MICROPROPAGATION IN SELECTED VARIETIES OF *Anthurium andreanum* Lind.

BY

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THESIS

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MASTER OF SCIENCE IN HORTICULTURE

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1996

DECLARATION

I hereby declare that this thesis entitled **"Micropropagation in selected varieties of** Anthurium andreanum **Lind."** 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 or other similar title, of any other University or Society.

Anita Thomas

Vellayani,
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CERTIFICATE

Certified that this thesis, entitled **"Micropropagation in selected varieties of** Anthurium andreanum Lind." is a record of research work done independently by Kum. **ANITA SUSAN THOMAS** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

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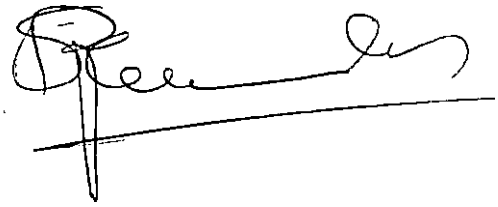
Dr. S. RAMACHANDRAN NAIR 

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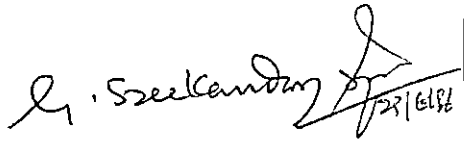
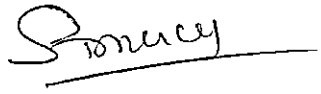
(1) Dr. G. SREEKANTAN NAIR 

(2) Dr. S. T. MERCY 

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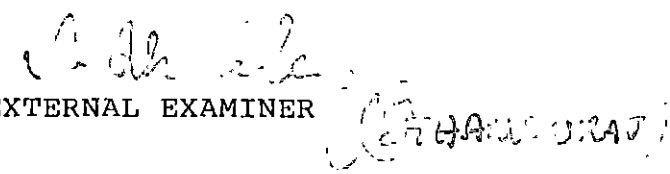
EXTERNAL EXAMINER 

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ANITA SUSAN THOMAS

CONTENTS

LIST OF TABLES

LIST OF PLATES

No.	Title
11	Shoot growth in medium supplemented with kinetin 0.5 mg/l + IAA 16.0 mg/l.
12	Rooted plantlets.
13	Planting out.

LIST OF ABBREVIATIONS

BA	-	Benzyl adenine
2ip	-	2 isopentenyl adenine
2,4-D	-	2,4-dichlorophenoxy acetic acid
NAA	-	Naphthalene acetic acid
IAA	-	Indole acetic acid
CH	-	Casein hydrolysate
CW	-	Coconut water
AC	-	Activated charcoal
MS	-	Murashige and Skoog (1962)
MMS	-	Modified Murashige and Skoog (1976)
SH	-	Schenk and Hildebrandt (1972)
LS	-	Linsmaier and Skoog (1965)
B5	-	Gamborg *et al*. (1968)

Introduction

1. INTRODUCTION

Recently, there has been an unprecedented awareness for flower cultivation in Kerala. Economic aspects of these ornamental plants are as important as the aesthetic ones. It serves as a primary occupation for different sectors of the society. Floriculture has self employment generating potential. Thus it helps to solve the unemployment problem to some extent.

Cut flowers constitute 45 per cent of the total world trade in floriculture products. India grows various types of subtropical and tropical flowers. However cut flower export from India is neglible. The market for tropical flowers in the global trade remains unsaturated. Cut flower export offers India a valuable avenue for earning foreign exchange.

The Government of India has identified Kerala as the Product Specific Intensive Floriculture Zone for anthurium and orchids. The Central Government has also announced the creation of nine model floriculture centres in nine states of the country. One such centre for anthurium and orchid is proposed to be set up at Trivandrum. Anthurium is an exotic floricultural plant, well suited for

large scale cultivation under the humid warm conditions prevailing in Kerala. Efforts have started for its widespread cultivation in both urban and rural areas of the state. In several centres in the state, voluntary agencies have now started sponsoring such programmes. The newly formed Federation of Indian Floriculturists is a pioneering effort in this direction.

Anthurium constitutes the largest genus of the family Araceae and includes about 600 species. The important species include Anthurium andreanum, A.scherzerianum, A. crystallinum and A. vetchii. They are valued both for their are colourful long lasting flowers and handsome foliage. Among the floriferous ones (cut flower types), A. andreanum Lind. is the most popular and economically important cultivated species.

Anthurium was unknown in the international cut flower market three decades ago. Now it occupies a top position in the global cut flower trade. There is also an increasing demand for cut flowers in the domestic market. Cut flowers are becoming a part of our culture and are used for decorating homes, business houses and hotels.

One of the main constraints in the large scale

cultivation of anthurium in Kerala is the insufficiency of quality planting materials. Anthuriums are conventionally propagated by seeds. They are naturally cross pollinated as plants are protogynous. Hence the seed propagated progeny are not true to type and are not uniform in their performance. Due to this problem, approximately one-third of the seedlings have to be discarded before flowering (Geier, 1990). The performance of seedling plants cannot be predicted. The other disadvantages of seed propagation are long period of seed maturity, short viability of seeds and long juvenile period. Vegetative propagation methods are available. They include division of plant suckers, topping and nodal stem cuttings. Since the number of propagules in these methods are few, the rate of multiplication is low. Hence it is insufficient to meet the large demand for planting material.

In this context, an *in vitro* propagation system with its high rate of multiplication becomes relevant. Methods of *in vitro* propagation through enhanced release of axillary buds (Kunisaki, 1980), somatic organogenesis (Pierik *et al.*, 1979 a, 1974 b; Pierik *et al.*, 1974; Kuehnle and Sugii, 1991) and somatic embryogenesis (Geier and Reuther, 1981; Kuehnle *et al.*, 1992; Rajasekaran and

Mohankumar, 1994) have been developed in anthurium. Somatic organogenesis has been found to be the most promising route for in vitro propagation of anthurium.

The in vitro response of many plants is highly dependant on the genotype. This effect is pronounced in the case of anthurium. Differences in morphogenetic potential of genotypes of anthurium have been observed by Pierik(1975), Leffring et al. (1976b) and Eapen and Rao (1985). Protocols have been evolved only for a few genotypes of A.andreanum

Variety is an important criterion in the cut flower market. To enter into the global flower trade, our varieties should be on par with the international standards. In the highly competitive market there is a demand for new hybrids and new varieties which remain in market only for a short period. An in vitro system of propagation can meet this demand in a short period.

The present studies were taken up for evolving the protocols for in vitro propagation in a few leading varieties of Anthurium andreanum namely, Dragon's Tongue, Flaking, Pompon Red, Honeymoon Red and Nitta.

