean of five observations
Culture period - four weeks

phytohormones and the type of explant used which indicated their capacity to establish in the culture. The results are presented below.

2.1.1. Effect of NAA and BA

Establishment of culture occurred in all the 16 combinations of NAA and BA tried (Table 6). For the establishment of culture shoot tip explants took 8 to 23 days, eye bud explants, about 9 to 24 days and floral apex explants 14 to 26 days.

All the three explants tried, namely shoot tip, eye bud and floral apex, took only minimum number of days (8, 9 and 14 respectively) for the establishment of culture at the treatment combination involving NAA 0.5 ppm + BA 3.0 ppm.

2.1.2. Effect of IAA and BA

Establishment of culture occurred in all the eight combinations of IAA and BA tried (Table 7). Number of days taken for culture establishment varied from 11 to 23 days for shoot tip explant, 12 to 23 days for eye bud explant and 21 to 26 days for floral apex explant.

The treatment combination involving IAA 1.0 ppm + BA 3.0 ppm was found to be the best compared to the remaining treatment combinations, as it was observed that at this combination of IAA

Table 6. Effect of NAA + BA on the establishment of culture using different banana explants (cv. Red banana)

Medium: MS

Treatments	Shoot tip	Eye bud	Floral apex
	Time taken for culture establishment (days)	Time taken for culture establishment (days)	Time taken for culture establishment (days)
NAA 0.25 ppm + BA 0.5 ppm	21	23	25
,, + ,, 1.0 ppm	17	20	24
,, + ,, 2.0 ppm	16	17	23
,, + ,, 3.0 ppm	15	17	22
NAA 0.5 ppm + BA 0.5 ppm	23	23	23
,, + ,, 1.0 ppm	19	21	23
,, + ,, 2.0 ppm	14	17	20
,, + ,, 3.0 ppm	8	9	14
NAA 1.0 ppm + BA 0.5 ppm	21	23	25
,, + ,, 1.0 ppm	19	21	25
,, + ,, 2.0 ppm	16	18	23
,, + ,, 3.0 ppm	11	13	17
NAA 2.0 ppm + BA 0.5 ppm	21	24	26
,, + ,, 1.0 ppm	20	22	24
,, + ,, 2.0 ppm	20	21	22
,, + ,, 3.0 ppm	9	19	20
CD (5%)	1.66	1.66	1.66
SEm±	0.82	0.82	0.82

Mean of three observations
Culture period - four weeks

Table 7. Effect of IAA + BA on the establishment of culture using different banana explants (cv. Red banana)

Medium: MS

Treatments	Shoot tip	Eye bud	Floral apex
	Time taken for culture establishment (days)	Time taken for culture establishment (days)	Time taken for culture establishment (days)
IAA 0.5 ppm + BA 0.5 ppm	21	21	23
,, + ,, 1.0 ppm	22	21	24
,, + ,, 2.0 ppm	21	21	22
,, + ,, 3.0 ppm	20	20	22
IAA 1.0 ppm + BA 0.5 ppm	23	23	26
,, + ,, 1.0 ppm	20	21	24
,, + ,, 2.0 ppm	17	18	21
,, + ,, 3.0 ppm	11	12	21
CD (5%)	1.73	1.73	1.73
SEm±	0.81	0.81	0.81

Mean of three observations
Culture period - four weeks

and BA, all the three explants tried viz. shoot tip, eye bud and floral apex took the minimum number of days (11, 12 and 21 respectively) for the establishment of culture.

2.1.3. Effect of GA and BA

In all the nine combinations of GA and BA tried as shown in Table 8, explants turned green, but the time taken for the establishment of culture was more than that was observed in the initial trials conducted using NAA/BA and IAA/BA combinations. The number of days taken for the establishment of culture was minimum (23) at the combination of BA 1.0 ppm + GA 0.5 ppm.

2.1.4. Effect of explant sources

From the initial trials conducted to study the effect of different combinations of phytohormone on the establishment of culture, the treatment combination NAA 0.5 ppm + BA 3.0 ppm and IAA 1.0 ppm + BA 3.0 ppm were found to be the best. In order to study the effect of explant sources on the establishment of culture of banana cultivars, MS medium containing NAA 0.5 ppm + BA 3.0 ppm was used (Table 9).

In all the three banana cultivars used for the study, it was noted that floral apex explants took maximum number of days (13-18) for establishment while shoot tip explants the minimum number of days (8-11) for culture establishment. Similarly, when

Table 8. Effect of GA + BA on the establishment of culture using different banana explants (cv. Red banana)

Medium: MS

Treatments	Shoot tip	Eye bud	Floral apex
	Time taken for culture establish-ment (days)	Time taken for culture establish-ment (days)	Time taken for culture establish-ment (days)
BA 0.25 ppm + GA 0.5 ppm	27	27	28
,, + ,, 1.0 ppm	27	27	29
,, + ,, 2.0 ppm	28	28	29
BA 0.5 ppm + GA 0.5 ppm	25	26	28
,, + ,, 1.0 ppm	25	27	28
,, + ,, 2.0 ppm	26	28	29
BA 1.0 ppm + GA 0.5 ppm	23	24	25
,, + ,, 1.0 ppm	24	25	26
,, + ,, 2.0 ppm	25	26	28
CD (5%)	1.72	1.84	1.72
SEm±	0.82	0.87	0.82

Mean of three observations
Culture period - four weeks

Table 9. Comparative performance of shoot tip, eye bud and floral apex explants of three banana cultivars on the establishment of culture

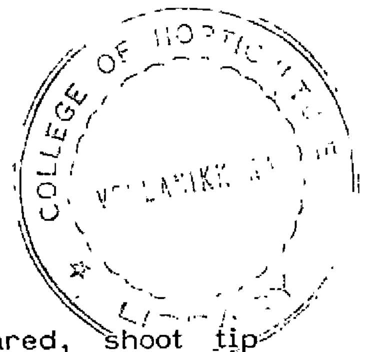
Medium: MS + BA 3.0 ppm + NAA 0.5 ppm

Explant	Nendran		Palayankodan		Red banana	
	Time taken* for culture establishment (days)	Culture** established (per cent)	Time taken* for culture establishment (days)	Culture** established (per cent)	Time taken* for culture establishment (days)	Culture** established (per cent)
Shoot tip	8.0	90	11.0	90	8.0	90
Eye bud	9.0	80	12.0	80	9.0	80
Floral apex	18.0	80	13.0	90	14.0	90
CD (5%)	2.00		NS		2.00	
SEm±	0.81		0.81		0.81	

* Mean of three observations

** Mean of ten observations

Culture period - four weeks



the percentage of culture established were compared, shoot tip explants recorded maximum percentage (90) in all the three banana cultivars tried.

In banana cv. Palayankodan, no significant difference was observed between the three explants tried with respect to the number of days taken for the establishment of culture. In the other two cultivars Red banana and Nendran, except floral apex explant, the other explants behaved in a similar manner as they took only eight to nine days for the establishment of culture. In Nendran, when shoot tip explant recorded 90 per cent of culture establishment, eye bud and floral apex explant recorded only 80 per cent culture establishment. In the case of Palayankodan and Red banana, both shoot tip and floral apex explants recorded 90 per cent of culture establishment, while eye bud explant recorded only 80 per cent of culture establishment.

2.1.5. Discolouration of media and explant in *in vitro* culture of banana

Adding ascorbic acid into the culture medium, resulted in maximum percentage of culture survival (70-90) and maximum percentage of culture without media and explant discolouration (60-90), than all the other treatments (Table 10). Among the different concentration of ascorbic acid tried, ascorbic acid 50 mg/l was found to be the best as cent per cent of the culture survived

Table 10. Effect of various treatments on the discolouration of media and explant in *in vitro* culture of banana (cv. Red banana)

Medium: MS + BA 3.0 ppm + NAA 0.5 ppm

Treatment		Culture survival (per cent)	Culture without media and explant discolouration (per cent)
1. Keeping in running tap water for	½ h	60	0
,,	1 h	80	10
,,	1½ h	60	20
,,	2 h	50	20
2. Rinsing in cysteine-HCl solution	25 mg/l	60	10
,,	50 mg/l	60	20
,,	100 mg/l	60	40
3. Rinsing in PVP solution	250 mg/l	50	10
,,	500 mg/l	50	10
,,	1000 mg/l	50	10
4. Adding ascorbic acid into the media	10 mg/l	70	60
,,	30 mg/l	80	80
,,	50 mg/l	90	90

Mean of ten observations
Culture period - four weeks

showed no signs of media and explant discolouration. Rinsing the explants in cysteine-HCl solution (100 mg/l) just before inoculation resulted in 60 per cent culture survival and 40 per cent cultures without media and explant discolouration.

2.1.6. Physical injury treatments

The results of the trial conducted by giving five types of physical injury to shoot tip explants (Table 11), showed variation in the percentage of established cultures (75-95 per cent). When the percentage of cultures exhibiting growth were compared, apical dome which was injured by giving a longitudinal cut exhibited the maximum percentage (90), which was on par with the other types of physical injury tried (60-75 per cent). In the visual rating of growth response, dividing the apical dome into two halves was found to be best as the explant greening was observed within a period of one to two week and from the half portion three to four adventitious buds were found to develop (Plate 1). In the case of the explant receiving a longitudinal cut, though the explant turned green within one to two weeks, only four to five adventitious buds developed (Plate 2). When explant with intact apical dome was used, only a single shoot developed (Plate 3).

2.2. Culture proliferation medium

The results of the detailed trials conducted to modify the

Table 11. Response of shoot tip explants to different type of physical injury treatments (cv. Red banana)

Medium: MS + BA 3.0 ppm + NAA 0.5 ppm

Treatment	Culture established (per cent)	Culture exhibiting growth (per cent)	Remarks
1. Apical dome intact	90	70	+
2. Apical dome cut longitudinally	95	90	++
3. Apical dome with '+' cut	75	60	+++
4. Half portion of the apical dome	80	75	++++
5. Quarter portion of the apical dome	80	60	+++++

Mean of twenty observations

Culture period - four weeks

+ - Explant turned green within 2 weeks, with only a single bud developing

++ - Explant turned green within 1-2 weeks and 4-5 adventitious buds developed

+++ - Explant turned green within 3 weeks, but exhibited black colour along the cut surface due to phenol oxidation

++++ - Explant turned green within 1-2 week and 3-4 adventitious buds developed

+++++ - Explant turned green within 2 week and 1-2 adventitious buds developed

Plate 1. Culture showing adventitious bud formation from half portion of the apical dome

Plate 2. Culture showing adventitious bud formation from an apical dome cut longitudinally

Plate 3. Single bud development from an apical dome intact shoot tip explant

PLATE 1

PLATE 2

PLATE 3

establishment medium and to arrive at a suitable basal proliferation medium for the induction and growth of axillary shoots using different combinations of auxins (NAA, IAA) and cytokinins (BA, kinetin) in MS (semi-solid) medium and the use of BA alone in Knudson's C (semi-solid) medium using various explants namely shoot tips, eye buds and floral apices are presented in Table 12, 13 and 14.

2.2.1. Shoot tip

The independent influence of BA was found to be significant to most of the combinations of NAA and BA tried (Table 12). It was found that as the BA level was increased there was a corresponding increase in the number of axillary shoots induced. In the combination of NAA and BA tried, similar trend was noticed. Addition of NAA along with BA was found to have no influence on the induction of axillary shoots as the number of shoots produced by using the same concentration of BA, alone in the medium was found to be more than the number produced in most of the combinations of NAA and BA tried. All the 16 treatment combinations recorded cent per cent culture proliferation (ie. all the cultures developed multiple shoots, the number ranging from 2.25 to 11.00) (Plate 4a and 4b). The maximum number of shoots (11.00) was produced by the treatment involving BA at 10.0 ppm and also at the treatment combination NAA 1.0 ppm + BA 10.0 ppm.

Table 12. Effect of BA and combinations of NAA and BA on the induction of axillary shoots in *in vitro* culture of banana (cv. Red banana)

Medium: MS

Treatment	Shoot tip		Eye bud		Floral apex	
	Percentage of cultures developed shoots	Shoots per culture	Percentage of cultures developed shoots	Shoots per culture	Percentage of cultures developed shoots	Shoots per culture
BA 4.0 ppm	100	3.00	100	3.50	100	2.25
,, 6.0 ppm	100	5.25	100	5.00	100	6.00
,, 8.0 ppm	100	9.75	100	9.50	100	9.75
,, 10.0 ppm	100	11.00	100	11.00	100	11.50
NAA 0.5 ppm + BA 4.0 ppm	100	2.25	100	3.75	100	3.00
,, + ,, 6.0 ppm	100	4.75	100	5.50	100	5.00
,, + ,, 8.0 ppm	100	8.00	100	9.00	100	9.00
,, + ,, 10.0 ppm	100	9.75	100	11.25	100	12.00
NAA 1.0 ppm + BA 4.0 ppm	100	4.25	100	3.50	100	3.00
,, + ,, 6.0 ppm	100	6.00	100	6.00	100	6.00
,, + ,, 8.0 ppm	100	9.00	100	9.00	100	8.50
,, + ,, 10.0 ppm	100	11.00	100	10.75	100	10.00
NAA 1.5 ppm + BA 4.0 ppm	100	2.50	100	2.75	100	3.60
,, + ,, 6.0 ppm	100	5.25	100	5.00	100	4.75
,, + ,, 8.0 ppm	100	7.75	100	6.75	100	7.50
,, + ,, 10.0 ppm	100	9.75	100	10.00	100	9.00
CD (5%)		1.23		1.12		1.18
SEm±		0.61		0.56		0.59

Mean of four observations
Culture period - four weeks

Table 13. Effect of kinetin and combination of NAA and kinetin on the induction of axillary shoots in *in vitro* culture of banana (cv. Red banana)

