IN VITRO MULTIPLICATION AND STANDARDISATION OF HARDENING TECHNIQUES IN PINEAPPLE (*Ananas comosus* (L.) Merr.)

By

J. PRABHA

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 and Floriculture
COLLEGE OF HORTICULTURE
Vellanikkara - Thrissur

1993

DECLARATION

I hereby declare that this thesis entitled "*In vitro* multiplication and standardisation of hardening techniques in pineapple (*Ananas comosus* (L.) Merr.)" 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.

Vellanikkara
15-12-1993

J. PRABHA

Dr.R. KESHAVACHANDRAN
Assistant Professor
Department of Pomology & Floriculture

College of Horticulture
Vellanikkara
Dated: 15 -12-1993

CERTIFICATE

Certified that the thesis entitled "In vitro multiplication and standardisation of hardening techniques in pineapple (Ananas comosus (L.) Merr.)" is a record of research work done independantly by **Mrs.J.PRABHA,** 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.

R. Keshavachandran

Dr.R. KESHAVACHANDRAN
Chairman
Advisory Committee

CERTIFICATE

We, the undersigned members of the Advisory Committee of **Mrs .J.Prabha,** a candidate for the degree of Master of Science in Horticulture, agree that the thesis entitled "In vitro multiplication and standardisation of hardening techniques in pineapple (Ananas comosus (L.) Merr.)" may be submitted by Mrs.J.Prabha in partial fulfilment of the requirements for the degree.

Chairman : **Dr.R.Keshavachandran**
Assistant Professor
Dept. of Pomology & Floriculture
College of Horticulture
Vellanikkara

Members : 1. **Dr.P.K.Rajeevan**
Professor & Head (i/c)
Dept. of Pomology & Floriculture
College of Horticulture
Vellanikkara

2. **Dr.N.K.Vijayakumar**
Associate Professor
College of Forestry
Vellanikkara

3. **Dr.P.K.Sudhadevi**
Assistant Professor
Pineapple Research Station
Vellanikkara

J. B. M. MD. ABDUL KHADER

ACKNOWLEDGEMENT

I am extremely thankful and with great pleasure, I express my indebtedness to Dr.R.Keshavachandran, Assistant Professor, Department of Pomology and Floriculture and Chairman of my Advisory Committee for his deep interest, sharp comments, sustained and valuable guidance throughout the course of this investigation and preparation of the manuscript.

I gratefully recollect the co-operation and help extended to me by late Sri.M.K.Mammen, Professor and Head, Department of Pomology and Floriculture, for the successful completion of this work.

Sincerely, I wish to place on record my profound sense of gratitude to Dr.P.K.Rajeevan, Professor and Head (i/c), Department of Pomology and Floriculture, for his constant help and co-operation which contributed much to the timely completion of the programme.

My heartfelt thanks are due to Dr.N.K.Vijayakumar, Associate Professor, College of Forestry and member of the Advisory Committee for the help rendered to me in clearing the doubts during the course of this work.

I gratefully acknowledge the help rendered to me by Dr.Sudhadevi, Assistant Professor, Pineapple Research Station, Vellanikkara and member of the Advisory Committee during the course of the study and for permitting the collection of explants for the investigations.

The co-operation and help extended by the staff members of the Department of Pomology and Floriculture is acknowledged with very many thanks.

The immense help and valuable guidance rendered to me by Sri.P.V.Prabhakaran, Professor and Head, Department of Agrl. Statistics for the analysis of data and interpretation of results is acknowledged with a deep sense of gratitude. I am thankful to Miss.C.Chandrika and staff members of the Department of Agrl. Statistics for their help in the analysis of the data.

The assistance and co-operation extended to me by Sri.V.K. Raju, Professor and Head (i/c), Department of Processing Technology and Dr.A.V.R.Kesava Rao, Assistant Professor, Department of Agrl. Meteorology is gratefully acknowledged.

The encouragement given by my friends during the course of this study will always be remembered. I am also thankful to Sri.Muthu, Smt.Elsy, Smt.Kowjumma and Smt.Pathumma for their timely help.

My sincere thanks are due to Sri.Joy for the neat typing of the manuscript.

The award of fellowship by the Kerala Agricultural Univeristy is gratefully acknowledged.

I am totally indebted to my husband who has been a constant source of inspiration to me throughout the course of this work. I wish to express my heartfelt thanks to my parents, whose blessings were always with me in this endeavour.

J. PRABHA

ABBREVIATIONS

BAP	benzylamino purine
cv	cultivar
2,4-D	2,4-dichlorophenoxy acetic acid
IAA	Indole-3-acetic acid
IBA	Indole-3-butyric acid
KIN	Kinetin; N^6-furfuryl adenine
MS	Murashige and Skoog's (1962) medium
NAA	α-naphthalene acetic acid
Mg l^{-1}	milligrams per litre
mM	millimolar
uM	micromolar
v/v	volume in volume
w/v	weight in volume

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

CONTENTS

Introduction

INTRODUCTION

Pineapple (Ananas comosus (L.) Merr.), belonging to the family Bromeliaceae, is one of the most important commercial fruit crops in the world, being mainly utilised for its processed products. In India, it is cultivated in an area of 83,490 ha, the main areas of cultivation being Kerala and the North Eastern regions. In Kerala, pineapple is grown in 4724 ha with an annual production, of 46265 tonnes (Farm Guide, 1993).

Pineapple is normally propagated vegetatively through the use of suckers, slips and to a lesser extent crowns. One of the major problems in the cultivation of pineapple is the limited availability of planting materials (Mathews, 1988). The main commercial variety Kew has a shy suckering habit and under Kerala conditions, slip production in this variety is rare. The other constraints in the cultivation of this crop are the non-uniform growth and asynchronous flowering among the individual plants in a field (Mathews, 1988). Often, very high rates of spontaneous mutations occur in the field which make it difficult to obtain true to type propagules from a desirable parent plant or a selected variety (Wakasa, 1989).

The use of in vitro techniques for clonal propagation has become the most widely used application of tissue culture technology in horticulture in the recent years (Thorpe, 1990). By adopting

micropropagation techniques, clonal plants could be produced at a very rapid rate compared to conventional methods (Chaturvedi and Sharma, 1988).

Micropropagation can be achieved by enhancing axillary bud breaking, production of adventitious buds directly or indirectly via callus and somatic embryogenesis directly or indirectly (Murashige, 1974). Following the successful rapid multiplication of orchids by shoot meristem culture (Morel, 1965) there has been, in recent years, an increasing interest in the application of micropropagation techniques as an alternate means of vegetative propagation of horticultural plants.

The *in vitro* derived plantlets have to be acclimatised to the entirely different environment prevailing *in vivo* as they are poorly adapted to resist the low relative humidity, higher light intensity and temperature met with, in the outside. A period of hardening is considered essential for the newly transferred plantlets to adapt to the outside environment during which the plantlets undergo morphological and physiological adaptation enabling them to ensure maximum survival when planted out (Sutter, 1985).

The major cost of producing *in vitro* plants is in the rooting and hardening stages (Rajeevan and Pandey, 1986). The shoots produced *in vitro* could be rooted *ex vitro* by treating with growth regulators and keeping under high humidity conditions in a mist chamber. Rooting under *ex vitro* conditions also facilitates the

combining of the rooting stage with acclimatization (George and Sherrington, 1984).

Encapsulation of differentiating tissue in a biodegradable synthetic polymer is a method to facilitate mechanised handling of the propagules. In addition, formation of the synthetic seeds also help in economising the time, space and cost involved in normal micropropagation procedures (Mathur et al., 1989).

In the light of this background, the present study was conducted with the following objectives.

1. To standardise the method of rapid multiplication of pineapple through in vitro methods.
2. To standardise the techniques of hardening of in vitro derived plantlets.
3. To determine the optimum container type and potting mix for maximum establishment of in vitro plantlets.
4. To develop a method of encapsulating pineapple shoots.

Review of Literature

REVIEW OF LITERATURE

2.1 *In vitro* culture

The concept of totipotency which is inherent in the cell theory of Schleiden (1838) and Schwann (1839), is the foundation for plant tissue culture. The first person to isolate fully differentiated cells was Haberlandt (1902) and he postulated that the isolated cell is capable of resuming uninterrupted growth. Hannig (1904) excised nearly mature embryos of some crucifers and successfully grew them to maturity on mineral salts and sugar solution. Van Overbeek *et al.* (1941) for the first time demonstrated the stimulatory effect of coconut milk on embryo development and callus formation. The first successful report of continuously growing cultures of tomato root tips was made by White (1943).

Skoog and Miller (1957) put forth the concept of hormonal control of organ formation and showed that the differentiation of roots and shoots was a function of the auxin-cytokinin ratio and that organ differentiation could be regulated by changing the relative concentrations of these two substances in the medium.

Morel (1960) applied the technique of shoot tip culture for rapid propagation of orchids and it became possible to produce large numbers of genetically identical plants from a single bud within a short time. Murashige (1977a) developed methods

for *in vitro* propagation of several species ranging from ferns to foliage, flower and fruit plants. He also developed the first completely defined nutrient medium along with Skoog in 1962. Wickson and Thimman (1958) showed that the growth of axillary buds which remained dormant in the presence of terminal buds can be initiated by the exogeneous application of cytokinin. Since then, plant tissue culture has evolved as a powerful research tool in the fundamental and applied aspects of agriculture.

Several aspects of tissue culture are currently being applied to agriculture. The main commercial applications so far has been in the production of clonal plants at a very rapid rate compared to the conventional methods through tissue culture techniques. These plants are reported to grow faster and mature earlier than seed propagated plants (Vasil and Vasil, 1980).

As per Murashige (1974), there are three possible routes available for *in vitro* propagule multiplication

a) Enhanced release of axillary buds

b) Production of adventitious shoots through organogenesis

c) Somatic embryogenesis

Shoot tip culture ensures genetic uniformity while somatic organogenesis through a callus phase may be useful for recovery of useful variant lines. Somatic embryogenesis though limited to a few plant species is the most rapid mode of plant regeneration (Evans *et al.*, 1981).

2.2 *In vitro* studies in pineapple

Pineapple (*Ananas comosus* (L.) Merr.) is a fruit crop which originated in the tropical regions of America. There are many varieties of pineapple and the important ones cultivated in Kerala are Kew and Mauritius. Pineapple is conventionally propagated vegetatively by the use of slips, suckers and crowns. One of the major problems in the cultivation of pineapple is the limited source of planting materials. Production of slips in the commercial variety Kew is rare under Kerala conditions and it has a shy suckering habit. Among the other constraints in the cultivation of this crop are the non-uniform growth and asynchronous flowering among the individual plants in the field. Very high rate of spontaneous mutations occur in the field which make it difficult to obtain true to type propagules from a desirable parent plant (Mathews, 1968).

2.2.1 Explants

The explants used for *in vitro* propagation include crowns, axillary buds from crowns, suckers, slips and syncarp slices. Aghion and Beauchesne (1960) obtained a few plantlets from crowns cultured *in vitro*. Mapes (1973) reported numerous plantlets and protocorm like bodies from the shoot tips of pineapple cv. Smooth Cayenne. Lakshmisita *et al*. (1974) were able to obtain plantlets from *in vitro* shoot tip culture of pineapple using explants from 1-1.5 month old slips of cv. Kew. Teo (1974) cultured meristem

7

tissues excised from pineapple plants when callus tissue formed after 30 days and callus differentiated into plantlets after a further thirty days.

Mathews et al. (1976) reported that pineapple plantlets could be raised in large numbers by in vitro culture of the terminal or axillary buds isolated from the crown. Supernumerary shoots arose from single buds after callus formation or originated de novo in the axils of leaves when grown in appropriate media.

Pannatier and Lanaud (1976) reported two methods of in vitro propagation of A. comosus cv. Smooth Cayenne using crown axillary leaf buds. In the first method, the plantlet obtained is sectioned horizontally or longitudinally and the fragments replanted on a similar medium. In the second method, a single bud can theoritically provide about a million plants after two years. The bud is grown for 10 days to a size of about 3 mm. It is then detached from the mother fragment and transferred to another medium.

Mathews and Rangan (1979) reported the formation of multiple plantlets in lateral bud cultures of pineapple. Plantlets were obtained from dormant axillary buds excised from the crown.

Wakasa et al. (1978) reported that various explants such as young syncarp, axillary buds from suckers or slips, small

crown and small slips in culture produced a mass, similar to a protocorm that grew vigorouly and subsequently differentiated into plantlets. Zepeda and Sagawa (1981) used axillary buds from crowns of mature fruit for in vitro propagation of pineapple plants. Rao et al. (1981) obtained differentiation of plantlets in 'Kew x Queen' hybrid embryo callus of pineapple. Embryonic callus was initiated by planting seeds with hybrid embryos. Cabral et al. (1984) reported micropropagation of pineapple by axillary buds from off shoots of cv. Smooth Cayenne.

Fitchet (1987) reported micropropagation of pineapple using basal buds from pineapple crown leaves in cvs. Queen and Cayenne. About 2000 plants could be obtained from one crown (yielding 40 buds) within a year.

Evans and Moore (1987) have suggested a means of mass production of pineapple propagules using axillary buds of Smooth Cayenne and Cambray pineapples. Axillary buds were initially cultured whereby numerous shoots were regenerated from each bud. Shooting material was produced continuously with approximate shoot removal and subculturing.

Liu et al. (1987) reported the in vitro propagation of spineless Red Spanish pineapple. Excised lateral buds (0.4-0.8 mm) and meristem tips from crowns of a promising spineless form of this normally spiny cultivar were rapidly propagated. Rosa-Marquez

et al. (1987) have reported callus induction and regeneration from tissue explants of Red Spanish pineapple. Dewald (1988) reported production of large number of viable plantlets of A. comosus and A. bracteatus var. tricolor. A system was employed utilising shake cultures derived from axillary buds of crown, slips and stems.

2.2.2 Surface sterilization of explants

The explants collected from the field, harbour numerous microorganisms which, when inoculated on to a nutrient medium contaminates the entire in vitro system. Hence, surface sterilization is resorted to prior to inoculation of explants. The efficiency of the sterilants used are found to vary depending upon the type of chemical, concentration, time of exposure etc.

Sodium hypochlorite (0.5 - 2.0 per cent w/v), calcium hypochlorite (filtered 5 - 10 per cent w/v) and mercuric chloride (0.05 - 0.1 per cent w/v) are the most commonly used surface sterilants. Since the sterilants are toxic to plant cells, the tissues have to be washed thoroughly with sterile, distilled water to ensure dilution of the chemical (Hu and Wang, 1983). Sommer and Caldas (1981) reported that for softer tissues, dilution to a lower strength may be needed.

Lakshmisita et al. (1974) reported surface sterilization of meristem tips of pineapple slips cv. Kew. The tiny growing

apex was sterilised with 75 per cent alcohol for 5 minutes and then rinsed in sterile distilled water and immersed in chlorine water for 5 to 10 minutes. Almost all the scale leaves visible to the naked eye were removed, leaving shoot tip to a very small size measuring about 3 - 5 mm in size.

Wakasa et al. (1978) used various explants such as suckers, slips, crowns and syncarps for in vitro propagation of pineapple. All materials were washed well with tap water, the leaves of suckers or slips were carefully removed and the small dormant axillary buds at the base of the leaves were excised. These buds alongwith a part of the shoot were sterilised in 5 per cent sodium hypochlorite solution for 10 to 20 minutes. The material was rinsed with sterile water several times before the colour of the buds turned brownish. This condition was not enough for complete sterilization; however, these parts were so sensitive to sodium hypochlorite solution that a stronger condition could not be used. The young syncarps about 3.5 to 4.5 cm in length, after removing the sepals, petals, stamens and pistils were immersed in 20 per cent sodium hypochlorite solution for 10 to 20 minutes. After further removing the outside layer thinly, the material was sliced by 1 to 2 mm thickness horizontally at first, then cut into pieces small enough to be placed into test tubes. These slices, including ovaries, pericarps and floral axes were thoroughly rinsed with sterile water. The same procedure was applied for culture

of small crowns and slips taken from the top or base of young syncarps.

Mathews and Rangan (1979) have reported surface sterilisation of dormant lateral buds from crowns of pineapple, with 0.1 per cent mercuric chloride for 3 minutes and then rinsed several times in distilled water.

Zepeda and Sagawa (1981) excised lateral buds from crowns of mature fruit. Crowns were disinfected in 0.5 per cent sodium hypochlorite (0.1% clorox) with 3 drops of wetting agent (Tween 20/100 ml) for 60 minutes. Axillary buds were then excised and disinfected in 1 per cent clorox for about 20 minutes.

Cabral et al. (1984) disinfected pineapple axillary buds with sodium hypochlorite (0.5 to 2.0%) and this treatment was reported to give better results than buds not disinfected.

Dewald et al. (1988) have reported surface sterilisation of crowns and stems of various cultivars. Crowns and stems were rinsed in water, defoliated and surface sterilised by agitation in a 20 per cent clorox solution (1% sodium hypochlorite) with 2 - 3 drops of a surfactant (Tween 20) for 20 minutes, followed by 3 rinses of 10 minutes each in sterile water. Terminal and axillary buds were then excised asceptically and surface sterilised in 2 per cent clorox solution for 10 minutes, followed by three 10 minute rinses in sterile water.

Zee and Munekata (1992) excised the axillary bud pieces (1 cm^3) from mature green house grown plants of A. comosus L. Merr, and disinfected in 15 per cent clorox solution (5.25% sodium hypochlorite) and Tween-20 (two drops per 100 ml) for 15 minutes followed by 10 per cent clorox and Tween-20 for 10 minutes. Pieces were then trimmed to 5 mm^3 in 5 per cent clorox and Tween-20 and soaked for 1 hour in 1 per cent clorox solution. Buds were rinsed in 1 ml of sterile water for 10 minutes.

2.2.3 Culture medium

One of the most important factors governing the growth and morphogenesis of plant tissues is the composition of the media. Murashige and Skoog's (1962) medium developed for tobacco has been used for in vitro culture of many plant species (Bonga, 1980). The MS medium has the highest salt content and some workers found it beneficial to reduce the strength by half (Skirvin, 1980, Griffis et al., 1981).

For in vitro propagation of pineapple, the different media used include Knudson's basal media with Nitsch's micro elements (Lakshmisita et al., 1974), MS medium (Mapes, 1973; Pannatier and Lanaud, 1976; Mathews and Rangan, 1979; Wakasa et al., 1978; Zepeda and Sagawa, 1981; Cabral et al., 1984; Ramirez, 1984; Evans and Moore, 1987; Liu et al., 1987; Rosa-Marquez, 1987; Dewald et al., 1988; Marchal and Alvard, 1988; Zee and Munekata, 1992 and Bordoloi and Sarma, 1993) and the Murashige and Tucker's

medium (Fitchet, 1989).

2.2.4 Growth regulators

The correct growth regulator used in optimum concentration influences the success of an in vitro system (Krikorian, 1982). Skoog and Miller (1957) showed in tobacco cultures that the type of morphogenesis could be influenced by the concentration of auxin and cytokinin in the culture medium. Shoot induction is promoted when the cytokinin level is higher than that of the auxin. Root induction is promoted when the concentration of cytokinin is less/ low relative to that of auxin, but at intermediate concentrations, unorganised callus growth results. Though the types of auxin and cytokinin and their concentrations may vary, this basic concept has been used to regenerate a wide variety of plants (Murashige, 1974).

For axillary shoot proliferation, cytokinin has been used to overcome the apical dominance of shoots and to enhance the branching of lateral buds from leaf axils (Murashige, 1974). Hu and Wang (1983) made a study of the growth regulators used for meristem and shoot tip culture and found that among the cytokinin containing culture establishment medium, 68 per cent contained BAP, 23 per cent Kinetin and 9 per cent 2iP, zeation and SD 8339. The effectiveness of the cytokinin varies with the plant species.

Lo et al. (1980) reported that high cytokinin content was deleterious to the initiation and elongation of roots of plants. Ancora et al. (1981) observed that some times, the residual cytokinin from stage II cultures was found to be high enough to suppress root formation. Hu and Wang (1983) described the young shoot apex as an active site for auxin biosynthesis and that for culture establishment, exogenous auxins were not always needed. However, though auxins do not promote axillary shoot proliferation, culture growth has been improved by their presence (Wang and Hu, 1980). One of the possible roles of auxin in culture proliferation medium is to nullify the suppressive effect of high cytokinin concentrations on axillary shoot elongation thereby restoring normal shoot growth (Lundergan and Janick, 1980). Too high a concentration of auxin may not only inhibit axillary bud branching but also induce callus formation (Hasegawa, 1980).

Mapes (1973) cultured explants from shoot tip in MS basal medium plus adenosine at 30 ppm or adenine at 20 ppm. Lakshmisita et al. (1974) inoculated shoot tips measuring about 3.0 - 5.0 mm in size on Knudson's basal media with Nitsch's micro elements. Clusters of thick roots and leaves developed on the medium with 1 ppm NAA. At the 4th leaf stage, the plantlets were subcultured into a medium of same constitution and were allowed to grow under increased light intensity. At the end of a 12th week period, these developed into dark green healthy plants with leaves measuring about 30-35 mm in length.

Pannatier and Lanaud (1976) have reported culture of crown axillary leaf buds in MS medium containing BA and NAA. In the first method, the plantlet obtained is sectioned horizontally or longitudinally and the fragments replanted on a similar medium. In the second method, in which a single bud can theoritically provide about a million plants after 2 years; the bud is grown for 10 days to a size of about 3 mm. It is then detached from the mother fragment and transferred to a medium containing BA, IBA and 2 per cent sucrose, where intense ramification occurs, with the formation of numerous buds.

Mathews and Rangan (1979) obtained in vitro plantlets from dormant axillary buds excised from the crown and grown on MS medium supplemented with NAA, IBA and KIN. Shoot buds appeared from lateral bud explants after 8-10 weeks on MS + NAA (1.8 mg l^{-1}) + IBA (2.0 mg l^{-1}) + KIN (2.1 mg l^{-1}). These regenerated buds, when transferred to fresh medium, developed a number of multiple buds and this cycle was repeatable. Such buds cultured on MS + NAA (0.18 mg l^{-1}) + IBA (0.4 mg l^{-1}) developed roots and grew into complete plantlets in 6-8 weeks. Shaking the culture flasks during growth, significantly increased the number of multiple shoots formed. A three fold increase in the number of multiple shoots per culture as compared to stationary cultures was observed. An average of 62 multiple shoots per culture were produced in shake cultures, as compared to 26 per culture

16

in stationary cultures. The multiple shoots when isolated and transplanted to a fresh medium of the same composition, with continued agitation again developed multiple shoots and large number of plants were reported to be obtained.

Wakasa et al. (1978) have reported culture of various explants in MS medium (Murashige and Skoog, 1962) supplemented with NAA and BA. The dormant axillary buds were initially cultured on MS medium with NAA 2 mg l^{-1} and BA 1-2 mg l^{-1} for formation of globular bodies. All the buds were transferred to the same composition medium after 45 days. The axillary buds that did not produce globular bodies on this medium even after two months formed the bodies when transferred to medium with 2 mg l^{-1} of NAA and BA. When very small crowns were cultured on the medium with 2 mg l^{-1} of NAA and 1 mg l^{-1} of BA, the growth pattern was similar to that of axillary buds. The growing crown when transferred to the medium with 2 mg l^{-1} of NAA and BA, numerous small leaves and buds developed first at the base of the crown and then all over. The process of differentiation from the cultured slip was similar to that from the crown. A number of leaves were produced on the medium with 1 mg l^{-1} of NAA and BA, within 4 months of incubation. Slices of syncarp when inoculated on media containing NAA and BA, produced lumpy tissues. The frequency of lumpy tissue formation was 10 to 20 per cent on media containing 2 mg l^{-1} of NAA, ten to 50 per cent

with 10 mg l^{-1} NAA and 60 per cent on medium with 10 mg l^{-1} each of NAA and BA. Of the different types of lumpy tissues subcultured to media containing 2 mg l^{-1} of NAA and 1 mg l^{-1} of BA, the nodular bodies showed further growth and these on transfer to the differentiation medium with 2 mg l^{-1} of BA and 2 mg l^{-1} of NAA formed shoots and leaves. On medium with 1 mg l^{-1} of NAA, the nodular bodies produced only roots.

Culture of axillary buds from crowns of mature fruit has been done in MS medium with 25 per cent coconut water (Zepeda and Sagawa, 1981). This was subcultured after two weeks on half strength MS with 25 per cent coconut water. Solitary shoots with 5-8 leaves were obtained within two months after initial culture. These were subcultured with 0.5, 1.0, 3.0 or 5.0 mg l^{-1} of BA. At least 3 axillary shoots were produced in cultures of 0.5 or 1.0 mg l^{-1} BA within 30 days. Further multiplication was obtained by separation of axillary shoots and subculturing four times in half strength MS medium with BA. Individual shoots were then transferred to solid half strength MS medium. An average of 16 shoots were obtained from 23 axillary buds per crown.

Rao et al. (1981) obtained differentiation of plantlets from embryonic callus initiated by planting seeds with hybrid embryos on medium containing BA or IBA (both at 0.1 mg l^{-1}). The radicle emerged out of seeds in about 7-15 days, callus formation started from the radicles and eventually enveloped the entire embryos.

Repeated subculturing resulted in the establishment of a continuously growing callus. Clumps of shoots with developed roots formed when callus pieces were subcultured to the basal MS medium plus NAA (2.0 mg l^{-1}) + IBA (2.0 mg l^{-1}) + BA (2.5 mg l^{-1}). Root formation started about 3 weeks from subculturing while after another 3 weeks, roots and shoots were fully developed. Individual plantlets were separated and planted on the basal medium. On an average, each hybrid embryo gave rise to 20-25 fully developed plants, averaged over 2 subcultures.

Cabral et al. (1984) used MS medium with 30 g sucrose per litre for initial culture of pineapple axillary buds from offshoots of cv. Smooth Cayenne, for initial rapid growth. MS + 30 g sucrose per litre + 1.8 mg NAA + 2.0 mg IAA + 2.1 mg BA and MS + 30 g sucrose per litre + 0.18 mg NAA + 0.4 mg BA induced multiple bud growth and rooting.

Ramirez (1984) reported that the most suitable medium for plantlet development was Murashige and Skoog's supplemented with NAA and KIN. Evans and Moore (1987) initially cultured axillary buds of Smooth Cayenne and Cambray pineapples in MS medium. Numerous shoots were regenerated from each bud. Shooting material was produced continuously with approximate shoot removal and subculturing. Ramirez (1987) has studied various modifications of MS medium for the in vitro culture of pineapple buds. Bud

growth was best on a medium containing 0.25 mg l^{-1} BA and 0.2 mg l^{-1} NAA.

Liu et al. (1987) on culturing excised lateral buds and meristem tips found that, MS medium without hormone, MS medium + 0.1 mg l^{-1} 2,4-D and AZ medium with 0.1 mg l^{-1} NAA, were the best for shoot differentiation. Modified liquid MS medium with 2,4-D at 0.1 mg l^{-1} and benzyl adenine at 0.5 mg l^{-1} was best for culturing the plantlets derived. Shoot differentiation and proliferation took 4-8 weeks.

Rosa-Marquez et al. (1987) used basal MS medium with benzyl adenine for induction of callus from tissue explants. Plant regeneration was best on MS medium with NAA added at 4 mg per litre. Callus regenerated into green plantlets after 2-3 weeks.

Dewald et al. (1988) cultured bud explants in MS medium, supplemented with 3 per cent sucrose, 0.8 per cent Difco Bacto-agar, 0.57 mM inositol, 1.2 uM thiamine HCl, 10.8 uM NAA and 8.8 uM BA. Proliferating explants were multiplied in 50 ml liquid cultures of the same medium in 125 ml Erlenmeyer flasks, maintained at 100 rpm on rotary shakers. Liquid shake cultures were subcultured at approximately 4 week intervals, when flasks had become tightly packed with plantlets.

Marchal and Alvard (1988) reported the effect of frequency of renewal of solutions in in vitro culture of pineapple in liquid

media. Pineapple explants were grown for 40 days on MS medium in petridishes with renewal of the culture solution after 10, 20 or 30 days. Growth in liquid medium was less rapid than on solid media. Renewal of the medium after 20 or 30 days improved plantlet growth.

Fitchet (1989) initiated callus from apical dome region of cv. Queen crowns and cultured on Murashige and Tucker's medium, supplemented with 400 mg caseine hydrolysate per litre, 15 per cent coconut milk, 40 mg l^{-1} NAA and 3 per cent sucrose.

Zee and Munekata (1992) cultured the excised buds of A. comosus L. Merr cv. on initiation medium containing full strength MS salts plus 100 mg l^{-1} myo-inositol, 2 mg l^{-1} glycine, 0.5 mg l^{-1} nicotinic acid, 0.5 mg l^{-1} pyridoxine HCl, 0.4 mg l^{-1} thiamine HCl, 2 mg l^{-1} BA, 2 mg l^{-1} NAA, 30 g sucrose and 9 g Bacto agar.

Bordoloi and Sarma (1993) found that MS medium containing IAA, IBA and KIN (5 mg l^{-1} each) was suitable for establishment of solitary shoots from the axillary buds of pineapple. Multiple shoot initiation was observed on MS medium supplemented with IBA (10 mg l^{-1}), KIN (5 mg l^{-1}) and casein hydrolysate (200 mg l^{-1}). On MS medium containing IAA, IBA and KIN (5 mg l^{-1} each), callus developed on the surrounding of the base of each shoot and after 15 days of culture, the calli started initiating shoot buds.