Review of Literature

2. REVIEW OF LITERATURE

Clonal multiplication through conventional methods of asexual propagation is not rapid enough to meet the sharp increase in demand for tropical ornamental plants. So the use of tissue culture technique is widely adopted for mass multiplication of selected materials. In vitro propagation is possible via enhanced release of axillary buds, somatic organogenesis and somatic embryogenesis (Murashige, 1974). Morel (1960) was the pioneer in clonal multiplication through in vitro techniques. He was successful in cloning the orchid Cymbidium through meristem culture. Since then in vitro clonal multiplication of the ornamental plants has gained momentum.

Several studies have been conducted in the in vitro propagation of Anthurium andreanum and Anthurium scherzerianum. Plantlet regeneration has been obtained via callus from cultured embryos and other explants like leaf lamina, petiole, inflorescence stalk, spathe and spadix, or without intervening callus from embryo and axillary bud explants. Methods of in vitro propagation of anthurium through the three different methods have been studied by many workers authors. Of the three different routes,

anthuriums are most successfully propagated *via* somatic organogenesis. This review highlights the research on the various techniques of *in vitro* propagation of anthuriums with special emphasis on somatic organogenesis.

2.1 Enhanced release of axillary buds

Many herbaceous horticultural species have been successfully multiplied using this technique. The success is partially due to the weak apical dominance and strong root regenerating capacities of many herbaceous plants (Hu and Wang, 1983). In "axillary shoot proliferation", cytokinin is utilized to overcome the apical dominance of shoots and to enhance the branching of lateral buds from leaf axils. This enhanced release of axillary buds with cytokinins was discovered by Wickson and Thimann (1958).

Kunisaki (1980) obtained higher yields of viable cultures of *A. andreanum* with the use of small explants of vegetative buds. Explants were grown into plantlets in a modified Murashige and Skoog (MS) medium supplemented with 15 per cent coconut water. He also observed that the best result was obtained when stem sections (two nodal) from asepetically grown plantlets were cultured in medium containing benzyl adenine (BA) 0.2 mg/l. Higher BA

concentrations produced more callus growth and stunted shoots. However, the major difficulty encountered with this route of propagation was the non availability of sterile cultures of initial explants. The high rate of contamination necessitated the use of minute explants of vegetative buds, which resulted in greater mortality of the cultures.

Sreelatha (1992) used shoot tips from in vitro grown seedlings as explants for the enhanced release of axillary buds. The maximum number of shoots were observed in a medium containing one fourth strength of MS major nutrients and full strength of minor nutrients supplemented with kinetin 2.0 mg/l as well as BA 1.0 mg/l. Light was necessary for the enhancement of axillary buds.

2.2 Somatic embryogenesis

Somatic embryogenesis is the development of embryos from somatic cells (Mascarenhas, 1989). Direct embryogenesis proceeds from the pre-embryogenically determined cells, while indirect embryogenesis requires the re-determination of differentiated cells, callus proliferation and differentiation of embryogenic determined cells (Kato and Takeuchi, 1966). This mode of regeneration

occurs only sporadically in anthurium and the factors required for its consistent induction are yet to be defined.

Plantlet regeneration from spadix derived embryogenic callus was reported by Geier and Reuther (1981) and Geier (1982).

Kuehnle *et al*. (1992) used whole leaf blade derived from *A.andreanum* plantlets grown *in vitro* as explants for somatic embryogenesis. Translucent embryogenic calli were formed at the basal ends of the explant within one month of culture in dark. Secondary somatic embryos developed frequently without an intervening callus on the surfaces of primary embryos. Embryogenesis was induced in modified half MS supplemented with 2,4-dichlorophenoxyacetic acid (2,4-D) (1.0-4.0 mg/l) and Kinetin (0.33-1.0 mg/l). A combination of two per cent sucrose with one per cent glucose favoured embryogenesis.

Rajasekaran and Mohankumar (1994) could successfully induce somatic embryogenesis from leaf explants collected from the field using Nitsch & Nitsch (1965) as the basal medium.

2.3 Somatic organogenesis

Somatic organogenesis can be direct or callus mediated

(Evans et al., 1981) and is useful in inducing genetic variability or to recover pre-existing natural genetic variability. In direct somatic organogenesis, adventitious shoots arise directly from the tissues of the explant followed by root formation. Indirect somatic organogenesis requires the re-determination of the differentiated cells leading to callus formation. Seperate shoot and root initials are characteristically formed in callus cultures (George and Sherrington, 1984).

Pioneering studies were first conducted by Pierik et al. (1974 a,b). They succeeded in the induction of regeneration, first from embryo and seedling tissues and later from nonmeristematic parts of mature plants. A modified MS medium supplemented with cytokinin PBA [6-(benzylamino)-9-(2-tetrahydropyranyl)-9H-purine] was used. Optimum growth of the callus tissues was obtained at 25^{o}C in darkness.

Callus multiplication was observed best in a liquid medium (Pierik, 1975; Pierik et al., 1975). For this, leaf pieces with the callus were transferred to a liquid medium and placed on a shaker rotating at 120 rpm. Based on the detailed studies, a scheme was proposed for the micropropagation of A.andreanum and A.scherzerianum by

Pierik and Steegmans (1975), Pierik (1976) and Pierik *et al.*, (1979).

Pierik (1976) proposed a micropropagation scheme based on callus multiplication. Alternative schemes developed by Leffring and Soede (1978; 1979 a,b), makes use of shoot proliferation as a means of multiplication. In this process, leaf callus derived shoots were isolated and induced to proliferate.

2.3.1 Factors affecting somatic organogenesis

2.3.1.1 GENOTYPE

The growth of cultured tissues or organs, and *in vitro* morphogenesis, are probably more influenced by genotype than by any other factor. Even closely related varieties of plants are found to differ in their cultural requirements (George and Sherrington, 1984). The most critial factor in anthurium tissue culture is the genotype. (Geier, 1990).

Pierik *et al.* (1974 a,b) reported that only thirty per cent of the adult genotypes studied were capable of forming callus. Pierik *et al.* (1975) studied 38 genotypes of

A.andreanum. They observed moderate to strong callus formation from leaf segments in thirty one types, very poor callus in four types and no response in three genotypes.

According to Pierik (1975) there were striking differences in the growth rate among subcultured callus clones of A.andreanum. Leffring et al. (1976 a) and Leffring and Hoogstrate (1977) found a close correlation between the ability of leaf explants to form callus and the further growth in subcultures. They also reported that in most genotypes callus growth was too slow and inconsistent to be exploited for large scale micropropagation.

Regeneration ability of A.scherzerianum was also strongly dependent on the genotype. Pierik and Steegmans (1975, 1976) reported that three out of four genotypes responded positively. Geier (1986) studied eighteen genotypes and found that three genotypes did not show any regeneration, five formed callus only and ten produced caulogenic callus. In the last group, the average number of shoots per explant after twenty weeks of cultivation was more than ten in two genotypes, one to ten in three genotypes and less than one in five genotypes.