Medium: MS

Treatments	Shoot tip		Eye bud		Floral apex	
	Percentage of cultures developed shoots	Shoots per culture	Percentage of cultures developed shoots	Shoots per culture	Percentage of cultures developed shoots	Shoots per culture
Kinetin 4.0 ppm	100	1.25	75	0.75	50	0.75
,, 6.0 ppm	100	1.75	100	1.75	100	1.25
,, 8.0 ppm	100	2.50	100	2.25	100	2.25
,, 10.0 ppm	100	3.50	100	3.25	100	3.50
NAA 0.5 ppm + Kinetin 4.0 ppm	75	1.00	75	0.75	75	0.75
,, + ,, 6.0 ppm	100	2.00	100	1.75	100	1.75
,, + ,, 8.0 ppm	100	2.25	100	2.00	100	2.25
,, + ,, 10.0 ppm	100	4.50	100	4.25	100	4.50
NAA 1.0 ppm + Kinetin 4.0 ppm	100	1.25	75	1.00	75	1.00
,, + ,, 6.0 ppm	100	1.25	100	1.50	100	1.75
,, + ,, 8.0 ppm	100	2.75	100	2.50	100	2.75
,, + ,, 10.0 ppm	100	4.00	100	3.75	100	3.75
NAA 1.5 ppm + Kinetin 4.0 ppm	75	0.75	50	0.75	75	1.00
,, + ,, 6.0 ppm	100	1.50	100	1.25	100	1.50
,, + ,, 8.0 ppm	100	2.50	100	2.25	100	2.75
,, + ,, 10.0 ppm	100	4.00	100	3.50	100	3.75
CD (5%)		0.88		0.85		0.85
SEm±		0.31		0.30		0.30

Mean of four observations
Culture period - four weeks

Table 14. Effect of BA and combinations of IAA and BA on the induction of axillary shoots in *in vitro* culture of banana (cv. Red banana)

Medium: MS

Treatments	Shoot tip		Eye bud		Floral apex	
	Percentage of cultures that developed shoots	Shoots per culture	Percentage of cultures that developed shoots	Shoots per culture	Percentage of cultures that developed shoots	Shoots per culture
BA 0.5 ppm	0	0	0	0	0	0
,, 1.0 ppm	75	0.75	75	0.75	50	0.50
,, 2.0 ppm	75	1.00	75	1.00	100	1.00
,, 3.0 ppm	100	2.50	100	2.50	100	2.00
IAA 0.5 ppm + BA 0.5 ppm	0	0	0	0	0	0
,, + ,, 1.0 ppm	50	0.75	50	0.50	50	0.50
,, + ,, 2.0 ppm	100	1.00	75	0.75	75	0.75
,, + ,, 3.0 ppm	100	3.00	100	3.00	100	3.00
IAA 1.0 ppm + BA 0.5 ppm	0	0	0	0	0	0
,, + ,, 1.0 ppm	50	0.50	25	0.25	25	0.25
,, + ,, 2.0 ppm	75	0.75	50	0.50	50	0.50
,, + ,, 3.0 ppm	100	3.50	100	3.75	100	3.50
CD (5%)		0.94		0.88		0.77
SEm±		0.32		0.30		0.26

0 - No response
Mean of four observations
Culture period - four weeks

Plate 4. Multiple shoot formation from shoot tip ex plant

a. MS medium containing NAA 0.5 ppm + BA 4.0 ppm

b. MS medium containing BA 10.0 ppm

PLATE 4a

PLATE 4b

Among the treatment combinations involving NAA and kinetin, NAA 0.5 ppm + kinetin 10.0 ppm recorded the highest number of shoots produced in culture (4.5 shoots/culture) (Table 13). The number of shoots produced in the rest of the treatments were four per culture or less. The addition of NAA was found to enhance the influence of kinetin at the highest concentration tried (10.0 ppm) in the induction of axillary shoots. The number of shoots produced when only kinetin was used at 10.0 ppm concentration was 3.50 shoots/culture, whereas the number of shoots produced ranged from 4.00 to 4.50 shoots/culture in the treatment combinations involving NAA at three levels ie. 0.5 ppm, 1.0 ppm, 1.5 ppm and kinetin 10.0 ppm. Among the 16 treatment combinations, 14 treatment combinations recorded cent per cent culture proliferation but the two treatment combinations (NAA 0.5 ppm + kinetin 4.0 ppm and NAA 1.5 ppm + kinetin 4.0 ppm) recorded only 75 per cent culture developing shoots.

Among the 12 treatment combinations tried, involving BA and IAA, IAA 1.0 ppm + BA 3.0 ppm recorded the maximum number of shoots per culture (3.50 shoots) (Table 14). No shoot production was observed when BA was used at 0.5 ppm concentration. The addition of IAA was found to improve the activity of BA as the number of shoots produced at the treatment combination IAA 1.0 ppm + BA 3.0 ppm was 3.5 which was on par with the number of shoots produced (2.5 shoots/culture) when BA alone was used

at 3.0 ppm concentration. Considering the percentage of cultures developing shoots, all the treatments involving BA at 3.0 ppm and the treatment combination (IAA 0.5 ppm + BA 2.0 ppm) recorded cent per cent culture proliferation.

The results of the trials conducted so far indicated that shoot tips can be chosen as a suitable explant in the field of rapid in vitro propagation of banana as it can be successfully used for the induction of numerous shoots within a short period. Of the 44 treatments tried, maximum number of shoots (11.00) per culture was produced in two treatments viz. BA 10.0 ppm and NAA 1.0 ppm + BA 10.0 ppm. The influence of these two treatments in inducing multiple shoots was found to be far superior to rest of the 42 treatments tried. When lower concentrations of cytokinins were considered, the treatment combination involving IAA and BA was found to be superior to all the other combinations involving NAA and BA, NAA and Kinetin, kinetin alone or BA alone.

2.2.2. Eye bud

As in the case of shoot tip culture, in the case of eye bud explant, the independent influence of BA was found to be more than the combinations of NAA and BA tried (Table 12). An increase in the number of axillary shoots was observed when the level of BA was increased. A similar trend was noticed in the

combination of BA and NAA tried. As the number of shoots produced in the combinations were more or less similar to the number produced when BA was used alone at the same concentration, adding NAA along with BA cannot be considered to have any influence on the induction of axillary shoots. All the cultures used for the particular study recorded cent per cent culture proliferation, the number of shoots produced ranging from 2.75 to 11.25. Contrary to shoot tip explant, the maximum number of shoots (11.25/culture) in the case of eye bud explant was recorded at the treatment NAA 0.5 ppm + BA 10.0 ppm, which was closely followed by the treatment involving BA alone at 10.0 ppm (11.00/ culture).

Among the treatment combinations involving NAA and kinetin (Table 13), NAA 0.5 ppm + kinetin 10.0 ppm recorded the highest number of shoots produced in culture (4.25 shoots/culture). The number of shoots produced in the rest of the treatments were 3.75 or less. As observed in the case of shoot tip explants, the addition of NAA was found to enhance the influence of kinetin at the highest concentration tried (10.0 ppm) in the induction of axillary shoots. When kinetin alone was used at 10.0 ppm concentration, the number of shoots produced was 3.25/culture, but in the treatment combinations involving NAA at three levels 0.5 ppm, 1.0 ppm, 1.5 ppm and kinetin at 10 ppm more number of shoots were found to be produced, the number varying from 3.50

to 4.25 shoots/culture. Considering the percentage of developing shoots, all the treatments except treatments involving kinetin at 4.0 ppm recorded cent per cent culture proliferation.

Among the 12 treatments (Table 14), treatment involving IAA 1.0 ppm + BA 3.0 ppm recorded the maximum number of shoots per culture (3.75 shoots) as in the case of shoot tip explant. The treatments involving BA at 0.5 ppm failed to induce any axillary shoot in culture. In the case of eye bud explant, the addition of IAA was found to improve the activity of BA as the number of shoots produced at the treatment combination of IAA 1.0 ppm + BA 3.0 ppm, was 3.75 which was far superior to the number of shoots produced (2.50/culture) when BA alone was used in the culture at 3.0 ppm concentration. All the treatments with BA 3.0 ppm recorded cent per cent culture proliferation.

In general, the effectiveness of the 44 treatments on eye bud explants indicated it as an useful explant for the induction of multiple shoots. Of the treatments tried, the highest number of shoots (11.25) was produced at the treatment combination of NAA 0.5 ppm + BA 10.0 ppm which was followed closely by BA at 10.0 ppm (11.00 shoots/culture). With regard to the influence of lower concentration of cytokinins, the treatment combinations involving IAA and BA was found to be superior to all the other combinations involving NAA and BA, NAA and kinetin, kinetin alone

or BA alone. The results obtained indicated that the response obtained for eye bud culture is more or less similar to that of shoot tip culture.

2.2.3. Floral apex

When the level of BA used in the culture medium was increased a corresponding increase in the number of axillary shoots was observed (Plate 5), a trend which was noticed in shoot tip and eye bud explant. A similar trend was noticed in the case of NAA and BA combinations. Except in few combinations (NAA 0.5 ppm + BA 4.0 ppm; NAA 1.0 ppm + BA 4.0 ppm; NAA 1.5 ppm + BA 4.0 ppm; NAA 0.5 ppm + BA 10.0 ppm), in all the other combinations addition of NAA was found to negatively influence the activity of BA in the induction of axillary shoots (Table 12). As observed in the case of other explants, cent per cent culture proliferation was recorded for all the culture tried, with the number of shoots produced ranging from 2.25 to 12.00 per culture. As in the case of eye bud explant, floral apex explant also recorded the maximum number of shoots (12.00/culture) at the treatment combination of NAA 0.5 ppm + BA 10.0 ppm, which was followed by the treatment involving BA alone at 10.0 ppm (11.50 shoots/culture).

As in the previous trials conducted with shoot tip and eye bud explant, among the treatment combinations involving NAA and

Plăte 5. Floral apex showing proliferation of buds in MS *medium* containing higher levels of BA

PLATE 5

kinetin, NAA 0.5 ppm + kinetin 10.0 ppm recorded the highest number of shoots (4.50) produced in culture (Table 13). The number of shoots produced in the rest of the treatments were 3.75 or less. As in the case of other explants, addition of NAA was found to enhance the influence of kinetin at the highest concentration tried (10.0 ppm) in the induction of multiple shoots. When only kinetin was added at 10.0 ppm concentration, the number of shoots produced was 3.50, whereas in the treatment combinations involving NAA at three levels 0.5 ppm, 1.0 ppm, 1.5 ppm and kinetin 10.0 ppm, the number of shoots produced were found to be more, the number varying from 3.75 to 4.50 shoots per culture. When the percentage of cultures developing shoots were considered, all the treatments except those with kinetin at 4.00 ppm concentration registered cent per cent culture proliferation.

Among the 12 treatment combinations tried involving IAA and BA, the treatment combination, IAA 1.0 ppm + BA 3.0 ppm (Table 14) recorded the maximum number of shoots produced in culture (3.50 shoots/culture). No axillary shoot induction was observed in the treatments containing BA at 0.5 ppm. As observed in the case of shoot tip and eye bud explants, the addition of IAA was found to positively influence the activity of BA as the number of shoots produced at the treatment combination IAA 1.0 ppm + BA 3.0 ppm was 3.50 which was on par with the number

of shoots produced (2.00/culture) when BA alone was used at 3.0 ppm concentration. Considering the percentage of cultures developing shoots, the treatments involving BA at 3.0 ppm and the treatment involving BA alone at 2.0 ppm concentration recorded cent per cent culture proliferation.

2.2.4. Effectiveness of Knudson's C medium

A trial was conducted to know whether modified Knudson's C medium can be used for inducing multiple shoots in in vitro culture of banana along with BA the most prominent phytohormone identified from the earlier studies conducted, in enhancing the release of axillary buds. The details of the trial are given in Table 15.

The results of the trial indicated that Knudson's C medium can be successfully used instead of MS medium for the induction and growth of axillary shoots as the shoot proliferation was more or less similar. In Knudson's C medium at BA 10 ppm, the number of shoots produced in shoot tip explants was 12.50 shoots/culture as against 11.00 shoots/culture in MS medium. In the case of eye bud explants also, similar trend was noticed as the number of shoots produced was 11.50 shoots/culture in Knudson's C medium containing BA 10.0 ppm, as against 11.00 shoots/culture in MS medium. In contrast to these two explants, floral apex explants

Table 15. Influence of Knudson's C medium containing BA on the formation of multiple shoots in *in vitro* culture of banana (cv. Red banana)

Treatments	Shoot tip		Eye bud		Floral apex	
	Percentage of cultures that developed shoots	Shoots per culture	Percentage of cultures that developed shoots	Shoots per culture	Percentage of cultures that developed shoots	Shoots per culture
Control (no BA)	0	0	0	0	0	0
BA 2.0 ppm	100	2.00	100	2.00	100	1.75
,, 4.0 ppm	100	4.25	100	4.00	100	3.50
,, 6.0 ppm	100	6.75	100	6.50	100	6.00
,, 8.0 ppm	100	9.50	100	9.25	100	8.50
,, 10.0 ppm	100	12.50	100	11.50	100	10.25
CD (5%)		0.91		1.01		1.32
SEm±		0.30		0.34		0.44

0 - No response
Mean of four observations
Culture period - four weeks

recorded a lower number of shoot production (10.25 shoots/culture) in Knudson's C medium with BA 10.0 ppm as against 11.50 shoots per culture in MS medium. Except the treatment with no BA, all the other treatments recorded cent per cent culture proliferation. It was also observed that as the level of BA was increased, there was a corresponding increase in the number of axillary shoots produced.

2.2.5. Effect of optional additive

The results indicated that no significant difference existed between the treatments tried (CW at 0, 50, 100, 150 and 200 ml/litre) on the culture survival and in the induction of axillary shoots in *in vitro* culture of banana using the three different explants viz. shoot tip, eye bud and floral apex (Table 16). In the visual rating for studying the growth response, KC medium containing BA 4.0 ppm supplemented with CW 15 per cent (v/v) was found to be performing well than all the other four levels.