2.3 Root induction

2.3.1 Rooting of shoots in vitro

In vitro plants must have a strong and functional root system. The shoots can be rooted under in vitro conditions and the rooted plantlets transferred to soil or the propagules could be treated like microcuttings and rooted ex vitro in a non-sterile, highly humid, low light environment. Hu and Wang (1983) observed that three phases are involved in rhizogenesis viz. induction, initiation and elongation.

Among the auxins, IBA and NAA have been most effective for root induction (Ancora et al., 1981). All cytokinins inhibit root induction and BA which is widely used for shoot multiplication does so particularly strongly that roots are delayed even after transferring to cytokinin free media (Yeoman, 1986). The root elongation phase is very sensitive to auxin concentration. High concentrations of auxin inhibited root elongation (Thimman, 1977).

Lakshmisita et al. (1974) obtained clusters of thick roots in in vitro pineapple plantlets, when inoculated in Knudson's basal media with Nitsch's micro elements with 1 ppm NAA. Mathews and Rangan (1979) subcultured multiple shoots into MS solid medium with NAA 0.18 mg l^{-1} + IBA 0.4 mg l^{-1} and obtained rooting. These developed into complete plantlets which could be transplanted to soil. Rao et al. could obtain rooting in in vitro

plantlets of pineapple after 3 weeks of subculturing in MS medium supplemented with NAA 2 mg l^{-1} + IBA 2 mg l^{-1} + BA 2.5 mg l^{-1}. Cabral et al. (1984) found that subculturing of explants to MS medium with 30 g sucrose per litre + 1.8 mg NAA l^{-1} + 0.18 mg NAA l^{-1} + 0.4 mg BA l^{-1} induced multiple bud growth and rooting. Wakasa (1989) obtained rooting of shoots induced in vitro, from various explants in medium without any growth regulators. Bordoloi and Sarma (1993) obtained maximum rooting on half strength MS medium containing 2 mg l^{-1} IBA.

2.3.2 Rooting of shoots ex vitro

The cost of production of in vitro plantlets could be reduced by changing the rooting stage from an in vitro step to an ex vitro one. The major cost of producing in vitro plants lies in the rooting and hardening stages (Rajeevan and Pandey, 1986). For rooting under ex vitro conditions, the shoots for rooting could be handled as microcuttings without using aseptic conditions. By adopting such a technique, the sterile tissue culture phase would end with the proliferation of shoots and rooting could be considered as conventional propagation. Rooting under ex vitro conditions also facilitates the combining of the rooting stage with acclimatization which is an essential part of the micropropagation procedure (George and Sherrington, 1984).

The formation of adventitious roots on a microcutting is a crucial step in commercial micropropagation (McClelland et al., 1990). The type of root system formed depends on the physical characteristic of the rooting environment (Nemeth, 1986), as well as the species and quality of the microcutting (George and Sherrington, 1984).

2.3.2.1 Methods of ex vitro rooting

Two-step process

Micropropagated shoots of Pinus radiata were pre-treated for rooting by inserting them into water agar medium containing auxins for five days after which they were moved to a potting mix of peat and pumice for rooting under high humidity (Aitken-Christie and Thorpe, 1984).

Maene and Deberg (1985) described a simpler technique of adding a layer of liquid medium over the agar surface in proliferating cultures and in vitro proliferated shoots of Magnolia soulangeana were successfully rooted ex vitro by this method. They further reported that addition of water, auxin solution, sucrose solution or a combination of auxin and sucrose helped in forming roots in several herbaceous species.

One-step process

The in vitro produced shoots are inserted into rooting media such as peat, perlite, vermiculite or a mixture of these

compounds. The microcuttings are then placed in a high humidity environment for rooting (George and Sherrington, 1984).

Treating the cut basal ends of micropropagated shoots with an auxin carried on talc powder before inserting them into a rooting medium and then placing them under mist or high humidity conditions were successful with black berry and blue berry (Zimmerman and Broome, 1980) and apple (Zimmerman and Broome, 1980; Simmonds, 1983).

2.4 Hardening and planting out

Wainwright (1988) observed that the environment in a tissue culture container is that of very high humidity, low light level and usually a constant temperature. Leaves on shoots or plantlets leaving this environment are as a result, very poorly adapted to resist the low relative humidity, higher light levels and more variable temperature found in vivo.

Langford and Wainwright (1987) observed that physiologically, the leaves grown in vitro are incapable of significant photosynthesis. The stomata are unable to close and as cuticular wax on the leaf surface is minimal, are unable to control water loss. Improper development of vascular connections between the shoot and the roots may also cause poor establishment of the plantlets.

26

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. Three methods of controlling relative humidity are the polythene tent, misting and fogging. In polythene tent, as the aerial weaning environment is closed, it is possible to take advantage of carbondioxide enrichment during hardening (Lakso et al., 1986).

Standardization of rhizosphere environment is also necessary for getting better growth of plantlets (Zimmerman and Fordham, 1985). Kyte and Briggs (1979) found that a porous potting mixture of peat, perlite and composted bark (1:1:1) was the best for rooting in vitro cultured Rhododendrons.

Ramesh et al. (1993) found that sand supported 53.3 per cent survival of in vitro produced jack plantlets. Vermiculite, peat and sand + soil mixture were found to record 40 per cent survival.

Nutrition of the micro propagules during rooting and hardening has been shown to be species dependent and Scott (1987) has shown that inclusion of fertilizer during hardening can be detrimental for Kalmia, improve plant quality of Rhododendron and is essential for quality Magnolia. Wong (1986) recommended addition of 3 g of nutricate (14:14:14 NPK) to each pot one week after

transplanting to get healthier in vitro banana plantlets for planting in the main field. Mathur et al. (1988) initially irrigated in vitro derived Java citronella plantlets with Hoagland and Arnon (1950) nutrient solution for one week. Keshavachandran (1991) obtained cent per cent establishment of Vetiver plantlets with the application of half strength MS nutrients as well as an NPK fertilizer solution (10:5:10 g l^{-1}) at weekly intervals. However better vigour of the plantlets was observed by application of the latter solution. Ramesh et al. (1993) found that application of half strength MS basal medium was found to be the best for maximum survival of jack plantlets.

2.5 Encapsulation studies

The term artificial seed was first coined by Murashige (1977c) and in simple terms it means a somatic embryo entrapped in a bio-degradable synthetic polymer coating that acts as an artificial seed coat. Kamada (1985) broadened the scope of artificial seed technology by defining these somatic propagules as a capsule prepared by coating a cultured matter such as a piece of tissue or an organ which can grow into a complete plant body along with nutrients with an artificial covering.

Redenbaugh et al. (1986) encapsulated somatic embryos of alfalfa, celery and cauliflower as single beads to produce individual somatic artificial seeds. The somatic embryos were

28

mixed in 3.2 per cent (w/v) sodium alginate, dropped individually into a 50 mM solution of calcium chloride and allowed to undergo complexation for 30 minutes to form an alginate bead.

Kitto and Janick (1985) produced synthetic seeds by encapsulating somatic embryos of carrot. Synthetic seed coats were applied to asexual embryos of carrot, by mixing equal volumes of embryo suspension and a 5 per cent (w/v) solution of polyethylene oxide (Polyox WSR-N 750) and dispensing 0.2 ml drops of this mixture into teflon sheets. The drops got dried to form detachable wafers consisting of embryo suspension embedded in polyox.

Bapat et al. (1987) encapsulated axillary buds of mulberry in alginate and agar to produce individual beads. The beads could be stored at 4°C for 45 days without loss of viability. Among the encapsulating agents tested, sodium alginate was found to be the best. Encapsulated buds regenerated complete plantlets on an appropriate medium.

Bapat and Rao (1988) encapsulated somatic embryos of sandalwood in an alginate matrix. Encapsulated single embryos germinated to form plants with roots and shoots. Embryogenic cell suspensions encapsulated and stored at 4°C for 45 days produced embryos when recultured as suspensions.

Mathur et al. (1989) encapsulated apical and axillary shoot buds of Valeriana wallichii in calcium alginate beads and plantlet development was observed under both in vitro (98%) and in vivo (64%) conditions in pots with vermiculite under glasshouse conditions. An assessment of various concentrations of sodium alginate and calcium chloride for the formation of beads indicated that a 6 per cent solution of sodium alginate upon complexation with 75 mM of $CaCl_2.2H_2O$ solution gave optimal firm and round beads within an ion exchange complexation duration of 30 minutes.

Keshavachandran et al. (1993) reported that differentiating calli of vetiver could be successfully encapsulated into beads using sodium alginate at 2.5 per cent and calcium chloride at 75 mM. The firm and round beads formed could be successfully stored at 4°C on cotton wool moistened with MS basal salts for 45 days without losing their regeneration capacity.

Materials and Methods

30

MATERIALS AND METHODS

The present studies on *in vitro* multiplication and standardisation of hardening techniques in pineapple (*Ananas comosus* (L.) Merr.) were carried out in the Tissue Culture Laboratory of the All India Co-ordinated Floriculture Improvement Project attached to the Department of Pomology and Floriculture, College of Horticulture, Vellanikkara during 1991 to 1993.

3.1 Materials

The major and minor elements required for the preparation of media were of analytical grade and procured from M/s.BDH Laboratories, Sisco Research Laboratories (SRL) and Merck. The amino acids, vitamins and plant growth regulators were obtained from M/s.Merck, SRL and Sigma Chemicals, USA.

3.1.1 Glasswares

Borosilicate glasswares of Corning/Borosil brand were used for the study. They were cleaned by initially soaking in potassium dichromate solution in sulphuric acid for 12 hours, followed by thorough washing with jets of tap water in order to completely remove all traces of dichromate solution. They were then soaked in detergent solution (Teepol 0.1%) overnight, thoroughly washed with tap water and rinsed twice with double distilled water. The glasswares were then dried in a hot air oven at 100°C for 24

31

hr. They were then stored in cupboards away from contaminants and dust until used.

3.1.2 Culture medium

3.1.2.1 Composition of medium

Murashige and Skoog (MS) medium (1962) was used in the present study. The nutrient medium included inorganic salts, vitamins, growth regulators, organic supplements and a carbon source. The composition of the medium is given in Table 1.

3.1.2.2 Preparation of medium

The stock solutions for micronutrients, vitamins, Fe-EDTA and growth regulators were prepared with sterile distilled water as given in Table 2a and b.

To make up one litre of medium, the required quantity of each stock solution was added into a glass beaker. A known quantity of double distilled water was added into the beaker and the required quantity of sucrose was weighed, added as solids and dissolved fully. The pH of the solution was adjusted using an electronic pH meter to 5-7 using 0.1 N HCl or 0.1 N NaOH. The volume was made upto one litre. Agar (0.7%) was weighed out, added to the medium and melted by keeping the solution in a waterbath maintained at 90-95°C. The medium was distributed to test tubes (150 mm x 25 mm) at the rate of 15 ml each or to 100 ml Erlenmeyer flasks at the rate of 25 ml each. The test

32

Table 1. Chemical composition of MS media

Constituents	Quantity (mg/l)
Major elements	
$CaCl_2.2H_2O$	440.000
$FeSO_4.H_2O$	27.800
KNO_3	1900.000
KH_2PO_4	170.000
$MgSO_4.7H_2O$	370.000
NH_4NO_3	1650.000
Na_2.EDTA	37.300
Minor elements	
$CaCl_2.6H_2O$	0.025
$CuSO_4.5H_2O$	0.025
H_3BO_3	6.200
KI	0.830
$MnSO_4$	22.300
$Na_2MOO_4.2H_2O$	0.250
$ZnSO_4$	8.600
Organic constituents	
Glycine	2.000
Myo-inositol	100.000
Nicotinic acid	0.500
Pyridoxine. HCl	0.500
Thiamine HCl	0.100
Sucrose	30.000 g
Agar	7.000 g

Table 2a. Composition and preparation of stock solutions of MS medium

Constituents	Concentration of stock (mg/100 ml)	Volume of stock l^{-1} of medium	Storage of stock solution
Micro nutrients			
H_3BO_3	620		
$MnSO_4$	2230		
$ZnSO_4$	860	1.0	4°C
Na_2MoO, $2H_2O$	25		
$CuSO_4$, $5H_2O$	2.5		
$CoCl_2$, $6H_2O$	2.5		
Vitamin			
Myo-inositol	10000		
Nicotinic acid	50		
Pyridoxine HCl	10	1.0	In the freezer
Ca pantothenate	100		
Glycine	200		
Folic acid	100		
Riboflavine	100		
$CaCl_2$, $2H_2O$	15		
KI	83		4°C in amber coloured bottle
Iron source			
$FeSO_4$, $7H_2O$	2780	1.0	4°C in amber coloured bottle
Na_2EDTA	3725		

Preparation of Iron stock solution.
The quantity of $FeSO_4$, $7H_2O$ and Na_2EDTA were dissolved separately in 40 ml each of distilled water. Na_2EDTA solution was boiled and then added to hot solutions of $FeSO_4$, $7H_2O$ gently.

Table 2b. Preparation of stock solutions of plant growth regulators

Sl. No.	Growth regulator	Common abbreviation	Quantity for 50 ml stock (mg)	Preparation	Concentration range
1	Auxins				
	a. α-Napthalene acetic acid	NAA	50	Dissolved in 2-5 ml of 1N NaOH with slight heating and gradually diluted to 50 ml with double distilled water	1 mg/ml
	b. Indole-3-butyric acid	IBA	50		
2	Cytokinins				
	a. 6-Benzyl adenine	BA	50	Dissolved in 2-5 ml of 1N NaOH with slight heating and gradually diluted to 50 ml with double distilled water	1 mg/ml
	b. Kinetin	KIN	50		
	c. 6 γ,γ Dimethyl allyl amino purine	2iP	50		

tubes or flasks were plugged with non-absorbent cotton and autoclaved at 121°C and 15 psi (1.06 kg/cm^2) for 20 minutes (Dodds and Roberts, 1982). The medium was allowed to cool to room temperature and stored in the culture room until used.

3.1.3 Transfer area and aseptic manipulations

All the aseptic manipulations such as surface sterilization of explants, preparation and inoculation of explants and subsequent subculturing were carried out under the hood of a clean air laminar flow cabinet (Klenzaids). The working table of the laminar airflow cabinet was first surface sterilised with absolute alcohol and then by putting on the U.V. light for 30 minutes. The petridishes as well as the instruments used for inoculation were first steam sterilised in an autoclave at 15 psi at 121°C for 30 minutes and then flame sterilised before each inoculation. The hands were also scrubbed with alcohol before each inoculation.

3.1.4 Culture room

The cultures were incubated at 27°±1°C in an air conditioned culture room with a 16 hour photoperiod (2000 lux) supplied by cool day light fluorescent tubes.

3.1.5 Source of explants

The explants were collected from the Pineapple Research Station, Vellanikkara, Thrissur. Crowns and suckers of pineapple varieties Kew and Mauritius were used as the explants.

Plate 1. Explant inoculated into establishment medium

3.2 Methods

3.2.1 Preparation of explants

The crowns used as explants were collected from mature fruits after harvest while the suckers of 1.5 to 2 months age were collected from the field. The leaves from crowns were removed one by one without damaging the growing point. The explants were reduced to a size of 0.5-0.8 mm^3 by trimming the four sides and the base of the stem portion. In case of suckers, leaves and the base with the attached roots were removed and the explants trimmed to a size of 0.5-0.8 mm^3. The cut explants were thoroughly washed in running tap water and then with distilled water. The explants were then subjected to surface sterilization treatments.

3.2.2 Culture establishment

3.2.2.1 Standardisation of surface sterilization

The explants were subjected to the surface sterilants at different concentration and for different durations as detailed in Table 3.

The observations on percentage of cultures contaminated and explant survival were recorded 3 weeks after inoculation.

3.2.2.2 Effect of season of explant collection on the rate of contamination

The explants were collected month wise from January to

37

December in all the months in which they were available. The explants were subjected to surface sterilization by treating with 0.1 per cent Emisan for 30 minutes followed by 0.1 per cent mercuric chloride for ten minutes. The surface sterilised explants were thoroughly washed with sterile distilled water and inoculated on MS establishment medium with BA 5 mg l^{-1} and NAA 1 mg l^{-1}.

Observations on the percentage of cultures contaminated and the explant survival were recorded 3 weeks after inoculation.

3.2.2.3 Effect of different cytokinins and auxins on establishment of explants

Shoot tips isolated from the crowns of varieties Kew and Mauritius were cultured in MS medium supplemented with the following cytokinins at different concentration with or without auxin.

1. BA 5.0 mg l^{-1}
2. BA 5.0 + NAA 0.5 mg l^{-1}
3. BA 5.0 + NAA 1.0 mg l^{-1}
4. BA 10.0 mg l^{-1}
5. 2iP 5.0 mg l^{-1}
6. 2iP 5.0 + NAA 0.5 mg l^{-1}
7. 2iP 5.0 + NAA 1.0 mg l^{-1}
8. 2iP 10.0 mg l^{-1}
9. Kinetin 5.0 mg l^{-1}
10. Kinetin 5.0 + NAA 0.5 mg l^{-1}

11. Kinetin 5.0 + NAA 1.0 mg l^{-1}
12. Kinetin 10 mg l^{-1}
13. Basal medium

Observations on the percentage of cultures established, percentage of cultures showing initiation of growth and the number of days taken for greening of the explants were recorded three weeks after initial culture.

3.2.3 Regneration of *in vitro* plantlets

3.2.3.1 Proliferation of globular structures

The greenish elongated shoot tips after 3-4 weeks of culture were transferred to MS media with 3 per cent sucrose and 0.7 per cent agar, supplemented with different cytokinins with or without auxin at different concentrations as given below, for formation of globular structures.

1. Kinetin 5.0 mg l^{-1}
2. BA 5.0 mg l^{-1}
3. 2iP 5.0 mg l^{-1}
4. Kinetin 10.0 mg l^{-1}
5. BA 10.0 mg l^{-1}
6. 2iP 10.0 mg l^{-1}
7. Kinetin 5.0 + NAA 0.5 mg l^{-1}
8. BA 5.0 + NAA 0.5 mg l^{-1}
9. 2iP 5.0 + NAA 0.5 mg l^{-1}

10. BA 5.0 + NAA 1.0 mg l^{-1}

11. Basal medium

Observations on the number of days taken for proliferation of the globular structures and the intensity of globular structures formed were recorded three weeks after transfer.

3.2.3.2 Effect of cytokinin and auxin on shoot production

The effect of different concentrations of various cytokinins and auxins on proliferation of shoots was studied. Approximate 500 mg of the globular structures formed were subcultured into MS media, with 3 per cent sucrose and agar 0.7 per cent, supplemented with different cytokinins and auxins at various concentrations as given below.

1. Kinetin 5.0 mg l^{-1}
2. Kinetin 10.0 mg l^{-1}
3. Kinetin 5.0 + NAA 0.5 mg l^{-1}
4. BA 5.0 mg l^{-1}
5. BA 10.0 mg l^{-1}
6. BA 5.0 + NAA 0.5 mg l^{-1}
7. 2iP 5.0 mg l^{-1}
8. 2iP 10.0 mg l^{-1}
9. 2iP 5.0 + NAA 0.5 mg l^{-1}
10. Basal medium

40

There were three replications per treatment. Observations on the percentage of cultures developing shoots and the number of shoots produced per culture were recorded 45 days after transfer.

3.2.4 Induction of rooting

3.2.4.1 Standardisation of auxin concentration and media consistency

The effect of auxins, IBA and NAA on induction of rooting in pineapple was studied in an experiment with solid MS media having an agar concentrations of 0.7 per cent and liquid medium without agar using filter paper supports. The varying concentrations of auxins tried were as follows.

1. IBA 1.0 mg l^{-1}
2. IBA 2.0 mg l^{-1}
3. NAA 1.0 mg l^{-1}
4. NAA 2.0 mg l^{-1}
5. Control - Basal medium

Individual shoots regenerated and having attained 4-9 cms length were separated and put in different rooting treatments. There were two replications per treatment.

Observations on percentage of cultures initiating roots, the number of days taken for root initiation, the number of roots formed per shoot and the nature of roots formed were recorded 40 days after subculturing for both solid and liquid cultures.

3.2.4.2 Effect of agar concentrations on root initiation and elongation

The effect of varying concentrations of agar on root initiation and elongation was tested in an experiment. The agar concentrations tried were

1. 0.70 per cent
2. 0.65 per cent
3. 0.60 per cent
4. 0.50 per cent

Individual shoots 4-9 cms in length obtained from the proliferation cultures were separated and used for the study. Observations on the number of roots formed and the mean length of roots produced per culture in each treatment were recorded, three weeks after transfer. There were 14 cultures per treatment.

3.2.4.3 Ex vitro rooting

The shoots formed in the proliferation stage were used for the study. Shoots of various sizes (2.0 to 7.0 cm) in length were treated with the following concentrations of growth regulators.

1. IBA 100 mg l^{-1}
2. IBA 200 mg l^{-1}
3. NAA 100 mg l^{-1}
4. NAA 200 mg l^{-1}

5. Rooton powder*

6. Control

The growth regulator solutions were prepared in ethanol. The shoots were dipped in the solutions for a period of 30 seconds and then planted in plastic pots filled with moistened vermiculite. For treatment with Rooton powder, the shoots were first dipped in distilled water and then in the powder before planting in vermiculite. The pots were then placed in the mist chamber.

Observations on the percentage of rooting, number of roots and mean length of roots were made fifteen days after planting.

3.2.5 Hardening and planting out

3.2.5.1 Hardening treatments

To standardise the procedure for hardening of *in vitro* derived plantlets, different hardening treatments were compared. Uniform plantlets of about 3-12 cms in height with well developed roots were utilised for the study. There were five replications per treatment.

3.2.5.2 Transfer of plantlets

The *in vitro* plantlets formed with a mean number of 5-12 leaves and 5-11 roots were carefully removed from the culture

* A rooting compound in powder form produced by M/s.B.K.Distillery (P) Ltd., Allahabad

vessels. The agar adhering to the roots was carefully removed by washing with distilled water. The plantlets were then subjected to various pre-transfer treatments.

3.2.5.3 Pre-transfer treatments

Two pre-transfer treatments were tried to study their comparative effectiveness on subsequent plant establishment.

1. Immersing the roots of plantlets in sterile water for 18 hrs prior to transferring
2. Coating the leaves of the plantlets with paraffin oil on both sides before transferring

The plantlets were transferred into different containers filled with potting media, individually covered with polyethylene covers and kept under shade.

The number of plantlets found to establish in these two treatments were recorded 15 days after imposing the treatments.

3.2.5.4 Standardisation of containers and potting media

In order to standardise the type of containers and the potting media to be used, 4 types of containers and 8 different media were used for the study.

Containers	Potting media
1. Mud pot (7.0 cm diameter)	1. Potting mixture (1:1:1 of red earth, sand and FYM)
2. Plastic pot (7.0 cm dia.)	2. Perlite
3. Pro-tray (with 64 wells)	3. Soil rite*
4. Polythene bags (11.0 x 8.5 cm)	4. Cocopeat**
	5. Biofibe***
	6. Sand
	7. Vermiculite
	8. Vermiculite + soil (1:1 ratio)

* Soil rite - A mixture of peat moss, vermiculite and perlite marketed by M/s.KEL Perlite, Bangalore

** Cocopeat - A proprietory compound containing decomposed peat and coir dust produced by M/s.Langalee Ltd., Bangalore

*** Biofibe - A proprietory mixture of vermiculite, perlite rice husk and coir dust, produced by M/s.Red Sanders Ltd., Bangalore

Uniform sized plantlets of 3-12 cms length and with well developed roots were used for the study. The plantlets were transferred into different containers filled with the different media. The plantlets were covered with plastic covers and kept under shade for two weeks.

Fungicidal sprays with 0.1 per cent Bavistin were given 2 days after transplanting and subsequently at fortnightly intervals.

The polythene covers covering the plantlets were removed for a period of one hr. for the first few days and then for two, three and four hr. on the subsequent days. Two weeks after keeping in shade, the plantlets were transferred to the net house.

The transferred plantlets were watered on alternate days with tap water. A nutrient solution was prepared by dissolving 5 gm per litre of a fertilizer (N:P:K - 10:5:20) mixture and applied once a week to the plantlets, at the rate of 8 ml per plantlet in mud pots, plastic pots and poly bags and 3 ml per plantlet in protrays.

The plantlets on transfer to the net house were covered with a frame made of polythene sheets, in order to simulate an improvised mist chamber.

Observations on the plant height, number of leaves per plantlet and the width of largest leaf were recorded at the time of transfer and subsequently at 15 days interval upto 45 days after transfer.

3.2.5.5 Effect of nutrient starter solution

Five nutrient starter solutions were tried on established pineapple plantlets in plastic pots (7.0 cm dia.) containing sand. The nutrient starter solutions tried were as follows.

1. Fertilizer solution (N, P, K - 10:5:20) @ 5 gm/litre applied once a week

2. Fertilizer solution (N, P, K - 10:5:20) @ 5 gm/litre applied twice a week
3. MS nutrient solution at $\frac{1}{2}$ strength with pH adjusted to 5.7, once a week
4. MS nutrient solution at $\frac{1}{4}$ strength with pH adjusted to 5.7, once a week
5. Hoagland's nutrient solution at $\frac{1}{2}$ strength once a week
6. Tap water as control

Watering of each pot with 5 ml each of the nutrient starter solutions was done. On alternate days, watering was carried out with tap water.

Observations were recorded on the plant height, number of leaves and width of the largest leaf initially and thereafter at 15 days interval upto 45 days after transplanting.

3.2.6 Encapsulation studies

The globular bodies formed in MS medium supplemented with 5 mg l^{-1} BA and 1 mg l^{-1} NAA were used for encapsulation studies in order to form beads which could be stored without loss of the ability to differentiate.

3.2.6.1 Encapsulation in alginate

Gels of sodium alginate (Sigma) were prepared in MS basal salt solution at concentrations of 2.0, 2.5, 3.0, 3.5 and 4.0 per cent. The differentiating globular bodies were broken into

individual bits and mixed thoroughly with the alginate gel. Calcium chloride solutions were prepared at concentrations of 25, 50, 75 and 100 mM. Sterilised glass tubes of 3 mm diameter were used to pick up the globular bodies along with the sodium alginate solution. Sodium alginate drops were allowed to fall into $CaCl_2$ solution at the rate of 30-40 drops per minute. The formation of round firm beads were noted. The resultant beads (0.5 to 0.7 cm in diameter) were left in the $CaCl_2$ solutions for 30 minutes on a rotary shaker (80 rpm for complexation). Beads were removed, washed three times with sterile distilled water and put in regeneration media or subjected to storage studies.

3.2.6.2 Storage of alginate beads

The alginate beads formed were put into 100 ml flasks on cotton wool, moistened with MS basal salts or distilled water and stored at room temperature and at 4°C.

The beads were removed at intervals of 5 days and put on MS regeneration media to find out the period to which the beads could be stored without losing the regeneraton capacity.

3.2.7 Statistical analysis

Statistical analysis of the data recorded was carried out in completely randomised design, wherever necessary, following Panse and Sukhatme (1985). Angular as well as $\sqrt{x}$ transformations were carried out, wherever necessary.

Results

48

RESULTS

The results of the studies on in vitro multiplication and standardisation of hardening techniques in pineapple conducted at the Tissue Culture Laboratory of the All India Co-ordinated Floriculture Improvement Project attached to the Department of Pomology and Floriculture of the College of Horticulture, Vellanikkara are presented in this chapter.

4.1 Standardisation of surface sterilisation of explants

The results of the experiments on surface sterilisation of pineapple explants are presented in Table 4a.

Among the different treatments tried, T_5 (Emisan 0.1 per cent for 35 minutes followed by treatment with mercuric chloride 0.1 per cent for 10 minutes) was found to give the least percentage of contamination (47.1). However, T_5 was on par with T_4 (Emisan 0.1 per cent for 30 minutes and mercuric chloride 0.1 per cent for 10 minutes), though there was a slight increase in the percentage of contamination (50.3%). Reduction in the time of treatment with Emisan increased the rate of contamination.

The duration of exposure of the explants to the sterilising treatments was found to influence the survival of the explants in the media. The highest percentage of survival (45.8) was observed in T_4 followed by that in T_5 (42.2), which were on

49

Table 3. Concentration and duration of treatment of surface sterilants on pineapple explants

Treatment No.	Sterilants	Concentration (%)	Duration of treatment (mts)
1	Emisan +	0.1	15
	$HgCl_2$	0.1	10
2	Emisan +	0.1	20
	$HgCl_2$	0.1	10
3	Emisan +	0.1	25
	$HgCl_2$	0.1	10
4	Emisan +	0.1	30
	$HgCl_2$	0.1	10
5	Emisan +	0.1	35
	$HgCl_2$	0.1	10
6	Emisan +	0.1	30
	$HgCl_2$ +	0.1	10
	Norfloxacin	0.1	30
7	Emisan +	0.1	30
	$HgCl_2$ +	0.1	10
	Norfloxacin	0.2	30

Table 4a. Effect of surface sterilant treatments on pineapple explants

Explant	Treatment No.	Sterilants	Concentration (%)	Duration (mts)	Contamination (%)	Survival (%)
Crown and sucker	1	Emisan + $HgCl_2$	0.1 0.1	15 10	91.8	9.6
	2	Emisan + $HgCl_2$	0.1 0.1	20 10	81.4	18.6
	3	Emisan + $HgCl_2$	0.1 0.1	25 10	71.8	22.1
	4	Emisan + $HgCl_2$	0.1 0.1	30 10	50.3	45.8
	5	Emisan + $HgCl_2$	0.1 0.1	35 10	47.1	42.2
	6	Emisan + $HgCl_2$ + Norfloxacin	0.1 0.1 0.1	30 10 30	85.4	14.5
	7	Emisan + $HgCl_2$ + Norfloxacin	0.1 0.1 0.2	30 10 30	71.9	11.7
		CD			6.24	6.75
		SEm±			2.06	2.22

Observations recorded three weeks after inoculation

50

51

par. Hence, considering both the percentage of contamination and percentage of survival, the treatment with Emisan 0.1 per cent for 30 minutes, followed by treatment with mercuric chloride 0.1 per cent for 10 minutes was found to be the best.