Kuehnle and Sugii (1991) and Nirmala and Singh

(1993) also reported that different genotypes showed varied response for *in vitro* propagation.

2.3.1.2 EXPLANT

George and Sherrington (1984) reported that the kind of explant chosen, its size, age and the manner in which it is cultured, can all affect the *in vitro* response. The use of immature tissues as explant material is an absolute requirement for successful induction of regeneration in anthurium. Substantial amounts of young and soft tissue are most freely obtained from young leaves although immature petioles, infloresence stalks, spathe and spadix can also be used as opined by Geier (1990).

Pierik *et al*. (1974a,b) found that all non meristematic parts of mature plants used as explants formed callus which spontaneously, or after subculturing gave rise to adventitious shoots.

Novak and Nepustil (1980) obtained *A.andreanum* callus clones with a high capacity for regeneration from leaf explants of flowering plants.

According to Geier (1982), spadix fragments of *A. scherzerianum* had a much higher capacity for regeneration

when compared to segments of leaf, petiole, infloresence stalk and spathe. However, these spadix-derived cultures showed relatively higher stability of floral determination during early stages of culture. Callus and shoot formation rarely occured in primary cultures. They were induced and promoted after repeated dissection and subculturing of the tissue.

Singh and Sangama (1993) reported that spadix explants had a better capacity for regeneration than leaf segments on the modified Nitsch medium. The plantlets derived from spadix explants were less variable.

Kuehnle and Sugii (1991), Sreekumar et al. (1992) and Singh (1994) found that both leaf and petiole explants had the same regeneration potential.

2.3.1.2.1 Leaf explant

According to Leffring (1976b) and Sreekumar et al. (1992), the morphogenetic response of the leaf lamina segment was accelerated if it contained parts of the midrib and major veins. However, Nirmala and Singh (1993) obtained the same response for leaf sections with and without midrib. They also found that leaf sections were the best explants

for multiplication.

Leffring et al. (1976b) studied the response from different portions of the leaf. They found that tissue from the apical half of the leaf produced callus better than that from the basal half. Sreelatha (1992) reported that the basal portions of the leaf responded more than the distal portions. However, Sreekumar et al. (1992) obtained the same response from both the basal and distal portions of the leaf explant.

The optimal size of the leaf used as explant is half to two-thirds of the final length (Geier, 1990). Sreelatha (1992) obtained good results when leaves 3-4 days after unfurling were used as explants. Satheeshkumar and Seeni (1994) found that three week old and fully expanded leaf gave optimum response.

2.3.1.3 SURFACE STERILIZATION

Contamination of cultures due to micro organisms, particularly moulds and bacteria is one of the major problems to be tackled during the intial stages of micropropagation. Sodium hypochlorite or common household bleach is largely used for this purpose (Hu and Wang, 1983).

However, in humid tropical countries, where there is the presence of microbial spores in the enviornment throughout the year, milder sterilant such as sodium hypochlorite will not always be effective. In many crops, mercuric chloride is reported to be an effective surface sterilant.

The blight pathogen Xanthomonas campestris pv. dieffenbachiae survived in or on callus derived from A. andreanum for over four months without producing symptoms or turbidity in the medium. The pathogen survived for more than one year on shoots without producing symptoms and was successively transferred three times as latently infected shoots were multiplied.

Organs of adult plants were sterilized with 96 per cent alcohol for few seconds followed by 30 minutes in one per cent sodium hypochlorite with a few drops Tween 20 (Pierik et al. (1974a). According to Pierik et al. (1979) the number of adventitious sprouts was increased when Tween 20 was added.

The spadix explants were surface sterilized with spathe still unopened by disinfecting in 70 per cent ethanol followed by three per cent sodium hypochlorite (10-20 minutes). After removal of the spathe, the spadix was again

disinfected in the same manner (Geier, 1982).

Sreekumar et al. (1992) and Satheeshkumar and Seeni (1994) cleaned the leaf and petiole explants with one per cent 'Labolene', then disinfected them by immersion in 70 per cent ethanol for 30 seconds and five per cent sodium hypochlorite solution for 15-20 minutes, and further treated with 0.5 per cent mercuric chloride for four minutes.

According to Lightbourn and Prasad (1992), the leaves were soaked in 32.5 per cent Benlate (benomyl) solution prior to sterilizaion with sodium hypochlorite.

2.3.1.4 BASAL MEDIUM

In most studies of in vitro culture of anthurium, basic media derived from the MS formula have been used (Pierik et al., 1975). Pierik (1976) introduced a sequence of MS modifications with changing concentrations of individual salts for the successive stages of callus induction, multiplication and plantlet regeneration.

According to Kuehnle and Sugii (1991) leaf explants produced callus most successfully on a modified Pierik medium whereas petiole explants produced callus on Pierik, modified Pierik and van Staden media.

Among the various nutrient formulations tried, Nitsch and Nitsch (1969) medium was the most effective to induce caulogenic callus formation (Sreekumar et al., 1992). However Sreelatha (1992) found modified MS medium (Pierik, 1976) as the most effective medium.

Nirmala and Singh (1993) found that callusing of leaf sections was best observed on Nitsch medium whereas callusing in spadix segments was induced by repeated subculturing in MS medium and vegetative buds callused on liquid Vacin and Went medium.

Full strength MS medium had the best inductive effect on bud formation while half strength MS medium was best for root formation.

2.3.1.5 PLANT GROWTH SUBSTANCES

Growth regulator concentrations in the culture medium are critical to the control of growth and morphogenesis (Skoog and Miller, 1957). Generally, a high concentration of auxin and a low concentration of cytokinin in the medium provide abundant cell proliferation with the formation of callus, whereas low auxin and high cytokinin concentration in the medium result in the induction of shoot

morphogenesis. Auxin, alone or in combination with a very low concentration of cytokinin is important in the induction of root primordia.

2.3.1.5.1. **Callus induction**

Pierik *et al.* (1974 a,b) found that cytokinin was essential for callus induction from sections of leaf lamina, petiole, spathe and infloresence stalk and he employed PBA as the cytokinin. Supplementing the media with a low level of auxins in addition to cytokinin was reported to be suitable for callus formation and further growth and regeneration (Pierik *et al.*, 1975; Pierik *et al.*, 1979; Hauzinska, 1976; Finnie and van Staden, 1986).

Pierik *et al.* (1979) compared four cytokinins in various concentrations and showed that optimal callus and sprout formation occured when zeatin was added at 1 mg/l.