2.2.6. Effect of continuous subculturing

At four week interval continuous subculturing was carried out to assess the rate of increase or decrease in the multiplication of axillary shoots in shoot tip culture, the details of which are given in Table 17. It was found that the rate of multiplication increased in all the subculture starting from the first subculture

Table 16. Effect of coconut water on the establishment of culture, growth of culture and induction of axillary shoots in *in vitro* culture on banana (cv. Red banana)
Medium: KC + BA 4 ppm

Treatments	Shoot tip			Eye bud			Floral apex		
	Culture* survival (per cent)	Culture growth	Shoots** per explant	Culture* survival (per cent)	Culture growth	Shoots** per explant	Culture* survival (per cent)	Culture growth	Shoots** per explant
CW - 0 ml	90	+	5.25	80	+	4.00	90	+	3.50
,, - 50 ml	90	++	4.20	80	+	4.00	90	+	3.20
,, - 100 ml	90	++	4.00	85	++	4.00	90	++	3.00
,, - 150 ml	95	+++	4.00	90	+++	3.80	95	++	3.00
,, - 200 ml	95	++	3.80	90	++	3.80	95	++	2.80
CD (5%)			NS			NS			NS
SEm±			.50			.56			.46

* Mean of twenty observations
** Mean of five observations
Culture period - four weeks

\+ Explant doubled in size in three weeks
++ Explant doubled in size in two weeks
+++ Explant thribled in size in two weeks

Table 17. Influence of continuous subculturing at four week interval on the multiplication rate of shoot tip explants of three banana cultivars

Medium: MS + BA 5.0 ppm

Serial subculture number	Nendran		Palayankodan		Red banana	
	Shoots per culture	Increase in number of shoots over the initial culture (per cent)	Shoots per culture	Increase in number of shoots over the initial culture (per cent)	Shoots per culture	Increase in number of shoots over the initial culture (per cent)
1	4.00	-	4.00	-	3.75	-
2	4.25	6.25	4.50	12.50	4.75	26.67
3	5.00	25.00	5.00	25.00	4.75	26.67
4	5.25	31.25	5.00	25.00	4.75	26.67
5	5.75	43.75	5.50	37.50	5.50	46.67
6	6.00	50.00	6.25	56.25	6.50	73.33
7	6.75	68.75	7.00	75.00	6.50	73.33
8	6.75	68.75	6.75	68.75	6.25	66.67
9	6.75	68.75	6.75	68.75	6.25	66.67
10	6.50	62.50	6.50	62.50	6.75	80.00
Mean	5.70		5.73		5.58	
CD (5%)	1.22		1.03		0.92	
SEm±	0.42		0.36		0.32	

Mean of four observations
Culture period - four weeks

itself. The percentage increase in number of shoots over the initial culture also increased and the maximum value (68.75) was obtained at the seventh, eighth and nineth subcultures for Nendran, (75.00) was obtained at the seventh subculture for Palayankodan and (80.00) was obtained at the tenth subculture for Red banana. On an average, the shoot multiplication rate increased by 5.70, 5.73, 5.58 due to subculturing in Nendran, Palayankodan and Red banana respectively. The variation in the multiplication rate among subcultures were found to be significant. No apparent reduction was observed in the growth and vigour of the cultures due to continuous subculturing. A diagrammatic summary of the various steps involved in the production of clonal banana plantlets by enhancing axillary bud release is presented in Fig. 4.

3. Culture conditions

3.1. Light

Duration and intensity of light was found to effect axillary shoot induction and growth of shoots in _in vitro_ culture of banana.

Number of axillary shoots

It was noticed that as the light intensity increased, axillary shoots produced also correspondingly increased. Light at higher intensities (80 W and 120 W) had significant effect on the number of axillary shoots induced in _in vitro_ culture of banana (Table 18).

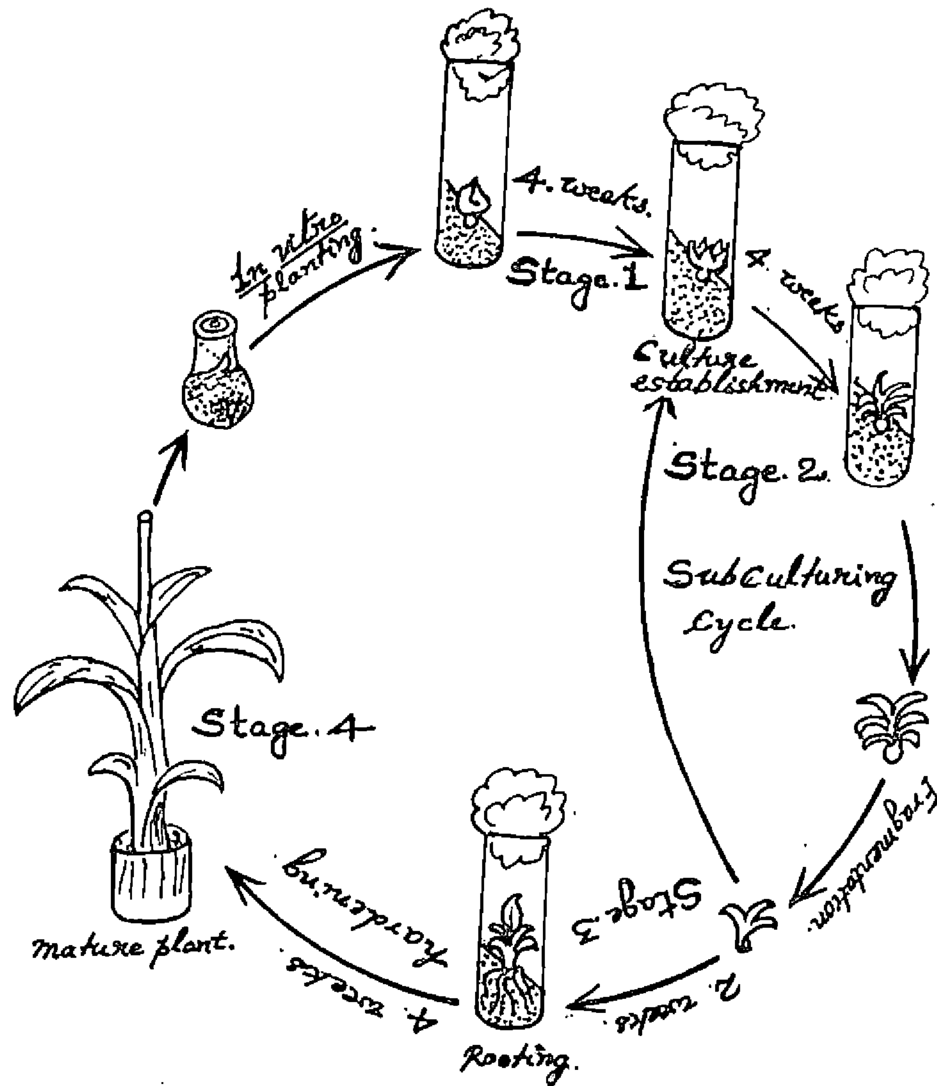


Fig:4. Diagrammatic summary of steps involved in rapid *in vitro* clonal multiplication of banana.

Table 18. Effect of light intensity on the formation of multiple shoots and growth of shoots in *in vitro* culture of banana (cv. Red banana)

Medium: MS + BA 5.0 ppm

Treatment	Shoot tip		Eye bud		Floral apex		Remarks
	Shoots per explant	Length of longest leaf (cm)	Shoots per explant	Length of longest leaf (cm)	Shoots per explant	Length of longest leaf (cm)	
No light	1.60	3.00	1.60	2.88	1.20	2.66	+
20 W	3.00	4.24	3.00	4.24	2.60	3.96	+
40 W	3.60	3.76	3.40	3.58	2.80	3.36	++
80 W	4.20	3.16	4.00	2.98	3.20	2.98	+++
120 W	4.20	2.96	3.80	2.74	3.00	2.76	+++
CD (5%)	1.02	0.41	0.90	0.50	0.81	0.70	
SEm±	0.49	0.20	0.43	0.24	0.39	0.33	

Mean of five observations
Culture period - four weeks

\+ - Tender, pale green in colour, less vigorous
++ - Robust, green in colour, with medium vigour
+++ - Dwarf, stout, dark green in colour, highly vigorous

In shoot tip culture, maximum number of shoots (4.2/explant) was produced at 80 W and 120 W light intensities and were significantly superior to 20 W and no light (dark) treatments. In the case of eye bud and floral apex culture maximum number of shoots (4.0/explant) and (3.2/explant) was produced at 80 W light intensity respectively and were significantly superior to 20 W and no light (dark) treatments. Thus in *in vitro* culture of banana, 80 W light intensity was found to be the best for inducing maximum number of shoots. Dark treatment was found to have only very little influence in the induction of axillary shoots or in their further growth and development. The treatment was avoided after the initial trials.

Length of the longest leaf

In general, leaf length was found to increase gradually on reducing the light intensities, except in the case of the dark treatment (Table 18). Maximum leaf length (4.24 cm) was registered by 20 W light intensity in shoot tip and eye bud culture. In floral apex culture, maximum leaf length (3.96 cm) was obtained at 20 W light intensity.

General vigour

In the visual rating of growth response, when the vigour of the shoots were compared no significant difference was noticed

among the three explants tried (Table 18). It was found that at 20 W light intensity and dark treatment, the shoots produced were less vigorous (Plate 6). As the light intensity level increased, the shoots showed a gradual improvement in their vigour. Highly vigorous, stout and dwarf shoots were produced at 80 W and 120 W light intensity (Plate 7).

4. *In vitro* rooting

Standardisation of basal rooting medium

Knudson's C medium (semi-solid) containing full strength of both inorganic and organic growth factors was found to be better than MS medium (semi-solid) containing full strength of both inorganic and organic growth factors and MS medium (semi-solid) containing half strength of inorganic salts and full strength of organic growth factors in rooting of banana shoots produced *in vitro*, particularly in the number of days taken for root initiation and the number of roots produced per shoot (Table 19).

Among the 16 treatment combinations tried with different levels of NAA and IBA for rooting of banana shoots produced *in vitro*, all treatments (except MS medium without any auxin) registered cent per cent of cultures initiating roots.

Number of roots

Maximum number of roots (6.75/shoot) (Plate 8) were

Plate 6. Nature of *in vitro* produced shoots under 20 W light intensity

Plate 7. Nature of *in vitro* produced shoots under 120 W light intensity

PLATE 6

PLATE 7

Table 19. Effect of auxins (NAA and IBA) on the rooting of banana shoots (cv. Red banana) *in vitro*

Treatment	Root initiation (per cent)	Roots per shoot	Time taken for root initiation (days)	Nature of roots formed
1. MS[a] (with no auxin)	75	1.50	13.25	Small, thin roots
,, + NAA 5.0 ppm	100	5.50	10.50	Thin, fibrous roots
,, + ,, 10.0 ppm	100	5.00	7.50	Thin, long roots
,, + ,, 15.0 ppm	100	3.50	7.00	Thick, short roots
,, + IBA 5.0 ppm	100	5.25	10.75	Thin, long roots
,, + ,, 10.0 ppm	100	3.25	8.00	Thick, short roots
2. MS[b] (with no auxin)	100	2.25	12.25	Long, thin roots
,, + NAA 5.0 ppm	100	5.75	9.25	Thin, long fibrous roots
,, + ,, 10.0 ppm	100	5.25	7.25	Thin, long roots
,, + ,, 15.0 ppm	100	4.25	6.75	Thick, very short roots
,, + IBA 5.0 ppm	100	5.50	9.25	Thin, long roots
,, + ,, 10.0 ppm	100	4.25	7.00	Thick, very short roots
3. KC (with no auxin)	100	6.00	6.25	Thin, long, fibrous roots
,, + NAA 5.0 ppm	100	6.75	6.00	
,, + ,, 10.0 ppm	100	4.00	7.00	Thick, short roots
,, + ,, 15.0 ppm	100	3.25	7.50	Thick, very short roots
CD (5%)		1.12	1.15	
SEm±		0.57	0.59	

Mean of four observations
Culture period - two weeks

MS[a] - MS medium with full strength of both inorganic salts and organic growth factors

MS[b] - MS medium with half strength of inorganic salts and full strength of organic growth factors

KC - Knudson's C medium with full strength of both inorganic salts and organic growth factors

Plate 8. Nature of roots produced *in vitro* in KC medium containing 5 ppm NAA

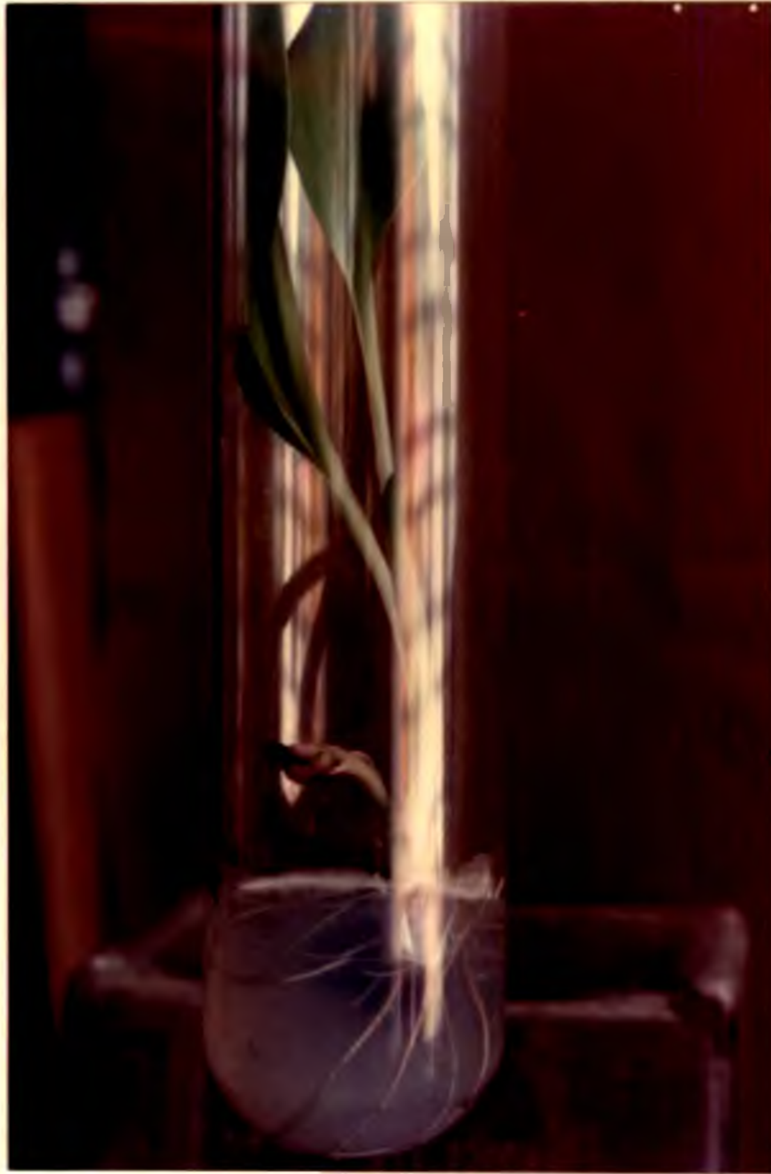


PLATE 8

recorded at the treatment KC + NAA 5.0 ppm which was closely followed by KC without any auxin (6 roots/shoot) (Table 19). Beyond 5.0 ppm NAA concentration, a fall in the number of roots produced were observed. Knudson's C medium was found to induce 6.0 roots per shoot even without the presence of any auxin, which was on par with the number of roots produced (1.50 and 2.25 roots/shoot) by MS^a and MS^b media respectively (Plate 9a and 9b).