4.2 Influence of season on explant contamination

The data with respect to the month wise details of the percentage of contamination of the explants in the different months is presented in Table 4b and Fig. 1.

The percentage contamination observed for 12 months showed distinct difference in the different months. The lowest percentage of contamination recorded was in the months of January (48.0) and February (50.0). The explants used in April and November showed the maximum contamination (86.0% and 84.6%, respectively). The highest percentage of survival of the explants was observed in the months of January (44.0), May (41.5), February (37.5) and October (37.5).

4.3 Standardisation of media and growth regulators for culture establishment

The data on the percentage of establishment of the explants in media supplemented with different concentrations of cytokinins and auxins are presented in Table 5 and Fig. 2. Maximum establishment (47.72%) of the crown explants was observed in

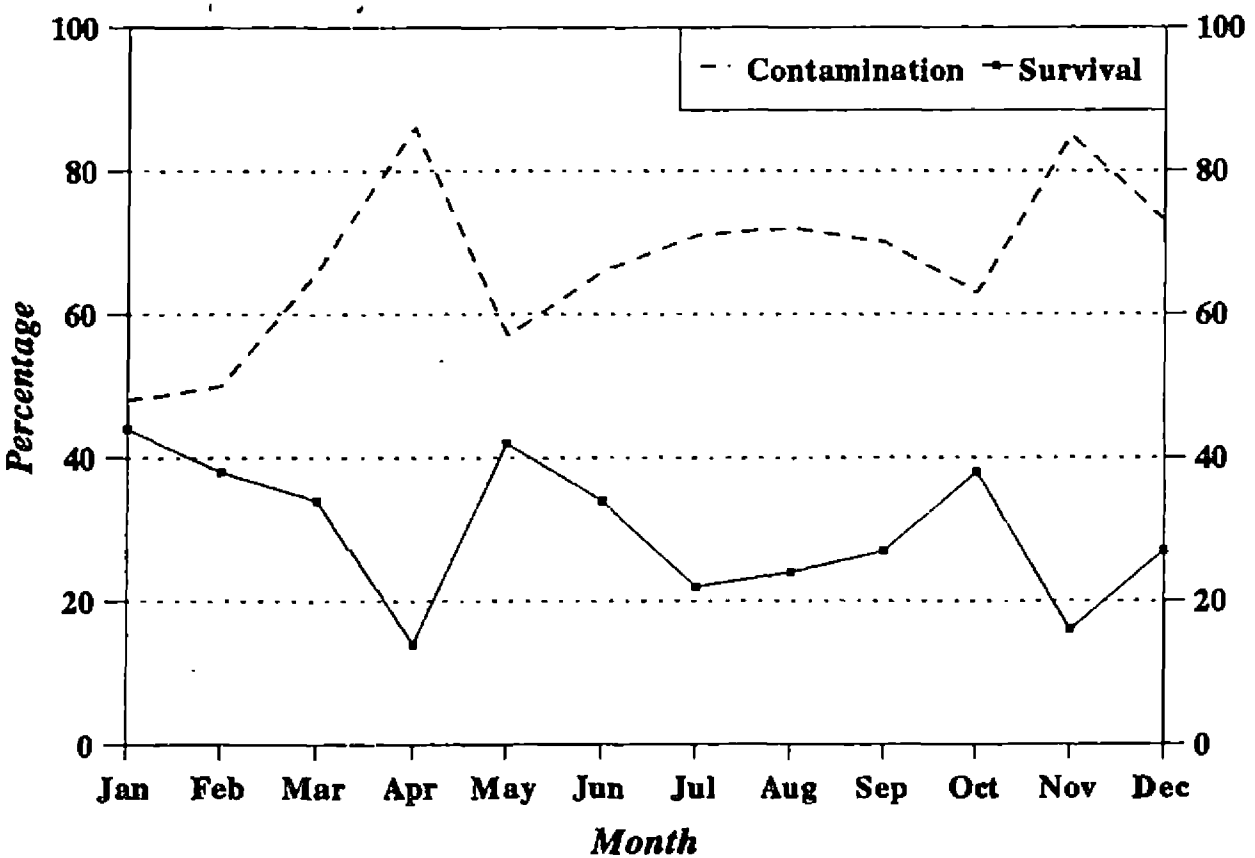
52

Table 4b. Influence of season on explant contamination

Month	Contamination (%)	Survival (%)
January	48.00	44.00
February	50.00	37.50
March	66.02	33.97
April	86.00	13.81
May	56.95	41.48
June	65.62	34.38
July	70.83	21.73
August	72.08	24.20
September	70.00	26.66
October	62.50	37.50
November	84.61	15.39
December	73.33	26.66

Surface sterilization of the crown explants was carried out by treatment with Emisan (0.1%) for 30 mts followed by in $HgCl_2$ (0.1%) for 10 mts.

Fig. 1. Influence of season on explant contamination



53

treatments T_1 (BA 5.0 mg l^{-1}) and T_3 (BA 5.0 + NAA 1.0 mg l^{-1}) (Plate 1). 2iP when added into the media was found to reduce the percentage of establishment. In treatment T_7 (2iP 5.0 mg l^{-1} + NAA 1.0 mg l^{-1}), none of the explants could be established. Among the cytokinins, the highest percentage of establishment was observed when BA was added to the media alone or in combination with NAA. When the concentration of BA was increased to 10 mg l^{-1}, there was a reduction in the percentage of establishment. The establishment of explants was better with KIN when compared with 2iP. With KIN also, at higher concentration of 10 mg l^{-1} there was a reduction in the percentage of establishment (26.14). Explants cultured on basal media also showed reduced establishment (26.14%).

4.3.1 Cultures showing growth

The data pertaining to the cultures showing growth after establishment are presented in Table 5.

The percentage of cultures showing growth after successful establishment in the media was found to follow the same pattern as for the establishment with respect to the concentrations of cytokinins and auxins added into the media. The highest percentage of cultures showing initiation of growth (47.72) were observed with treatment T_1 (BA 5.0 mg l^{-1}) and T_3 (BA 5.0 mg l^{-1} + NAA 1.0 mg l^{-1}). This was followed by T_{10} (KIN 5.0 mg l^{-1} + NAA 0.5 mg l^{-1})

54

in which 39.01 per cent of the cultures initiated growth. The higher concentration of the cytokinins tried namely, 10 mg l^{-1} had adverse effect on the percentage of cultures initiating growth. Among the cytokinins, BA was found to be the most effective for initiating growth of the established cultures followed by KIN and 2iP. The percentage of cultures initiating growth in the basal media was also low (26.14).

4.3.2 Time taken for greening

The data relating to the time taken for greening of the established explants are presented in Table 5.

The treatments with BA added to the media showed the fastest response in respect of the time taken for greening of the established explants (Plate 2). The shortest time for this response (4.37 days) was observed in treatments T_1 (BA 5.0 mg l^{-1}) and T_3 (BA 5.0 mg l^{-1} and NAA 1.0 mg l^{-1}). With 10 mg l^{-1} BA, the explants took more time for greening (6.84 days). The response of the explants cultured in media to which KIN was added was significantly slower compared to the BA treatments. With respect to the KIN treatments, the fastest response (6.00 days) was observed with the treatment T_{11} (KIN 5.0 mg l^{-1} and NAA 1.0 mg l^{-1}). The response of the explants was slowest in media supplemented with 2iP. Higher concentration of the cytokinin on the whole increased the time taken for greening. However, the

Table 5. Effect of different concentrations of cytokinins and auxins on the establishment of pineapple explants in MS medium

Treatment No.	Growth regulator	Concentration (mg l^{-1})	Establishment (%)	Cultures showing growth (%)	Time for greening (days)
1	BA	5.0	47.72	47.72	4.37
2	BA + NAA	5.0 + 0.5	30.30	26.14	5.67
3	BA + NAA	5.0 + 1.0	47.72	47.72	4.37
4	BA	10.0	26.14	26.14	6.84
5	2iP	5.0	21.59	21.59	7.17
6	2iP + NAA	5.0 + 0.5	30.30	26.14	11.50
7	2iP + NAA	5.0 + 1.0	0.00	0.00	0.00
8	2iP	10.0	26.14	26.14	9.34
9	KIN	5.0	30.30	30.30	7.25
10	KIN + NAA	5.0 + 0.5	39.01	39.01	7.68
11	KIN + NAA	5.0 + 1.0	21.59	21.59	6.00
12	KIN	10.0	26.14	26.14	8.83
13	Control (Basal medium)	-	26.14	26.14	5.34
	CD (0.05)		4.65	4.16	0.54
	SEm±		1.52	1.36	0.18

Observations recorded three weeks after inoculation

Fig.2. Effect of cytokinins and auxins on establishment of pineapple explants

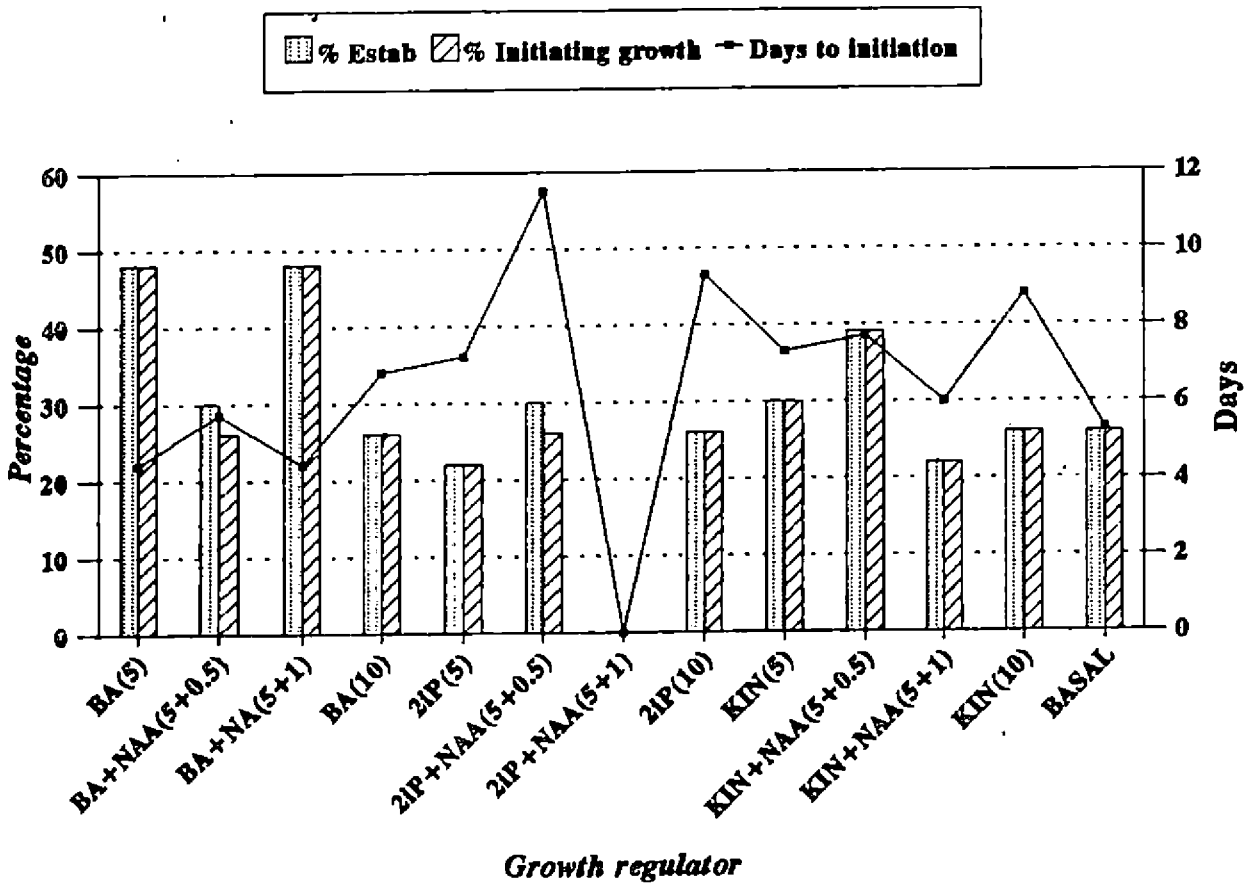


Plate 2. Greening of the primary explant

56

slowest response of 11.5 days was observed with the treatment T_6 (2iP 5.0 mg l^{-1} + NAA 0.5 mg l^{-1}). In the basal media, the time taken for greening was 5.34 days, but the number of cultures getting established and initiating growth was found to be low as compared to the other treatments.

4.4 Proliferation of the explants

4.4.1 Standardisation of cytokinins and auxins for proliferation of globular structures

The results of the experiment on the effect of different concentrations of cytokinins such as BA, 2iP and KIN with different concentrations of NAA on proliferation of the explants are presented in the Table 6 and Plates 3a to 4b.

4.4.1.1 Time taken for formation of the globular structures

Among the different treatments tried for proliferation of the globular structures, the treatment T_{10} (BA 5.0 mg l^{-1} + NAA 1.0 mg l^{-1}) showed the fastest response of 5.96 days, followed by T_2 (BA 5.0 mg l^{-1}) with 6.95 days. In the basal media (T_{11}), it took an average of 7.45 days for the formation of the globular structures. Treatments T_{10}, T_2 and T_{11} were however, statistically on par. The maximum number of days taken for the formation of the globular structures was observed in treatment T_9 (2iP 5.0 mg l^{-1} + NAA 0.5 mg l^{-1}) where it took an average of 16.94 days for the response.

57

Comparing the three cytokinins tried, BA showed the fastest response followed by KIN and 2iP. The globular structures formed with 2iP, however, had characteristic narrow leafy proliferation. In general, at the higher concentration of 10 mg l^{-1}, the time taken for formation of the globular structures increased. Likewise, when NAA was added at a lower concentration of 0.5 mg l^{-1}, the days taken for the formation of globular structures was found to increase.

4.4.1.2 Intensity of formation of the globular structures

The intensity of the globular structures formed showed variation with the different treatments. Maximum formation of the globular structures was obtained in treatment T_2 (BA 5.0 mg l^{-1}) and T_{10} (BA 5.0 mg l^{-1} + NAA 1.0 mg l^{-1}) followed by T_1 (KIN 5.0 mg l^{-1}) and T_7 (KIN 5.0 mg l^{-1} and NAA 0.5 mg l^{-1}). The lowest intensity of the globular structures was formed in the basal medium. Among the cytokinins, higher intensity of globular structures was observed with BA followed by KIN and 2iP. The formation of globular structures was better at the lower concentration of 5.0 mg l^{-1} of the cytokinin. Addition of 0.5 mg l^{-1} of NAA also resulted in less number of globular structures being formed. However, 1.0 mg l^{-1} of NAA in combination with BA 5.0 mg l^{-1} was found to produce high intensity of globular structures.

Table 6. Effect of different concentrations of cytokinins and auxins on formation of globular structures in pineapple

Treatment No.	Growth regulator	Concentration (mg l^{-1})	Time taken for response (days)	Intensity of nodular structures formed	Remarks
1	KIN	5.0	8.08	+++	
2	BA	5.0	6.95	++++	
3	2iP	5.0	12.62	+++	Narrow leafy proliferations
4	KIN	10.0	8.09	++	
5	BA	10.0	8.13	++	
6	2iP	10.0	13.91	++	Narrow leavy proliferations
7	KIN + NAA	5.0 + 0.5	10.54	+++	
8	BA + NAA	5.0 + 0.5	9.79	+++	
9	2iP + NAA	5.0 + 0.5	16.49	++	Very narrow leafy proliferations
10	BA + NAA	5.0 + 1.0	5.96	++++	
11	Control (Basal medium)	-	7.45	++	
	CD (0.05)		1.96		
	SEm±		0.63		

Observations recorded three weeks after transfer

Plate 3a. Formation of globular bodies from the primary explant

Plate 3b. Further proliferation of globular bodies

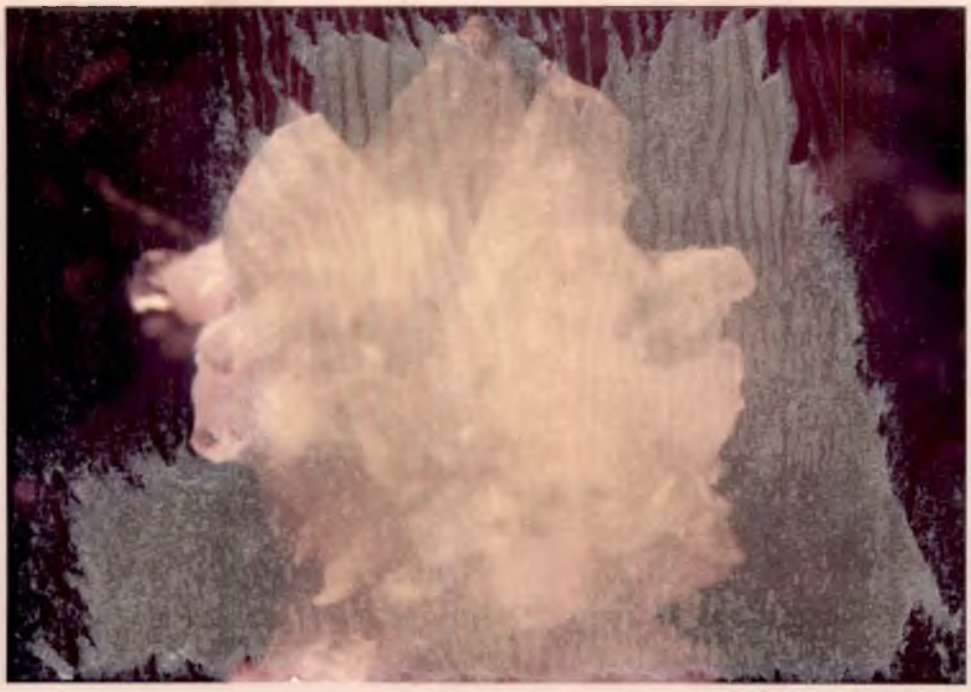


Plate 4a. Close up view of globular bodies formed

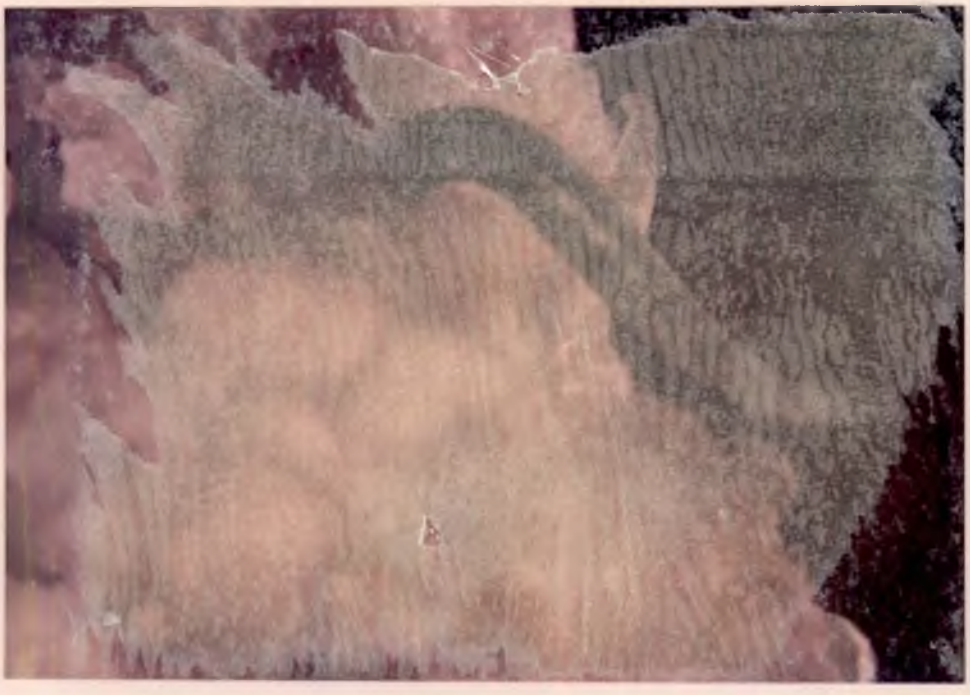


Plate 4b. A magnified view of a shoot formed from the globular bodies

59

4.4.2 Standardisation of cytokinins and auxins for shoot proliferation

The data with respect to the study on standardisation of different concentrations of cytokinins and auxins on shoot production is presented in Table 7 and Fig. 3.

4.4.2.1 Percentage of cultures developing shoots

The different treatments showed significant difference with respect to the percentage of cultures developing shoots. The highest percentage of cultures forming shoots was observed in treatment T_{10} (basal MS media) where cent per cent of the cultures developed vigorous, dark green long shoots (Plate 5). The treatments T_2 (KIN 10.0 mg l^{-1}) with 91.1 per cent and T_1 (KIN 5.0 mg l^{-1}) with 74.8 per cent, ranked next in order. With treatment T_5 (BA 10.0 mg l^{-1}), there was no formation of shoots. Among the different cytokinins, the highest percentage of cultures developing shoots was observed when KIN was added to the media followed by 2iP. With respect to KIN and 2iP, addition of NAA at 0.5 mg l^{-1} was found to reduce the percentage of cultures developing shoots. However, with BA and increase in the percentage of cultures developing shoots was observed when 0.5 mg l^{-1} of NAA was added.

4.4.2.2 Number of shoots formed per culture

The highest number of shoots formed per culture was observed with treatment T_{10} (basal MS media) where a mean of

Table 7. Effect of different concentrations of cytokinins and auxins on shoot production in pineapple

Treatment No.	Growth regulator	Concentration (mg l^{-1})	Cultures developing shoots (%)	Shoots per culture (No.)	Remarks
1	KIN	5.0	74.8	6.6	Broad short leaved rosette type shoots
2	KIN	10.0	91.1	4.4	Short, thick leaved shoot initials
3	KIN + NAA	5.0 + 0.5	49.6	3.9	Shoots light green in colour
4	BA	5.0	28.0	1.8	-
5	BA	10.0	0.0	0.0	-
6	BA + NAA	5.0 + 0.5	72.1	1.5	Shoots light green in colour
7	2iP	5.0	69.8	3.5	-
8	2iP	10.0	53.4	3.1	Shoots pale green in colour
9	2iP + NAA	5.0 + 0.5	43.7	1.3	-
10	Control (Basal medium)	-	100.0	11.9	Vigorous, greenish longer shoots
	CD (0.05)		5.19	1.41	
	SEm±		1.76	0.48	

Observations recorded 45 days after transfer

Fig. 3. Effect of cytokinins and auxins on shoot production in pineapple

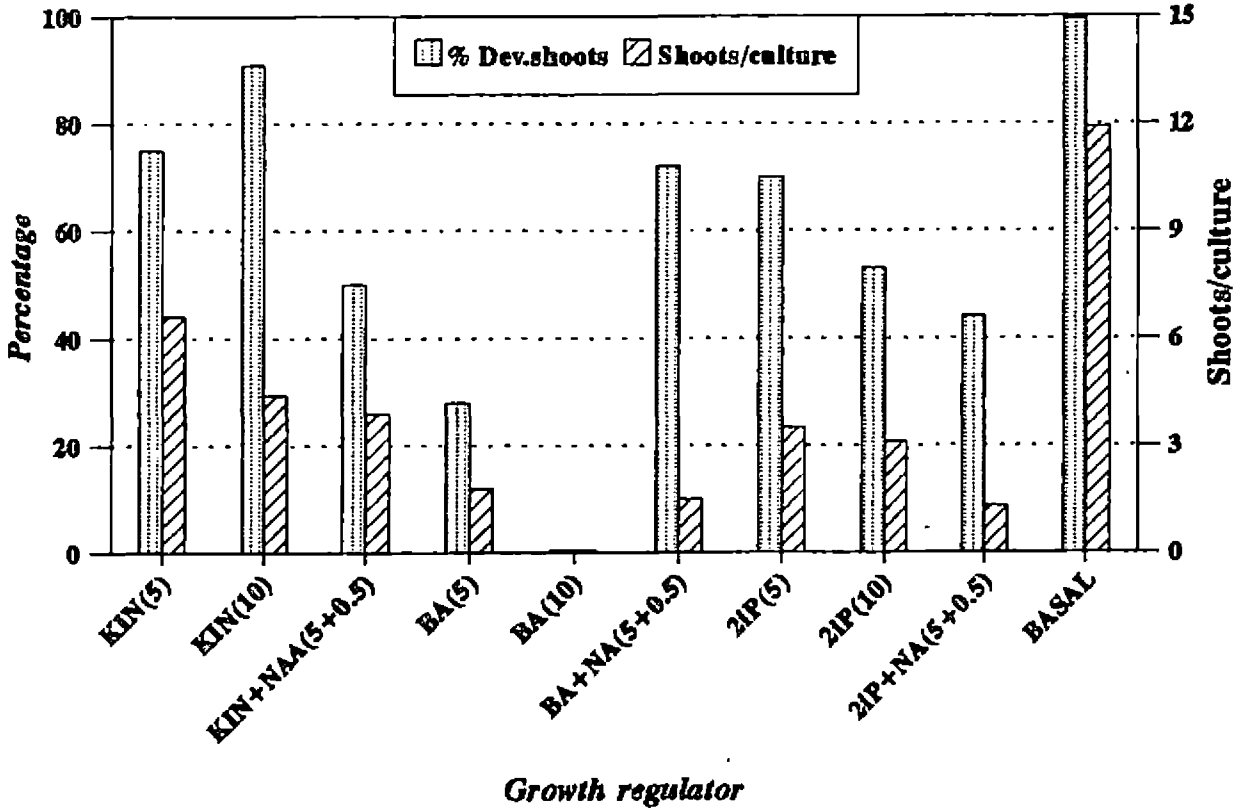


Plate 5. Formation of multiple shoots

11.9 shoots were formed, followed by treatment T_1 (KIN 5.0 mg l^{-1}) with 6.6 shoots and T_2 (KIN 10.0 mg l^{-1}) with 4.4 shoots per culture. The lowest number of shoots formed was in treatment T_9 (2iP 5.0 mg l^{-1} + NAA 0.5 mg l^{-1}), where only a mean number of 1.3 shoots were formed per culture. In general, among the cytokinins, more number of shoots were formed with KIN followed by 2iP. The lower concentration of the cytokinin (5.0 mg l^{-1}) was more effective than the higher concentration (10.0 mg l^{-1}). Likewise addition of NAA (0.5 mg l^{-1}) alongwith the cytokinins also reduced the number of shoots formed per culture.

The appearance of the shoot formed also varied slightly with the treatments. In the basal media, vigorous dark green coloured larger shoots were formed. The shoots formed with KIN were in general shorter, broader and thicker, while those formed with 2iP and BA were pale green in colour.

4.5 Induction of rooting

The data relating to the experiment on induction of rooting are presented in Table 8 and Fig. 4.

4.5.1 In vitro rooting

4.5.1.1 Percentage of rooting

There was no significant difference with respect to percentage of root initiation in the different treatments. In all the treat-

62

ments including basal MS medium, cent per cent rooting was obtained (Plate 6a to c).

4.5.1.2 Days to root initiation

The treatments showed significant difference with respect to the number of days taken for initiation of rooting. The minimum number of days for rooting was observed in basal MS medium (8.54 days). Among the auxins tried, faster rooting occurred when IBA was added into the media (22.16 days) when compared to NAA (30.20 days). Considering the two concentrations of auxins alone, the higher concentrations of 2 mg l^{-1} was found to produce faster rooting (25.72 days) compared to a mean of 26.66 days when 1 mg l^{-1} was used. Considering the two auxins, and the two concentrations together, the fastest rooting was observed with IBA 2 mg l^{-1} (21.25 days).

The physical state of the media had a significant effect on the number of days taken for rooting. Irrespective of the other treatments, considering the physical nature of the media alone, rooting in liquid media was found to be significantly faster. Rooting in liquid media occurred in a mean of 24.84 days while in solid media it took a mean number of 27.53 days for root initiation. Comparing the two auxins along with the media consistency, the fastest rooting occurred with IBA in liquid media (21.14 days) followed by IBA in solid media (23.2 days).

63

Comparing the two auxins along with their concentrations and the media consistency together, the fastest rooting was observed when IBA 2 mg l^{-1} was added into liquid medium (19.81 days) followed by IBA 1 mg l^{-1} in liquid medium (22.47 days) and IBA 2 mg l^{-1} in solid medium (22.69 days). When IBA 1 mg l^{-1} was added to solid medium, it took a mean number of 23.71 days for rooting. Among the NAA treatments, when 2 mg l^{-1} was added to liquid medium, it took a mean number of 28.49 days for rooting. The maximum number of days taken for rooting was a mean of 31.88 days, when NAA 2 mg l^{-1} was added to solid medium.

4.5.1.3 Number of roots

The different treatments showed significant difference with respect to the number of roots formed.

Comparing the two auxins alone, irrespective of the concentrations and the media consistency, more number of roots were formed with IBA (6.06) compared to NAA (4.17). Comparing the two concentrations of the auxins used alone, the higher concentration of 2 mg l^{-1} was significantly superior with a mean of 5.44 roots being produced compared to 4.79 roots at 1.0 mg l^{-1}. Comparing the two auxins and their concentrations together, the treatments with IBA showed significant difference. The maximum number of roots was observed with IBA 2 mg l^{-1} (6.41).