Geier (1986) found BA 1 mg/l + 2,4-D 0.1 mg/l, whereas Sreelatha (1992) found BA 1 mg/l + 2,4-D 0.08 mg/l as the most efficient in supporting callus development

Caulogenic callus can be obtained by using the cytokinin BA alone at 1.5 - 2.0 mg/l concentration, whereas high concentrations induced extensive proliferation of

relatively non-caulogenic callus. (Sreekumar et al., 1992). According to them, calli obtained in media supplemented with Naphthaleneacetic acid (NAA) and combinations of BA and NAA and BA and 2,4-D rarely regenerated shoots in the next passage.

.3.1.5.2. Shoot regeneration

Shoot proliferation is generally achieved by subculturing under low illumination on media supplemented with cytokinins (Geier, 1990).

Leffring and Soede (1979 b) reported that kinetin 0.2 mg/l produced optimum branching whereas BA and 2ip caused less branching but at the same time produced more callus. This was confirmed by Kunisaki (1980) who therefore recommended a low level of 0.2 mg/l BA to induce shoot proliferation without concomitant callus development.

Geier (1985) reported that of different cytokinins, BA at 0.2 or 0.5 mg/l most effectively stimulated shoot production resulting in about 30 fold multiplication within 15 weeks. According to Lightbourn and Prasad (1992) good shoot multiplication (3-5 shoots/callus) was obtained with BA 0.2-0.8 mg/l in all the

four cultivars studied.

Sreelatha (1992) reported that shoot regeneration and growth of the shoots were best in MS medium with BA 0.5 mg/l and IAA 2.0 mg/l.

The callus was subcultured to a hormone-free medium for shoot differentiation (Sreekumar et al., 1992). Cen et al. (1995) proved that BA 1 mg/l had the best inductive effect on bud formation.

2.3.1.5.3 Rooting

According to Geier (1990) rooting of shoots may occur spontaneously when cultures are allowed to stand for long periods under illumination. More rapid and consistent rooting is achieved however by isolating shoots and transferring them to a special rooting medium. With Anthurium andreanum, modified MS medium devoid of hormones was mostly used for this purpose. Although not necessary, auxins have been included in low concentrations in the medium in order to enhance rooting (Pierik and Steegmans, 1975, 1976; Fersing and Lutz, 1977).

Sreekumar et al. (1992) and Singh (1994) showed that hormones were not required for rooting. According to

Cen et al. (1995) half strength MS medium supplemented with 0.1 ppm NAA was best for inducing root formation.

2.3.1.6. CARBON SOURCE

Pierik et al. (1974b) and Perik et al. (1979) employed glucose two to four per cent as the carbon source.

However Sreelatha (1992) found that sucrose three per cent had better effect than glucose three per cent. A sucrose level of two per cent was used by Sreekumar et al. (1992), Rajasekaran and Mohankumar (1994) and Satheeshkumar and Seeni (1994).

Cen et al. (1995) compared the effects of different levels of sucrose and glucose and found that three per cent glucose had the greatest inductive effect on callus formation.

2.3.1.7. AMMONIUM NITRATE.

The importance of lowering the ammonium nitrate level was first demonstrated by Pierik et al. (1975) and Pierik (1976). Later Pierik et al (1979) showed that this effect is caused by the ammonium ion and not by the nitrate ion.

Of the various media factors tested, the NH_4NO_3 level had the most significant effect on callus and shoot formation (Geier, 1986).

Zens and Zimmer (1988) found that shoot productivity decreased with increasing NH_4NO_3. A low level of NH_4NO_3 (825 mg/l) was found optimum for callusing whereas a higher level of 1650 mg/l was optimum for further regeneration (Sreelatha, 1992).

According to Lightbourn and Prasad (1992) root formation was not affected by varying concentrations of ammonium nitrate. However larger leaves and more prolific leaf production occured with increased ammonium nitrate level.

Nirmala and Singh (1993) and Singh (1994) reported that a lower concentration of NH_4NO_3 (200 mg/l) in the medium was essential for callusing.

Satheeshkumar et al. (1994) reported that ammonium free medium was optimum.

2.3.1.8 CULTURE CONDITIONS

Most workers observed optimum callus formation and

subsequent growth in continuous darkness at temperature around 25°C. However, Finnie and van Staden (1986) and Cen et al. (1995) showed that light was superior to darkness for induction and growth of callus.

The formation and development of adventitious sprouts was enhanced by transferring the callus cultures from darkness to light (Pierik and Steegmans, 1976). According to Pierik et al. (1979) combinations of 16 weeks darkness + 4 weeks light, 12+8 or 8+12 are the most optimal dark-light regimes for regeneration. Sreekumar et al. (1992) and Singh (1994) achieved callus induction and subculturing in total darkness at 25°c while regeneration and shoot formation was best done under light.

2.4 CYTOLOGICAL STUDIES

Numerical or structural changes in chromosomes are reported to be associated with in vitro regeneration of plants (Larkin and Scowcroft, 1981). In Anthurium scherzerianum, Geier (1985,1988) observed a very small precentage of tetraploid variants in plants regenerated from spadix and leaf segments. However, the extent of variability was less than that usually observed in seed propagated cultivars.

Cytological examinations of the root tip squashes made on random number of plantlets at planting out, showed a normal diploid chromosome count (Sreelatha, 1992).

2.5 Planting out and acclimatization

According to Ajith kumar (1993) in vitro plantlets of anthurium with at least 2.5-3cm length (with 3-4 leaves and two or more roots) had the highest survival rate (up to 100 per cent) irrespective of media and containers. Larger plants performed better than smaller ones.

Acclimatization is necessary in the case of micropropagated plants because in vitro plant material is not adapted for ex vitro conditions (Brainerd and Fuchigami, 1981). The success in acclimatization of micropropagated plants is largely dependant upon not only the post-transfer growth conditions, but also upon the pre transfer culture conditions (Ziv,1986). Tissue cultured plants are very poorly adapted to resist the low relative humidity, higher light levels and more variable temperatures prevailing outside (Wainwright, 1988).

Light, temperature and relative humidity are the three major factors to be controlled during acclimatization. Hu and Wang (1983) suggested a period of humidity acctimatization for the newly transferred plants.

Materials and Methods

3. MATERIALS AND METHODS

Studies were carried out for standardising the micropropagation of selected varieties of Anthurium andreanum at the Plant Tissue Culture Laboratory, Department of Horticulture, College of Agriculture, Vellayani during 1994 - 1995.

The materials and methods tried for the micropropagation via indirect somatic organogenesis are described in this chapter.

3.1 Varieties

Five anthurium varieties (Dragon's Tongue, Flaking, Pompon Red, Honeymoon Red and Nitta) were used for the study.

3.2 Explants

Explants of anthurium were taken from actively growing adult plants.

Segments of tender leaves, petiole and immature spadix were the explants used for culture.