Days for root initiation

The treatment KC + NAA 5.0 ppm registered the minimum number of days for root initiation (6.00) which was followed closely by KC without any auxin (6.25) (Table 19). The maximum number of days for root initiation was recorded at the treatment MS^a without any auxin (13.25) and MS^b without any auxin (12.25). The treatment KC containing NAA at 5.0 ppm and KC without any auxin was found to be highly superior to all the other treatments. Even without using any auxin KC medium alone can be successfully used for inducing roots in minimum number of days.

Nature of roots

When NAA was used at 5.0 ppm concentration the roots produced were thin, long and fibrous. As the concentration was increased the length was found to decrease and the roots become thickened. When IBA was used at 5.0 ppm concentration thin and long roots were produced. As the IBA concentration was increased

Plate 9. Production of roots *in vitro* in MS medium

a. MS medium with full strength of both inorganic salts and organic growth factors without any auxin

b. MS medium with half strength of inorganic salts and full strength of organic growth factors without any auxin

PLATE 9a

PLATE 9b

roots become thicker and short. When the plantlets were planted after hardening, the plantlets with long, thin fibrous roots survived better than those with thick, short roots.

Comparative performance of banana cultivars

To know whether there exists any difference among banana cultivars in their ability to produce roots *in vitro*, an experiment was conducted with three banana cultivars namely Nendran, Palayankodan and Red banana the details of which are given in Table 20. Based on the results obtained from the initial trials conducted with banana cv. Red banana, the treatment identified best for this cultivar (KC medium containing 5.0 ppm NAA) was used for the rest two cultivars namely Nendran and Palayankodan.

Considering the percentage of cultures capable of initiating roots, no difference was observed among the three cultivars (all cultivars recorded root initiation in all the cultures tried). No significant difference was observed among the cultivars in the number of days taken for root initiation. However, among the three cultivars (Plate 10a, 10b and 10c), Palayankodan recorded the maximum number of roots (8.0/shoots) and minimum number of days for root initiation (5.5).

4.2. Standardisation of medium supplements

An experiment was conducted to study whether any other

Table 20. Comparative performance of banana cultivars on the rooting of banana shoots produced *in vitro*

Medium: KC + NAA 5.0 ppm

Cultivar	Root initiation (per cent)	Roots per shoot	Time taken for root initiation (days)
1. Nendran	100	6.5	5.75
2. Palayankodan	100	8.0	5.50
3. Red banana	100	6.75	6.00
CD (5%)		1.27	NS
SEm±		0.56	0.46

Mean of four observations
Culture period - two weeks

Plate 10. Comparative performance of three banana cultivars in the *in vitro* rooting of banana shoots

a. Palayankodan

b. Red banana

c. Nendran

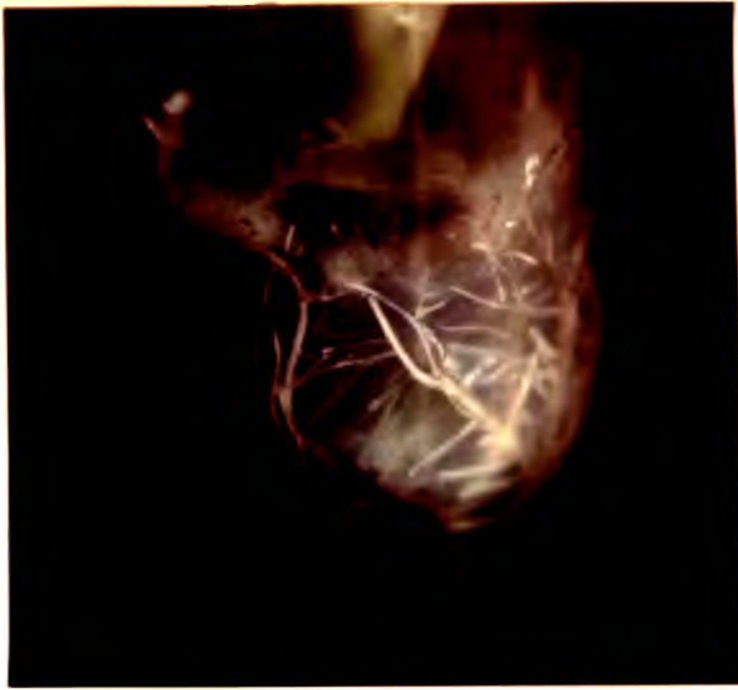


PLATE 10a

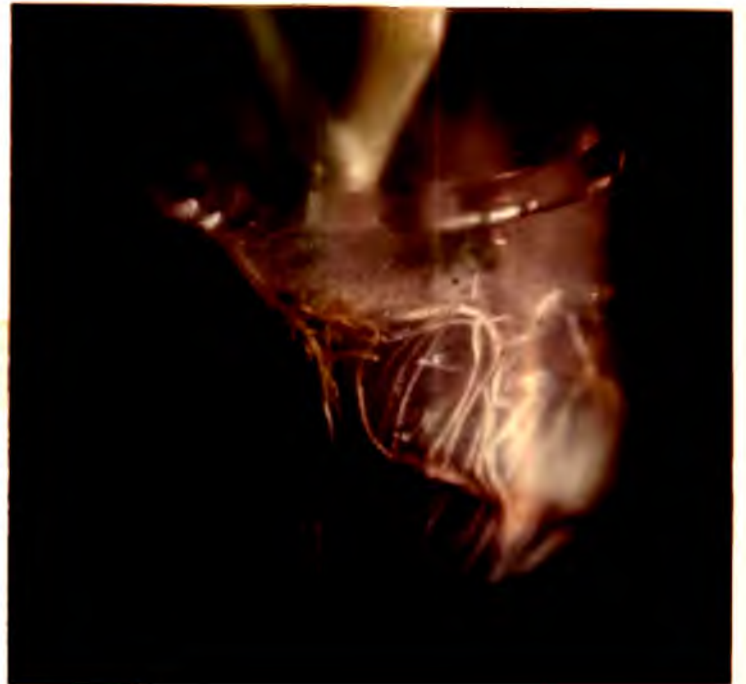


PLATE 10b

PLATE 10c

compounds (sucrose and activated charcoal) other than growth regulators, has got any effect on the rooting of banana shoots (cv. Red banana) produced *in vitro*. The results are presented in Table 21.

Sucrose

In all the four levels (1.5, 3.0, 4.5 and 6.0 per cent) of sucrose tested, cent per cent of the cultures showed root initiation. The lowest level (1.5 per cent) produced maximum number of roots (8.75/shoot) and the highest level (6.0 per cent) produced the least number of roots (5.75/shoot). Minimum number of days for root initiation (5.5) was recorded at the lowest level and the maximum number of days (10.25) at the highest level tried. Thicker and shorter roots were produced at the higher levels (4.5 and 6.0 per cent) whereas the lower levels (1.5 and 3.0 per cent) produced thin and long roots. The plantlets with thin and long roots recorded better survival and growth after planting out.

Activated charcoal

Addition of AC to the culture medium was found to remarkably influence the number of roots produced. All the four levels (0.025, 0.05, 0.1 and 0.2 per cent) tested enabled root initiation in cent per cent of the cultures. Maximum number of roots (10.75/ shoot) was recorded by AC at 0.025 per cent (Plate 11a) and

Table 21. Effect of sucrose and activated charcoal on the *in vitro* rooting of banana shoots (cv. Red banana)

Medium: KC + NAA 5.0 ppm

Treatments	Root initiation (per cent)	Roots per shoot	Time taken for root initiation (days)
Sucrose 1.5%	100	8.75	5.50
,, 3.0%	100	6.75	6.00
,, 4.5%	100	6.00	8.00
,, 6.0%	100	5.75	10.25
CD (5%)		1.11	1.24
SEm±		0.51	0.57
Activated charcoal 0.025%	100	10.75	7.00
,, 0.050%	100	8.00	7.00
,, 0.100%	100	7.50	6.25
,, 0.200%	100	6.75	5.00
CD (5%)		0.94	0.74
SEm±		0.43	0.34

Mean of four observations
Culture period - two weeks

Plate 11. Nature of roots produced *in vitro* in AC containing medium

a. AC at 0.025 per cent level

b. AC at 0.2 per cent level

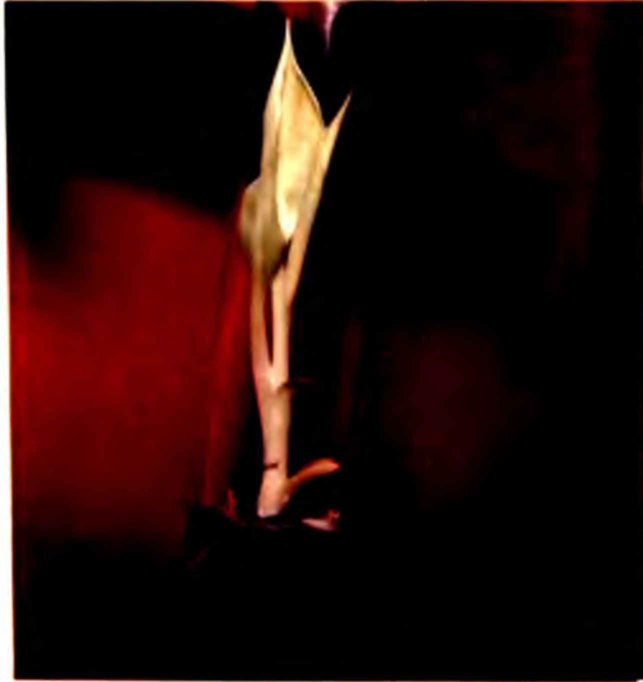


PLATE 11a

PLATE 11b

the minimum number of roots (6.75/shoot) was produced by AC at 0.2 per cent (Plate 11b). The least number of days (5.0) required for root initiation was registered by AC at 0.2 per cent. As far as the nature of roots produced in AC containing medium was concerned, they were white and thin. And the plantlets with white and thin roots recorded better survival percentage after planting out.

5. Acclimatization/hardening

5.1. Standardisation of hardening treatments

Control of temperature and maintenance of relative humidity during acclimatization was achieved by covering the plantlets with transparent plastic microscope covers having 60 cm height and 45 cm diameter at the base. This was done based on the reports of Rajmohan (1985) and Reghunath (1989). Spraying of cold water (12 ± 2°C) at an interval of three hours during the day time using a hand sprayer with fine mist nozzle enabled to maintain high relative humidity (90-100 per cent) and mild temperature (26 ± 3°C) inside the plastic cover.

Both pre-transfer and post-transfer hardening treatments were found to influence the survival of planted out plantlets (Plate 12a and 12b). Keeping in distilled water for 8 h followed by 8 h in MS liquid medium (containing 1/10th strength of inorganic salts, with no sucrose) under microscope cover and then covering

Table 22. Influence of hardening treatments, potting mixtures and containers on the survival of the *in vitro* produced banana plantlets (cv. Red banana)

	Pre-transfer treatments	Post-transfer treatments	Survival of plantlets (per cent)
A. Hardening treatments			
1.	Direct planting into the potting mixture	Keeping in the open	0
2.	,,	Covering the plantlets and pots with moistened plastic cover for 4 weeks	50
3.	Keeping in distilled water for 8 h and then for 8 h in MS solution of 1/10th strength, under microscope cover	Keeping in the culture room	10
4.	,,	Covering the plantlets and pots with moistened plastic cover for 4 weeks	90
5.	Keeping in MS solution of 1/10th strength for 2 days under microscope cover	Keeping in the culture room	0
6.	,,	Covering the plantlets and pots with moistened plastic covers for 4 weeks	80
B. Potting mixture			
1.	Sand		80
2.	Vermiculite : Sand (1 : 1)		90
3.	Red earth : Sand : Soil : Compost (1:1:1:1)		70
C. Containers			
1.	White plastic cups		80
2.	Black plastic cups		80
3.	Tubular polythene bags		90
D. Chemical sterilants			
1.	Mercuric chloride 0.2%		60
2.	Bavistin 0.1%		50
3.	Emesan 0.1%		100

Mean of ten observations
Culture period - four weeks

Plate 12. Humidity acclimatization of rooted banana shoots - different stages

a. Plantlet kept in distilled water

b. Plantlets kept under microscope cover

PLATE 12a

PLATE 12b

the planted out plantlets and pots with moistened plastic cover for four weeks recorded 90 per cent plantlet survival (Table 22). This hardening treatment was closely followed by a plantlet survival percentage of 80 obtained by keeping the plantlets in MS liquid medium (containing 1/10th strength of inorganic salts, with no sucrose) for two days under microscope cover and then covering the plantlets and pots with moistened plastic cover for four weeks. Keeping the planted out plantlets without any humidity cover in the open or in the culture room resulted in poor establishment of the plantlets.

5.2. Potting mixture

The most suitable potting mixture was found to be a 1:1 (v/v) mixture of vermiculite and sand (Table 22). It registered maximum survival (90 per cent) of the plantlets. Use of sterile sand was also found suitable, but recorded only 80 per cent survival of the plantlets. The potting mixture containing red earth : sand : soil : compost 1 : 1 : 1 : 1 (v/v) was also found suitable though it recorded only 70 per cent plantlet survival.

5.3. Containers

Tubular polythene bags were found to be the best container as it registered 90 per cent plantlet survival (Table 22). White and black plastic cups were also found suitable, as the plantlet survival percentage observed was 80 per cent.

5.4. Chemical sterilant

Out of the three chemical sterilants used for sterilizing the soil, emesan 0.1 per cent registered cent per cent plantlet survival. The effect of emesan as a chemical sterilant in sterilizing the potting mixture, was found to be highly superior to that of mercuric chloride (0.2 per cent) and bavistin (0.1 per cent).