64

Considering the media consistency alone, irrespective of the other factors, more number of roots were observed in liquid media (5.35). Comparing the two auxins alongwith the media consistency, irrespective of the auxin concentrations, the maximum number of roots was obtained with IBA in liquid medium (6.30) followed by IBA in solid medium (5.82).

Comparing the two auxins, at the two concentrations in solid and liquid media, the highest number of roots were observed with IBA 2 mg l^{-1} in liquid media followed with IBA 2 mg l^{-1} in solid media (6.28) and IBA 1 mg l^{-1} in liquid media (6.07). Among the NAA treatments, the highest number of roots was observed with 2 mg l^{-1} in liquid media (4.77) and the lowest number of roots with NAA 1 mg l^{-1} in solid media (3.72). In basal media, a mean number of 4.78 roots per culture was observed.

4.5.1.4 Appearance of the roots

The appearance of the roots formed with the two auxins and in the basal medium showed visible difference. The roots formed when IBA was added into the media were characterised by short and stubby appearance (Plate 7a). Thin long branched roots were formed when the in vitro formed shoots were cultured in basal MS media (Plate 7b). The roots formed in NAA treatments were characterised by their short, thick and hairy appearance. Proliferation of callus like mass was also observed at the base

Table 8. Effect of different concentrations of NAA and IBA on rooting of *in vitro* plantlets of pineapple

Treatment No.	Growth regulator	Concentration (mg l^{-1})	Media	Root initiation (%)	Root initiation (days)	Roots per shoot (No.)	Nature of roots
1	NAA	1	Liquid	100	28.59	4.03	Short, thick brownish hairy roots with mass of tissue at base of plantlets
2	NAA	1	Solid	100	31.86	3.72	,,
3	NAA	2	Liquid	100	28.49	4.77	Brownish coiled roots with mass of tissue at base of plantlets
4	NAA	2	Solid	100	31.88	4.17	
5	IBA	1	Liquid	100	22.47	6.07	Short brownish roots
6	IBA	1	Solid	100	23.71	5.35	,,
7	IBA	2	Liquid	100	19.81	6.53	,,
8	IBA	2	Solid	100	22.69	6.28	
9	Basal MS	-	Solid	100	8.54	4.78	Thin, long branched roots
	CD (0.05)				3.27	1.85	
	SEm±				2.09	0.67	

Observations recorded 40 days after subculture

Fig. 4. Effect of auxins on rooting of pineapple plantlets

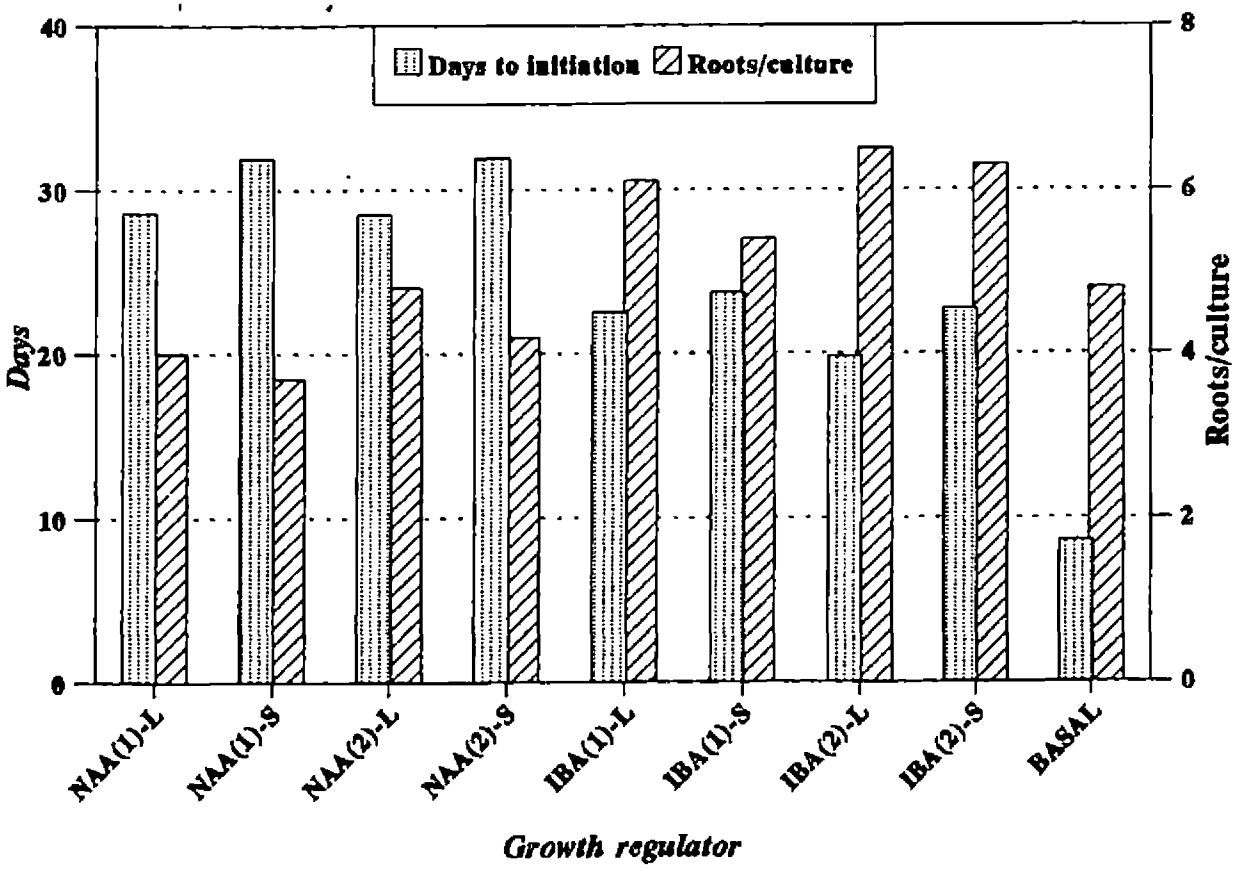


Plate 6a. In vitro rooting with IBA

Plate 6b. In vitro rooting with NAA

Plate 6c. *In vitro* rooting in basal medium

Plate 7a. Nature of roots formed with IBA

Plate 7b. Nature of roots formed in basal medium

of the shoots (Plate 6b). At the higher concentration of 2 mg l^{-1}, roots showed characteristic coiled nature.

4.5.1.5 Effect of agar concentration on root initiation and elongation

The data pertaining to the study on standardisation of the agar concentration for optimum root initiation and elongation are presented in Table 9.

4.5.1.5a Number of roots

Among the different concentrations of agar used, the maximum number of roots was observed with 0.65 per cent where a mean number of 4.7 roots were observed followed by 4.0 in media solidified with 0.6 per cent agar. With 0.5 per cent agar a mean number of 3.9 roots were observed. The lowest number of 3.0 roots per culture was observed for 0.7 per cent agar.

4.5.1.5b Length of roots

The treatments showed statistical significance with respect to the length of roots. The maximum length of 2.5 cm was observed with 0.65 per cent agar followed by 2.1 cm with 0.50 per cent agar and 2.0 cm with 0.6 per cent agar. The length of the roots was minimum (1.4 cm) when 0.7 per cent agar was used.

4.5.2 Ex vitro rooting

The data relating to the experiment on induction of ex

67

Table 9. Effect of different concentrations of agar on the initiation and elongation of roots in MS media (basal)

Treatment No.	Concentration of agar (%)	Number of roots	Length of roots (cm)
1	0.70	3.0	1.4
2	0.65	4.7	2.5
3	0.60	4.0	2.0
4	0.50	3.9	2.1
CD (0.05)		NS	0.51
SEm±		0.18	0.18

Observations recorded after 21 days

vitro rooting is presented in Table 10. The shoots of different sizes (Plate 8a) kept for rooting (Plate 8b) under the different treatments showed differences in their rooting.

4.5.2.1 Percentage of rooting

The highest percentage of rooting was obtained for treatment with Rooton powder (84) followed by control (70), IBA 200 mg l^{-1} (62) and IBA 100 mg l^{-1} (59). The shoots treated with NAA at both the concentrations failed to root.

4.5.2.2 Days to rooting

The fastest induction of rooting was obtained by treatment with Rooton powder (11.2 days) followed by IBA 200 mg l^{-1} (11.4 days). The days taken for induction of rooting both for control and IBA 100 mg l^{-1} were the same (14.6 days, each).

4.5.2.3 Number of roots

The highest number of roots was obtained for treatment with IBA 200 mg l^{-1} (9) followed by that with Rooton powder (7). For control and IBA 100 mg l^{-1} treatments, a mean of 6 roots, each were obtained.

4.5.2.4 Length of roots

The mean length of the roots differed widely with respect to the different treatments (Plate 8c). The maximum length was

Table 10. Effect of auxins and rooting formulations on ex vitro rooting of pineapple

Treatment No.	Treatments	Rooting (%)	Days to rooting	No. of roots	Mean length of roots (cm)
1	IBA 100 mg l^{-1}	59	14.6	6	0.72
2	IBA 200 mg l^{-1}	62	11.4	9	0.77
3	NAA 100 mg l^{-1}	Nil	-	-	-
4	NAA 200 mg l^{-1}	Nil	-	-	-
5	Rooton powder	84	11.2	7	2.15
6	Control	70	14.6	6	2.00

Observations taken 21 days after treatment

8a

Plate 8a. Shoots of different sizes kept for *ex vitro* rooting

8b

Plate 8b. *In vitro* formed shoots kept for *ex vitro* rooting under different treatments

Plate 8c. Ex vitro rooting of shoots

70

observed for treatment with Rooton powder (2.15 cm) followed by that for control (2.00 cm). The roots produced with IBA treatments were comparatively short and stubby. With IBA 200 mg l^{-1}, the mean length of the roots was 0.77 cm and with IBA 100 mg l^{-1}, 0.72 cm.

4.6 Hardening of plantlets

The effect of certain pre-transfer treatments such as immersing the roots of the plantlets in distilled water for 18 hr before transferring to pots (Plate 9) and coating the leaves of the plantlets with paraffin oil was combined with different types of containers and potting media. The data are presented in Tables 11 to 28b. The plantlets were then transferred into different types of containers with different media (Plate 10) covered individually in the plastic bags and kept in shade for two weeks following which they were transferred into the net house (50% light) and kept in an improvised mist chamber (Plate 11).

4.6.1 Effect of containers

4.6.1.1 Number of leaves

Fifteen days after transplanting in the case of the plantlets subjected to pretreatment 1, among the different types of containers tried, the plantlets grown in plastic pots had the highest mean number of leaves (9.8). The number of leaves

Plate 9. Plantlets kept in distilled water prior to transfer

Plate 10. Plantlets transferred into different containers with different media

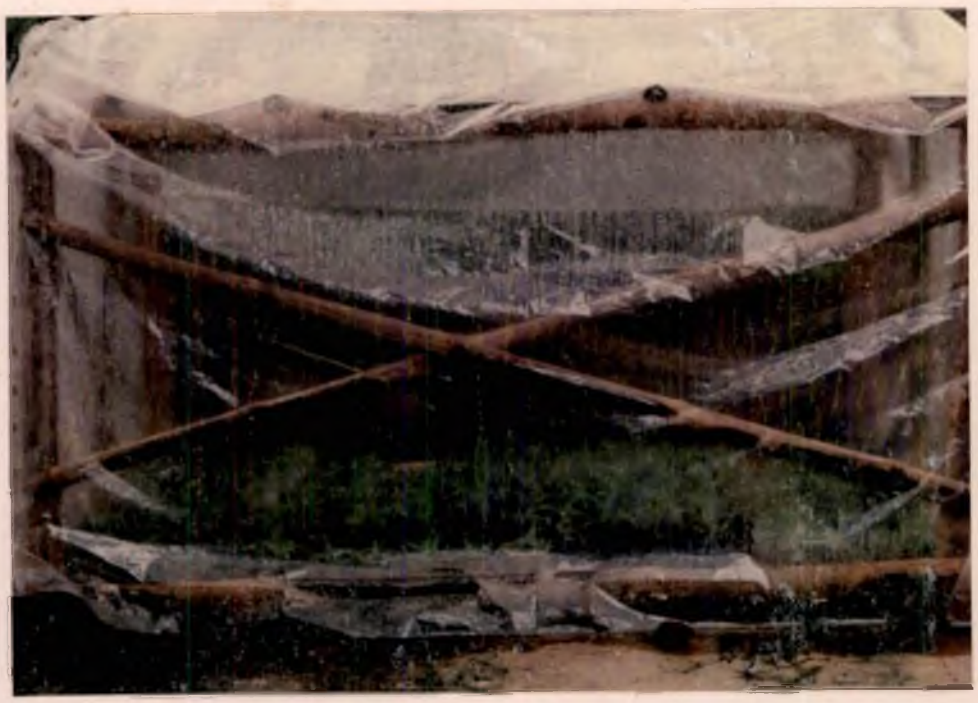


Plate 11. Plantlets kept in an improvised mist chamber for hardening

recorded for plantlets grown in mud pots ranked next with a mean of 9.4. The lowest number of leaves were observed for plants grown in pro-trays (7.3).

Thirty days after transplanting, the same trend was observed with respect to the number of leaves of the plantlets grown in different containers. The highest number was observed for plantlets grown in plastic pots and mud pots (11.5) while the lowest number was observed in the case of plantlets grown in pro-trays (8.9).

Forty five days after transplanting, the number of leaves recorded for the plantlets grown in different types of containers showed the same trend. The highest number (13.4) was obtained for plantlets in plastic pots followed by (13.1) for those in mud pots. The number of leaves observed for plantlets grown in pro-trays was the lowest (10.6).

The number of leaves recorded for the plantlets subjected to pretreatment 2 also showed the same trend. Fifteen days after transplanting the highest number of leaves (8.9) was observed in the case of plantlets in plastic pots, followed by a mean of 8.2 observed for plantlets in polybags and a mean of 7.6 for those in mud pots. The lowest number of leaves were observed for the plantlets in pro-trays (5.9). The same trend was noticed 30 and 45 days after transplanting.

72

Thus, considering the type of containers, irrespective of the media used, the number of leaves recorded at 15, 30 and 45 days after transplanting was the highest in the case of plantlets grown in plastic pots, followed closely by those in mud pots and polybags (Plate 12a and Fig. 5a). The lowest number of leaves was observed in all the cases with the plantlets grown in pro-trays (Plate 12b). Considering the percentage of increase in the production of leaves taking into account the different types of containers alone, 30 days after transplanting, the maximum increase was observed for the plantlets subjected to pretreatment 1, in pro-trays (23.6%), followed by for those in mud pots (22.3%), polybags (21.3%) and plastic pots (17.3%). At 45 days after transplanting the maximum increase was observed in pro-trays (19.1%) followed by plastic pots (16.5%), polybags (15.3%) and mud pots (14.8%).

The percentage of increase in the number of leaves in the case of the plantlets subjected to pretreatment 2 was comparatively less as compared to the increase observed in the case of the plantlets subjected to pretreatment 1. The maximum increase at 30 days after transplanting was observed for the plantlets in pro-trays (3.3%), followed by polybags (1.2%) and plastic pots (1.1%). At 45 days after transplanting also, the maximum increase in the number of leaves was observed in the case of plantlets in pro-trays (16.1%) followed by in polybags (9.6%) and plastic pots (4.4%).

Table 11. Effect of different containers on the number of leaves of pineapple plantlets

Treatment No.	Containers	Number of leaves					
		Pre treatment 1*			Pre treatment 2**		
		15 days after trans-planting	30 days after trans-planting	45 days after trans-planting	15 days after trans-planting	30 days after trans-planting	45 days after trans-planting
1	Mud pot	9.4 (3.1)***	11.5 (3.4)	13.1 (3.6)	7.6 (2.8)	7.6 (2.8)	7.9
2	Plastic pot	9.8 (3.1)	11.5 (3.4)	13.4 (3.7)	8.9 (2.9)	9.0 (3.0)	9.4
3	Pro tray	7.3 (2.7)	8.9 (2.9)	10.6 (3.3)	5.9 (2.4)	6.2 (2.5)	7.2
4	Poly bag	8.9 (2.9)	10.8 (3.3)	12.5 (3.5)	8.2 (2.9)	8.3 (2.9)	9.1
	CD (0.05)	0.10	0.11	0.12	0.12	0.12	
	SEm±	0.04	0.04	0.04	0.04	0.04	

* Dipping the roots of the plantlets for 18 hr in sterile water, prior to transplanting

** Coating the leaves of the plantlets with paraffin oil on both sides prior to transplanting

*** The values in parenthesis denote $\sqrt{x}$ transformed ones

**** Not statistically analysed due to plant mortalities

Table 12. Effect of different containers on the percentage increase in the number of leaves of pineapple plantlets

Treatment No.	Containers	% increase in number of leaves			
		Pre-treatment 1*		Pre-treatment 2**	
		30 days after transplanting	45 days after transplanting	30 days after transplanting	45 days after transplanting
1	Mud pot	22.3	14.8	0.0	3.9
2	Plastic pot	17.3	16.5	1.1	4.4
3	Pro-tray	23.6	19.1	3.3	16.1
4	Poly bag	21.3	15.7	1.2	9.6

* Dipping the roots of plantlets 18 hr in sterile water, prior to transplanting

** Coating the leaves of the plantlets on both sides with paraffin oil prior to transplanting

Fig.5a Effect of containers on growth of pineapple plantlets

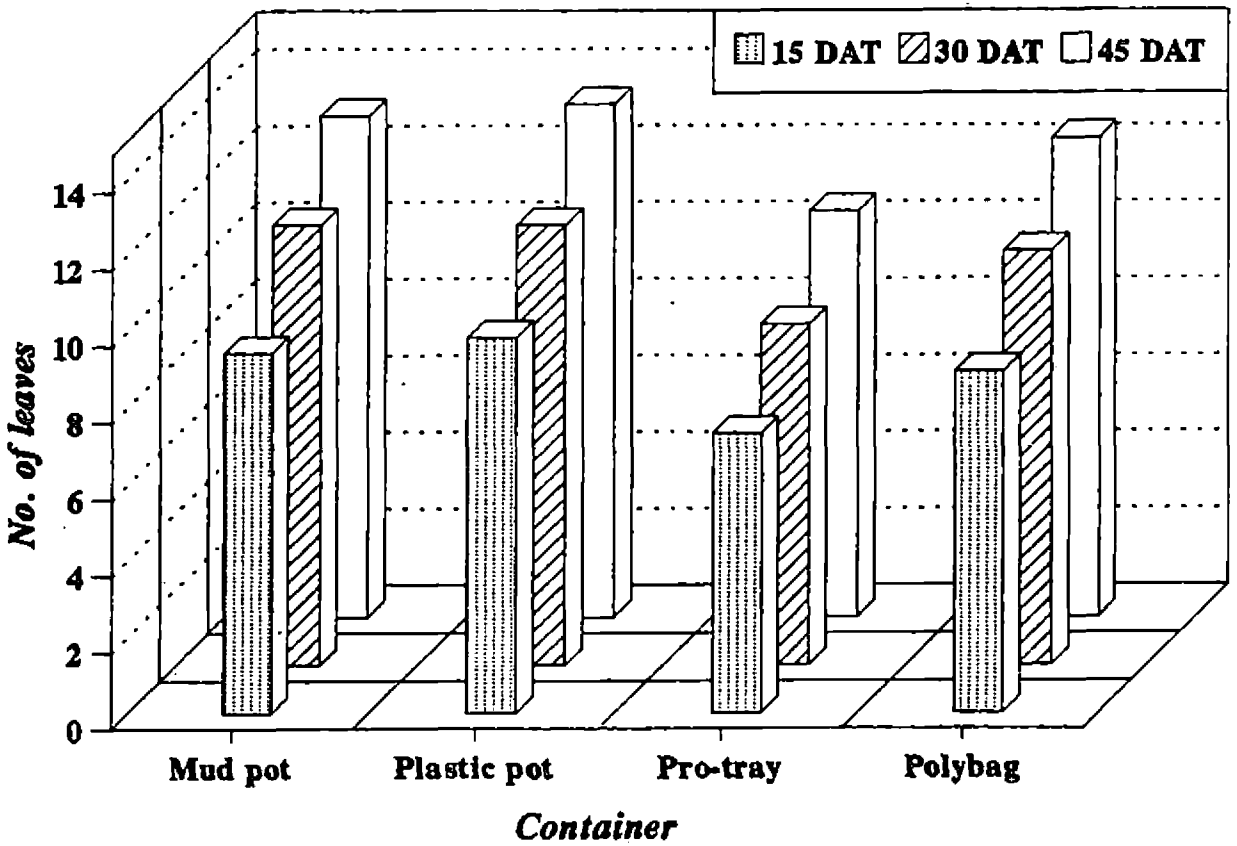


Plate 12a. Plantlets growing in different containers (i) Plastic pot (ii) Mudpot (iii) Poly bag

Plate 12b. Plantlets growing in different containers (iv) Pro-tray

75

4.6.1.2 Height of the plant

In the case of plantlets subjected to pre-treatment 1, 15 days after transplanting, the maximum height was observed for the plantlets grown in plastic pots (8.2 cm) followed by polybags (7.5 cm) and mud pots (7.0 cm). Thirty days after transplanting, the same trend was observed with the plantlets in plastic pots having a height of 8.5 cm, followed by those in polybag (7.7 cm) and in mud pots (7.3 cm). Forty five days after transplanting, maximum height was again recorded for plants in plastic pots (8.9 cm) followed by those in polybag (8.0 cm) and mud pots (7.6 cm). At all these intervals, the lowest height was recorded for the plantlets in pro-trays (Fig. 5b).

In the case of plantlets subjected to pretreatment 2 also, the maximum height was recorded for plantlets grown in plastic pots, followed by those in polybags and mud pots. Fifteen days after transplanting, the maximum height observed for plantlets in plastic pots was 7.9 cm followed by 6.8 cm for those in polybags and 6.5 cm for those in mud pots.

Thirty days after transplanting, the maximum height recorded for the plantlets in plastic pots was 8.1 cm followed by 6.9 cm for those in polybags and 6.6 cm for those in mud pots. Forty five days after transplanting, the maximum height observed for the plantlets in plastic pots was 8.4 cm followed

Table 13. Effect of containers on the height of pineapple plantlets

Treatment No.	Containers	Height of plantlets (cm)					
		Pre-treatment 1*			Pre-treatment 2**		
		15 days after trans-planting	30 days after trans-planting	45 days after trans-planting	15 days after trans-planting	30 days after trans-planting	45 days*** after trans-planting
1	Mud pot	7.0	7.3	7.6	6.5	6.6	7.1
2	Plastic pot	8.2	8.5	8.9	7.9	8.1	8.4
3	Protray	4.1	4.2	4.6	4.1	4.2	4.7
4	Poly bag	7.5	7.7	8.0	6.8	6.9	7.7
	CD (0.05)	0.55	0.55	0.59	0.67	0.67	
	SEm±	0.19	0.19	0.22	0.24	0.24	

* Dipping the roots of the plantlets for 18 hr in sterile water, prior to transplanting

** Coating the leaves of the plantlets with paraffin oil on both sides, prior to transplanting

*** Not statistically analysed due to plant mortality

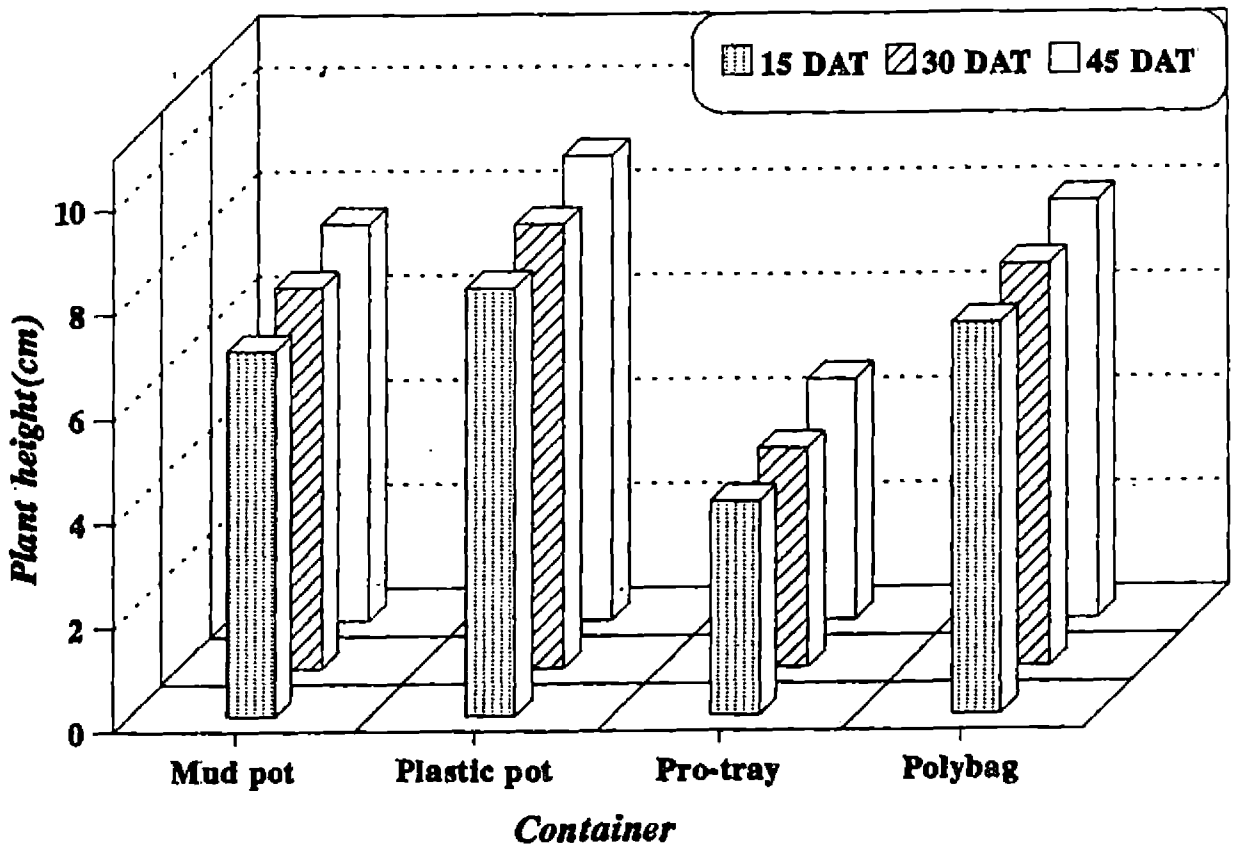
Table 14. Effect of containers on the percentage increase in the height of pineapple plantlets

Treatment No.	Containers	% increase in the height of the plantlets			
		Pre-treatment 1*		Pre-treatment 2**	
		30 days after transplanting	45 days after transplanting	30 days after transplanting	45 days after transplanting
1	Mud pot	4.3	4.1	1.5	7.6
2	Plastic pot	3.7	4.7	2.5	3.7
3	Pro-tray	2.4	9.5	2.4	11.9
4	Poly bag	2.7	3.9	1.4	11.6

* Dipping the roots of the plantlets 18 hr in sterile water prior to transplanting

** Coating the leaves of the plantlets on both sides with paraffin oil prior to transplanting

Fig.5b Effect of containers on growth of pineapple plantlets



by 7.7 cm for those in polybags and 7.1 cm for those in mud pots. The lowest height at all the intervals was recorded for the plantlets in pro-trays.

Thirty days after transplanting, the percentage of increase in the height of the plantlets subjected to pretreatment 1 was maximum for the plantlets in mud pots (4.3%) followed by those in plastic pots (3.7%) and polybags (2.7%). Forty five days after transplanting, the percentage of increase in height was maximum for the plantlets in pro-trays (9.5%) followed by those in plastic pots (4.7%) and mud pots (4.1%).

In the case of the plantlets subjected to pretreatment 2 and 30 days after transplanting, the maximum height was observed for the plantlets in plastic pots (2.5%) followed by for those in pro-trays (2.4%). The lowest increase was observed for the plantlets in polybags. At 45 days after transplanting, the maximum increase in height was observed for the plantlets in pro-trays (11.9%) followed by for those in polybags (11.6%) and mud pots (7.6%).

4.6.1.3 Width of the largest leaf

In the case of plantlets subjected to pretreatment 1, 15 days after transplanting, the maximum width of the largest leaf was observed in the case of plantlets in plastic pots (6.4 mm) followed by the plantlets in mud pots (5.9 mm) and polybags (5.8 mm).

Table 15. Effect of containers on the width of the largest leaf of pineapple plantlets

Treatment No.	Containers	Width of the largest leaf (mm)					
		Pre-treatment 1*			Pre-treatment 2**		
		15 days after trans-planting	30 days after trans-planting	45 days after trans-planting	15 days after trans-planting	30 days after trans-planting	45 days*** after trans-planting
1	Mud pot	5.9	6.4	11.9	5.2	5.2	5.3
2	Plastic pot	6.4	7.1	12.4	6.5	6.5	6.5
3	Protray	4.3	4.7	8.8	3.9	4.0	4.4
4	Poly bag	5.8	6.3	11.7	5.9	5.9	6.4
	CD (0.05)	0.42	0.48	1.05	0.59	0.60	
	SEm±	0.15	0.17	0.38	0.21	0.22	

* Dipping the roots of the plantlets for 18 hr in sterile water prior to transplanting

** Coating the leaves of the plantlets with paraffin oil on both sides, prior to transplanting

*** Not statistically analysed due to plant mortality

Thirty days after transplanting, the maximum width was observed for the plantlets in plastic pots (7.1 mm) closely followed by those in mud pots (6.4 mm) and those in polybags (6.3 mm).