3.3 Collection and preparation of explants

Tender leaves (light coppery brown in colour), 2-3 days after unfurling were selected and the leaves were excised along with their petioles. The petioles were immediately immersed in water in a 250 ml conical flask. Culturing was done within 2-6 hours after excision. The leaves were first washed gently in running tap water. The petioles were then removed and the leaves washed in distilled water containing a few drops of the wetting agent 'Labolene'. They were further washed two to three times with distilled water to remove the traces of 'Labolene'.

The distal portion of the petiole and immature spadix with spathe still unopened were also used as explants. These explants were also collected and prepared as in the case of tender leaves.

During the monsoon season a high rate of microbial contamination was observed. To overcome this the washed explants were treated with the systemic fungicide Bavistin (0.1 per cent) for one hour prior to surface sterilization.

3.4 Surface sterilization

Surface sterilization of the explants material was

carried out inside a laminar air flow chamber. The leaves were wiped with cotton wool dipped in 70.0 per cent ethyl alcohol. The leaf margins were cut off with a sharp sterile blade. They were then cut into smaller pieces of 5cm square and surface sterilized using sodium hypochlorite (1.0 per cent) for 15 minutes in sterile 500 ml conical flasks with constant shaking. The explants after surface sterilization were rinsed 4 to 5 times with sterile double glass distilled water. The petiole and spadix explants were sterilized in the same manner. The immature spadix along with the unopened spathe was first surface sterilized and after removal of the spathe, the spadix was again sterilized in the same manner.

The variety Dragon's Tongue was used for further refinement of the surface sterilization techniques using sterilants as per Table 1. All the treatments were replicated twelve times.

3.5 Inoculation and incubation

All the inoculation operations were carried out in a laminar air flow chamber.

The tools (blades, forceps etc) and glasswares

Table 1. Surface sterilization treatments tested on *A. andreanum* explants var: Dragon's Tongue

Sterilant	Concentration (per cent)	Time of treatment (minutes)	Explant	Code No.
mercuric chloride	0.1	5	leaf	S_1
mercuric chloride	0.1	6	leaf	S_2
mercuric chloride	0.1	7	leaf	S_3
mercuric chloride	0.1	8	leaf	S_4
mercuric chloride	0.1	9	leaf	S_5
sodium hypochlorite	1.0	15	leaf	S_6
sodium hypochlorite	1.0	16	leaf	S_7
sodium hypochlorite	1.0	17	leaf	S_8
sodium hypochlorite	1.0	20	leaf	S_9
mercuric chloride	0.1	10	petiole	S_{10}
mercuric chloride	0.1	15	spadix	S_{11}

required for inoculation were washed thoroughly, rinsed with double glass distilled water, covered with aluminium foil and autoclaved at 121°C and 1.06 kg/cm^2 pressure for 40 minutes.

The leaf explants after surface sterilization were given a fresh cut along the margins to remove the portions which had become brown due to sterilization. Such pieces were further cut into square pieces of 1 cm^2. Pieces with and without midrib or major veins were used for inoculation. Similarly, the two ends of the spadix and petiole were given a fresh cut. They were further cut into segments of 0.8 mm and 1 cm length, respectively. The explants were finally dried on a sterilized blotting paper placed over a sterile petri dish.

The explants after preparation were used for inoculation. The cotton plugs of the test tubes and flasks were removed for a while and the mouth was flamed. The leaf, spadix and petiole explants were then inoculated into the medium and the mouth of the culture vessels were again closed with plugs after flaming. The inoculated culture vessels were then kept either in light or darkness at $26 \pm 1^{\circ}$ c.

3.6 Media

The basal media used for the study were MS (Murashige and Skoog, 1962), modified MS [MMS] (Pierik,1976), B5 (Gamborg et al., 1968), Nitsch's (Nitsch and Nitsch,1969), LS (Linsmaier and Skoog, 1965) and SH (Schenk and Hildebrandt, 1972). The chemicals used for the preparation of the culture media were of analytical grade from the British Drug House (BDH, Bombay), Sisco Research Laboratory (SRL, Bombay), Merck (Bombay) and Sigma (U.S.A).

Standard procedures were followed for the preparation of media (Thorpe, 1980). Stock solutions of major and minor nutrients, vitamins and amino acids were prepared by dissolving the required quantity of chemicals in specific volume of double glass distilled water, whereas plant growth substances were initially dissolved in dilute acid/alcohol and further volume made up with double glass distilled water. The stock solutions were stored under refrigerated conditions. The stock solutions of plant growth substances were stored only for a period of one week whereas the other stock solutions were maintained in refrigerated conditions upto one month.

All glasswares used for the preparation of the

media were washed with water containing a few drops of 'Labolene' and rinsed with double glass distilled water. Specific quantities of stock solutions were pipetted into a 1000 ml beaker. Sucrose (30.0 g/l) and myo-inositol were added fresh and dissoloved. For specific treatment requirements glucose, casein hydrolysate (CH) and glutamine were added fresh. Coconut water (CW) when used, was collected from freshly harvested coconut (approximately eight months old). The volume was made up to 950 ml using double glass distilled water. With an electronic pH meter (Philips make, model PP 9046) the pH value of the solution was adjusted between 5.7 and 5.8 using 0.1N NaOH or 0.1N HCl. Agar (0.6 g/l) as well as activated charcoal [AC] (in specific treatments) were then added to the medium and the final volume was made upto 1000 ml.

The medium was then heated by placing the beaker on a heating mantle and stirred thoroughly for uniform mixing till the agar dissolved. The medium was then poured into pre-sterilized culture vessels which were rinsed with double glass distilled water. Corning brand test tubes (25 x 150 mm) and Erlenmeyer flasks (100 ml, 150 ml, 250 ml) were used as culture vessels. The test tubes and Erlenmeyer flasks were filled with 15.0 ml, 30.0 ml, 45.0 ml and

60.0 ml of the medium respectively. The culture vessels containing the medium were plugged tightly with cotton. They were then autoclaved at 121°C and 1.06 kg/cm^2 pressure for 20 minutes and after cooling, were used for inoculation as and when needed.

3.7 Somatic Organogenesis

3.7.1 The five varieties, namely, Dragon's Tongue, Honeymoon Red, Flaking, Pompon Red and Nitta were used for the initial study. The leaf explants of these five varieties were subjected to nine treatments as shown in Table 2. The basal medium used was MMS (modified MS) with quarter strength of ammonium nitrate (200 mg/l) as reported by Nirmala and Singh (1993) and Singh (1994). The treatments were replicated thirty times. The influence of these treatments on callus initiation was studied. The number of cultures initiating callus in the five varieties in the different treatments was recorded.