6. Planting out and evaluation

With respect to the survival of rooted plantlets under mist and in the open the cv. Palayankodan excelled Nendran and Red banana as it recorded 100 per cent plantlet survival under mist and 91.7 per cent survival after eight weeks of planting out (Table 23) (Plate 13a, 13b and 13c). Twelve weeks after planting out the percentage survival registered by Palayankodan and Red banana were the same (83.3 per cent). The cv. Nendran recorded lower survival rate than Palayankodan cultivar in all the stages of growth.

When the height of the plantlets of the three cultivars were compared, Nendran was found to be taller than the other two cultivars during the early stages, but by about 12 weeks after planting out Palayankodan was found to be taller than Nendran. In all the three stages, no significant difference in plant height was observed between Nendran and Palayankodan but Red banana

Table 23. Comparative performance of *in vitro* plantlets of banana cultivars on the survival and growth under mist and open

Parameters	Cultivars			CD (5%)
	Nendran	Palayankodan	Red banana	
1. After four weeks under mist				
Survival** (%)	91.7	100	91.7	
Plant height* (cm ± SE)	1.54 ± 0.16	1.20 ± 0.16	0.80 ± 0.16	0.35
Leaves per plant* (No ± SE)	3.40 ± 0.33	3.20 ± 0.33	3.40 ± 0.33	NS
Longest leaf length* (cm ± SE)	5.06 ± 0.14	4.84 ± 0.14	5.82 ± 0.14	0.30
2. Eight weeks after planting out				
Survival** (%)	83.3	91.7	83.3	
Plant height* (cm ± SE)	3.42 ± 0.13	3.36 ± 0.13	2.40 ± 0.13	0.29
Leaves per plant* (No ± SE)	5.60 ± 0.41	5.80 ± 0.41	5.60 ± 0.41	NS
Longest leaf length* (cm ± SE)	8.24 ± 0.10	7.92 ± 0.10	8.96 ± 0.10	0.22
3. Twelve weeks after planting out				
Survival** (%)	75.0	83.3	83.3	
Plant height* (cm ± SE)	6.16 ± 0.14	6.30 ± 0.14	5.22 ± 0.14	0.30
Leaves per plant* (No ± SE)	9.80 ± 0.50	9.00 ± 0.50	8.80 ± 0.50	NS
Longest leaf length* (cm ± SE)	10.54 ± 0.13	10.42 ± 0.13	10.98 ± 0.13	0.29

* Mean of three observations

** Mean of twelve observations

Potting mixture : Sand : Soil : Red earth : Dried and powdered cattle manure (1:1:1:1 v/v)

Plate 13. Planted out plantlets of banana - different stages

a. Two week old

b. One month old

c. Two month old

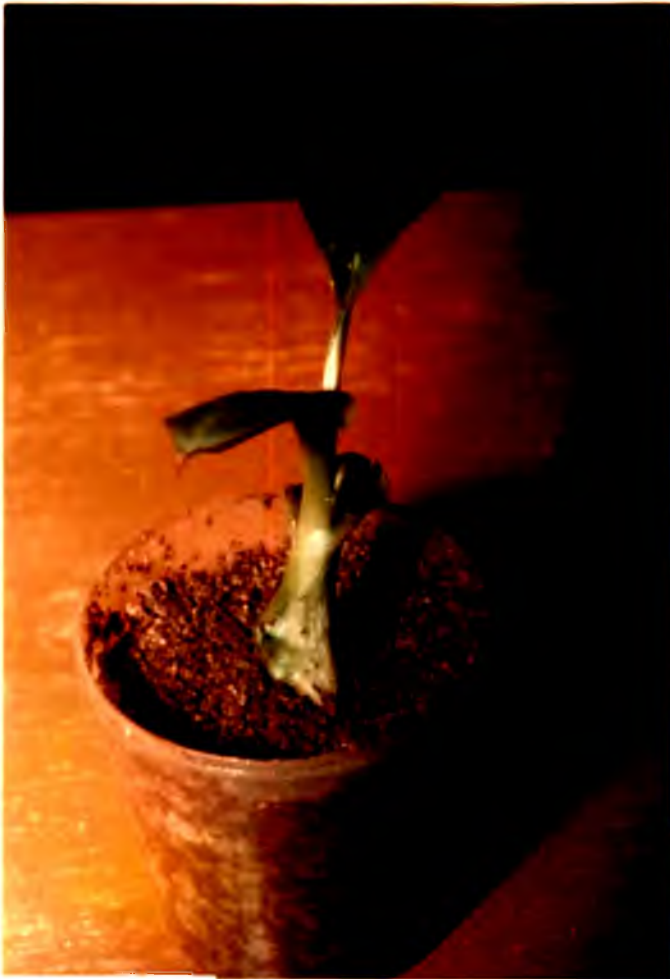


PLATE 13a

PLATE 13b

PLATE 13c

was found to be significantly lower in height than the other two cultivars.

In the case of number of leaves produced by plantlets no significant difference was noticed among the cultivars. However, the number of leaves produced by Nendran (9.8) was found to be more than Palayankodan (9.0) and Red banana (8.8), 12 weeks after planting out. Regarding the length of the longest leaf produced by the plantlets, significant differences were observed among the three cultivars at all stages of growth. Red banana recorded the maximum value at all the stages of growth as compared to Palayankodan and Nendran. When all the characters taken for study were considered, no cultivar could be said to be significantly superior.

DISCUSSION

DISCUSSION

Banana, is propagated vegetatively through suckers. In recent years there has been an increasing interest in the application of tissue culture techniques as a rapid means of asexual propagation of economically important plants. The propagation of various cultivars of banana by conventional methods has been studied by a number of authors in the past (Ascenso, 1967; De Langhe, 1961 and Hamilton, 1965). These methods are laborious and time consuming as far as the production of a large number of homogenous plants are concerned. Moreover, the rate of propagation of banana by this method is rather low. Tissue culture techniques ensure an extremely rapid rate of multiplication which is not season dependent and requires only a limited quantity of plant tissue as the initial explant. Tissue culture techniques can also aid in the production of disease free plants and in the cryopreservation of germplasm. In view of the above facts, there has been an upsurge of interest in the field of rapid clonal propagation of banana plants applying tissue culture technique (Cronauer and Krikorian, 1984a; Bakry et al., 1985; Gupta, 1986; Fitchet and Winnaar, 1987; Mateille and Foncelle, 1988 and Aravindakshan, 1989). However, reports on the standardisation of micropropagation techniques in the case of important cultivars of banana still lacks complete details. Though the private firm, A.V. Thomas and

Company has successfully produced banana plants by *in vitro* techniques in large scale, the technology adopted is not made known. The present investigations carried out at the College of Horticulture, Vellanikkara were mainly aimed to standardise the *in vitro* propagation technique for selected commercially important banana cultivars of Kerala. The route adopted for *in vitro* propagule multiplication was via enhanced release of axillary buds. The most remarkable advantage of this method is the genetic stability of the plantlets produced.

In micropropagation the organs and tissues are carried through a sequence of steps in which differential cultural and environmental conditions are provided. These steps have been indicated as different stages with stage I being physiological pre-conditioning of the explant and explant establishment, stage II rapid multiplication of shoots through increased axillary branching, stage III rooting and hardening of *in vitro* raised plants and stage IV acclimatization and transplantation to field conditions (Reghunath, 1989).

Banana being a monocotyledonous plant, never produces branches and the buds in the axils are dormant due to the 'apical dominance' shown by fast growing meristematic tip. The stage I is to activate these buds to form branches by suppressing the apical dominance. Physical injury treatments like scoring or killing

the bud apex was found to be favourable in the release of axillary buds. 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. 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) and then by Jarret et al. (1985). Dividing the apical dome into two halves and culturing each half separately was found to be the 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 was removed.

As the shoot tips and eye buds emerge from below the ground level, they accumulate lot of soil and dirt. This necessitates a thorough and effective surface sterilization of the explants before culturing. Out of the various sterilants tried mercuric chloride (0.05-0.2 per cent) was found to give better sterilization of explants than bleaching powder (Table 4). Though the use of sodium hypochlorite is more common, in the present studies it was found that mercuric chloride was more efficient especially when the explants were highly contaminated. An initial rinsing of floral apex explants with 95 per cent absolute alcohol for 30s followed by mercuric chloride treatment (0.05 per cent) for 10 min. resulted in least rate of contamination (10 per cent) and maximum

percentage of explant survival (85 per cent) and minimum percentage of explant mortality (5 per cent). For shoot tip and eye bud explants, the best sterilization treatment was found to be that using mercuric chloride (0.2 per cent) for 5 min. which resulted in very low contamination rate (5 per cent) and maximum percentage of explant survival (80 per cent) eventhough the rate of explant mortality was high (15 per cent). Enhancing the strength of mercuric chloride or extending the treatment duration resulted in still lesser rate of contamination but increased the rate of explant mortality.

Shoot tip collection in banana during November to April resulted in least contamination rate (30 to 40 per cent) and maximum explant survival (50 to 60 per cent) (Table 5). This may be because of low rainfall and humidity prevailing in Kerala during this period, which was incongenial for the growth of microbes. The season of explant collection was also found to have some influence on the rate of survival of cardamom explants (Reghunath, 1989).

In banana, tissue and media discolouration was found to be a serious problem in establishing a good and healthy culture. This discolouration resulted from the oxidation of polyphenols present in the explant tissues of banana. Several workers have attempted various methods to overcome this problem but no method

was found to result in complete success (Jarret et al. 1985; Vuylsteke and De Langhe, 1985 and Banerjee and De Langhe, 1985). It was found that by the addition of ascorbic acid into the media at the rate of 50 mg/l 90 percentage of the cultures were freed from this problem. The exact action of ascorbic acid in reducing media and explant discolouration is not known.

Jarret et al. (1985) reported that shoot tip cultures of two banana clones 'Saba' and 'Pelipita' established on a modified MS basal medium supplemented with IAA 1 ppm and BA 3.0 ppm. The results of the present study indicated that out of the 33 different treatments tried using MS (semi-solid) medium, the treatment involving NAA 0.5 ppm and BA 3.0 ppm was found to be the best with respect to the number of days taken for culture establishment. At this combination the three explants tried namely shoot tip, eye bud and floral apex took the least number of days (8, 9 and 14 respectively) for culture establishment which was on par with the other remaining treatments.

According to Krishnamoorthy (1981) GA treatment enhances the level of endogenous auxins in the plant. Addition of gibberellic acid (GA) to the culture medium was found to be highly unfavourable for culture establishment and growth in shoot tip, eye bud and floral apex culture.

In shoot tip culture on an average each explant released 11.00 axillary shoots when the BPM contained NAA 1.0 ppm and BA 10.0 ppm or BA 10 ppm (Table 12). In the case of eye bud and floral apex explants on an average each explant released 11.25 and 12.0 axillary shoots respectively, when the BPM contained NAA 0.5 ppm and BA 10.0 ppm.

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). In the present studies, auxins (NAA and IAA) were found to influence axillary shoot induction and growth at certain particular combinations. In most of the combinations involving NAA and BA, it was observed that the auxin NAA negatively influenced the activity of BA, but in most of the combinations tried involving NAA and kinetin, NAA was found to enhance the activity of kinetin, when it was added at 10 ppm concentration. In the case of IAA, it was found to enhance the activity of BA at 3.00 ppm concentration.

The basic phenomenon involved In the induction of axillary shoots and subsequent plantlet production _in vitro_ are reported

to be due to the action of plant hormones. Little is known about how hormones evoke a particular pattern of morphogenesis (Thorpe, 1980). One hypothesis is that hormone treatment starts the cells on a specific developmental pathway. The alternative view is that hormone responsive cells are already determined and that the hormones evoke the expression of the committed state. The available evidence suggests that the hormones act in both ways. Hence the observed difference in organogenesis in the different explants of the same plant would be attributed to the differential requirement of growth hormones for each explant. According to Skoog and Miller (1957), quantitative interaction between diverse growth factors may have a decisive role in organogenesis. When the performance of shoot tip, eye bud and floral apex explants were compared, floral apices took more time for culture establishment. Eye bud and shoot tip took more or less the same time. This difference in the performance of floral apex explant may be attributed to the difference in the level of endogenous phytohormones, nutrients and metabolites and interaction between various growth factors. This observation was found to be in confirmation with an earlier report (Drew *et al*., 1989) in which it was stated that sucker derived explants initiated shoots more readily than inflorescence derived explants. According to Cronauer and Krikorian (1985) the most possible reason suggested for the longer period taken by the floral apex explants is that

unlike the vegetative buds, the floral apices has to revert to the vegetative stage to get established in the medium and to initiate shoots. During the course of study it was clearly uderstood 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.

Several workers had indicated that the response of banana tissues in culture may vary depending on the genotype (Cronauer and Krikorian, 1984 a and b; Banerjee and De Langhe, 1985; Jarret et al., 1985; Vuylsteke and De Langhe, 1985; Wong, 1986). In the present studies also, it was found that different cultivars of banana responded differently to a fixed set of treatments. The reason for the observed difference in the behaviour of the 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 groups.

Another important observation was that when MS medium was compared with Knudson's C medium (having comparatively lower salt concentration), it was found that KC medium was as good as or even better than MS medium in inducing axillary buds and producing healthy shoots with good green leaves in shoot tip, eye bud and floral apex culture. In KC medium at BA 10.0 ppm the number of axillary shoots produced for shoot tip, eye

bud and floral apex explants were 12.5, 11.5 and 10.25 respectively, whereas in MS medium at the same concentration of BA, the number of axillary shoots produced were, 11.0, 11.0 and 11.5 respectively. Thus, it can be seen that except for floral apex, for the other two explants, KC medium can be successfully used for in vitro shoot production.

The addition of coconut water (CW) into the medium, which contains a number of cell division factors (Shantz and Steward, 1952; Letham, 1974) and a large number of free amino acids were found to have no significant influence on the number of axillary shoots induced in in vitro culture of banana.

The effect of serial subculturing of an explant stock continuously for a long period of time was also looked into. It was found that when subculturing was done at four week interval, the number of shoots produced per explant per culture vessel increased at a mean rate of 5.70, 5.73 and 5.58 per subculture in Nendran, Palayankodan and Red banana respectively. Increase in multiple shoot production continued from the first subculturing onwards. In Nendran, slight decline in shoot production was noticed after nineth subculture, whereas in Palayankodan decline was noticed after seventh subculture. Contrary to these two cultivars, Red banana produced maximum number of shoots at the tenth subculture

(Table 17). Several workers (Doreswamy et al., 1983; Damasco et al., 1984; Vuylsteke and De Langhe, 1985; Aravindakshan, 1989) had reported increased shoot multiplication rate achieved through serial subculturing in banana. It is supposed that continuous subculturing modifies the physiological state of the plant in such a way that it favours revitalisation of innate dormant vegetative buds (Litz and Conover, 1978; Franclet, 1979; David, 1982). Continuous subculturing was also found to reduce media and explant discolouration in banana in vitro.