Forty five days after transplanting, the same trend was observed at a higher magnitude. The mean width observed for the plantlets in plastic pots had increased to 12.4 mm, closely followed by the plantlets in mud pots (11.9 mm) and in polybags (11.7 mm). The minimum width at all these intervals was observed for the plantlets grown in pro-trays (Fig. 5c).

In the case of plantlets subjected to pretreatment 2, also, maximum width was observed for those grown in plastic pots, followed by those in polybags and mud pots, with the lowest values recorded for those in pro-trays.

Variation was observed with respect to the width of the largest leaf in the different types of containers without considering the interaction with the type of media. For plantlets subjected to pretreatment 1, 30 days after transplanting, the percentage increase was maximum for the plantets in plastic pots (10.9) followed by those in pro-trays (9.3), polybags (8.6) and mud pots (8.5). At 45 days after transplanting stage, the percentage increase in the width of the largest leaf was greater. The highest percentage increase was observed for the plantlets in mud pots

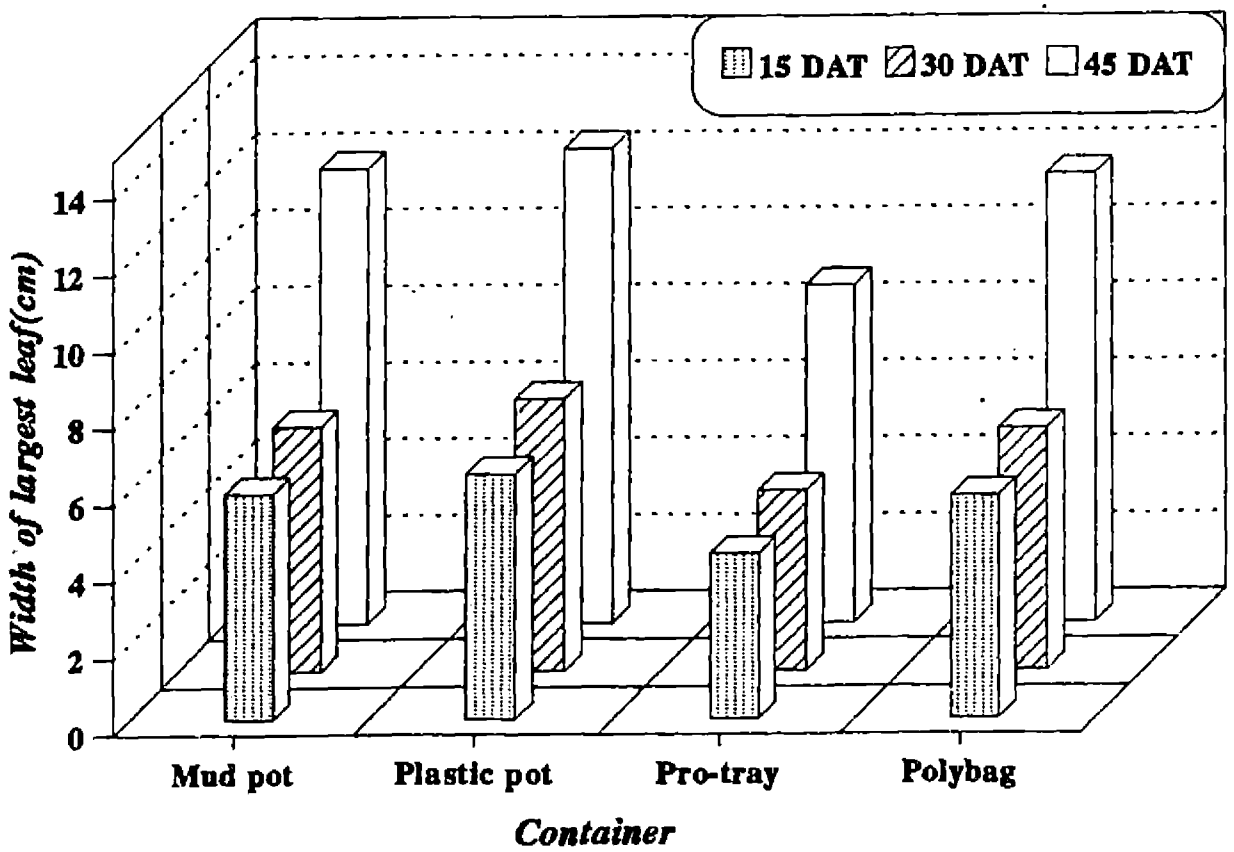
Table 16. Effect of containers on the percentage increase in the width of the largest leaf of the pineapple plantlets

Treatment No.	Containers	% increase in the width of the largest leaf			
		Pre-treatment 1*		Pre-treatment 2**	
		30 days after transplanting	45 days after transplanting	30 days after transplanting	45 days after transplanting
1	Mud pot	8.5	93.2	0.0	1.9
2	Plastic pot	10.9	74.6	0.0	0.0
3	Pro-tray	9.3	87.2	2.6	9.1
4	Poly bag	8.6	85.7	0.0	7.8

* Dippling the roots of the plantlets for 18 hr in sterile water, prior to transplanting

** Coating the leaves of the plantlets with paraffin oil on both sides, prior to transplanting

Fig.5c.Effect of containers on growth of pineapple plantlets



(93.2) followed by those in pro-trays (87.2), polybags (85.7) and plastic pots (74.6). For plantlets subjected to pretreatment 2, the maximum increase in width at 30 days after transplanting was observed for the plantlets in pro-trays (2.6%). Forty five days after transplanting, the maximum increase in width was observed for the plantlets in pro-trays (9.1%) followed by those in polybags (7.8%).

4.6.2 Effect of media

4.6.2.1 Number of leaves

The plantlets subjected to pretreatment 1 showed significant difference with respect to the number of leaves recorded in different media irrespective of the containers used, 15, 30 and 45 days after transplanting (Plate 12c and Fig. 6a). Fifteen days after transplanting, the highest number of leaves (9.9) was recorded for perlite followed by biofibe and normal potting mixture with a mean of 9.4 leaves and a mean of 9.2 for soilrite and cocopeat. The lowest number of leaves was recorded for the plantlets grown in a mixture of vermiculite and soil (7.2).

Thirty days after transplanting, the number of leaves recorded was highest for plantlets grown in cocopeat (11.5) followed by 11.1 for soilrite and 10.9 for perlite and normal potting mixture. The lowest number (9.1) was observed for plantlets grown in vermiculite and soil.

Table 17. Effect of different media on the number of leaves of pineapple plantlets

Treatment No.	Media	Number of leaves					
		Pre-treatment 1*			Pre-treatment 2**		
		15 days after trans-planting	30 days after trans-planting	45 days after trans-planting	15 days after trans-planting	30 days after trans-planting	45 days**** after trans-planting
1	Potting mixture 1:1:1	9.4 (3.0)***	10.9 (3.3)	12.8 (3.6)	7.4 (2.7)	7.6 (2.8)	7.8 (2.8)
2	Perlite	9.9 (3.2)	10.9 (3.3)	12.4 (3.5)	6.9 (2.6)	6.9 (2.6)	8.1 (2.9)
3	Soilrite	9.2 (3.0)	11.1 (3.3)	12.9 (3.6)	7.7 (2.8)	7.7 (2.8)	8.5 (2.9)
4	Cocopeat	9.2 (3.0)	11.5 (3.4)	13.5 (3.7)	8.1 (2.8)	8.2 (2.9)	8.7 (2.9)
5	Biofibe	9.4 (3.1)	10.4 (3.2)	12.1 (3.5)	7.2 (2.7)	7.3 (2.7)	7.5 (2.7)
6	Sand	8.4 (2.9)	10.7 (3.3)	12.2 (3.5)	7.7 (2.8)	7.9 (2.8)	8.4 (2.9)
7	Vermiculite	8.0 (2.8)	10.7 (3.3)	12.5 (3.5)	8.3 (2.9)	8.3 (2.9)	9.4 (3.1)
8	Vermiculite + soil	7.2 (2.7)	9.1 (3.0)	10.8 (3.3)	7.9 (2.8)	7.9 (2.8)	8.8 (3.0)
	CD (0.05)	0.15	0.15	0.17	NS	NS	
	SEm±	0.05	0.05	0.06	0.06	0.06	

* Dipping the roots of plantlets for 18 hr in sterile water, prior to transplanting
** Coating the leaves of the plantlets with paraffin oil on both sides, prior to transplanting
*** The values in parenthesis represent $\sqrt{x}$ transformed ones
**** Not statistically analysed due to plant mortality

Plate 12c. Plantlets growing in different media

Forty five days after transplanting, the highest number of leaves (13.5) was observed for the plantlets grown in cocopeat followed by 12.9 for soilrite and 12.8 for normal potting mixture. The lowest number of leaves (10.8) was observed for the plantlets grown in a mixture of vermiculite and soil.

The number of leaves recorded for the plantlets subjected to pretreatment 2 did not show any significant difference at any of the intervals of taking observations. Fifteen days after transplanting, a mean number of 8.3 leaves were produced for the plantlets grown in vermiculite, followed by 8.1 for cocopeat. The lowest value (6.9) was observed for perlite.

Thirty days after transplanting, the same trend was maintained. Highest number of 8.3 was recorded for the plantlets grown in vermiculite followed by 8.2 for cocopeat. The plantlets grown on perlite showed the least value (6.9).

Forty five days after transplanting, the highest number of leaves was observed in the case of vermiculite (9.4) followed by 8.8 for the mixture of vermiculite and soil, 8.7 for cocopeat and 8.5 for soilrite. The lowest value was recorded for the plantlets in biofibe (7.5).

Considering the percentage of increase in the number of leaves of the plantlets grown in the different media irrespective of the type of containers, for the plantlets subjected to pretransfer

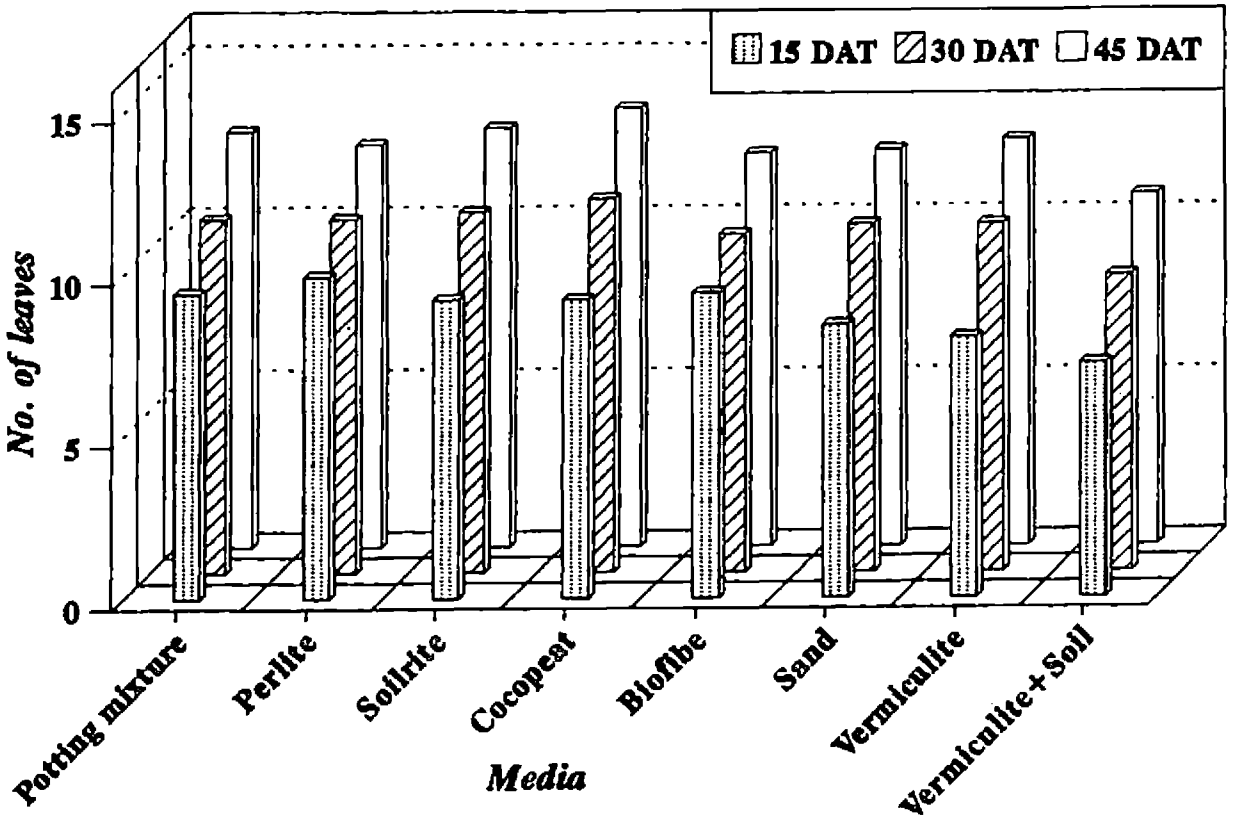
Table 18. Effect of different media on the percentage increase in the number of leaves of pineapple plantlets

Treatment No.	Media	% increase in number of leaves			
		Pre-treatment 1*		Pre-treatment 2**	
		30 days after transplanting	45 days after transplanting	30 days after transplanting	45 days after transplanting
1	Potting mixture 1:1:1	16.0	17.4	2.7	2.6
2	Perlite	10.1	12.1	0.0	17.4
3	Soilrite	20.7	16.2	0.0	10.4
4	Cocopeat	25.0	17.4	1.2	5.9
5	Biofibe	10.6	16.3	1.4	2.7
6	Sand	27.4	14.0	2.6	6.4
7	Vermiculite	33.8	16.8	0.0	11.8
8	Vermiculite + soil	26.4	18.7	0.0	11.4

* Dipping the roots of the plantlets 18 hr in sterile water prior to transplanting

** Coating the leaves of the plantlets with paraffin oil on both sides, prior to transplanting

Fig.6a.Effect of media on growth of pineapple plantlets



treatment 1, 30 days after transplanting the maximum increase (33.8%) was observed in the case of plantlets grown in vermiculite followed by in sand (27%) and in a mixture of vermiculite and soil (26.4%) and cocopeat (25%). The minimum increase was observed for the plantlets grown in biofibe (10.6%) and perlite (10.1%). Forty five days after transplanting, the percentage of increase in the number of leaves was maximum for the plantlets in a mixture of vermiculite and soil (18.7%) followed by those in cocopeat (17.4%), normal potting mixture (17.4%), vermiculite (16.8%), biofibe (16.3%) and soilrite (16.2%). The minimum increase (12.1%) was observed for the plantlets in perlite medium.

For the plantlets subjected to pretransfer treatment 2, the percentage of increase in the number of leaves was comparatively less. Thirty days after transfer, the maximum increase was observed for the plantlets in normal potting mixture (2.7%) followed by in sand (2.6%). Forty five days after transfer, the maximum increase was observed for the plantlets grown in perlite (17.4%) followed by for those in vermiculite (11.8), the mixture of vermiculite and soil (11.4%) and soilrite (10.4%).

4.6.2.2 Height of the plantlets

In the case of plantlets subjected to pretreatment 1, 15 days after transplanting, maximum height was observed for the plantlets grown in cocopeat (7.3 cm) closely followed by those

Table 19. Effect of different media on the height of pineapple plantlets

Treatment No.	Media	Height of plantlets (cm)					
		Pre-treatment 1*			Pre-treatment 2**		
		15 days after trans-planting	30 days after trans-planting	45 days after trans-planting	15 days after trans-planting	30 days after trans-planting	45 days*** after trans-planting
1	Potting mixture	6.7	6.9	7.3	6.4	6.5	6.9
2	Perlite	6.6	6.9	7.2	5.8	5.8	6.0
3	Soil rite	6.9	7.2	7.5	6.8	6.8	7.5
4	Cocopeat	7.3	7.5	7.9	6.8	6.9	7.4
5	Biofibe	7.0	7.1	7.3	5.6	5.7	6.3
6	Sand	5.9	6.1	6.4	6.8	6.9	7.2
7	Vermiculite	7.1	7.4	7.7	6.2	6.3	7.0
8	Vermiculite + soil	6.0	6.3	6.9	6.5	6.6	7.3
	CD (0.05)	0.77	0.78	0.84	NS	NS	
	SEm±	0.28	0.28	0.30	0.34	0.34	

* Dipping the roots of the plantlets for 18 hr in sterile water prior to transplanting

** Coating the leaves of the plantlets with paraffin oil on both sides, prior to transplanting

*** Not statistically analysed due to plant mortality

in vermiculite (7.1 cm), biofibe (7.0 cm) and soilrite (6.9 cm). Thirty days after transplanting, the maximum height was again observed for the plantlets grown in cocopeat (7.5 cm) followed by those in vermiculite (7.5 cm), soilrite (7.2 cm) and biofibe (7.1 cm). Forty five days after transplanting, the same trend was observed with the maximum height being observed for the plantlets grown in cocopeat (7.9 cm) followed by those in vermiculite (7.7 cm), soilrite (7.5 cm) and biofibe (7.3 cm). The plantlets grown in sand showed the minimum height at all the intervals (Fig. 6b).

In the case of plantlets subjected to pretreatment 2, 15 days after transplanting, the maximum height was recorded for the plantlets grown in cocopeat, soilrite and sand (6.8 cm), closely followed by those grown in the mixture of vermiculite and soil (6.5 cm).

Thirty days after transplanting the maximum height was observed for the plantlets grown in cocopeat and sand (6.9 cm), soilrite (6.8 cm) closely followed by those in a mixture of vermiculite and soil (6.6 cm) and in normal potting mixture (6.5 cm). Forty five days after transplanting, the maximum height was recorded for the plantlets grown in soilrite (7.5 cm), cocopeat (7.4) and mixture of vermiculite and soil (7.3 cm).

Considering the percentage increase in height for the plantlets subjected to pretransfer treatment 1, thirty days after

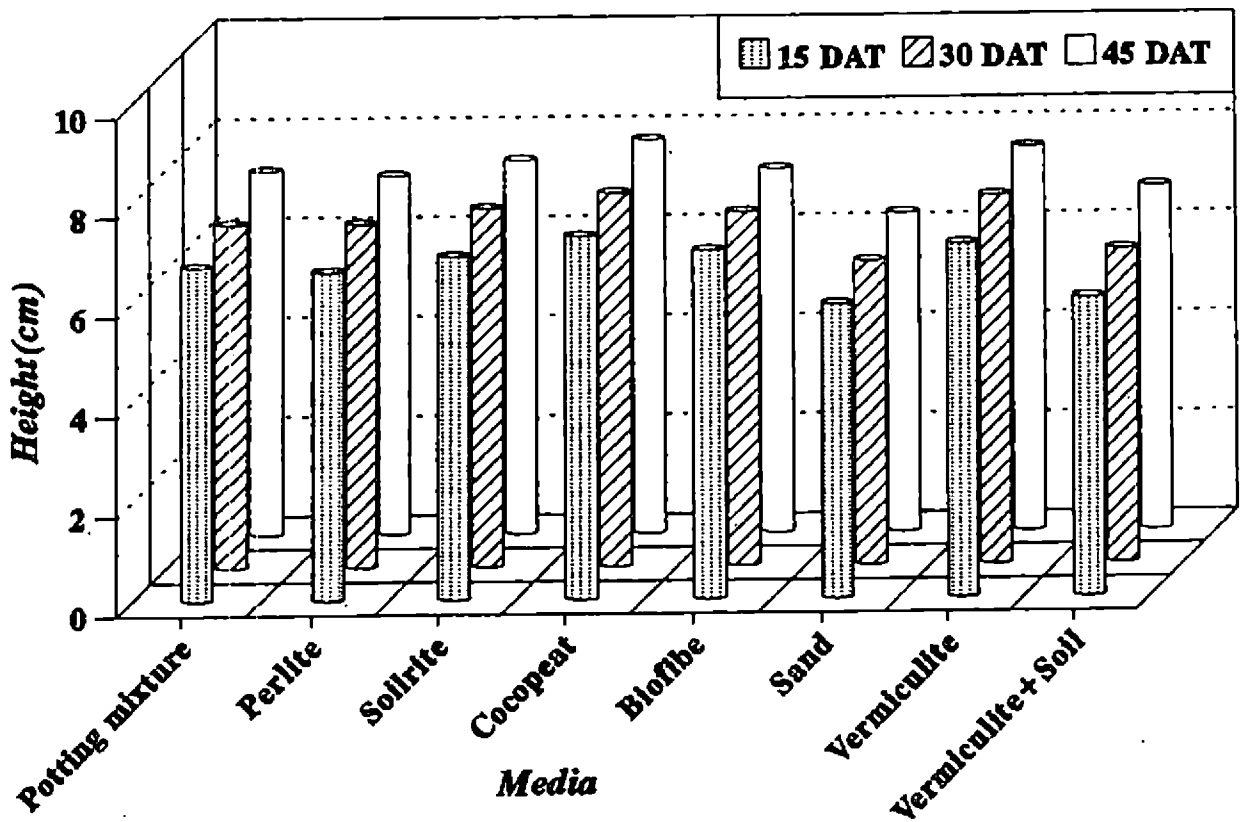
Table 20. Effect of different media on the percentage increase in the height of pineapple plantlets

Treatment No.	Media	% increase in height of plantlets			
		Pre-treatment 1*		Pre-treatment 2**	
		30 days after transplanting	45 days after transplanting	30 days after transplanting	45 days after transplanting
1	Potting mixture 1:1:1	3.0	5.8	1.6	6.2
2	Perlite	4.5	4.3	0.0	3.4
3	Soilrite	4.3	4.2	0.0	10.3
4	Cocopeat	2.7	5.3	1.5	7.2
5	Biofibe	1.4	2.8	1.8	10.5
6	Sand	3.4	4.9	1.5	4.3
7	Vermiculite	4.2	4.1	1.6	11.0
8	Vermiculite + soil	5.0	9.5	1.5	10.6

* Dipping the roots of plantlets 18 hr in sterile water prior to transplanting

** Coating the leaves of the plantlets with paraffin oil on both sides, prior to transplanting

Fig.6b Effect of media on growth of pineapple plantlets



transplanting, the maximum increase was observed for the plantlets in a mixture of vermiculite and soil (5%) followed by in perlite (4.5%), soilrite (4.3%) and vermiculite (4.2%). The least percentage increase was observed for the plantlets grown in biofibe (1.4%). Forty five days after transplanting, the maximum percentage of increase was observed for the plantlets in a mixture of vermiculite and soil (9.5%) followed by the plantlets grown in normal potting mixture (5.8%) and cocopeat (5.3%). The least increase was observed for those in biofibe medium (2.8%).

In the case of plantlets subjected to pretransfer treatment 2, thirty days after transplanting, the percentage increase in height of the plantlets was only slight. The maximum increase was observed for the plantlets in biofibe (1.8%) followed by those in normal potting mixture (1.6%), vermiculite (1.6%), cocopeat, sand and a mixture of vermiculite and soil (1.5%, each). Forty five days after transplanting, the maximum increase was observed for those in vermiculite (11%), a mixture of vermiculite and soil (10.6%), biofibe (10.5%) and soilrite (10.3%). The least increase was observed for the plantlets grown in sand (4.3%) and perlite (3.4%).

4.6.2.3 Width of the largest leaf

In the case of plantlets subjected to pretreatment 1, the width of the largest leaf showed significant difference at all the

Table 21. Effect of media on the width of the largest leaf of pineapple plantlets

Treatment No.	Media	Width of the largest leaf (mm)					
		Pre-treatment 1*			Pre-treatment 2**		
		15 days after trans-planting	30 days after trans-planting	45 days after trans-planting	15 days after trans-planting	30 days after trans-planting	45 days*** after trans-planting
1	Potting mixture	6.0	6.1	9.9	5.7	5.8	6.0
2	Perlite	5.9	6.4	11.6	5.2	5.2	5.6
3.	Soilrite	6.4	6.6	13.1	5.7	5.8	5.7
4	Cocopeat	5.5	6.6	13.2	6.0	6.1	6.1
5	Biofibe	5.8	6.3	10.6	5.0	5.1	5.4
6	Sand	4.8	5.6	9.7	4.9	4.9	5.1
7	Vermiculite	5.7	6.3	12.6	5.7	5.7	6.2
8	Vermiculite + soil	4.7	5.3	8.7	4.9	4.9	5.1
	CD (0.05)	0.59	0.68	1.48	NS	NS	
	SEm±	0.21	0.24	0.53	0.30	0.31	

* Dipping the roots of the plantlets for 18 hr in sterile water, prior to transplanting

** Coating the leaves of the plantlets with paraffin oil on both sides, prior to transplanting

*** Not statistically analysed due to plant mortality

intervals of taking observations. Fifteen days after transplanting, the maximum width was observed for the plantlets grown in soilrite (6.4 mm) followed by those in normal potting mixture (6.0 mm), perlite (5.9 mm), biofibe (5.8 mm).

Thirty days after transplanting, the maximum width was observed for the plantlets grown in cocopeat, and soilrite (6.6 mm) followed by those in perlite (6.4 mm) and vermiculite and biofibe (6.3 mm).

Forty five days after transplanting, maximum width was observed, in the case of plantlets grown in cocopeat (13.2 mm), soilrite (13.1 mm), vermiculite (12.6 mm) and perlite (11.6 mm). At all the intervals, the width recorded was minimum in the case of plantlets grown in mixture of vermiculite and soil (Fig. 6c).

In the case of plantlets subjected to pretreatment 2, the maximum width was observed for the plantlets grown in cocopeat at 15 and 30 days intervals and in vermiculite at 45 days interval, closely followed by normal potting mixture and soilrite.

The percentage increase thirty days after transplanting in the width of the largest leaf, in the case of the plantlets subjected to pretransfer treatment 1, was maximum for the plantlets grown in cocopeat (20) followed by for those in sand (16.7). Forty five days after transplanting, the percentage increase in width was considerably more. The maximum increase was observed for

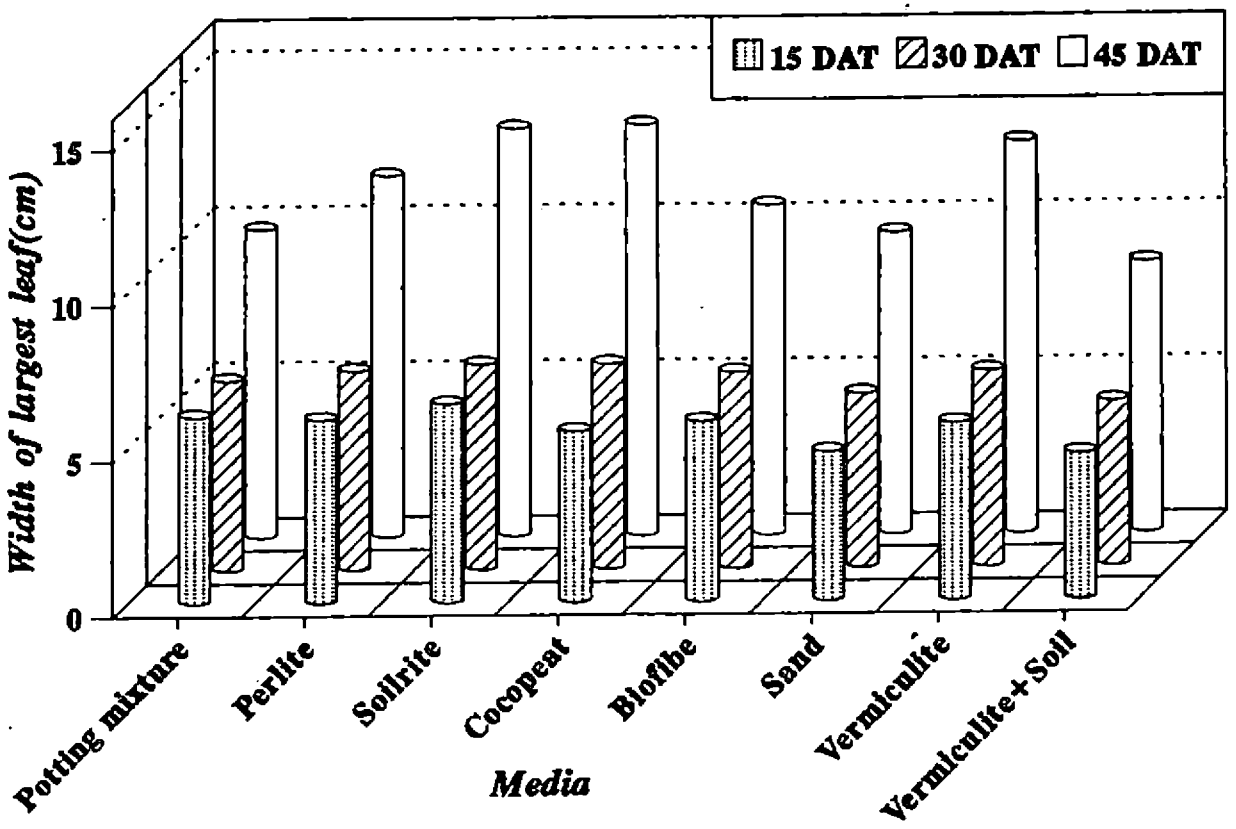
Table 22. Effect of different media on the percentage increase in the width of the largest leaf of pineapple plantlets

Treatment No.	Media	% increase in width of the largest leaf			
		Pre-treatment 1*		Pre-treatment 2**	
		30 days after transplanting	45 days after transplanting	30 days after transplanting	45 days after transplanting
1	Potting mixture 1:1:1	1.7	62.3	1.8	3.4
2	Perlite	8.5	81.3	0.0	7.7
3	Soilrite	3.1	98.5	1.8	0.0
4	Cocopeat	20.0	100.0	1.7	0.0
5	Biofibe	8.6	68.3	2.0	5.9
6	Sand	16.7	73.2	0.0	4.1
7	Vermiculite	10.5	100.0	0.0	8.8
8	Vermiculite + Soil	12.8	64.2	0.0	4.1

* Dipping the roots of the plantlets for 18 hr in sterile water, prior to transplanting

** Coating the leaves of the plantlets with paraffin oil on both sides, prior to transplanting

Fig.6c.Effect of media on growth of pineapple plantlets



the plantlets grown in cocopeat (100%) and vermiculite (100%) followed by in soilrite (98.5%). The percentage increase in the width of the largest leaf in the case of the plantlets subjected to pretransfer treatment 2 was much less. Thirty days after transplanting, the maximum increase in width was observed for the plantlets in biofibe medium (2%) followed by in soilrite and ordinary potting mixture (1.8%, each). Forty five days after transplanting, the maximum increase in width was observed for the plantlets in vermiculite (8.8%) and perlite (7.7%).