The calli obtained in all the five varieties were subcultured twice in the same medium for a period of two months for callus multiplication. They were then transferred to the regeneration medium developed by Sreelatha (1992) consisting of MS + BA 0.5 mg/l + Indole acetic acid (IAA)

Table 2 . Treatments tested on callus initiation in five varieties of A. andreanum

Basal medium: MMS* Explant: leaf

Treatment (mg/l)	Code No.
BA 0.5 + 2,4-D 0.06	T_1
BA 0.5 + 2,4-D 0.5	T_2
BA 1.0 + 2,4-D 0.06	T_3
BA 1.0 + 2,4-D 0.08	T_4
BA 1.0 + 2,4-D 0.5	T_5
BA 1.5 + 2,4-D 0.06	T_6
BA 1.5 + 2,4-D 0.5	T_7
BA 0.1 + Kinetin 1.0 + 2,4-D 0.1	T_8
BA 1.0 + NAA 1.0 + 2,4-D 1.0	T_9

MMS* - modified MS basal medium with quarter strength ammonium nitrate.

2.0mg/l + sucrose 30.0 g/l + agar 6.0 g/l.

After initial screening, Dragon's Tongue (Plate 1), the variety which showed regenerative potential was further subjected to different treatments for refinement of callus initation and shoot proliferation.

3.7.2 Callus initiation

The explants were subjected to different treatments for callus initiation. Each treatment was replicated 30 times (1 replication = 1 culture vessel).

3.7.2.1 Basal Media

Different basal media like MS, MMS, Nitsch's, SH, LS and B5 were tried to find their influence on callus initiation. The explant sources used were leaf (Plate 2a), petiole (Plate 2b) and spadix (Plate 2c).

Observations on the number of cultures initiating callus were recorded.

3.7.2.2 Ammonium nitrate

The influence of different levels of ammonium nitrate in the culture media on the initiation of callus was studied.

Observations were recorded on the number of

Plate 1

Anthurium andreanum var: Dragon's Tongue.

Plate 2a

Leaf explant cultured in callus initiation medium.

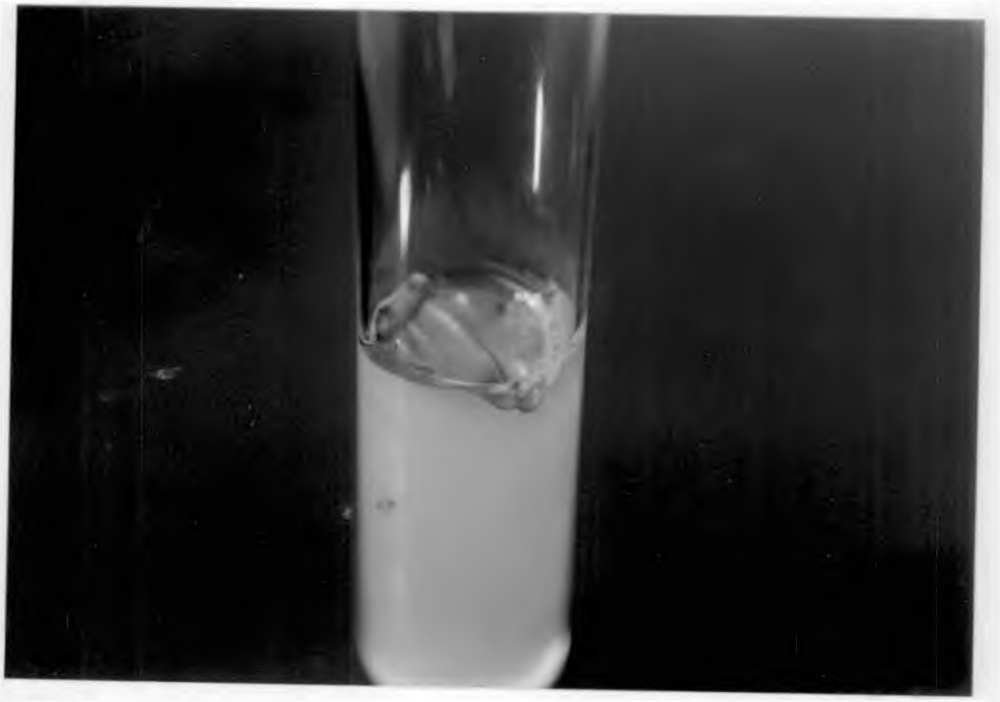
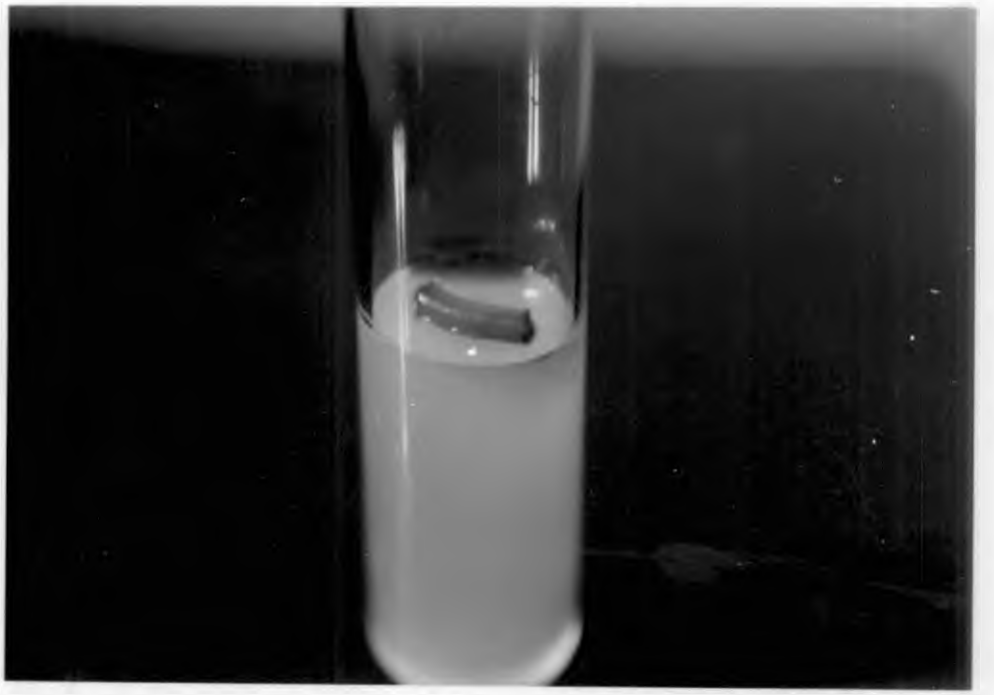