Light has got a significant influence on the induction of axillary shoots and growth of the shoots. A light intensity ranging between 80 W and 120 W for 16 h followed by 8 h dark period was found to be giving maximum growth and development of axillary shoots in shoot tip culture and a light intensity of 80 W was found to be best for eye bud and floral apex culture. In general, it has been observed that 80 W light intensity is best, for maximum induction of axillary shoots and the production of vigorous dwarf shoots. According to Murashige (1977) the optimum light period is 16 h.

Stage 3 involves de novo regeneration of adventitious roots from the shoots obtained in Stage 2. KC medium was identified to be the most suitable basal medium for rooting. So far only a single report (Berg and Bustamante, 1974) was available on the

usage of KC. Among the auxins (NAA and IBA) tried NAA 5.0 ppm was the best with respect to initiating rooting in maximum number of cultures (100 per cent) within the shortest period of time (6 days). Maximum number of roots (6.75/shoot) was also obtained by adding 5 ppm NAA to the basal rooting medium (Table 19). Lane (1979) reported that NAA usually gave rise to short thick roots. But in the present observation it was seen that NAA at 5.0 ppm concentration produced thin, long and fibrous roots, but as the concentration was increased the length was found to decrease and the roots become thickened. Same phenomenon was noticed when IBA was added. The concentration of inorganic salts in the basal medium influence the *in vitro* rooting regardless of the growth substances present. Several researchers had shown that *in vitro* rooting can successfully be achieved by reducing 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). 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). When the three banana cultivars namely Nendran, Palayankodan and Red banana were compared for their ability to produce roots *in vitro*, no significant difference was observed among the cultivars in the number of days taken for root initiation. Among the three cultivars, Palayankodan recorded the maximum number of roots

(8.0/shoot) and the minimum number of days for root initiation (5.5).

Sucrose at 1.5 per cent concentration was found optimum for the production of sufficient roots and normal plantlets. As a source of energy as well as a factor for osmoregulation for optimising the rooting response, sucrose has already been recognised (Chong and Pua, 1985). 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 (5.5). The favourable effect of reducing the sucrose concentration to half on in vitro rooting and shoot elongation has been reported by Mateille and Foncelle (1988). Addition of activated charcoal (AC) to the culture medium had remarkable influence in the initiation of roots as well as in the number of roots produced per shoot. Incorporation of 0.025 per cent AC to the basal rooting medium had resulted in the initiation of maximum number of roots (10.75/shoot) within a period of 7 days (Table 21). The effect was found to be due to AC alone because withdrawal of auxin from the medium did not make much difference. In AC containing medium, shoots produced white and thin roots. And the plantlets with white, thin roots established well after planting out. The favourable effects of AC in rooting had been reported by many workers (Wang and Huang, 1976; Banks and Hackett, 1978). The capacity of AC

to adsorb inhibiting compounds and excessive concentration of plant growth hormones and the property to darken the medium which mimics the soil conditions are the factors proposed in favour of the favourable effects of AC in rooting (Proskauer and Berman, 1970; Wang and Huang, 1976; Fridborg *et al*., 1976).

Debergh and Maene (1981) pointed out that rooting *in vitro* was the most labour intensive part of micropropagation. In the present investigation also, rooting of individual shoots *in vitro* after separation from the multiple shoot cluster was found to be the most time and labour consuming part. Yeoman (1986) suggested that wherever possible rooting and hardening processes may be combined and if practicable rooting of shoots may be attempted by direct planting into a conventional medium so as to eliminate a further costly transfer to a sterile medium. Reghunath (1989) made an attempt to induce roots by direct planting of cardamom shoots into a conventional medium and it resulted in failure, as cardamom was a monocotyledonous plant. Banana also being a monocotyledonous plant, no attempt was made to directly induce roots from shoots developed *in vitro*. Yeoman (1986) reported that transfer of stage 3 shoots to higher light levels and allowing adequate entry of carbondioxide into the culture vessels prior to planting out will enable the leaves to become quickly self-supporting by carrying photosynthesis. According to Murashige (1978) enhanced photosynthesis under the influence of high light

intensities helps in building up a high food reserve to be utilised during the transformation period from partially heterotrophic to autotrophic stage of the plantlets after transplantation. After the initiation of roots, keeping the plantlet in the medium itself for another two weeks, till the medium showed signs of dryness and then planting out resulted in better survival percentage than the transferring of the plantlets into the potting mixture soon after the production of all roots.

Hardening the _in vitro_ raised plantlets so as to make them adapt to the outside environment is a critical process due to their anatomical and physiological pecularities. On transplanting excessive water loss from the plants had been recorded which was attributed to the improper development of cuticle and slowness of stomatal response to water stress (Brainerd and Fuchigami, 1981; Fabbri _et al._, 1984). The problem may be aggravated if the vascular connection between root and shoot is improper. A period of humidity acclimatization was considered necessary for the newly transferred plantlets to adapt to the outside environment, during which the plantlets undergo morphological and physiological adaption enabling them to develop typical terrestrial plant water control mechanism (Grout and Aston, 1977; Sutter _et al._, 1985). High relative humidity (90 to 100 per cent) was maintained during the initial period of planting out with the help of microscope and plastic covers with intermittent cold water

sprays. Out of the various hardening treatments adopted, maximum percentage of plantlets survived when the plantlets after removal from the rooting media were kept in distilled water for 8 h and then for another 8 h in MS liquid medium of 1/10th strength under moistened microscope cover and after planting out into the potting mixture covering with moistened plastic cover for four weeks.

The most suitable potting medium for maximum plant survival (90 per cent) was found to be a 1:1 (v/v) mixture of vermiculite and sand. 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 sand might have helped in giving a better grip for the roots besides providing ample aeration. Addition of inorganic nutrients to the potting mixture is essential for the normal growth of the potted plants (Brown and Sommer, 1982; Amerson et al., 1985). Application of 5-10 ml nutrient solution containing MS inorganic salts at half concentration at weekly intervals enhanced the survival and promoted normal growth of the plantlets. Rajmohan (1985) and Reghunath (1989) also reported the use of a nutrient solution similiar to the above for the successful growth of in vitro raised jack and cardamom plantlets respectively. Type of containers were also found to influence the survival of plantlets. Tubular polythene bags were identified as the best container recording 90 per cent survival of plantlets.

In order to achieve cent per cent plantlet survival, the sterile potting mixture was once again sterilized using chemical sterilants. Emesan 0.1 per cent was found to be the best one out of the three chemical sterilants tried as it resulted in cent per cent survival of planted out plantlets.

When the growth performance of the plantlets of the three banana cultivars were compared, significant difference was found to exist between varieties in the case of plant height and the length of the longest leaf. No significant difference could be observed in the number of leaves produced per plant in all the stages of growth between the varieties. According to Smith *et al.* (1986) it was not the state of growth of a tissue under microculture that was important in its performance when planted out, but it was the environment provided afterwards which was more important. They had also observed that the photosynthetic capacity of the leaves of microcultured plants was less than half when compared to that of a normal green house grown plant and it varied considerably during the initial stages of planting out. However, this capacity was restored after non *in vitro* rooting and establishment of the microcultured plantlets. The same principle may be underlying in the performance of micropropagated plantlets of banana varieties.

A tissue culture method, to become commercially acceptable, has to ensure a reasonable percentage of plant survival in the field. The growth and yield of such plants should also be better than that of seedlings or other conventional materials of propagation. Banana being a perennial plant may take ten to sixteen months from planting to harvest depending on the cultivar (Karmacharya, 1984). Hence, in the present investigation a detailed field study could not be undertaken in the comparative performance between plants raised through tissue culture method and conventional method.

The salient findings of the present investigation have been discussed above, which indicates possibilities for developing shoot tip and eye bud culture technique as a means for rapid multiplication of the three banana cultivars on a commercial scale. For successful commercial exploitation of _in vitro_ propagation method some of the problems identified during the present investigation has to be solved for which intensified work is needed in specific areas. One of the critical factor which influences the rapid multiplication and establishment of _in vitro_ grown banana plants is the adoption of an economically and commercially feasible hardening method. Environmental factors like humidity, temperature, light etc. has found to influence the establishment of tissue culture derived banana plants. Eventhough the tissue culture method of propagation of banana plants has been standardised, further research

is needed to standardise an economically feasible hardening process, so that *in vitro* propagation method of banana can be undertaken on a commercial scale.

It is stated that tissue culture propagation ensures genetic stability. But it is possible that continuous subculturing might result in somaclonal variation. Research on this aspect will help to pinpoint the various aspects of this phenomenon. If found advantageous, it might be possible to commercially exploit this phenomenon. Certain novel approaches for improving banana through tissue culture are proposed in Fig. 5, the achievement of which will definitely help in the improvement of banana cultivation in India.

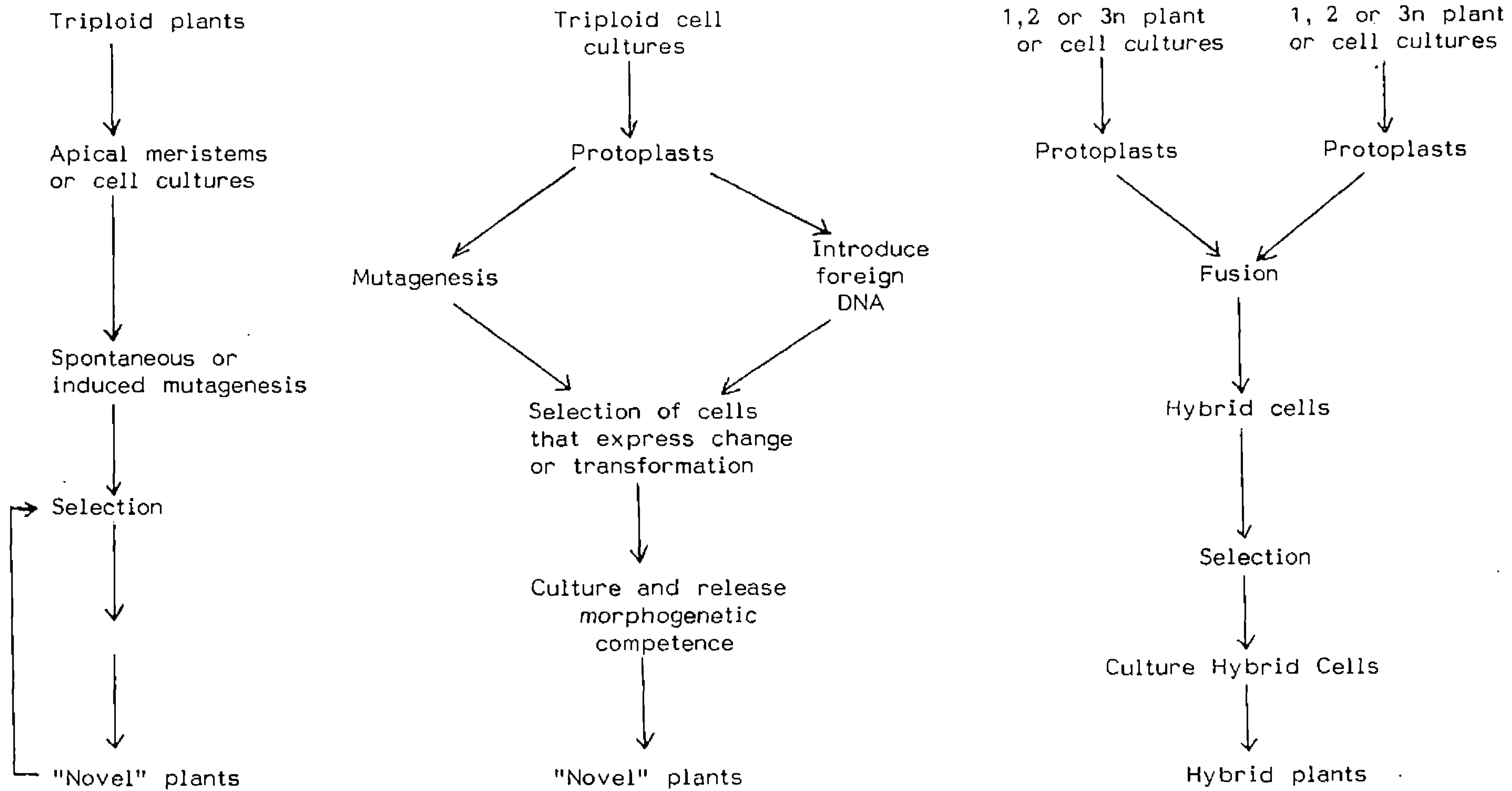


Fig. 5. Novel approaches for improving banana through tissue culture

SUMMARY

SUMMARY

Investigations on standardising the *in vitro* propagation technique in banana were carried out in the Plant Tissue Culture Laboratory, Department of Pomology and Floriculture, College of Horticulture, Vellanikkara during the period 1988-1990. The results of the study are summarised below..

1. Physically injuring the apical dome of shoot tip explants into two halves and culturing each half separately was found to be best in enhancing the release of axillary buds in culture.

2. For floral apex explants the sterilization treatment which resulted in least contamination was an initial rinsing of the explants with 95 per cent absolute alcohol for 30s' followed by mercuric chloride treatment (0.05 per cent) for 10 min. For shoot tip and eye bud explants the best sterilization treatment was found to be that using mercuric chloride (0.2 per cent) for five min.

3. Shoot tips collected during November to April recorded least contamination rate (30 to 40 per cent) in culture.

4. The addition of ascorbic acid into the media at the rate of 50 mg/l reduced media and explant discolouration due to polyphenol oxidation.

5. For the better and speedier explant establishment and growth of shoot tip, eye bud and floral apex explants, MS (semi-solid) medium containing NAA 0.5 ppm and BA 3.0 ppm was found to be the best.

6. Addition of gibberellic acid to the culture medium was found to be highly unfavourable for culture establishment and growth in shoot tip, eye bud and floral apex culture as evidenced by the time taken for establishment of culture.