4.6.3 Effect of containers and media

4.6.3.1 Number of leaves

In the case of plantlets subjected to pretreatment 1, considering the type of containers and media together, 15 days after transplanting, the highest number of leaves was observed for plantlets grown in plastic pots filled with perlite (11.6) followed by those in plastic pots with cocopeat (10.9) and polybags with soilrite (10.6). The least number of leaves was observed for those in pro-trays (5.8) filled with the mixture of vermiculite and soil.

Thirty days after transplanting, the highest number of leaves was observed for the plantlets in plastic pots with cocopeat (13.4) followed by those in polybags filled with soilrite (12.9) and mud pot with cocopeat and polybags with cocopeat (12.2,

each). The lowest number of leaves (6.6) was observed in the case of plantlets in pro-trays with the mixture of vermiculite and soil.

Forty five days after transplanting, the highest number of leaves was observed again in the case of plantlets grown in plastic pots with cocopeat (15.9), followed by those in mud pots with cocopeat (14.9) and polybags with soilrite (14.8). The lowest number of leaves was observed for the plants grown in pro-trays with a mixture of vermiculite and soil (9.1).

Considering the percentage increase in the number of leaves, 30 days after transplanting, among the plantlets subjected to pretreatment 1, the highest percentage increase was observed for the plantlets grown in polybags with vermiculite (46.2) followed by in pro-trays filled with vermiculite (43.8), pro-trays with normal potting mixture (38.3), mud pots with the mixture of vermiculite and soil (37.9) and mud pots with cocopeat (37.1). The lowest percentage increase was observed for plastic pots with perlite (2.6).

Forty five days after transplanting, the highest increase in the percentage number of leaves was observed for the plantlets in pro-trays with a mixture of vermiculite and soil (37.9) followed by polybags with normal potting mixture (24.2), pro-trays with vermiculite (23.9) and plastic pots with soilrite (23.2).

Table 23a. Effect of different potting media and containers on the number of leaves of pineapple plantlets

Containers/ Media	Number of leaves (Pretreatment 1*)											
	15 days after transplating				30 days after transplanting				45 days after transplanting			
	Mud pot	Plastic pot	Pro-tray	Poly-bag	Mud pot	Plastic pot	Pro-tray	Poly-bag	Mud pot	Plastic pot	Pro-tray	Poly-bag
Potting mixture	9.5 (3.1)**	10.1 (3.2)	8.1 (2.8)	9.9 (3.2)	10.2 (3.2)	11.3 (3.4)	11.2 (3.3)	10.8 (3.3)	12.4 (3.5)	13.8 (3.7)	12.8 (3.6)	12.3 (3.5)
Perlite	10.3 (3.2)	11.6 (3.4)	8.6 (2.4)	9.5 (3.1)	12.1 (3.5)	11.9 (3.5)	9.4 (3.1)	10.4 (3.2)	13.9 (3.7)	13.2 (3.6)	10.9 (3.3)	11.7 (3.4)
Soilrite	9.5 (3.1)	8.9 (2.9)	7.6 (2.8)	10.6 (3.2)	11.4 (3.4)	9.9 (3.1)	10.3 (3.2)	12.9 (3.6)	13.6 (3.7)	12.1 (3.5)	11.4 (3.4)	14.8 (3.8)
Cocopeat	8.9 (2.9)	10.9 (3.3)	7.1 (2.7)	9.9 (3.2)	12.2 (3.5)	13.4 (3.7)	8.5 (2.9)	12.2 (3.5)	14.9 (3.9)	15.9 (3.9)	9.9 (3.2)	13.4 (3.7)
Biofibe	10.2 (3.2)	10.5 (3.2)	7.9 (2.8)	9.2 (3.0)	11.6 (3.4)	11.5 (3.4)	8.5 (2.9)	10.1 (3.2)	12.4 (3.5)	13.7 (3.7)	10.4 (3.2)	12.2 (3.5)
Sand	9.2 (3.0)	9.4 (3.1)	6.6 (2.6)	8.8 (2.9)	11.7 (3.4)	11.8 (3.4)	7.9 (2.8)	11.5 (3.4)	12.6 (3.5)	13.5 (3.7)	9.2 (3.0)	13.7 (3.7)
Vermiculite	9.4 (3.1)	8.7 (2.9)	6.4 (2.5)	7.8 (2.8)	11.8 (3.4)	10.7 (3.3)	9.2 (3.0)	11.4 (3.4)	13.4 (3.7)	12.5 (3.5)	11.4 (3.4)	12.7 (3.6)
Vermiculite + soil	7.4 (2.8)	8.7 (2.9)	5.8 (2.4)	6.6 (2.6)	10.9 (3.3)	11.7 (3.4)	6.6 (2.6)	7.6 (2.8)	11.9 (3.5)	12.9 (3.6)	9.1 (3.0)	9.3 (3.1)
CD (0.05)	NS				0.30				0.33			
SEm±	0.11				0.11				0.12			

* Dipping the roots of the plantlets for 18 hrs in sterile water, prior to transplanting

** Values in parenthesis indicate $\sqrt{x}$ transformed ones

Table 23b. Effect of different potting media and containers on the number of leaves of pineapple plantlets

Containers/ Media	Number of leaves (Pretreatment 2*)											
	15 days after transplanting				30 days after transplanting				45 days after transplanting***			
	Mud pot	Plastic pot	Pro-tray	Poly-bag	Mud pot	Plastic pot	Pro-tray	Poly-bag	Mud pot	Plastic pot	Pro-tray	Poly-bag
Potting mixture	7.5 (2.7)**	8.5 (2.9)	5.8 (2.4)	8.0 (2.8)	7.8 (2.8)	8.5 (2.9)	5.9 (2.4)	8.2 (2.9)	8.5	8.0	7.0	8.3
Perlite	7.4 (2.7)	8.4 (2.9)	5.2 (2.3)	6.8 (2.6)	7.4 (2.7)	8.5 (2.9)	5.4 (2.3)	6.8 (2.6)	8.0	9.3	6.8	8.3
Soilrite	7.4 (2.7)	8.9 (2.9)	5.9 (2.4)	8.4 (2.9)	7.4 (2.7)	8.9 (2.9)	6.2 (2.5)	8.5 (2.9)	7.5	9.7	7.0	9.8
Cocopeat	7.8 (2.8)	9.6 (3.1)	5.8 (2.4)	9.6 (3.1)	7.8 (2.8)	9.6 (3.1)	6.2 (2.5)	9.6 (3.1)	8.3	9.6	7.0	10.0
Biofibe	7.3 (2.7)	8.1 (2.8)	5.6 (2.4)	7.8 (2.8)	7.4 (2.7)	8.3 (2.9)	5.8 (2.4)	7.8 (2.8)	8.7	8.4	6.7	7.8
Sand	7.4 (2.7)	9.1 (3.0)	6.4 (2.5)	8.2 (2.9)	7.4 (2.7)	9.1 (3.0)	6.8 (2.6)	8.4 (2.9)	7.4	9.5	7.0	9.8
Vermiculite	7.8 (2.8)	10.7 (3.3)	5.9 (2.4)	9.1 (3.0)	7.8 (2.8)	10.7 (3.3)	5.9 (2.4)	9.2 (3.0)	7.8	11.8	7.6	10.5
Vermiculite + soil	8.3 (2.9)	8.8 (2.9)	7.1 (2.7)	7.6 (2.8)	8.3 (2.9)	8.8 (2.9)	7.1 (2.7)	7.8 (2.8)	8.3	10.0	8.6	8.5
CD (0.05)	NS				NS							
SEm±	0.12				0.12							

* Coating the leaves of the plantlets with paraffin oil on both sides, prior to transplanting
** Values in parenthesis denote $\sqrt{x}$ transformed ones
*** Not statistically analysed due to plant mortality

Plantlets subjected to pretreatment 2, did not show significant difference with respect to the number of leaves, at any of the intervals of recording observations, while considering the type of containers and media together.

Fifteen days after transplanting, the highest number of leaves was observed for the plantlets in plastic pots with vermiculite (10.7) followed by in plastic pots with cocopeat and polybags with cocopeat (9.6). The lowest number of leaves was observed for the plantlets in pro-trays with perlite (5.2).

Thirty days after planting, the highest number of leaves was observed for the plantlets in plastic pots with vermiculite (10.7) followed by in plastic pots with cocopeat and polybags with cocopeat (9.6), polybags with vermiculite (9.2) and plastic pots with sand (9.1). The lowest number of leaves was observed for the plantlets in pro-trays with perlite (5.4).

Forty five days after transplanting, the highest number of leaves was observed for the plantlets in plastic pots with vermiculite (11.8) followed by polybags with vermiculite (10.5), polybags with cocopeat and plastic pots with a mixture of vermiculite and soil (10.0).

Considering the percentage increase in the number of leaves, the maximum increase 30 days after transplanting, was observed for the plantlets grown in pro-trays with cocopeat (6.9%), sand

Table 24a. Effect of different potting media and containers on the percentage increase in the number of leaves of pineapple plantlets

Containers/ Media	% increase in number of leaves - Pretreatment 1*							
	30 days after transplanting				45 days after transplanting			
	Mud pot	Plastic pot	Pro-tray	Poly bag	Mud pot	Plastic pot	Pro-tray	Poly bag
Potting mixture	7.4	11.9	38.3	9.1	21.57	22.1	14.3	24.2
Perlite	17.5	2.6	9.3	9.5	14.9	10.9	15.9	12.5
Soilrite	20.0	11.2	35.5	21.7	19.3	23.2	10.7	14.7
Cocopeat	37.1	22.9	19.7	23.2	22.1	18.7	16.5	9.8
Biofibe	13.7	9.5	7.6	9.8	6.9	19.1	22.4	20.8
Sand	27.2	25.5	19.7	30.7	7.7	14.4	16.5	19.1
Vermiculite	25.5	22.9	43.8	46.2	13.6	16.8	23.9	11.4
Vermiculite + soil	37.9	34.5	13.8	15.2	9.2	10.3	37.9	22.4

* Dipping the roots of plantlets for 18 hr in sterile water, prior to transplanting

Table 24b. Effect of different potting media and containers on the percentage increase in the number of leaves of pineapple plantlets

Containers/ Media	% increase in number of leaves - Pretreatment 2*							
	30 days after transplanting				45 days after transplanting			
	Mud pot	Plastic pot	Pro-tray	Poly bag	Mud pot	Plastic pot	Pro-tray	Poly bag
Potting mixture	4.0	0.0	1.7	2.5	2.6	0.0	18.6	1.2
Perlite	0.0	1.2	3.8	0.0	8.1	9.4	25.9	22.1
Soilrite	0.0	0.0	5.1	1.2	1.4	8.9	12.9	15.3
Cocopeat	0.0	0.0	6.9	0.0	6.4	0.0	12.9	4.2
Biofibe	1.4	2.5	3.6	0.0	17.6	1.2	15.5	0.0
Sand	0.0	0.0	6.3	2.4	0.0	4.4	2.9	16.7
Vermiculite	0.0	0.0	0.0	1.1	0.0	10.3	28.8	14.1
Vermiculite + soil	0.0	0.0	0.0	2.6	0.0	13.6	21.1	8.9

* Coating the leaves of the plantlets on both sides with paraffin oil, prior to transplanting

(6.3%) and soilrite (5.1%). Forty five days after transplanting, the maximum increase was observed for the plantlets grown in pro-trays with vermiculite (28.8%), perlite (25.9%), polybags with perlite (22.1%) and mud pots with biofibe (17.6%).

4.6.3.2 Height of the plant

In the case of plantlets subjected to pretreatment 1, there was significant difference with respect to the height of the plantlets, 15 days after transplanting. The maximum height was observed for the plantlets in plastic pots with cocopeat (9.8 cm) followed by those in polybag with soilrite (9.7 cm) and in plastic pots with biofibe and vermiculite (9.0 cm, each). The lowest value was recorded for the plantlets in pro-trays with mixture of vermiculite and soil (3.7 cm).

Thirty days after transplanting, the maximum height was observed for the plantlets in plastic pots with cocopeat (10.1 cm) followed by in polybag with soilrite (9.9 cm) and plastic pots with vermiculite (9.4 cm). The lowest value was recorded for the plantlets in pro-trays with soilrite, mixture of vermiculite and soil as well as normal potting mixture (3.9 cm, each).

Forty five days after transplanting, the maximum height was again observed for the plantlets in plastic pots with cocopeat (10.3 cm) followed by polybags with soilrite (10.0 cm) and plastic pot with vermiculite (9.7 cm). The lowest value was in

Table 25a. Effect of different potting media and containers on the height of pineapple plantlets

Containers/ Media	Height of plantlets (cm) - Pretreatment 1*											
	15 days after transplanting				30 days after transplanting				45 days after transplanting			
	Mud pot	Plastic pot	Pro-tray	Poly-bag	Mud pot	Plastic pot	Pro-tray	Poly-bag	Mud pot	Plastic pot	Pro-tray	Poly-bag
Potting mixture	6.9	8.4	3.8	7.8	7.1	8.6	3.9	7.9	7.2	8.9	5.0	7.9
Perlite	7.3	8.4	4.2	6.7	7.5	8.7	4.4	6.9	8.1	9.1	4.6	7.1
Soilrite	7.3	7.1	3.8	9.7	7.6	7.4	3.9	9.9	7.9	7.6	4.3	10.0
Cocopeat	7.7	9.8	4.0	7.7	7.9	10.1	4.1	7.9	8.2	10.3	4.8	8.3
Biofibe	7.2	9.0	4.6	7.3	7.3	9.1	4.8	7.3	7.6	9.3	4.8	7.6
Sand	6.5	6.3	4.2	6.4	6.5	7.0	4.3	6.5	6.8	7.5	4.3	7.0
Vermiculite	7.3	9.0	4.3	7.7	7.9	9.4	4.5	7.8	8.1	9.7	4.7	8.4
Vermiculite + soil	6.2	7.7	3.7	6.5	6.6	8.0	3.9	6.9	6.9	8.3	4.5	7.7
CD (0.05)	1.54				NS				NS			
SEm±	0.56				0.56				0.61			

* Dipping the roots of the plantlets for 18 hr in sterile water, prior to transplanting

Table 25b. Effect of different potting media and containers on the height of pineapple plantlets

Containers/ Medid	Height of the plantlets (cm) - Pretreatment 2*											
	15 days after transplanting				30 days after transplanting				45 days after transplanting**			
	Mud pot	Plastic pot	Pro-tray	Poly-bag	Mud pot	Plastic pot	Pro-tray	Poly-bag	Mud pot	Plastic pot	Pro-tray	Poly-bag
Potting mixture	6.3	7.4	4.7	7.3	6.4	7.4	4.8	7.3	6.6	7.6	5.7	7.7
Perlite	5.7	7.2	3.7	6.6	5.7	7.2	3.8	6.6	6.2	7.2	4.1	7.0
Soilrite	6.9	8.3	5.0	6.8	7.1	8.3	5.1	6.9	7.4	8.9	5.9	7.9
Cocopeat	8.4	8.3	3.5	6.8	8.5	8.4	3.7	6.9	9.8	8.4	3.8	8.1
Biofibe	4.9	7.6	3.7	6.2	5.0	7.7	3.9	6.2	6.2	7.7	4.9	6.7
Sand	6.5	8.0	4.5	8.2	6.6	8.2	4.7	8.3	6.7	8.2	4.7	8.9
Vermiculite	7.2	8.2	3.4	6.0	7.2	8.3	3.4	6.0	7.2	9.6	4.2	7.2
Vermiculite + soil	5.8	9.0	4.5	6.8	5.8	9.1	4.5	6.9	6.5	10.1	4.6	7.8
CD (0.05)	NS				NS							
SEm±	0.68				0.69							

* Coating the leaves of the plantlets on both sides with paraffin oil, prior to transplanting

** Not statistically analysed due to plant mortality

the case of plantlets in pro-trays with sand and also with soilrite (4.3 cm).

Considering the percentage increase in plantlet height 30 days after transplanting, the maximum increase was observed for the plantlets in mud pot with vermiculite (8.2) followed by in plastic pots with the mixture of vermiculite and soil (7.8). There was no increase in height upto 30 days in the case of plants in polybags with biofibe and mud pots with sand.

Forty five days after transplanting, the percentage increase in height was maximum for the plantlets in pro-trays with normal potting mixture (28.2) followed by in pro-trays with cocopeat (17.1), pro-trays with mixture of vermiculite and soil (15.4), polybags with mixture of vermiculite and soil (11.6). However, there was no increase in height with the plantlets in pro-trays with either biofibe or sand and in polybags with normal potting mixture.

Among the plantlets subjected to pretreatment 2, 15 days after transplanting, there was no significant effect of the type of container and the media. The maximum height (9.0 cm) was observed for the plantlets in plastic pots with the mixture of vermiculite and soil, followed by in mud pots with cocopeat (8.4 cm) and in plastic pots with soilrite (8.3 cm) as well as with cocopeat (8.3 cm) and with vermiculite and sand (8.2 cm). The

plantlets in pro-trays with vermiculite exhibited the lowest mean height (3.4 cm).

Thirty days after transplanting, the maximum height (9.1 cm) was observed for the plants in plastic pots with a mixture of vermiculite and soil followed by in mud pots with cocopeat (8.5 cm), plastic pot with cocopeat (8.4 cm), plastic pot with soilrite (8.3 cm), plastic pot with vermiculite (8.3 cm) and poly-bags with sand (8.3 cm). The plantlets grown in pro-trays with vermiculite exhibited the least height (3.4 cm).

Forty five days after transplanting, the maximum height was again recorded for plantlets in plastic pots (10.1 cm) followed by in mud pots with cocopeat (9.8 cm) and plastic pots with vermiculite (9.6 cm).

Considering the percentage increase in height, the highest increase was recorded 30 days after transplanting for the plantlets in pro-trays with cocopeat (5.7), with biofibe (5.4) and with sand (4.4). There was no mean increase in height, for the plantlets grown in plastic pots with ordinary potting mixture or with perlite or soilrite, as well as in polybags with potting mixture or perlite. Mud pots with vermiculite as well as with the mixture of vermi-culite and soil also exhibited no mean increase in height of the plantlets as was the case also with the plantlets in pro-trays with either vermiculite or the mixture of vermiculite and soil.

Table 26a. Effect of different potting media and containers on the percentage increase in the height of pineapple plantlets

Containers/ Media	% increase in height of the plantlets - Pretreatment 1*							
	30 days after transplanting				45 days after transplanting			
	Mud pot	Plastic pot	Pro-tray	Poly bag	Mud pot	Plastic pot	Pro-tray	Poly bag
Potting mixture	2.9	2.4	2.6	1.3	1.4	3.5	28.2	0.0
Perlite	2.7	3.6	4.8	2.9	8.0	4.6	4.5	2.9
Soilrite	4.1	4.2	2.6	2.1	3.9	2.7	10.3	1.0
Cocopeat	2.6	3.1	2.5	2.6	3.8	1.9	17.1	5.1
Biofibe	1.4	1.1	4.3	0.0	4.1	2.2	0.0	4.1
Sand	0.0	11.1	2.4	1.6	4.6	7.1	0.0	7.7
Vermiculite	8.2	4.4	4.7	1.3	2.5	3.2	4.4	7.7
Vermiculite + soil	6.5	7.8	5.4	6.2	4.5	3.7	15.4	11.6

* Dipping the roots of the plantlets for 18 hr in sterile water, prior to transplanting

Table 26b. Effect of different potting media and containers on the percentage increase in the height of pineapple plantlets

Containers/ Media	% increase in height of the plantlets - Pretreatment 2*							
	30 days after transplanting				45 days after transplanting			
	Mud pot	Plastic pot	Pro-tray	Poly bag	Mud pot	Plastic pot	Pro-tray	Poly bag
Potting mixture	1.6	0.0	2.1	0.0	3.1	2.7	18.8	5.5
Perlite	0.0	0.0	2.7	0.0	8.8	7.2	7.9	6.1
Soilrite	2.9	0.0	2.0	1.5	4.2	7.2	15.7	14.5
Cocopeat	1.2	1.2	5.7	1.5	15.3	0.0	2.7	17.4
Biofibe	2.0	1.3	5.4	0.0	24.0	0.0	25.6	8.1
Sand	1.5	2.5	4.4	1.2	1.5	0.0	0.0	7.2
Vermiculite	0.0	1.2	0.0	0.0	0.0	15.7	23.5	20.0
Vermiculite + soil	0.0	1.1	0.0	1.5	12.1	10.9	2.2	13.0

* Coating the leaves of the plantlets on both sides with paraffin oil, prior to transplanting

4.6.3.3 Width of the largest leaf

In the case of plantlets subjected to pretreatment 1, there was significant difference with respect to the width of the largest leaf at all the intervals of recording observations. At 15 days after transplanting, the maximum width (7.4 mm) was observed for the plantlets in plastic pots with verimiculite followed by those in plastic pots with normal potting mixture (7.2 mm), poly-bags with soilrite (7.2 mm) and mud pots with soilrite (6.8 mm). The lowest value was recorded for the plantlets in pro-trays with a mixture of vermiculite and soil (3.0 mm).

Thirty days after transplanting, the maximum width was observed for the plantlets in polybags with soilrite and plastic pots with cocopeat (8.0 mm) followed by plastic pots with biofibe (7.8 mm) and plastic pots with vermiculite (7.6 mm). The lowest value was for plants in pro-trays with sand (3.2 mm).

Forty five days after transplanting, the maximum width was observed also for the plantlets in polybags with soilrite (16.2 mm) followed by plastic pots with cocopeat (15.4 mm) and mud pot with soilrite (14.0 mm). The lowest width was found for the plantlets in pro-trays with a mixture of vermiculite and soil (5.0 mm).

Considering the percentage increase in the width of the leaves, 30 days after transplanting, the maximum increase was

Table 27a. Effect of different potting media and containers on the width of the largest leaf of pineapple plantlets

Containers/ Media	Width of the largest leaf (mm) - Pretreatment 1*											
	15 days after transplanting				30 days after transplanting				45 days after transplanting			
	Mud pot	Plastic pot	Pro-tray	Poly-bag	Mud pot	Plastic pot	Pro-tray	Poly-bag	Mud pot	Plastic pot	Pro-tray	Poly-bag
Potting mixture	5.8	7.2	5.0	6.0	6.2	7.2	5.0	6.0	8.4	11.4	10.0	9.6
Perlite	6.4	6.8	5.0	5.2	7.2	7.0	5.6	5.8	12.4	13.8	9.8	10.4
Soilrite	6.8	6.0	5.4	7.2	7.2	6.2	6.0	8.0	14.0	11.6	10.4	16.2
Cocopeat	6.2	5.6	4.4	5.6	6.4	8.0	5.6	6.4	12.8	15.4	11.0	13.4
Biofibe	6.2	6.4	4.4	6.2	6.6	7.8	4.4	6.4	11.4	12.0	6.4	12.4
Sand	5.2	5.4	3.2	5.4	6.4	6.6	3.2	6.0	10.8	10.2	6.4	11.4
Vermiculite	5.6	7.4	3.6	6.0	6.2	7.6	5.2	6.2	13.0	13.6	11.0	13.4
Vermiculite + soil	4.8	6.0	3.0	5.0	5.2	6.6	3.6	5.6	12.2	11.0	5.0	6.4
CD (0.05)	1.18				1.36				2.96			
SEm±	0.43				0.49				1.07			

* Dipping the roots of the plantlets for 18 hr in sterile water, prior to transplanting

Table 27b. Effect of different potting media and containers on the width of the largest leaf of pineapple plantlets

Containers/ Media	Width of the largest leaf (mm) - Pretreatment 2*											
	15 days after transplanting				30 days after transplanting				45 days after transplanting**			
	Mud pot	Plastic pot	Pro-tray	Poly-bag	Mud pot	Plastic pot	Pro-tray	Poly-bag	Mud pot	Plastic pot	Pro-tray	Poly-bag
Potting mixture	4.8	6.2	4.0	7.8	5.0	6.4	4.0	7.8	5.0	6.4	4.0	8.7
Perlite	5.4	6.2	3.4	5.6	5.4	6.2	3.6	5.6	5.6	6.3	3.8	6.7
Soilrite	5.4	7.4	3.8	6.0	5.4	7.4	4.0	6.2	5.5	7.4	4.3	6.5
Cocopeat	6.6	7.4	4.0	6.0	6.6	7.4	4.2	6.0	7.0	7.4	4.3	6.3
Biofibe	6.4	6.2	3.2	4.2	6.4	6.2	3.6	4.2	6.4	6.8	4.0	4.5
Sand	4.6	5.8	3.8	5.4	4.6	6.0	3.8	5.4	4.6	6.3	4.0	5.5
Vermiculite	4.2	7.0	4.6	7.0	4.2	7.0	4.6	7.0	4.2	7.0	5.8	8.0
Vermiculite + soil	4.2	6.0	4.4	5.2	4.2	6.0	4.4	5.2	4.6	6.0	4.6	5.2
CD (0.05)	1.67				NS							
SEm±	0.60				0.62							

* Coating the leaves of the plantlets on both sides with paraffin oil, prior to transplanting

** Not statistically analysed due to plant mortality

observed for the plantlets in pro-trays with vermiculite (44.4) followed by those in plastic pots with cocopeat (42.9). There was no increase in width for the plantlets in pro-trays with ordinary potting mixture or biofibe or sand, as well as in plastic pots and polybags with ordinary potting mixture.

Forty five days after transplanting, the maximum percentage increase was observed for the plantlets in polybags with vermiculite (116.1) followed by those in pro-trays with vermiculite (111.5), in mud pots with vermiculite (109.7) polybags with cocopeat (109.4), polybag with soilrite (102.5), mud pots with cocopeat (100.0) and pro-trays with ordinary potting mixture or sand (100.0).

Among the plantlets subjected to pretreatment 2, there was significant difference with respect to the type of containers and media in the width of the plantlet leaves. The maximum width was observed for the plantlets in polybags with potting mixture (7.8 mm) followed by plastic pots with soilrite or cocopeat (7.4 mm), plastic pots with vermiculite (7.0 mm) and polybags with vermiculite (7.0 mm).

The lowest values were recorded for the plantlets in pro-trays with perlite (3.4 mm) and with biofibe (3.2 mm).

Thirty days after transplanting, the maximum width was observed for the plantlets in polybags with potting mixture (7.8 mm) followed by those in plastic pots with soilrite or cocopeat

Table 28a. Effect of different potting media and containers on the percentage increase in the width of the largest leaf of pineapple plantlets

Containers/ Media	% increase in width of the largest leaf - Pretreatment 1*							
	30 days after transplanting				45 days after transplanting			
	Mud pot	Plastic pot	Pro-tray	Poly bag	Mud pot	Plastic pot	Pro-tray	Poly bag
Potting mixture	6.9	0.0	0.0	0.0	35.5	58.3	100.0	60.0
Perlite	12.5	2.9	12.0	11.5	72.2	97.1	75.0	79.3
Soilrite	5.9	3.3	11.1	11.1	94.4	87.1	73.3	102.5
Cocopeat	3.2	42.9	27.3	14.3	100.0	92.5	96.4	109.4
Biofibe	6.5	21.9	0.0	3.2	72.7	53.8	45.5	93.8
Sand	23.1	22.2	0.0	11.1	68.8	54.5	100.0	90.0
Vermiculite	10.7	2.7	44.4	3.3	109.7	78.9	111.5	116.1
Vermiculite + soil	8.3	10.0	20.0	12.0	134.6	66.7	38.9	14.3

* Dipping the roots of the plantlets for 18 hr in sterile water, prior to transplanting

Table 28b. Effect of different potting media and containers on the percentage increase in the width of the largest leaf of pineapple plantlets

Containers/ Media	% increase in width of the largest leaf - Pretreatment 2*							
	30 days after transplanting				45 days after transplanting			
	Mud pot	Plastic pot	Pro-tray	Poly bag	Mud pot	Plastic pot	Pro-tray	Poly bag
Potting mixture	4.2	3.2	0.0	0.0	0.0	0.0	0.0	11.5
Perlite	0.0	0.0	5.9	0.0	3.7	1.6	5.6	19.6
Soilrite	0.0	0.0	5.3	3.3	1.9	0.0	7.5	4.8
Cocopeat	0.0	0.0	5.0	0.0	6.1	7.4	2.4	5.0
Biofibe	0.0	0.0	12.5	4.2	0.0	9.7	11.1	7.1
Sand	0.0	3.4	0.0	0.0	0.0	5.0	5.3	1.9
Vermiculite	0.0	0.0	0.0	0.0	0.0	0.0	30.4	14.3
Vermiculite + soil	0.0	0.0	0.0	0.0	9.5	0.0	4.5	0.0

* Coating the leaves of the plantlets on both sides with paraffin oil, prior to transplanting

(7.4 mm) and plastic pots with vermiculite as well as polybags with vermiculite (7.0 mm). The lowest values were recorded for the plantlets in pro-trays with perlite or biofibe (3.6 mm).