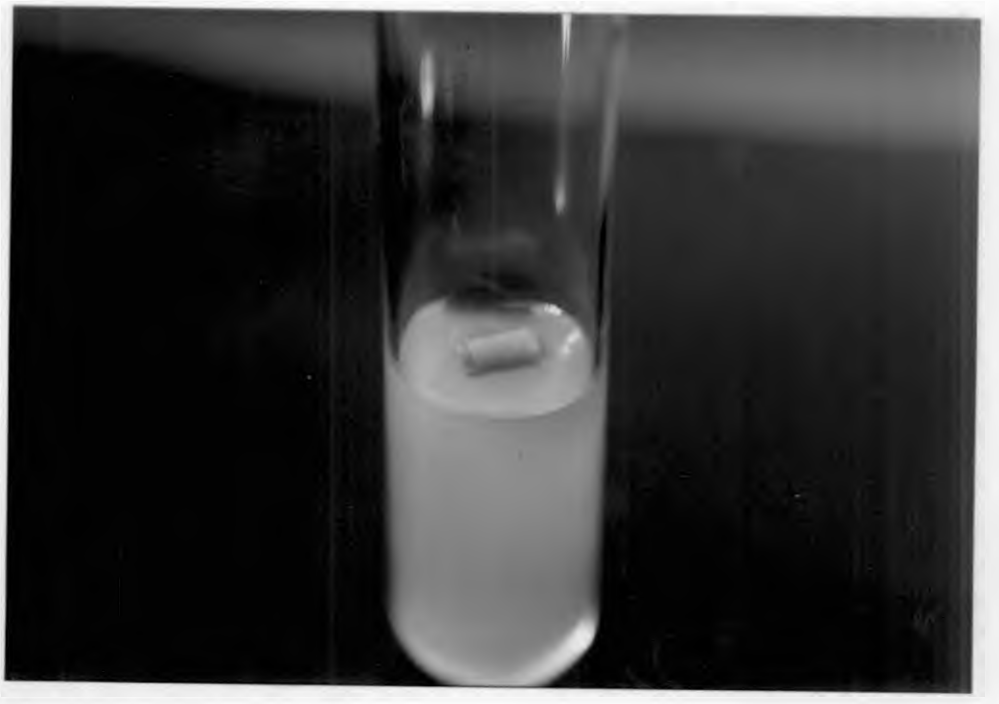
Plate 2b

Petiole explant cultured in callus initiation medium.

Plate 2c

Spadix explant cultured in callus initiation medium.





cultures initiating callus

3.7.2.3 Plant growth substances

Studies were conducted to find out the effect of different combinations of plant growth substances. The basal medium used was MMS with quarter strength of ammonium nitrate. The treatments involved combinations of BA (0.1 - 1.5 mg/l), kinetin (1.0 mg/l), 2,4-D (0.06-0.5 mg/l) and NAA (1.0 mg/l) as shown in Table 3.

The number of cultures initiating callus in these treatments was recorded

The effect of different plant growth substances supplementing the Nitsch's basal medium (NH_4NO_3 200 mg/l) was also studied. Different combinations of BA (0.5-1.0 mg/l), kinetin (0.5 mg/l) and 2,4-D (0.5 mg/l) were tried (Table 4).

The influence of these treatments on the number of cultures initiating callus was noted,

3.7.2.4 Carbon Source

The effect of replacing sucrose (30.0 g/l) by

Table 3. Plant growth substances incorporated into MMS medium for callus initiation

Basal medium : MMS* Explant : Leaf

Plant growth substances (mg/l)	Code No
BA 0.5 + 2,4-D 0.06	CP_1
BA 0.5 + 2,4-D 0.08	CP_2
BA 0.5 + 2,4-D 0.10	CP_3
BA 0.5 + 2,4-D 0.15	CP_4
BA 0.5 + 2,4-D 0.20	CP_5
BA 0.5 + 2,4-D 0.50	CP_6
BA 1.0 + 2,4-D 0.06	CP_7
BA 1.0 + 2,4-D 0.08	CP_8
BA 1.0 + 2,4-D 0.10	CP_9
BA 1.0 + 2,4-D 0.15	CP_{10}
BA 1.0 + 2,4-D 0.20	CP_{11}
BA 1.0 + 2,4-D 0.50	CP_{12}
BA 1.5 + 2,4-D 0.06	CP_{13}
BA 1.5 + 2,4-D 0.08	CP_{14}
BA 1.5 + 2,4-D 0.10	CP_{15}
BA 1.5 + 2,4-D 0.15	CP_{16}
BA 1.5 + 2,4-D 0.20	CP_{17}
BA 1.5 + 2,4-D 0.50	CP_{18}
BA 0.1 + Kinetin 1.0 + 2,4-D 0.1	CP_{19}
BA 1.0 + NAA 1.0 + 2,4-D 0.05	CP_{20}

*MMS - modified MS basal medium with quarter strength of ammonium nitrate

Table 4: Plant growth substances incorporated into the Nitsch's medium for callus initiation

Basal medium : Nitsch's* Explant : Leaf

Plant growth substances (mg/l)	Code No
BA 1.0 + 2,4-D 0.08	CP_{21}
Kinetin 0.5 + 2,4-D 0.5	CP_{22}
BA 0.5	CP_{23}
BA 0.7	CP_{24}
BA 0.9	CP_{25}
BA 1.1	CP_{26}
BA 1.5	CP_{27}

Nitsch's* - Nitsch's basal medium with ammonium nitrate 200 mg/l

glucose (30.0 g/l) was tested.

The number of cultures initiating callus was recorded.

3.7.2.5 Culture conditions

Studies were conducted to find out the effect of light on the initiation of callus. The control treatment (darkness) was provided either by placing the cultures in culture racks covered by black polythene sheets or by covering the culture vessels with aluminium foil. Light (photoperiod :16 h, intensity : 3000 lux) was provided by cool white flourescent tubes. The light intensity was measured using a luxmeter.

Observations were recorded on the number of cultures initiating callus.

3.7.3 Regeneration of shoots and roots

The cultures after callus multiplication were transferred to the regeneration medium developed by Sreelatha (1992). MS medium containing BA 0.5 mg/l, IAA 2.0 mg/l, sucrose 30.0 g/l and agar 6.0 g/l was used. The cultures were provided with a light intensity of 3000 lux

and a photoperiod of 16 hours.

3.7.4 Shoot proliferation

The regenerated shoots were subjected to various treatments for refinement of shoot proliferation. For this single shoots were subcultured to the different treatments. The treatments were replicated 3 times (1 replication = 1 culture vessel). The length of shoot and width of leaf were measured at the time of subculture using a sterile steel scale.

3.7.4.1 Basal media

The two different basal media ie, MS and Nitsch's were used to compare their effect on shoot proliferation.

The number of shoots, length of the longest shoot and width of the largest leaf in the two basal media were recorded after six weeks of culture.

3.7.4.2 Ammonium Nitrate

The cultures were transferred to varying levels of ammonium nitrate (200-1650 mg/l) to study the effect of ammonium nitrate concentration on shoot proliferation.