7. In shoot tip culture, on an average each explant released 11.00 axillary shoots when the BPM contained NAA 1.0 ppm and BA 10.0 ppm or BA 10.0 ppm. In the case of eye bud and floral apex explants on an average each explant released 11.25 and 12.0 axillary shoots respectively, when the BPM contained NAA 0.5 ppm and BA 10.0 ppm.

8. When the performance of shoot tip, eye bud and floral apex explants were compared, floral apices took more time for culture establishment. Eye bud and shoot tip explants took more or less the same time.

9. 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 culture.

10. The three different cultivars namely Nendran, Palayankodan and Red banana responded differently in culture with regard to the time taken for the establishment of culture and the percentage of culture established. The reason may be attributed to the difference in physiological conditions and the level of endogenous phytohormones present in these cultivars.

11. When MS medium was compared with KC medium, it was found that KC medium was as good as or even better than MS medium in inducing axillary buds and producing healthy shoots with good green leaves in shoot tip, eye bud and floral apex culture.

12. The addition of CW into the BPM was found to have no significant influence on the number of axillary shoots induced in _in vitro_ culture.

13. The number of axillary shoots produced per explant per culture vessel increased at a mean rate of 5.70, 5.73 and 5.58 per subculture in Nendran, Palayankodan and Red banana respectively when subculturing was carried out at four week intervals continuously for ten serial subculture.

14. A light intensity ranging between 80 W and 120 W for 16 h followed by 8 h dark period was found to be giving maximum growth and development of axillary shoots in shoot tip

culture and a light intensity of 80 W was found to be the best for eye bud and floral apex culture.

15. For *in vitro* rooting KC (semi-solid) medium containing NAA 5.0 ppm was found to be the best with respect to root initiation, producing maximum number of roots (6.75/shoot), in maximum number of culture (100 per cent) within the shortest period of time (6 days).

16. When the *in vitro* rooting ability of three banana cultivars were compared, the banana cv. Palayankodan recorded the maximum number of roots (8.0/shoot) and the minimum number of days for root initiation (5.5).

17. Rooting medium containing sucrose at 1.5 per cent concentration was found optimum for the production of sufficient roots and normal plantlets.

18. The addition of AC at 0.025 per cent to the basal rooting medium resulted in the initiation of maximum number of roots (10.75/shoot) within a period of seven days.

19. Ninety per cent of planted out plantlets survived when the plantlets after removal from the rooting media were kept in distilled water for 8 h and then for another 8 h in MS liquid medium of 1/10th strength under moistened microscope cover and then planting out into the potting mixture covered with moistened plastic cover for four weeks.

20. The most suitable potting medium giving maximum plant survival percentage (9.0) was found to be a 1:1 (v/v) mixture of vermiculite and sand.

21. Tubular polythene bags of 15 cm diameter and 20 cm length were identified as the best container recording 90 per cent survival of plantlets.

22. Emesan (0.1 per cent) was found to be the best chemical sterilant for sterilizing the potting mixture as it resulted in cent per cent survival of planted out plantlets.

23. When the growth performance of the plantlets of the three banana cultivars were compared significant difference was found to exist between varieties in the case of plant height and the length of the longest leaf. Red banana was found to be significantly lower in height than the other two cultivars. With regard to the length of the longest leaf, Red banana recorded the maximum value. No significant difference could be observed in the number of leaves produced per plant in all the stages of growth among the cultivars.

REFERENCES

REFERENCES

Amerson, H.V., Frampton, Jr. L.J., Mc Keand, S.E., Mott, R.L. and Weir, R.J. 1985. Loblolly pine tissue culture: Laboratory, green house and field studies. Henke, R.R., Hughes, K.W., Constantin, M.J. and Hollaender, A. (Ed.) Tissue Culture in Forestry and Agriculture. Plenum Press, New York, pp.271-287.

Ancora, G., Belli Donini, M.L. and Cuozzo, L. 1981. Globe artichoke plants obtained from shoot apices through rapid in vitro micropropagation. Sci. Hort. **14**:207-213.

Anderson, W.C. 1980. Mass propagation by tissue culture: Principles and techniques. In: Proceedings of Conference on Nursery Production of Fruit Plants through Tissue Culture Applications and Feasibility. U.S.D.A., Maryland, pp.1-10.

Aravindakshan, K. 1989. Studies on in vitro propagation of diploid banana hybrids and their parents. Ph.D. (Hort.) thesis submitted to Tamil Nadu Agricultural University, Coimbatore.

Arias, O. and Valverde, M. 1987. Production y variacion somaclonal de plantas de banano variedad Grande Naine producides por cultivo de tejidos, ASBANA. Revista de la Asociacion Bananera Nacional (Costa Rica) **11**(28):6-11.

Ascenso, J.C. 1967. A simple technique for the multiplication of banana planting material. Trop. Agric. **44**:243-244.

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.

Balakrishnamoorthy, G. and Sreerangaswamy, S.R. 1988. Regeneration of banana plantlet 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., De Langhe, E. 1987. Somatic embryogenesis in Musa. In: Proceedings of the Third Conference of International Association for Research on Plantain and Other Cooking Bananas (IARPCB). Abidjan, Ivory Coast. 27-31, May 1985. pp.13.

Banerjee, N., Vuylsteke, D. and De Langhe, E. 1986. Meristem tip culture of Musa; histomorphological studies of shoot bud proliferation. In: Withers, L.A. and Anderson, P.G. (Ed.). Plant Tissue Culture and its Agricultural Applications. Butterworths, London. pp.139-147.

Banks, M.S. and Hackett, W.P. 1978. Differentiation from Hedera helix. Pl. Physiol. Suppl. **61**:245.

Bapat, V.A. and Rao, P.S. 1977. Shoot apical meristem culture of Pharbitis nil. Pl. Sci. Lett. **10**:327.

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

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

Brown, C.L. and Sommer, H.E. 1982. Vegetative propagation of dicotyledonous trees. In: Bonga, J.M. and Durzan, D.J. (Ed.), Tissue Culture in Forestry. Martinus Nijhoff/Dr.W. Junk Publishers, London, pp.109-149.

Chand, S. and Roy, S.C. 1980. Shoot tip culture as a means of propagation of genetically alike plants. Sci. Cult. **46**: 307-308.

Chong, C. and Pua, E.C. 1985. Carbon nutrition of Ottawa-3 apple rootstock 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.) In: Bajaj, Y.P.S. (Ed.), Biotechnology in Agriculture and Forestry Vol.1. Trees. Springer-Verlag, Berlin, pp.233-252.

Damasco, O.P. and Barba, R.C. 1985. in vitro culture of Saba banana (Musa balbisiana cv. Saba (ABB). International Rice Research Institute (En. 6 ref.) 41-44.

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

David, A. 1982. In vitro propagation of gymnosperms. In: Bonga, J.M. and Durzan, D.J. (Ed.), Tissue Culture in Forestry. Martinus Nijhoff/Dr.W.Junk Publishers, London. pp.72-101.

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

De Guzman, E.V., Decena, A.C. and Ubalde, E.M. 1980. Plantlet regeneration from unirradiated and irradiated banana shoot tip tissues cultured 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 for induced mutation studies. In: Improvement of Vegetatively Propagated Plants and Tree Crops through Induced Mutations. International Atomic Energy Agency, Vienna. pp.33-54.

De Langhe, E. 1961. La Phyttotaxie du bananier it ses consequences pour la comprehension du systeme rijetonnant, Fruits **16**: 429-441.

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

Doreswamy, R. and Sahijram, L. 1989. Micropropagation of banana from male floral apices cultured in vitro. Sci. Hort. **40**: 181-188.

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

Evans, D.A., Sharp, W.R. and Flinck, C.E. 1981. Growth and behaviour of cell cultures. Embryogenesis and organogenesis. In: Thorpe, T.A. (Ed.), Plant Tissue Culture: Methods and Applications in Agriculture. Academic Press, New York, pp.45-114.

Fabbri, A., Sutter, E.G. and Dunston, S.K. 1984. Morphological adaptation of strawberry plant growth in vitro to growth chamber conditions. HortScience **19**:259.

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

Fitchet, M. and De Winnaar, W. 1987. Rapid multiplication of bananas. Inf. Bull. Citrus Subtropical Fruit Res. Inst. **179**: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. **187**:12-16.

*Franclet, A. 1979. Rejeunnissement des arbres adultes en vue de leur propagation vegetative. Micropropagation des Arbres Forestiers, Annales AFOCEL No.12, 6/79 AFOCEL, Nangis, France, pp.3-18.

Fridborg, G., Pedersen, M., Landstorm, L. and Eriksson, T. 1978. The effect of activated charcoal on tissue cultures: Absorption of metabolites inhibiting morphogenesis. Physiol. Plant. **43**:104-106.

Gamborg, O.L., Murashige, T., Thorpe, T.A. and Vasil, I.K. 1976. Plant tissue culture media. In Vitro **12**:473-478.

Gamborg, O.L. and Shyluk, J.P. 1981. Nutrition, media and characteristics of plant cell and tissue culture. In: Thorpe, T.A. (Ed.), Plant Tissue Culture: Methods and Applications in Agriculture. Academic Press, New York, pp.21-44.

George, E.F. and Sherrington, P.D. 1984. Plant Propagation by Tissue Culture: Handbook and Directory of Commercial Laboratories. Exegetics Ltd., England. pp.709.

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

Gupta, P.P. 1986. Eradication of mosaic disease and rapid clonal multiplication of bananas and plantains through meristem tip culture. Pl. Cell Tissue Organ 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 citriodera. Hook by tissue culture. Pl. Sci. Lett. 20:195-201.

*Haberlandt, G. 1902. Kulturversuche mit isolierten Pflanzenzellen S.B. Sber. Akad. Wiss. Wien, 111:69-92.

Hamilton, K.S. 1965. Reproduction of banana from adventitious buds. Trop. Agric. 42:69-73.

*Heller, R. 1953. Recherches sur la nutrition minerale des tissus vegetaux cultives in vitro. Ann. Sci. Nat. Bot. Biol. Veg. 14(11):1-223.

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

Hu, C.Y. and Wang, P.J. 1983. Meristem, shoot tip and bud cultures. In: Evans, D.A., Sharp, W.R., Ammirato, P.V. and Yamada, Y. (Ed.). Handbook of Plant Cell Culture Vol.I. Techniques for Propagation and Breeding. Macmillan, New York, pp.177-227.

Hussey, G. 1983. In vitro propagation of horticultural and agricultural crops. In: Mantell, S.H. and Smith, H. (Ed.). Plant Biotechnology. Cambridge University Press, Cambridge, pp.111-138.

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

Jarret, R.L., Fisher, J.B. and Litz, R.E. 1985. Organ formation in Musa tissue cultures. J. Pl. Physiol. 21:123-130.

Karmacharya, J.L. 1984. Pollen morphology, fertility and compatibility studies in banana. M.Sc.(Hort.) thesis submitted to Kerala Agricultural University, Vellanikkara, Trichur.

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.

Knudson, L. 1946. A new nutrient solution for the germination of orchid seed. Bull. Am. Orchid Soc. 15:214-217.

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

Krikorian, A.D. 1982. Cloning higher plants from aseptically cultured tissues and cells. Biol. Rev. 57:151-218.

Krikorian, A.D. 1989. In vitro culture of bananas and plantains: background, update and call for information. Trop. Agric. 66:194-200.

Krikorian, A.D. and Cronauer, S.S. 1984a. Bananas. In: Sharp, W.R., Evans, D.A., Ammirato, P.V. and Yamada, Y. (Ed.), Handbook of Plant Cell Culture, Vol.II. Crop Species. Macmillan, New York, pp.327-348.

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

Krishnamoorthy, H.N. 1981. Plant Growth Substances. Tata Mc Graw Hill, New Delhi, pp.79.

Kunisaki, J.T. 1980. In vitro propagation of Anthurium andreanum L. HortScience 15:508-509.

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

Letham, D.S. 1974. Regulators of cell division in plant tissues. XX. The cytokinins of coconut milk. Physiol. Plant. 32: 66-70.

Litz, R.E. and Conover, R.A. 1978. In vitro propagation of papaya. HortScience 13:241-242.

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

Mante, S. and Tepper, B. 1983. Propagation of Musa textilis Nee plants from apical meristem slices in vitro. Pl. Cell. Tissue Organ Cult. 2:151-159.

*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. Chinese Soc. hort. Sci. 20:6.

Mateille, T. and Foncelle, B. 1988. Micropropagation of Musa AAA cv. Poyo in the Ivory Coast. Trop. Agric. 65:325-328.

Mohanram, H.Y. and Steward, F.C. 1964. The induction of growth in explanted tissue of the banana fruit. Can. J. Bot. 42: 1559-1579.

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

*Murashige, T. 1977. Manipulation of organ initiation in plant tissue cultures. Bot. Bull. Acad. Sin. 18:1-24.

Murashige, T. 1978. Principles of rapid propagation. In: Hughes, K.W., Henke, R.R. and Constantin, M. (Ed.), Propagation of Higher Plants through Tissue Culture - A Bridge between Research and Application. Tech. Inf. Centre, U.S.D.E., Univ. Tennessee, U.S.A. pp.14-24.

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

Novak, F.J., Afza, R., Duren, M. van, Perea-Dallos, M., Conger, B.V. and Tang, X.L. 1989. Somatic embryogenesis and plant regeneration in suspension cultures of dessert (AA & AAA) and cooking (ABB) bananas (Musa spp.). Biotechnology 7(2): 154-159.

Panse, V.G. and Sukhatme, P.V. 1985. Statistical Methods for Agricultural Workers. 4th ed. I.C.A.R., New Delhi, pp.131-143.

Pollard, J.K., Shantz, E.M. and Steward, F.C. 1961. Hexitols in coconut milk: their role in nurture of dividing cells. Pl. Physiol. 36:492.

Pool, D.J. and Irizarry, H. 1987. "Off-type" banana plants observed in a commercial planting of Grand Nain propagated using the in vitro culture technique, In: Proceedings of the VII Meeting of A.C.O.R.B.A.T., San Josa, Costa Rica, 23-27 September, 1985 (Galindo, J.J. and Jaramillo, R. eds.), CATIE, Centro Agronomico Tropical de Investigacion y Ensenanza, Turrialba, Costa Rica, Boletin Tecnico, No.121. pp.99-102.