Forty five days after transplanting, the maximum width was observed for the plantlets in polybags with ordinary potting mixture (8.7 mm) or with vermiculite (8.0 mm) followed by plastic pots with vermiculite and mud pot with cocopeat (7.0 mm) and plastic pot with cocopeat or biofibe (6.8 mm).

The lowest value was observed for the plantlets in pro-trays with perlite (3.8 mm) or with sand or biofibe (4.0 mm).

Considering the percentage increase in the width of the largest leaf, the maximum increase was observed for the plantlets grown in pro-trays either with biofibe (12.5) or with perlite (5.9) or soilrite (5.3) or cocopeat (5.0).

4.6.4 Effect of nutrient starter solution

The data recorded relating to the effect of various nutrient starter solutions on the growth of the pineapple plantlets are presented in Table 29.

4.6.4.1 Number of leaves

The highest number of leaves was observed for the plantlets treated with the N:P:K fertilizer solution once a week at the 3 intervals of taking observations. Fifteen days after

transplanting, the plantlets treated with NPK fertilizer solution once a week had the mean number of 9 leaves followed by a mean of 7.8 leaves in the case of the plantlets to which Hoagland's solution was applied, closely followed by those which were treated with $\frac{1}{4}$ MS salts.

Thirty days after transplanting, the same trend was observed with the highest number of leaves being observed for the plantlets treated with fertilizer solution once a week (10.0) followed by those with Hoagland's solution (9.6) and $\frac{1}{4}$ MS salts (9.2).

Forty five days after transplanting, the maximum number of leaves observed for the plantlets treated with fertilizer solution once a week (11.0) closely followed by 10.6 for those treated with Hoagland's solution and 9.4 for those treated with $\frac{1}{4}$ MS salts.

Fifteen days after transplanting the percentage of increase in the number of leaves was maximum for the plantlets treated with the fertilizer solution once a week (7.1), while 30 days after transplanting, the percentage increase was maximum for those treated with $\frac{1}{4}$ MS (23.7) and with Hoagland's solution (23.07).

Forty five days after transplanting, the percentage increase in the mean number of leaves, was the highest for the plantlets treated with the fertilizer solution twice a week (15.0) followed by with Hoagland's solution (10.4).

4.6.4.2 Height of the plantlets

Fifteen days after transplanting, the maximum height (6.8 cm) was observed for the plantlets treated with ¼ MS salts followed by those for control (5.9 cm) and those treated with Hoagland's solution (5.7 cm). Thirty days after transplanting, the maximum height was recorded for the plantlets treated with Hoagland's solution (6.9 cm) followed by those in ¼ MS (6.8 cm). Forty five days after transplanting, the maximum height was observed for the plantlets treated with Hoagland's solution (7.5 cm) followed by those with ¼ MS (7.3 cm).

Considering the percentage increase in the height of the plantlets, 15 days after transplanting, the maximum height was observed with plantlets treated with ¼ MS salts (10.9) while at 30 days after transplanting, the plantlets treated with ½ MS salts (24.0) and Hoagland's solution (21.1) showed the maximum.

Forty five days after transplanting, the maximum percentage increase in the height of the plantlets was observed with the plantlets treated with Hoagland's solution (8.7) or ½ MS (8.1).

4.6.4.3 Width of the largest leaf

The maximum width 15 days after transplanting was observed for the control treatment (6.8 mm) followed by for those treated with NPK fertilizer solution once a week (6.2 mm), twice a week

Table 29. Effect of nutrient starter solutions on the growth of *in vitro* plantlets of pineapple

Treatment No.	Nutrient starter solution	Number of leaves			Height of the plantlet (cm)			Width of the largest leaf (mm)		
		15 days after transplanting	30 days after transplanting	45 days after transplanting	15 days after transplanting	30 days after transplanting	45 days after transplanting	15 days after transplanting	30 days after transplanting	45 days after transplanting
1	Fertilizer soultion applied once a week	9.0 (7.1)*	10.0 (11.1)	11.0 (9.1)	5.7 (3.6)	6.2 (8.8)	6.5 (4.8)	6.2 (3.3)	6.2 (0.0)	6.2 (0.0)
2	Fertilizer solution applied twice a week	6.6 (3.1)	8.0 (18.2)	9.2 (15.0)	5.6 (5.7)	6.3 (12.5)	6.8 (7.9)	6.0 (3.4)	6.6 (10.0)	6.6 (0.0)
3	MS salts - $\frac{1}{4}$ conc.	7.6 (2.7)	9.4 (23.7)	9.4 (0.0)	6.1 (10.9)	6.8 (11.5)	7.3 (7.4)	5.6 (0.0)	5.8 (3.6)	6.0 (6.0)
4	MS salts - $\frac{1}{2}$ conc.	7.0 (2.9)	8.2 (17.1)	8.8 (7.3)	5.0 (6.4)	6.2 (24.0)	6.7 (8.1)	5.0 (0.0)	5.6 (7.7)	5.6 (0.0)
5	Hoagland's nutrient solution	7.8 (0.0)	9.6 (23.1)	10.6 (10.4)	5.7 (3.6)	6.9 (21.1)	7.5 (8.7)	5.8 (0.6)	5.8 (0.0)	6.2 (6.9)
6	Control	7.0 (2.9)	8.0 (14.3)	8.8 (10.0)	5.9 (5.4)	6.6 (11.9)	6.9 (4.5)	6.8 (0.0)	6.8 (0.0)	6.8 (0.0)

Observation taken 45 days after transplanting

* Values in parenthesis indicate percentage increase

(6.0 mm) and Hoagland's solution (5.8 mm). Thirty days after transplanting, the maximum width recorded was for control (6.8 mm) followed by for NPK fertilizer solution twice a week (6.6 mm), once a week (6.2 mm), ¼ MS (5.8 mm) and Hoagland's solution (5.8 mm). Forty five days after transplanting, the maximum width was observed for control (6.8 mm) followed by those for the plantlets treated with fertilizer solution twice a week (6.6 mm), once a week (6.2 mm), Hoagland's solution (6.2 mm) and ¼ MS (6.0 mm).

The percentage increase in the width of the largest leaf 15 days after transplanting was maximum for the plantlets treated with the fertilizer solution twice a week (3.4) followed by fertilizer solution once a week (3.3). Thirty days after transplanting, the maximum increase was observed for the plantlets treated with NPK fertilizer solution once a week (10%) followed by ½ MS (7.7%) and ¼ MS (3.6%). Forty five days after transplanting, the maximum increase in width of the largest leaf was observed for the plantlets treated with Hoagland's solution (6.9%), followed by those with ¼ MS (3.4%).

4.6.5 Transfer to the field

The plantlets after 90 days in the net house with 10-12 cm height and a minimum of 12 leaves were transferred into soil (Plate 13). The plantlets were carefully removed from the containers and planted in trenches in double lines without disturbing the roots.

Plate 13. Plantlets transferred into the field

4.7 Encapsulation studies

An assessment of the various concentrations of sodium alginate (2-4 per cent w/v) and calcium chloride (25-125 mM) for the formation of beads is presented in Table 30. A 2.5 per cent solution of sodium alginate upon complexation with 75 mM of $CaCl_2.2H_2O$ solution gave optimal, firm and round beads within an ion exchange duration of 30 minutes (Plate 14a). The diameter of the beads ranged between 0.6 and 0.8 cm (Plate 14b). Lower concentration of sodium alginate (2%) resulted in the formation of very fragile beads, whereas concentration higher than 2.5 per cent proved to be too viscous for the free flow of the dispersed explants. Lower levels of $CaCl_2.2H_2O$ on the other hand, not only adversely affected the bead quality, but also prolonged the complexation time.

The encapsulated beads stored at room temperature (30°C) retained their regenerative capacity for 30 days when put on cotton wool moistened with MS basal salts (Plate 15).

The encapsulated beads when put on regeneration media, regenerated shoots in 15 days (Plate 16).

Table 30. Effect of different concentrations of sodium alginate and calcium chloride on bead formation in pineapple

$CaCl_2$, $2H_2O$ (mM)	Sodium alginate (% w/v)				
	2.0	2.5	3.0	3.5	4.0
25	++	+++	+	+	+
50	++	+++	+	+	+
75	+++	++++	+++	+	+
100	+++	++++	+++	+	+
125	+++	++++	+++	++	+

Observations were taken after 30 minutes complexation duration

+ refers to bead quality in terms of shape, size and firmness

Plate 14a. Encapsulated beads suspended in calcium chloride solution for complexation

Plate 14b. Encapsulated beads of pineapple

Plate 15. Storage of encapsulated beads

Plate 16. Shoots growing from encapsulated beads

Discussion

DISCUSSION

The present studies on *in vitro* multiplication and standardization of hardening techniques in pineapple (*Ananas comosus* (L.) Merr.) were carried out at the Tissue Culture Laboratory of the All India Co-ordinated Floriculture Improvement Project attached to the Department of Pomology and Floriculture, College of Horticulture, Vellanikkara from 1991 to 1993.

Pineapple, belonging to the family Bromeliaceae is one of the most important of the commercially cultivated fruit crops in the world. The fruit is mainly valued for its processed products such as juices, squashes, jams and canned slices.

Pineapple is propagated vegetatively by using slips, suckers or crowns. One of the major constraints in the cultivation of pineapple is the limited source of planting materials available especially when high density planting is being recommended. Slip production in the commercially important Kew variety is rare under Kerala conditions and it has a shy suckering habit. The non-uniform growth and asynchronous flowering among the individual plants in the field are the other constraints in the cultivation of pineapple. Obtaining true-to-type propagules from a desirable parent is also difficult due to the high rate of spontaneous mutations which occur in the field (Mathews, 1988).

Rapid propagation through tissue culture is the most useful technique in this context to facilitate faster multiplication of the selected parent in a shorter time compared to conventional methods (Murashige, 1974).

The in vitro raised plantlets need to be subjected to a series of hardening treatments before they can be successfully planted out in the open. The studies on the different methods of hardening and to determine the optimum size and age of the plantlets for planting in the field are essential to ensure maximum establishment in the field.

The present studies were carried out to standardise the in vitro technique for raising the maximum number of plantlets and to standardise the hardening techniques for maximum establishment of the plantlets in the field.

The results obtained in the studies are discussed in this chapter.

5.1 Standardisation of surface sterilization of explants

The explants collected from the field harbour a variety of microorganisms which have to be removed before inoculation on to the culture medium. Though general sterilization procedures have been outlined by various workers (Dodds and Roberts, 1982 and George and Sherrington, 1984), specific sterilization

procedures have to be evolved based on the tissues being handled. Hence, in the present study, the sterilization procedure with respect to the concentration and duration of exposure was standardised. Among the different treatments tried, the best sterilization treatment for crown explants was found to be a combination of a dip in Emisan 0.1 per cent for 30 minutes followed by treatment with 0.1 per cent mercuric chloride for 10 minutes which gave the least percentage of contamination and the maximum survival of explants. The reduction in the time of treatment with Emisan increased the rate of contamination while the increase in duration of exposure of the explants to the sterilizing treatments decreased the survival of the explants in the media.

Mathews (1988) used 0.1 per cent mercuric chloride for three minutes to surface sterilize buds excised from crowns of pineapple. In the present study, the same concentration of mercuric chloride (0.1%) but for an increased duration of treatment of 10 minutes was found to be optimum. However, a dip in 0.1 per cent Emisan for 30 minutes prior to treatment with mercuric chloride was found to be essential for reducing contamination percentage in the present study.

The percentage of contamination observed for the explants collected in the different months showed variation. The explants collected in the drier months of the year showed comparatively lower contamination while those collected in the wetter rainy

months showed higher contamination rates. The percentage of contamination was the lowest when the explants were collected in the months of January and February.

5.2 Standardisation of media and growth regulators for culture establishment

Among the media used for pineapple micropropagation, most of the workers have used MS medium (Mapes, 1973; Pannatier and Lanaud, 1976; Mathews and Rangan, 1979; Wakasa et al., 1978; Zepeda and Sagawa, 1981; Cabral et al., 1984; Ramirez, 1984; Evans and Moore, 1987; Liu et al., 1987; Rosa-Marquez, 1987; Dewald et al., 1988; Marchal and Alvard, 1988; Zee and Munekata, 1992 and Bordoloi and Sarma, 1993). However, Lakshmisita et al. (1974) have used Knudson's basal media with Nitsch's micro elements and Fitchet (1989), Murashige and Tucker's medium for pineapple.

In the present study, maximum establishment of the crown explants was observed in MS medium supplemented with BA 5.0 mg l^{-1} alone or in combination with NAA 1.0 mg l^{-1}. Among the cytokinins tried, BA was found to give the maximum percentage of establishment of explants followed by KIN and 2iP. At the higher concentration of the cytokinins, there was a reduction in the percentage of establishment. The same pattern was observed with respect to the percentage of cultures initiating growth. The

time taken for greening of the established explants was also shortest with BA 5.0 mg l^{-1} alone or in combination with NAA 1.0 mg l^{-1}. The response of the explants was slowest with 2iP. Higher concentrations of the cytokinins in general increased the time taken for greening of the explants.

Favourable effects of supplementing establishment media with cytokinins and auxins have been reported by many workers (Murashige, 1974; Bonga, 1982; Durzan, 1984 and Zimmerman, 1985). Various workers have used combinations of BA and NAA in the establishment media for pineapple (Pannatier and Lanaud, 1976; Wakasa et al., 1978; Ramirez, 1984; Rosa-Marquez et al., 1987 and Dewald et al., 1988).

5.3 Proliferation of explants

The explants which were established and started greening formed masses of globular structures when cultured on fresh MS media supplemented with cytokinins and auxins. Among the different cytokinins used, the fastest formation of the globular structures with the highest intensity occurred in media supplemented with BA with or without NAA followed by KIN and 2iP. The fastest response of formation of the globular structures was obtained with BA 5.0 mg l^{-1} + NAA 1.0 mg l^{-1}. The maximum formation of the globular structures was obtained with BA 5.0 mg l^{-1} alone or with NAA 1.0 mg l^{-1}. The globular structures formed with 2iP

also showed characteristic narrow leafy proliferation. The higher concentration of the cytokinins was found to increase the time needed for formation of the globular structures and also to reduce the intensity of the globular structures formed. However, NAA added at the higher concentration of 1.0 mg l^{-1} helped to induce faster formation and higher intensity of globular structures.

Wakasa (1989) obtained formation of globular bodies when explants such as crowns, slips and axillary buds excised from suckers or slips were used for culture on MS media supplemented with 2.0 mg l^{-1} each of NAA and BA. As in the present study, culture of whole crown explants resulted in the formation of numerous small leaves and buds at the base of the growing crowns followed by the formation of such globular bodies all over the explants.

5.4 Proliferation of shoots

The globular structures with small leaves and shoots when subcultured into fresh media formed complete shoots. The highest percentage of cultures developing into shoots as well as the highest number of multiple shoots formed was obtained with basal MS media. Among the cytokinins tried, KIN at 10.0 mg l^{-1} followed by KIN at 5.0 mg l^{-1} were effective in inducing formation of a high percentage of shoots in large numbers. Addition of NAA resulted in the reduction of the percentage of cultures developing shoots

as well as the number of multiple shoots formed with respect to KIN and 2iP. However, with BA, addition of NAA increased the percentage of cultures developing shoots. Though auxins do not promote axillary shoot proliferation, there are reports of improvement of culture growth by their presence (Wang and Hu, 1980). Lundergan and Janick (1980) observed that one of the possible roles of auxin in culture proliferation medium is to nullify the suppressive effect of high cytokinin concentration on axillary shoot elongation thereby restoring normal shoot growth.

Mathews (1988) also obtained the maximum number of multiple shoots of pineapple on MS medium supplemented with KIN, NAA and IBA though KIN alone or with NAA induced multiple shoot formation. Wakasa (1989) reported that when the globular bodies were subcultured to media with 2.0 mg l^{-1} BA, rapid growth was induced from the differentiated leaves and small shoots in the globular bodies. Further growth of these shoots was obtained as in the present study by transferring them to medium without growth regulators.

5.5 Induction of rooting

5.5.1 In vitro rooting

In the present study, cent per cent root initiation was obtained in the basal medium as well as with various concentrations of NAA and IBA. The shortest time for initiation of roots

(8.54 days) was obtained in basal medium though with lesser number of roots. The highest number of roots (6.53) was produced when IBA 2.0 mg l^{-1} was added to liquid stationary media.

The physical state of the medium had a significant effect on the number of days taken for rooting as well as the number of roots formed. Faster rooting as well as more number of roots were formed at different concentrations of IBA and NAA in liquid stationary media with the shoots being supported on filter paper supports. In the case of usage of solid media, the agar concentration could be reduced to 0.65 per cent without reduction in the number of roots formed and their mean length.

The roots produced in the basal medium were thin, long and branched while those produced with IBA and NAA were brownish, short and stubby.

Various workers have obtained rooting of pineapple shoots in a medium lacking phytohormones (Zepeda and Sagawa, 1981 and Wakasa, 1989). In the present study also, maximum rooting within the shortest time was observed in the basal medium while there was an increase in the number of roots formed and a prolonging of the time needed for rooting when auxins such as IBA or NAA are added into the media.

Mathews (1988) used a mixture of NAA (0.18 mg l^{-1}) and IBA (0.4 mg l^{-1}) while Bordoloi and Sarma (1993) obtained rooting

of pineapple shoots with 2.0 mg l^{-1} IBA.

The high percentage of rooting obtained in the basal medium may be because even before the shoots were transferred into the media, root initiation and formation would have already taken place in the regeneration media which, in addition to cytokinins also contains NAA.

Hence from the present study, *in vitro* rooting of pineapple shoots in basal MS medium or liquid MS medium supplemented with 2.0 mg l^{-1} of IBA is recommended.

5.5.2 *Ex vitro* rooting

Deberg and Maene (1981) observed that the most labour intensive part of micro-propagation is *in vitro* rooting while as per Rajeevan and Pandey (1986), the major cost of producing *in vitro* plants lies in the rooting and hardening stages. Separation of individual shoots from multiple shoot clusters and rooting them *in vitro* is both time and labour consuming. The shoots produced *in vitro* could be rooted under *ex vitro* conditions by treating the shoots as microcuttings without using aseptic conditions. Rooting under *ex vitro* conditions would also facilitate the combining of the rooting stage with the acclimatization (George and Sherrington, 1984).

In the present study, ex vitro rooting was attempted and found successful. The highest percentage of rooting, the fastest rooting and the maximum length of roots were obtained by treatment with the commercially available Rooton powder. The number of roots produced was maximum for treatment with IBA 200 mg l^{-1}. There was profuse rooting of pineapple shoots even when not treated with any growth regulator and the roots formed were comparatively long though it took slightly more number of days for initiation of rooting.

Dewald et al. (1988) reported that transfer to a rooting medium under in vitro conditions was not necessary for pineapple shoots. The shoots larger than 3.0 cm in length had 100 per cent survival when transferred to soil. In the present study, the in vitro produced shoots larger than 5.0 cm in length were successfully rooted when put in vermiculite without treatment with any growth regulators and kept in a mist chamber with high humidity. The shoots also formed profuse roots on treatment with Rooton powder or IBA 200 mg l^{-1}. Thus ex vitro rooting under non-aseptic conditions help to reduce the time and labour involved in in vitro rooting and in the subsequent hardening stages thereby substantially reducing the cost of production of in vitro plantlets.

5.6 Hardening and planting out

5.6.1 Hardening treatments

The *in vitro* derived plantlets have to be acclimatised to the entirely different environment prevailing *in vivo* as they are poorly adapted to resist the lower relative humidity, higher light intensity and temperature. A precise control of the weaning environment alone will ensure maximum survival rates (Grout and Aston, 1977; Langford and Wainwright, 1987 and Wainwright, 1988). A period of humidity acclimatization was considered essential for the newly transferred plantlets to adapt to the outside environment during which the plantlets undergo morphological and physiological adaptation enabling them to develop typical terrestrial plant water control mechanism (Grout and Aston, 1977; Sutter, 1985).

Selvapandian *et al.* (1988) reported a method of coating the leaves of *in vitro* plantlets with glycerol, paraffin wax or grease to avoid rapid water loss from the leaves of plants grown *in vitro* during their transplantation to the field to prevent their desiccation.

In the present study, immersing the roots of the plantlets for 18 hr in sterile water prior to transplanting was found to give cent per cent survival of the plantlets compared to sixty per cent for the plantlets whose leaves were coated with paraffin oil.

5.6.2 Type of containers

The type of containers used for transplanting the in vitro plantlets may affect the establishment and growth of the transplanted plantlets. In the present study, considering the number of leaves produced, the height of the plantlets and the width of the largest leaf at different intervals, the plantlets grown in plastic pots showed the highest vigour followed closely by the plantlets in mud pots and polybags and the minimum by those in pro-trays. However considering the percentage increase in the number of leaves, height of the plantlets and width of the largest leaf, the maximum increase in general, of these parameters was observed for the plantlets in pro-trays.

Various workers have used different types of containers, to transfer in vitro derived plantlets. Plastic pots have been used to transfer plantlets of different species (Canas and Benbadis, 1988; Reuveni et al., 1990 and Yadav et al., 1990). Ramesh et al. (1993) also used plastic pots for successful transfer of jack plantlets and reported mud pots to be the least effective. Balachandran (1990) transplanted plantlets of turmeric and ginger in trays. After 3-4 weeks, the plantlets were transplanted into pots. From the present study it can be said that the plantlets can be successfully transferred into pro-trays for rapid growth of more number of plantlets in the initial stages and then transplanted into plastic pots or mud pots for vigorous growth.

5.6.3 Effect of media

The type of potting media used to transplant *in vitro* grown plantlets is an important factor which determines the percentage of establishment. Media which maintain an optimum moisture level and sufficient aeration to the root zone of the plantlets promote better establishment and growth of the transplanted plantlets (Ramesh *et al.*, 1993).

In the present study, potting mixes such as cocopeat, soilrite, biofibe and vermiculite were found to facilitate better growth of the *in vitro* grown plantlets as manifested in the number of leaves produced, the height of the plantlet and the width of the largest leaf as well as the percentage increase in the same. Among these potting mixes, cocopeat is the most economical to use being the cheapest followed by biofibe and vermiculite, with soilrite mix being the costliest.

Various workers have used different media to successfully transplant *in vitro* plantlets. Keshavachandran (1991) found that a mixture of soilrite and potting mixture gave the highest establishment percentage and better vigour of vetiver plantlets. Ramesh *et al.* (1993) observed that for transferring jack plantlets, sand gave the highest survival followed by vermiculite and peat. Folliot and Marchal (1990) found that for transplanting pineapple plantlets, a peat based subtrate gave the best results followed

by a mixture of compost and perlite. Reuveni et al. (1990) used a mixture of peat and polystyrene shredded flakes for successfully transferring papaya plantlets. The better results obtained with potting mixes such as cocopeat, biofibe, vermiculite and soilrite in the present study may be due to the improved rhizosphere provided by these media having better ventilation and drainage which would have created a favourable condition for better root growth and the consequent vigorous growth of the plantlets (Wainwright, 1988).

5.6.4 Effect of containers and media

The containers and potting mixtures used for transplanting in vitro plantlets were found to influence the survival rate (Zimmerman and Fordham, 1985).

In the present study, based on the observations on the number of leaves produced, the height of the plant and the width of the largest leaf, the plantlets grown in plastic pots filled with cocopeat or plastic bags with soilrite showed in general, better vigour. However, the percentage increase in the number of leaves produced, the height of the plantlet and the width of the largest leaf were more for the plantlets grown in pro-trays with cocopeat, soilrite or vermiculite as media.

5.6.5 Nutrient starter solution

The beneficial effect of addition of inorganic nutrients to

the pot mixture has been reported by several workers (Brown and Sommer, 1982; Amerson et al., 1985; Rajmohan, 1985 and Keshavachandran, 1991). Wong (1986) recommended the addition of 3.0 g of nutricate mix (14:14:14 NPK) to each pot one week after transplanting to get healthier in vitro banana plantlets for planting in the main field. Mathur et al. (1988) initially irrigated in vitro derived Java Citronella plantlets with Hoagland and Arnon (1950) nutrient solution for a week.

In the present study, among the different treatments tried in the early stages, application of NPK solution once a week or ¼ strength MS salts were sufficient to induce healthy growth of the plantlets. At later stages of plantlet growth, better vigour of the plantlets was observed by the application of Hoagland's solution or the NPK fertilizer solution twice a week.

Kavanagh et al. (1987) found in Prunus serotina that application of full strength Hoagland's solution weekly produced the most vigorous plantlets. Dewald et al. (1988) reported that for best results with pineapple plantlets initial application of a fertilizer bi-weekly followed by 10 per cent MS salt solution in the latter stages was found to be the optimum.

5.7 Encapsulation studies

Differentiating globular bodies formed from crown explants, could be successfully encapsulated into beads using sodium alginate

at 2.5 per cent and calcium chloride at 75 mM with an ion exchange duration of 30 min. The beads formed were firm and round. The beads when put on regeneration media started differentiating into shoots within 15 days. The encapsulated beads could be successfully stored at room temperature on cotton wool moistened with MS basal salts without loosing their regeneration capacity for 30 days.

Mathur et al. (1989) have reported in Valeriana wallichii that encapsulation of apical and axial shoot buds has resulted in scaling up the micropropagation technique while at the same time economising upon the medium, space and time requirements. As per their report, encapsulated explants utilize only about 200 ml of MS medium for the preparation of nearly 350 beads each of which contains an explant, thereby producing 350 plantlets under in vitro conditions. When non-encapsulated explants were used, nearly 4.5 l of MS medium were required for producing the same number of plants. The culture space requirement can also be reduced to 1/6 in an Erlenmeyer flask of 250 ml capacity, which can accomodate only 5 non-encapsulated explants but can hold 30 beads. In addition, plants from encapsulated explants can be easily retrieved by picking with a forceps or just shaking the beads out. Also, the technique offers easy transportation of large number of plants in low bulk.

Thus clonal multiplication of pineapple is possible through in vitro culture of crown explants. The large number of leafy

globular structures formed from the primary explant could be induced to form shoots which in turn could be separated and individually rooted. The success of ex vitro rooting would help in reducing the cost of producing in vitro plantlets. The rooted plantlets after undergoing a period of hardening under a low light, high humidity atmosphere could be successfully transferred to soil. The standardisation of an in vitro propagation technique for pineapple would help in generating larger numbers of true to type planting material. The development of an encapsulation technique would further help in economising the time, labour and cost of producing in vitro plantlets.

Summary

SUMMARY

Studies were conducted on in vitro multiplication and standardisation of hardening techniques in pineapple at the Tissue Culture Laboratory of the All India Co-ordinated Floriculture Improvement Project, attached to the Department of Pomology and Floriculture of the College of Horticulture, Vellanikkara. The salient findings of the study are summarised in this chapter.

1. Among the different surface sterilization treatments tried, considering the contamination percentage and survival of explants, treatment with Emisan 0.1 per cent for 30 minutes followed with mercuric chloride 0.1 per cent for 10 minutes was found to be the best.

2. The explants collected in the months of January and February gave the least contamination and maximum survival percentages.

3. MS medium with BA 5.0 mg l^{-1} alone or in combination with NAA 1.0 mg l^{-1} was found to be the best for maximum establishment of the explants.

4. Higher concentrations of the cytokinins were found to reduce the percentage of establishment of the explants. BA gave the highest percentage of establishment followed by KIN and 2iP.

5. The percentage of cultures initiating growth was the highest and the time taken for greening of the explants was the shortest with BA 5.0 mg l^{-1} alone or in combination with NAA 1.0 mg l^{-1}.

6. The maximum intensity of globular structures with leaves and shoots were formed within the shortest time of 5.96 days on subculture to MS medium with BA 5.0 mg l^{-1} and NAA 1.0 mg l^{-1} or within 6.95 days with BA 5.0 mg l^{-1} alone.

7. Among the three cytokinins tried, the fastest explant response and the highest intensity of globular structures was formed with BA, followed by with KIN and 2iP. The structures formed with 2iP also had characteristic narrow leafy proliferations.

8. The maximum shoot proliferation (11.9 per culture) was obtained with basal MS medium with cent per cent of the cultures developing vigorous dark green shoots.

9. Among the cytokinins tried, KIN at 10.0 mg l^{-1} followed by KIN at 5.0 mg l^{-1} were effective in inducing formation of a high percentage of shoots. Addition of NAA resulted in the reduction of the percentage of cultures developing shoots as well as the number of multiple shoots formed with respect to KIN and 2iP. However, with BA, addition of NAA increased the percentage of cultures developing shoots.

10. Hundred per cent rooting was obtained in the basal MS medium as well with various concentrations of IBA and NAA. The fastest rooting (8.54 days) was observed in the basal medium.

11. Among the auxins tried, faster rooting and more number of roots were induced with IBA compared to NAA. The higher concentration of 2.0 mg l^{-1} was more effective.

12. Rooting was faster in stationary liquid media compared to solid media.

13. The roots formed in the basal medium were thin, long and branched; with IBA short, stubby and brown coloured and with NAA, short, thick and hairy.

14. In solid media, early root initiation and maximum length of roots was observed with 0.65 per cent agar concentration.

15. Ex vitro rooting of shoots over 5.0 cm in length was possible by placing the shoots under intermittant mist thereby facilitating savings of time and labour involved in in vitro rooting and the hardening stages.

16. The commercially available rooting powder Rooton induced the fastest rooting and the maximum length of the roots. The number of roots produced was highest for treatment with IBA 200 mg l^{-1} as quick dip.

17. Profuse rooting of in vitro derived shoots of pineapple was obtained even without treatment with growth regulators by keeping the shoots under mist.

18. Immersing the roots of the plantlets for 18 hr in sterile water prior to transplanting gave hundred per cent survival of the plantlets.

19. The plantlets grown in plastic pots in general showed the maximum vigour with respect to the number of leaves, height and the width of the largest leaf, followed by those in mud pots and polybags. The percentage increase in these parameters were, however, observed maximum for the plantlets in pro-trays.

20. The potting mixes such as cocopeat, soilrite, biofibe and vermiculite were found better for inducing vigorous growth of the *in vitro* grown plantlets.

21. The plantlets grown in plastic pots with cocopeat or plastic bags with soilrite mix in general induced more vigorous growth of the plantlets. In pro-trays, cocopeat, soilrite or vermiculite were more suitable for inducing vigorous growth of the seedlings.

22. A nutrient starter solution of NPK fertilizer solution once a week or one fourth strength basal MS salts were sufficient to induce healthy growth of the transplanted plantlets in the early stages of growth. Application of the NPK fertilizer solution twice a week or Hoagland's solution once a week was found to induce better growth of the plantlets in the later stages.

23. The plantlets with a minimum of 10 cm height and 12 leaves were successfully transferred after 90 days to soil and planted in trenches.

24. The differentiating globular bodies formed from the primary explants could be successfully encapsulated into beads using sodium alginate at 2.5 per cent and calcium chloride at 75 mM with an ion exchange duration of 30 min.

25. The encapsulated beads could be successfully stored at room temperature on cotton wool moistened with MS basal salts without loosing their regeneration capacity for 30 days.

References

REFERENCES

*Aghion, D. and Beauchesne, G. 1960. Utilisation de la technique de culture steriled' organes pour obtenir des clones d' ananas. Fruits d' Outre Mer 15:444-446

Aitken-Christie, J. and Thorpe, T.A. 1984. Clonal propagation gymnosperms. In: Cell culture and somatic cell genetics of plants. Vol.I, Laboratory procedures and their applications. Vasil, I.K. (Ed.). Academic Press, Orlando: 82-95

Amerson, H.V., Frampton, L.I., Mckeand, S.E., Mott, R.L. and Weir, R.J. 1985. Loblolly pine tissue culture: Laboratory, greenhouse and field studies. In: Tissue Culture in Forestry and Agriculture. Henke, R.R., Hughes, K.W., Constantin, M.J. and Hollaender, A. (Ed.). 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

Balachandran, S.M., Bhat, S.R. and Chandel, K.P.S. 1990. In vitro clonal multiplication of turmeric (Curcuma Spp.) and ginger (Zingiber officinale Rosc.). Pl. Cell Rept. 8:521-524

Bapat, V.A., Mhatre, M. and Rao, P.S. 1987. Propagation of Morus indica L. (Mulberry) by encapsulated shoot buds. Pl. Cell. Rept. 6:393-395

Bapat, V.A. and Rao, P.S. 1988. Sandalwood plantlets from synthetic seeds. Pl. Cell Rept. 7:434-436

Bonga, J.M. 1980. Plant propagation through tissue culture emphasising woody species. In: Plant Cell Cultures : Results and Perspectives. Sala, F., Parisi, B., Cella, R. and Ciferi, O. (Ed.). Elsevier, North Biomedical Press, New York. pp.253-264

Bonga, J.M. 1982. Vegetative propagation in relation to juvenility, maturity and rejuvenation. In: Tissue Culture in Forestry. Bonga, J.M. and Durzan, D.J. (Ed.). Martinus Nijhoff/ Dr.W.Junk Publishers, London. pp.150-181

Bordoloi, N.D. and Sarma, C.M. 1993. Effect of plant growth regulators on in vitro micropropagation and post-transplant survival of plantlets of pineapple. Proceedings of the Golden Jubilee Symposium, Bangalore. p.213

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

*Cabral, J.R.S., Cunha, G.A.P. and DA. Rodrigues, E.M. 1984. Pineapple micropropagation. Agropecuaria 1:124-127

Canas, L.A. and Benbadis, A. 1988. In vitro plant regeneration from cotyledon fragments of the olive tree (Olea europaea L.). Pl. Sci. 54:65-74

Chaturvedi, H.C. and Sharma, M. 1988. Successful exploitation of tissue culture for rapid clonal multiplication. Proceedings of the National Seminar on Plant Tissue Culture, CPCRI, Kasaragod. pp.17-22

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

Dewald, M.G. 1988. Tissue culture and electrophoretic studies of pineapple (A. comosus) and related species. Dissertation Abstracts International, B. 48(8):2165-B

Dewald, M.G., Moore, G.A., Sherman, W.B. and Evans, M.H. 1988. Production of pineapple plants in vitro. Pl. Cell. Rept. 7(7):535-537

Dodds, J.H. and Robert, L.W. 1982. Experiments in Plant Tissue Culture. Cambridge University Press, London. p.178

Durzan, D.J. 1984. Fibre and Wood: Adult vs. juvenile explants. In: Handbook of Plant Cell Culture Vol.2, Crop Sciences. Sharp, W.R., Evans, D.A., Ammirato, P.V. and Yamada, Y. (Ed.). Macmillan Publishing Co., New York. pp.471-505

Evans, M.H. and Moore, G.A. 1987. Production and characterisation of pineapple plants produced in vitro propagation via bud culture. HortSci. 22(5):Sect.2. 1067

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

Farm Guide, 1992. Farm Information Bureau, Government of Kerala. p. 9, 15.

Fitchet, M. 1985. Tissue culture of pineapples. Inf. Bull. **149**:1-2

Fitchet, M. 1987. Review on propagation by means of tissue culture techniques - cloning of pineapples. Inf. Bull. **175**:20-24

Fitchet, M. 1989. Preliminary radioimmuno assay studies on pineapple callus. Appl. Pl. Sci. **3**(2):127-128

*Folliot, M. and Marchal, J. 1990. Influence of culture substrate on the rate of growth of pineapple in vitro plants during hardening off-phase. Fruits. **45**(4):367-376

George, E.F. and Sherrington, P.D. 1984. Plant propagation by Tissue Culture : Handbook and Directory of Commercial Laboratories. Exegetics Limited. pp.184-308

Griffis, J.L. Jr., Sheehan, T.J. and Dennis, B. 1981. Excised floral tissues of coconut palm (Cocos nucifera L.) cultured in vitro. HortSci. **16**:460

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

*Haberlandt, G. 1902. Culturversuche mit isolatierten pfkonzenzollen sitz-Ber. Mat-Hat. KI-Kas Akad Wiss Wien. **111**:69-92

*Hannig, E. 1904. Zur physiologie pflanzenlicher. Embryonen 1. Uber Die Kultur Von Crucifereen Embryonen auster halb der embryosackes. Bot. Zeit. 62:45-80

Hasegawa, P.M. 1980. Factors affecting shoot and root initiation from cultured rose shoot tips. J. Am. Soc. Hort. Sci. 105:216-220

Hoagland, D.R. and Arnon, D.I. 1950. The water culture method for growing plants without soil. Calif. Agr. Exp. Sta. Circ., 347

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

*Kamada, H. 1985. Artificial seed. In: Practical Technology on the mass production of clonal plants. Tanaka, R. (Ed.). CMS Publishers, Tokyo. p.48

Kavanagh, K.L., Maynard, C.A. and Drew, A.P. 1987. Tissue culture propagation of mature trees of Prunus serotina: Acclimatization effect of gibberellin, fertilizer and cold treatment on dormancy. In Vitro. 23(2):71B

Keshavachandran, R. 1991. In vitro propagation studies in vetiver (Vetiveria zizanioides (L.) Nash) and Cashew (Anacardium occidentale L.). Ph.D. thesis submitted to Tamil Nadu Agricultural University, Coimbatore.

Keshavachandran, R., Md. Abdul Khader and Sudhakar Johnson. 1993. Propagation of Vetiveria zizanioides (L.) Nash through synthetic seeds. Abstracts of the Golden Jubilee Symposium, Bangalore. p.251

Kitto, S.L. and Janick, J. 1985. Hardening treatments increase survival of synthetically coated asexual embryos of carrot. J. Am. Soc. Hort. Sci. **110**:283-286

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

Kyte, L. and Briggs, 1979. A simplified entry into tissue culture production. Proceedings of International Plant Propagator Society. p.290

Lakshmisita, G., Singh, R. and Iyer, C.P.A. 1974. Plantlets through shoot tip cultures in pineapple. Curr. Sci. **43**(22):724-725

Lakso, A.N., Reisch, R.I., Mortensen, J. and Roberts, M.N. 1986. Carbondioxide enrichment for stimulation of growth of in vitro propagated grapevines after transfer from culture. J. Am. Soc. Hort. Sci. **111**:636-638

Langford, P.J. and Wainwright, H. 1987. Effects of sucrose concentration of photosynthetic ability of rose shoots in vitro. Ann. Bot. **60**:633-640

Liu, L.J., Rosa-Marquez, E. and Lizardi, E. 1987. In vitro propagation of Spineless Red Spanish pineapple. Phytopathol. **77**(12):1711

Lo, O.F., Chen, C.J. and Ross, J.G. 1980. Vegetative propagation of temperate foliage grasses through callus culture. Crop Sci. **20**:363-367

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

Maene, L.J. and Deberg, P.C. 1985. Liquid medium additions to established cultures to improve elongation and rooting *in vivo*. *Pl. Cell Tissue Organ Cult.* 5:23-33

Mapes, M.O. 1973. Tissue culture of Bromeliads. *Combined Proceedings of the International Plant Propagators' Society*. 23:47-55

*Marchal, J. and Alvard, D. 1988. The effect of frequency of renewal of solutions in *in vitro* culture of pineapple in liquid media. *Fruits*. 43(12):701-707

Mathews, V.H. 1988. Propagation of pineapple through tissue culture. In: *Proceedings of the National Seminar on Plant Tissue Culture*. CPCRI, Kasaragod. pp.54-61

*Mathews, V.H., Rangan, T.S. and Narayanaswamy, S. 1976. Micropropagation of *Ananas sativus* *in vitro*. *Z. Pflanzenphysiol.* 79:450-454

Mathews, V.H. and Rangan, T.S. 1979. Multiple plantlets in lateral bud and leaf explant *in vitro* cultures of pineapple. *Sci. Hort.* 11:319-328

Mathur, A.K., Ahuja, P.S., Pandey, B., Kukreja, A.K. and Mandal, S. 1988. Screening and evaluation of somaclonal variations for quantitative and qualitative traits in an aromatic grass, *Cymbopogan winterianus* Jowitt. *Pl. Breeding*. 101:321-334

Mathur, A.K., Ahuja, P.S., Lal, N. and Mathur, A.K. 1989. Propagation of *Veleriana wallichii* D.C. using encapsulated apical and axial shoot buds. *Pl. Sci.* 60:111-116

Mc Clelland, M.T., Smith, M.A.L. and Carothers, Z.B. 1990. The effects of in vitro and ex vitro root initiation on subsequent microcutting root quality in three woody plants. Pl. Cell Tissue Organ Cult. 23:115-123

Morel, G. 1960. Producing virus-free cymbidiums. Am. Orchid Soc. Bull. 29:495-497

*Morel, G. 1965. Clonal propagation of orchids by meristem culture. Cymbidium Soc. News 20(7):3-11

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

Murashige, T. 1977a. Clonal crops through tissue culture. In: Plant Tissue culture and its Biotechnological Applications. Barz, W., Reinhard, E. and Zenk, M.H. (Ed.). Springer-Verlag, Berlin and New York. pp.392-403

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

Murashige, T. 1977c. Plant cell and organ culture as horticultural practices. Acta Hort. 78:17

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

Nemeth, G. 1986. Induction of rooting. In: Biotechnology in Agriculture and Forestry, Vol.I. Bajaj,Y.P.S. (Ed.). Springer-Verlag, Berlin. pp.49-64

*Pannetier, C. and Lanaud, C. 1976. Divers aspects de l' utilisation possible des cultures "in vitro" pour la multiplication vegetative de l' Ananas comosus L. Merr. variete 'Cayennelisse'. Fruits d' Outre Mer. 31:739-750

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

Rajeevan, M.S. and Pandey, R.M. 1986. Economics of mass propagation of papaya through tissue culture. In: Plant Tissue culture and its Agricultural Applications. Withers,L.A. and Alderson, P.G. (Ed.). Butterworths, London. pp.211-215

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

Ramesh, B., Rajmohan, K. and Mohanakumaran, N. 1993. Maximising the ex vitro establishment of jack plantlets : Basic and applied aspects. Proceedings of the Fifth Kerala Science Congress, Kottayam. pp.106-108

*Ramirez, A.L. 1984. Reproduction of pineapple (Ananas comosus L. Merr.) by tissue culture. Cultivos Tropicales. 6(3): 681-697

*Ramirez, A.L. 1987. Study of in vitro growth of pineapple (Ananas comosus (L.) Merr.) cv. Smooth Cayenne buds. Cultivos Tropicales 9(2):32-35

Rao, N.K.S., Doreswamy, R. and Chacko, E.K. 1981. Differentiation of plantlets in hybrid embryo callus of pineapple. Sci. Hort. 15:235-238

Redenbaugh, K., Paasch, B.D., Nichol, J.W., Kossler, M.E., Viss, P.R. and Walker, K.A. 1986. Somatic seeds: Encapsulation of asexual plant embryos. Biotechnology. 4:497-801

Reuveni, O., Shlesinger, D.R. and Lavi, U. 1990. In vitro clonal propagation of dioecious Carica papaya. Pl. Cell Tissue Organ Cult. 20:41-46

Rosa-Marquez, E., Liu, L.J. and Lizardi, E. 1987. Callus induction and regeneration from Red Spanish pineapple. Phytopathol. 77(12):1711

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

*Schwann, T. 1839. 'Mikroskopische Untersuchungen Uber die Ubereinstimmung in der Strukker und dem Wachstume der Tiere and Pfanzen.' (Ostwalds Klassiker der exaketen Wissenschaften', No.176, Engelmann, Leipzing, 1910).

Scott, M.A. 1987. Weaning of cultured plants. In: Alderson,P.G. and Dulforce,W.M. (Ed.). Micropropagation in Horticulture: Practice and Commercial Problems. Proc. Inst. Hort. Symp. Uni. Nottingham. pp.173-182

Selvapandian, A., Subramani, J., Bhatt, P.N. and Mehta, A.R. 1988. A simple method for direct transplantation of cultured plants to the field. Pl. Sci., 56:81-83

Simmonds, J. 1983. Direct rooting of micropropagated M.26 apple root stocks. Sci. Hort. 21:233-241

Skirvin, R.M. 1980. Fruit crops. In: Cloning Agricultural Plants via in vitro techniques. Conger, B.V. (Ed.). CRS Press, Boca Raton, Florida. pp.51-139

*Skoog, F. and Miller, C.O. 1957. Chemical regulation of growth and organ formation in plant tissues cultured in vitro. The Biological Action of Growth Substances. Symp. Soc. Exp. Biol. **11**:118-131

Sommer, H.E. and Caldas, L.S. 1981. In vitro methods applied to forest trees. In: Plant Tissue culture : Methods and Applications in Agriculture. Thorpe,T.A. (Ed.). Academic Press, New York. pp.349-358

Sutter, E.G. 1985. Morphological, physical and chemical characteristics of epicuticular wax on ornamental plants regenerated in vitro. Ann. Bot. **55**:321-329

*Teo,C.K.H. 1974. Clonal propagation of pineapple (Ananas comosus) by tissue culture. Planter **50**(575):58-59

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

Thorpe, T.A. 1990. The current status of plant tissue culture. In: Plant Tissue Culture : Applications and Limitations. Bhojwani,S.S. (Ed.). Elsevier Science Publishing Company, New York. pp.1-22

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

Vasil, I.K. and Vasil, V. 1980. Clonal propagation. Int. Rev. Cytol. Suppl. **11A**:145-173

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

Wakasa, K. 1989. Pineapple (Ananas comosus (L.) Merr.). In: Biotechnlogy in Agriculture and Forestry. Vol.5. Trees II. Bajaj, Y.P.S. (Ed.) Springer-Verlag, Berlin. pp.13-29

Wakasa, K., Yoshiaki, K. and Massaki, K. 1978. Differentiation from in vitro cultures of Ananas comosus. Jap. J. Breed. **28**:113-121

Wang, P.J. and Hu, C.Y. 1980. Regeneration of virus free plants through in vitro culture. In: Advances in Biochemical Engineering Vol.18. Fiechter,A. (Ed.). Springer-Verlag, New York. pp.61-99

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

*Wickson, M. and Thimman, K.V. 1958. The antagonism of auxin and kinetin in apical domiance. Physiol. Plant. **11**:62-74

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

Yadav, U., Lal, M. and Jaiswal, V.S. 1990. In vitro micropropagation of the tropical fruit tree Syzygium cuminii L. Pl. Cell Tissue Organ Cult. **21**:87-92

Yeoman, M.M.. 1986. Plant cell culture technology. Blackwell Scientific Publications, Melbourne. p.33

Zee, F.T. and Munekata, M. 1992. In vitro storage of pineapple (Ananas spp.) germplasm. HortSci. **27**(1):57-58

Zepeda, C. and Sagawa, Y. 1981. In vitro propagation of pineapple. HortSci. **16**(4):495

Zimmerman, R.H. 1985. Applications of tissue culture propagation to woody plants. In: Tissue culture of Forestry and Agriculture. Henke, R.R., Hughes, K.W., Constantin, M.M.J. and Hollaender, A. (Ed.). Plenum Press, New York. pp.165-178

Zimmerman, R.H. and Broome, O.C. 1980. Blueberry micropropagation. In: Proceeding of the Conference on nursery production of Fruit plants through Tissue culture - Application and Feasibility. Agric. Res. Sci. Educ. Admn., U.S.D.A. Beltsville. pp.44-47

Zimmerman, R.H. and Fordham, I. 1985. Simplified method for rooting apple cultivars in vitro. J. Am. Soc. Hort. Sci. **110**:34-38

* Originals not seen

Appendices

APPENDIX-I

Meteorological parameters of the experimental site at the College of Horticulture, Vellanikkara, for the period from January to December 1992

Month	Mean temperature (°C)		Mean relative humidity (%)	Rainfall (mm)	Number of rainy days	Mean sunshine (hours)
	Maximum	Minimum				
January	32.6	20.9	53	0	0	9.0
February	34.5	21.8	65	0	0	9.2
March	36.9	22.8	61	0	0	9.2
April	36.3	24.4	65	48.6	3	8.8
May	33.8	24.8	73	90.6	6	7.4
June	30.1	23.7	84	976.8	22	3.3
July	28.8	22.7	87	874.5	26	2.1
August	28.9	22.3	88	562.9	25	2.7
September	30.1	23.1	82	302.9	17	4.1
October	30.1	22.9	82	386.7	14	4.6
November	31.0	23.1	77	376.7	12	5.5
December	31.1	22.3	61	2.0	0	8.9

APPENDIX-II

Abstract of analysis of variance for the effect of different treatments

Sl. No.	Character	Treatment DF	Treatment MSS	Error DF	Error MSS	Level of significance
1	2	3	4	5	6	7
In vitro multiplication						
(1)	Effect of surface sterilant treatments					
a.	Contamination	6	373.45	14	12.69	0.05
b.	Survival	6	288.74	14	14.84	0.05
(2)	Effect of cytokinins and auxins on establishment of pineapple explants					
a.	Establishment	12	153.57	13	4.64	0.05
b.	Percentage of cultures showing growth	12	152.69	13	3.71	0.05
c.	Days for greening	12	15.78	13	0.06	0.05
(3)	Effect of cytokinins and auxins on formation of nodular structures					
a.	Days for response	10	21.44	11	0.79	0.05
(4)	Effect of cytokinins and auxins on shoot production					
a.	Percentage cultures developing shoots	9	1283.50	20	9.29	0.05
b.	Number of shoots per culture	9	51.46	20	0.68	0.05
(5)	Effect of NAA and IBA on in vitro rooting					
a.	Days for root initiation	8	45.95	9	2.06	0.05
b.	Number of roots per shoot	8	35.95	9	0.67	0.05

Contd

APPENDIX-II

Abstract of analysis of variance for the effect of different treatments

Sl. No.	Character	Treatment DF	Treatment MSS	Error DF	Error MSS	Level of significance
1	2	3	4	5	6	7
In vitro multiplication						
(1)	Effect of surface sterilant treatments					
a.	Contamination	6	373.45	14	12.69	0.05
b.	Survival	6	288.74	14	14.84	0.05
(2)	Effect of cytokinins and auxins on establishment of pineapple explants					
a.	Establishment	12	153.57	13	4.64	0.05
b.	Percentage of cultures showing growth	12	152.69	13	3.71	0.05
c.	Days for greening	12	15.78	13	0.06	0.05
(3)	Effect of cytokinins and auxins on formation of nodular structures					
a.	Days for response	10	21.44	11	0.79	0.05
(4)	Effect of cytokinins and auxins on shoot production					
a.	Percentage cultures developing shoots	9	1283.50	20	9.29	0.05
b.	Number of shoots per culture	9	51.46	20	0.68	0.05
(5)	Effect of NAA and IBA on in vitro rooting					
a.	Days for root initiation	8	45.95	9	2.06	0.05
b.	Number of roots per shoot	8	35.95	9	0.67	0.05

Contd.

1	2	3	4	5	6	7
(6)	Effect of different concentration of agar on the initiation and elongation of roots in MS medium					
a.	Length of roots	3	2.34	52	0.45	0.05
b.	Number of roots	3	0.33	52	0.18	NS
	HARDENING					
(7)	Effect of different containers on the number of leaves of pineapple					
a.	Pretreatment 1					
	i) 15 days after transplanting	3	1.52	128	0.06	0.05
	ii) 30 days after transplanting	3	1.48	128	0.06	0.05
	iii) 45 days after transplanting	3	1.35	128	0.07	0.05
b.	Pretreatment 2					
	i) 15 days after transplanting	3	2.24	128	0.08	0.05
	ii) 30 days after transplanting	3	1.99	128	0.08	0.05
	(not analysed)					
(8)	Effect of containers on the height of pineapple plantlets					
a.	Pretreatment 1					
	i) 15 days after transplanting	3	132.45	128	1.55	0.05
	ii) 30 days after transplanting	3	140.05	128	1.58	0.05
	iii) 45 days after transplanting	3	135.66	128	1.85	0.05

Contd.

1	2	3	4	5	6	7
	b. Pretreatment 2					
	i) 15 days after transplanting	3	104.66	128	2.30	0.05
	ii) 30 days after transplanting	3	102.05	128	2.36	0.05
(9)	Effect of containers on the width of the largest leaf of pineapple plantlets					
	a. Pretreatment 1					
	i) 15 days after transplanting	3	33.45	128	0.91	0.05
	ii) 30 days after transplanting	3	42.01	128	1.19	0.05
	iii) 45 days after transplanting	3	107.14	128	5.69	0.05
	b. Pretreatment 2					
	i) 15 days after transplanting	3	50.72	128	1.82	0.05
	ii) 30 days after transplanting	3	47.63	128	1.89	0.05
(10)	Effect of media on the number of leaves of pineapple plantlets					
	a. Pretreatment 1					
	i) 15 days after transplanting	7	0.47	128	0.05	0.05
	ii) 30 days after transplanting	7	0.25	128	0.06	0.05
	iii) 45 days after transplanting	7	0.26	128	0.07	0.05
	b. Pretreatment 2					
	i) 15 days after transplanting	7	0.15	128	0.08	NS
	ii) 30 days after transplanting	7	0.14	128	0.08	NS

Contd.

1	2	3	4	5	6	7
(11)	Effect of media on the height of pineapple plantlets					
a.	Pretreatment 1					
	i) 15 days after transplanting	7	5.38	128	1.55	0.05
	ii) 30 days after transplanting	7	5.01	128	1.58	0.05
	iii) 45 days after transplanting	7	4.56	128	1.85	0.05
b.	Pretreament 2					
	i) 15 days after transplanting	7	4.23	128	2.30	NS
	ii) 30 days after transplanting	7	4.56	128	2.36	NS
(12)	Effect of media on the width of largest leaf of pineapple plantlets					
a.	Pretreatment 1					
	i) 15 days after transplanting	7	6.56	128	0.91	0.05
	ii) 30 days after transplanting	7	4.81	128	1.19	0.05
	iii) 45 days after transplanting	7	59.35	128	5.69	0.05
b.	Pretreatment 2					
	i) 15 days after transplanting	7	3.64	128	1.82	NS
	ii) 30 days after transplanting	7	3.77	128	1.89	NS
(13)	Effect of different media and containers on the number of leaves of pineapple plantlets					

Contd.

1	2	3	4	5	6	7
a.	Pretreatment 1					
	i) 15 days after transplanting	21	0.06	128	0.06	NS
	ii) 30 days after transplanting	21	0.17	128	0.06	0.05
	iii) 45 days after transplanting	21	0.14	128	0.07	0.05
b.	Pretreatment 2					
	i) 15 days after transplanting	21	0.05	128	0.08	NS
	ii) 30 days after transplanting	21	0.05	128	0.08	NS
(14)	Effect of different media and containers on the height of pineapple plantles					
a.	Pretreatment 1					
	i) 15 days after transplanting	21	2.86	128	1.55	0.05
	ii) 30 days after transplanting	21	2.58	128	1.58	NS
	iii) 45 day after transplanting	21	2.31	128	1.85	NS
b.	Pretreatment 2					
	i) 15 days after transplanting	21	2.43	128	2.30	NS
	ii) 30 days after	21	2.52	128	2.36	NS
(15)	Effect of different media and containers on the width of the largest leaf of pineapple plantlets					
a.	Pretreatment 1					
	i) 15 days after transplanting	21	1.56	128	0.91	0.05

Contd.

1	2	3	4	5	6	7
	ii) 30 days after transplanting	21	2.00	128	1.19	0.05
	iii) 45 days after transplanting	21	14.23	128	5.69	0.05
b.	Pretreatment 2					
	i) 15 days after transplanting	21	3.29	128	1.82	0.05
	ii) 30 days after transplanting	21	3.02	128	1.89	NS

ABSTRACT

Studies were conducted on *in vitro* multiplication and standardisation of hardening techniques in pineapple (*Ananas comosus* (L.) Merr.) at the Tissue Culture Laboratory of the All India Co-ordinated Floriculture Improvement Project attached to the Department of Pomology, College of Horticulture, Vellanikkara during 1991-'93.

Surface sterilization treatment was standardised for crown explants. Among the different treatments tried, treatment with Emisan 0.1 per cent for 30 minutes followed with mercuric chloride 0.1 per cent for 10 minutes was found to be the best. Explants collected in the months of January and February gave the least contamination and maximum survival percentage.

MS medium with BA 5.0 mg/l alone or in combination with NAA 1.0 mg/l gave maximum establishment of the explants. The globular structures were formed at maximum intensity within the shortest time of 5.96 days in MS medium supplemented with BA 5.0 mg/l and NAA 1.0 mg/l. Among the three cytokinins tried, the fastest response and the highest intensity of globular structures was obtained with BA followed by KIN and 2iP.

Maximum shoot proliferation (11.9 per culture) was obtained with basal MS medium in which cent per cent of the cultures developed vigorous dark green shoots.

Rooting of the *in vitro* derived shoots was obtained in *in vitro* as well as *ex vitro* conditions. Hundred per cent *in vitro* rooting was obtained in basal MS medium as well as in media supplemented with various concentrations of IBA and NAA. The fastest rooting (in 8.54 days) was obtained in the basal medium. Rooting was also faster in liquid medium compared to solid medium. In solid medium, early root initiation and the maximum length of roots were observed with 0.65 per cent agar concentration. Among the *ex vitro* rooting treatments tried, treatment with the rooting powder Rooton resulted in the fastest rooting and the maximum length of the roots. Profuse rooting of the shoots was obtained without using growth regulators by keeping them in a mist chamber.

Treatments were standardised for successful transfer of the plantlets to the outside environment. Hundred per cent survival of the plantlets was obtained by immersing the roots of the plantlets in sterile water for 18 hr prior to transplanting. Among the different containers tried, plantlets grown in plastic pots, in general showed maximum vigour with respect to the number of leaves, height and width of the largest leaf, followed by those in mud pots and poly bags. The maximum percentage increase in these parameters was observed for the plantlets in pro-trays. Potting mixes such as cocopeat, soilrite, biofibe and vermiculite were found to be better in inducing vigorous growth of the plantlets. Plantlets grown in plastic pots with cocopeat or plastic bags with soilrite mix, in general, grew more vigorously.

A nutrient starter solution of NPK fertilizer solution once a week or one fourth strength basal MS salts was found to be sufficient to induce healthy growth of the transplanted plantlets in the early stages of growth. To induce better growth of the plantlets in the later stages, application of the NPK fertilizer solution twice a week or Hoagland's solution once a week was found to be better.

Encapsulated beads were successfully formed with the differentiating globular bodies formed from the primary explants. The globular bodies could be encapsulated using 2.5 per cent sodium alginate and 75 mM calcium chloride with a complexation time of 30 minutes.

The plantlets after 90 days of growth in the greenhouse with a minimum height of 10 cm and 12 leaves were successfully transferred to soil.