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

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

Ramcharan, C., Gonzalez, A. and Knausenberger, W.I. 1987. Performance of plantains (Musa AAB Group) using tissue cultured plantlets in St. Croix, U.S. Virgin Islands, In: Proceedings of the Third Conference of International Association for Research on Plantain and Other Cooking Bananas (IARPCB), Abidjan, 27-31 May 1985. pp.36-39.

Rao, A.N. and Lee, S.K. 1986. An overview of the in vitro propagation of woody plants and plantation crops. In: Withers, L.A. and Alderson, F.G. (Ed.), Plant Tissue Culture and Its Agricultural Applications. Butterworths, London, pp.123-138.

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.

Reghunath, B.R. 1989. In vitro studies on the propagation of cardamom (Elettaria cardamomum Maton). Ph.D.(Hort.) thesis submitted to Kerala Agricultural University, Vellanikkara, Trichur.

*Reinert, J. 1958. Morphogenese und ihre kontrolle an gewebekulturen aus karotten. Naturwissenschaften 45:344-345.

Reuveni, O., Israeli, Y., Eshdat, Y. and Degani, H. 1986. Genetic variability in banana plants multiplied via in vitro techniques. Report to the International Board for Plant Genetic Resources, Rome.

Robinson, J.C. 1990a. Vegetative morphology and phenology of banana tissue culture plants. Ban. Newsl. No.13:9.

Robinson, J.C. 1990b. Tissue culture banana plants versus conventional suckers - components of yield. Ban. Newsl. No.13:10.

Rodriguez, G.A., Martin, J.R.L. and Enriquez, M.J.R. 1987. In vitro propagation of Canary island banana (Musa accuminata Colla AAA var. Dwarf Cavendish). Studies of factors affecting culture obtention, preservation and conformity of the plants. Acta Hort. 212:577-583.

Rowe, P.R. 1984. Breeding banana and plantains. Pl. Breed. Rev. 2:135-155.

*Rowe, P.R. and Richardson, D.C. 1975. Breeding bananas for disease resistance, fruit quality and yield. Trop. Agri. Res. Serv. (SIATSA). Bull. No.2:41pp.

*Schleiden, M.J. 1838. Beitrage zur Phytogenesis, Muller. Arch. Ant. Physiol. Wiss. Med. pp.137-176.

Shantz, E.M. and Steward, F.C. 1952. Coconut milk factor: The growth promoting substances in coconut milk. J. Am. Chem. Soc. **74**:6133-6135.

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

Skoog, F. and Miller, C.O. 1957. Chemical regulation of growth and organ formation in plant tissues cultured in vitro. In: Porter, A.K. (Ed.). The Biological Action of Growth Substances. Exptl. Biol. No.11, pp.118-131.

Smith, M.A.L., Palta, J.P. and Mc Cown, B.H. 1986. Comparative anatomy and physiology of microcultured, seedling and green house grown Asian white birch. J. Am. Soc. hort. Sci. **111**:437-442.

Steward, F.C. and Mapes, M.D. 1958. Growth and organized development of cultured cells. III. Interpretations of the growth from free cell to carrot plant. Am. J. Bot. **45**: 705-708.

Sun, Y.F. 1985. Propagation of various Musa sp by tissue culture. J. agric. Assoc. China No.130:52-57.

Sutter, E.G., Fabbri, A. and Dunston, S. 1985. Morphological adaptation of leaves of strawberry plants grown in vitro after removal from culture. In: Henke, R.R., Hughes, K.W., Constantin, M.J. and Hollaender, A. (Ed.). Tissue Culture in Forestry and Agriculture. Plenum Press, New York, pp.358-359.

Thimann, K.V. 1935. On the growth hormone produced by Rhizopus. J. Biol. Chem. 103:279-291.

*Thimann, K.V. 1977. Hormone Action in the Whole Life of Plant. University of Massachusetts Press, Amherst.

Thorpe, T.A. 1978. Physiological and biochemical aspects of organogenesis in vitro. In: Thorpe, T.A. (Ed.), Frontiers of Plant Tissue Culture. University of Calgary Press, Canada, pp.49-58.

Thorpe, T.A. 1980. Organogenesis in vitro: structural, physiological and biochemical aspects. Int. Rev. Cytol. Suppl. 11A:71-111.

Thorpe, T.A. and Patel, K.R. 1984. Clonal propagation: Adventitious buds. In: Vasil, I.K. (Ed.), Cell Culture and Somatic Cell Genetics. I. Laboratory Procedures and Their Applications. Academic Press, Orlando, pp.49-57.

Valsalakumari, P.K. 1984. Cytotaxonomical studies on banana cultivars. Ph.D. (Hort.) thesis submitted to the Kerala Agricultural University, Vellanikkara, Trichur.

Van Overbeek, J., Conklin, M.E. and Blakeslee, A. 1941. Factors in coconut milk essential for growth and development of very young Datura embryos. Science **94**:350.

Vanstaden, J. and Drewes, S.E. 1974. Identification of cell division inducing compounds from coconut milk. Physiol. Plant. **32**:347.

Vasil, I.K., Ahuja, M.R. and Vasil, V. (Ed.). 1979. Advances in Genetics. Academic Press, New York, pp.127-215.

Vessey. J.C. and Rivera, J.A. 1981. Meristem culture of bananas. Turrialba **31**(2):162-163.

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., Wilson, G.F. and De Langhe, E. 1988. Phenotypic variation among in vitro propagated plantain (Musa sp. cv. AAB). Sci. Hort. **36**:79-88.

Wainwright, H. 1988. Overcoming problems in establishing micro-propagules - guidelines for growers. Prof. Hort. **2**(3): 67-72.

Wang, P.J. 1978. Clonal multiplication of Cryptomeria japonica D. Don in vitro. Studies and Essays in Commemoration of the Golden Jubilee of Academia Sinica, Taipei pp.559-566.

Wang, P.J. and Huang, L.C. 1976. Beneficial effects of activated charcoal on plant tissue and organ cultures. In Vitro **12**:260.

Weatherhead, M.A., Burdon, J. and Henshaw, 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.

*White, P.R. 1943. Nutrient deficiency studies and an improved inorganic nutrient for cultivation of excised tomato roots. Growth **7**:53-65.

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

Yeoman, M.M. (Ed.). 1986. Plant Cell Culture Technology. Blackwell Scientific Publications, Melbourne, pp.33.

Ziv, M. 1986. In vitro hardening and acclimatization of tissue culture plants. In: Withers, L.A. and Alderson, P.G. (Ed.), Plant Tissue Culture and its Agricultural Applications, Butterworths, London, pp.187-196.

* Originals not seen

APPENDIX

APPENDIX-I

Analysis of variance for different characters

Treatment/Character	Mean square	
	Treatment	Error
1	2	3
A. Physiological pre-conditioning of the explant		
1. Explant establishment		
i. Collection and preparation of explants - influence of month of collection		
a. Number of shoots	1.65	0.60**
B. Enhanced release of axillary buds		
1. Culture establishment		
i. NAA + BA		
a. Days taken for culture establishment/shoot tip	48.40	1.00**
b. Days taken for culture establishment/eye bud	48.60	1.00**
c. Days taken for culture establishment/floral apex	30.20	1.00**
ii. IAA + BA		
a. Days taken for culture establishment/shoot tip	43.66	1.00**
b. Days taken for culture establishment/eye bud	34.23	1.00**
c. Days taken for culture establishment/floral apex	8.94	1.00**

Contd.

Appendix-I. Continued

1	2	3
iii. GA + BA		
a. Days taken for culture establishment/shoot tip	7.58	1.00**
b. Days taken for culture establishment/eye bud	4.98	1.14**
c. Days taken for culture establishment/floral apex	5.83	1.00**
iv. Varietal performance		
a. Days taken for culture establishment/Nendran	121.33	1.66**
b. Days taken for culture establishment/Palayankodan	4.00	1.66 NS
c. Days taken for culture establishment/Red banana	41.33	1.66**
2. Basal proliferation medium		
i. BA : NAA + BA		
a. Number of shoots/shoot tip	36.89	0.74**
b. Number of shoots/eye bud	36.21	0.62**
c. Number of shoots/floral apex	40.99	0.69**
ii. Kinetin: NAA + kinetin		
a. Number of shoots/shoot tip	5.32	0.38**
b. Number of shoots/eye bud	5.02	0.35**
c. Number of shoots/floral apex	5.67	0.35**

Contd.

Appendix-I. Continued

1	2	3
iii. BA : IAA + BA		
a. Number of shoots/shoot tip	5.21	0.41**
b. Number of shoots/eye bud	6.61	0.37**
c. Number of shoots/floral apex	5.81	0.27**
3. Medium supplements		
i. Knudsons medium		
a. Number of shoots/shoot tip	69.12	0.36**
b. Number of shoots/eye bud	58.95	0.45**
c. Number of shoots/floral apex	48.62	0.76**
ii. Coconut water		
a. Number of shoots/shoot tip	0.14	0.62 NS
b. Number of shoots/eye bud	0.06	0.78 NS
c. Number of shoots/floral apex	0.14	0.52 NS
4. Serial subculturing in BPM		
a. Number of shoots per shoot tip in Nendran	4.32	0.71**
b. Number of shoots per shoot tip in Palayankodan	4.52	0.50**
c. Number of shoots per shoot tip in Red banana	4.16	0.40**
5. Physical conditions		
i. Light		
a. Number of shoots/shoot tip	5.86	0.60**

Contd.

Appendix-I. Continued

1	2	3
b. Longest leaf/shoot tip	2.46	0.09**
c. Number of shoots/eye bud	4.54	0.46**
d. Longest leaf/eye bud	1.72	0.14**
e. Number of shoots/floral apex	3.14	0.38**
f. Longest leaf/floral apex	1.40	0.27**
C. *In vitro* rooting and hardening		
1. Basal rooting medium		
a. Number of roots/shoot	8.20	0.66**
b. Days for root initiation	19.12	0.68**
2. Varietai performance		
a. Number of roots/shoot	2.58	0.63**
b. Days for root initiation	0.25	0.41 NS
3. Medium supplements		
i. Sucrose		
a. Number of roots/shoot	7.39	0.52**
b. Days for root initiation	18.73	0.64**
ii. AC		
a. Number of roots/shoot	12.16	0.37**
b. Days for root initiation	5.56	0.22**

Contd.

1	2	3
D. Planting out and evaluation		
1. Varietal performance		
i. After four weeks under mist		
a. Plant height	0.68	0.06**
b. Leaves/plant	0.07	0.26 NS
c. Longest leaf	1.32	0.04**
ii. Eight weeks after planting out		
a. Plant height	1.63	0.04**
b. Leaves/plant	0.07	0.43 NS
c. Longest leaf	1.41	0.02**
iii. Twelve weeks after planting out		
a. Plant height	1.72	0.04**
b. Leaves/plant	1.40	0.63 NS
c. Longest leaf	0.43	0.04**

**Significant at 1 per cent level of probability

STANDARDISATION OF *IN VITRO* PROPAGATION TECHNIQUE IN BANANA

By

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ABSTRACT OF A THESIS

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ABSTRACT

Investigations were carried out at the Plant Tissue Culture Laboratory of the College of Horticulture, Vellanikkara during 1988-90 to standardise the *in vitro* propagation technique in banana.

Three banana cultivars namely Nendran (AAB), Palayankodan (AAB) and Red banana (AAA) were utilised for the study. For standardising the explant, three types of explants were used namely shoot tip, eye bud and floral apex. For culture establishment, axillary shoot initiation and *in vitro* rooting studies different types of growth regulators were made use of. They were auxins (NAA, IAA and IBA), gibberellin (GA) and cytokinins (BA and kinetin). The plantlets produced *in vitro* were subjected to different types of hardening treatments to secure a better establishment of planted out plants.

For shoot tip and eye bud explants, surface sterilization using mercuric chloride (0.2 per cent) for 5 min. was found to be the best, but for floral apex explant an initial rinsing of the explants with 95 per cent absolute alcohol for 30s followed by mercuric chloride treatment (0.05 per cent) for 10 min. was found to be best. Better and speedier explant establishment and growth of shoot tip, eye bud and floral apex explant was observed in MS (semi-solid) medium containing NAA 0.5 ppm and BA 3.0 ppm

Gibberellic acid was found to have unfavourable effect on culture establishment and growth.

Shoot tips collected during November to April recorded maximum survival percentage. Among the physical injury treatments for enhancing the release of axillary buds in culture splitting the apical dome of shoot tip longitudinally into two halves and culturing each half separately was found to be the best. The addition of ascorbic acid into the media at the rate of 50 mg/l reduced media and explant discolouration due to polyphenol oxidation. When the performance of the three explants were compared, floral apex explants took more time for culture establishment. The three banana cultivars used for the study responded differently in culture.

In shoot tip culture, on an average each explant released 11.00 axillary shoots when the basal proliferation medium contained NAA 1.0 ppm and BA 10.0 ppm or BA 10.0 ppm. In the case of eye bud and floral apex explants on an average each explant released 11.25 and 12.00 axillary shoots respectively, when the basal proliferation medium contained NAA 0.5 ppm and BA 10.0 ppm. Out of the two cytokinins tried (BA and kinetin), BA was found to be the most efficient one for the induction of axillary shoots. It was found that KC medium containing BA can be used for inducing axillary shoots. The addition of CW into the basal

proliferation medium was found to have no significant influence on the induction of axillary shoots. Axillary shoots produced per explant per culture vessel increased at a mean rate of 5.70, 5.73 and 5.58 per subculture in Nendran, Palayankodan and Red banana. Optimum light intensity for axillary shoot induction and proliferation was 80 W for a period of 16 h per day.

For *in vitro* rooting KC (semi-solid) medium containing NAA 5.0 ppm, AC 0.025 per cent and sucrose 1.5 per cent was found to be the best. Cent per cent of the planted out plantlets survived when the plantlets after removal from the rooting media were hardened by keeping in distilled water for 8 h and then for another 8 h in MS liquid medium of 1/10th strength under moistened microscope cover and then planting out into potting mixture (1:1 v/v mixture of vermiculite and sand) which was sterilised using emesan 0.1 per cent in tubular polythene bags covered with moistened plastic covers for four weeks. When the growth performance of the plantlets of the three banana cultivars were compared significant difference was found to exist between varieties in the case of plant height and the length of the longest leaf.