The influence of ammonium nitrate concentration on

the number of shoots, length of the longest shoot and width of the largest leaf was recorded six weeks after culture.

3.7.4.3 Organics

The organics in MS basal medium were incorporated (Thiamine. HCl, Pyridoxine. HCl, Nicotinic acid, Glycine) at quarter, half and full strength to study their effect on shoot proliferation.

Observations were made after six weeks on the number of shoots, length of the longest shoot and width of the largest leaf.

3.7.4.4 Carbon Source

A comparison of the effect of two different carbon sources on shoot proliferation was made by transferring the cultures to media containing either glucose or sucrose at 20, 30, 40 and 50 g/l (Table 5).

Observations were made after six weeks on the number of shoots, length of the longest shoot and width of the largest leaf.

3.7.4.5 Other supplements

Different media supplements like casein hydrolysate

Table 5. Carbon sources tested on shoot proliferation.

Culture Medium: MS + BA 0.5 mg/l + IAA 2.0 mg/l + agar 6.0 g/l.

Carbon source (g/l)	Code No.
Sucrose 10.0	RC_1
Sucrose 20.0	RC_2
Sucrose 30.0	RC_3
Sucrose 40.0	RC_4
Sucrose 50.0	RC_5
Glucose 10.0	RC_6
Glucose 20.0	RC_7
Glucose 30.0	RC_8
Glucose 40.0	RC_9
Glucose 50.0	RC_{10}

(CH) (50-150 mg/l), glutamine (50-150 mg/l) and coconut water (CW) (100-250 ml/l) were tried to study the effect of various supplements at different concentrations on shoot proliferation shown in Table 6.

The effect of these treatments on the number of shoots, length of the longest shoot, width of the largest leaf was recorded six weeks after culture.

3.7.4.6 Plant growth substances

The effect of plant growth substances like BA (0.5 - 1.5 mg/l), IAA (2.0 - 4.0 mg/l), Kinetin (0.5 - 1.5 mg/l) NAA (2.0 - 4.0 mg/l) was studied by incorporating them in various combinations in the shoot proliferation media as shown in Table 7 (i).

3.7.4.7 Activated Charcoal

Some of the cultures were transferred to a hormone free MS media supplemented with activated charcoal (AC) at 1.0 gm/l. Observations were made on the increase in the number of shoots and number of leaves six weeks after culture.

After one month, the above cultures were subcultured to treatment combinations of kinetin and IAA as shown in Table 7 (ii).

Table 6. Different media supplements tested on shoot proliferation

Culture medium : MS + BA 0.5 mg/l + IAA 2.0 mg/l + sucrose 30.0g/l + agar 6.0 g/l

Treatment	Code No.
CH 50 mg/l	RS_1
CH 100 mg/l	RS_2
CH 150 mg/l	RS_3
Glut 50 mg/l	RS_4
Glut 100 mg/l	RS_5
Glut 150 mg/l	RS_6
CW 100 ml/l	RS_7
CW 150 ml/l	RS_8
CW 200 ml/l	RS_9
CW 250 ml/l	RS_{10}

Table 7(i): Plant growth substances tested on shoot proliferation

Basal medium : MS

Plant growth substances (mg/l)	Code No
BA 0.5 + IAA 2.0	RP_1
BA 0.5 + IAA 3.0	RP_2
BA 0.5 + IAA 4.0	RP_3
BA 1.0 + IAA 2.0	RP_4
BA 1.0 + IAA 3.0	RP_5
BA 1.0 + IAA 4.0	RP_6
BA 1.5 + IAA 2.0	RP_7
BA 1.5 + IAA 3.0	RP_8
BA 1.5 + IAA 4.0	RP_9
Kinetin 0.5 + IAA 2.0	RP_{10}
Kinetin 0.5 + IAA 3.0	RP_{11}
Kinetin 0.5 + IAA 4.0	RP_{12}
Kinetin 1.0 + IAA 2.0	RP_{13}
Kinetin 1.0 + IAA 3.0	RP_{14}
Kinetin 1.0 + IAA 4.0	RP_{15}
Kinetin 1.5 + IAA 2.0	RP_{16}
Kinetin 1.5 + IAA 3.0	RP_{17}
Kinetin 1.5 + IAA 4.0	RP_{18}
BA 0.5 + NAA 2.0	RP_{19}
BA 0.5 + NAA 4.0	RP_{20}
BA 1.5 + NAA 2.0	RP_{21}
BA 1.5 + NAA 4.0	RP_{22}

Table 7 (ii) Plant growth substances tested on shoot proliferation from regenerated shoots precultured on medium containing AC

Basal Medium: MS

Plant growth substances (mg/l)	Code No.
Kinetin 0.5 + IAA 4.0	RP_{23}
Kinetin 0.5 + IAA 8.0	RP_{24}
Kinetin 0.5 + IAA 16.0	RP_{25}
Kinetin 1.0 + IAA 4.0	RP_{26}
Kinetin 1.0 + IAA 8.0	RP_{27}
Kinetin 1.0 + IAA 16.0	RP_{28}
Kinetin 0.0 + IAA 8.0	RP_{29}

Table 8. Gelling agents tested on shoot proliferation.

Basal medium : MS

Treatment(g/l)	Code No.
agar 4.0	RG_1
agar 5.0	RG_2
agar 6.0	RG_3
agar 7.0	RG_4
agar 8.0	RG_5
agarose 6.0	RG_6
agarose 7.0	RG_7

The number of shoots, length of the longest shoot and width of the largest leaf were observed six weeks after culture.

3.7.4.7. Gelling agents

Two gelling agents, (agar and agarose) at various concentrations were used to study their effect on shoot proliferation (Table 8).

Observations were made after six weeks on the number of shoots, length of the longest shoot and width of the largest leaf.

3.7.5 Planting out and hardening

Rooted plantlets as well as shoots without any roots were carefully removed from the containers and washed thoroughly to remove the adhering agar. The plantlets were then treated with a systemic fungicide Bavistin (2.0 g/l). They were further transferred to sterilized sand medium (moisture: 19 per cent) in plastic pots.

The potted plants were kept in plastic basins and covered with a polythene sheet to maintain high humidity. For lowering the humidity during the later period of

acclimatization, the polythene sheet was gradually uncovered.

Observations were made on the number of plantlets survived after 30 days of planting out.

Results

4. RESULT

Investigations were carried out for developing protocols for *in vitro* propagation of selected varieties of *Anthurium andreanum* suitable for commercial cultivation. The varieties used for the study were Dragon's Tongue, Flaking, Pompon Red, Honeymoon Red and Nitta. The results obtained are presented in this chapter.

4.1 Surface sterilization

Surface sterilization with mercuric chloride 0.1 per cent for eight minutes (S_4) was found to be very effective for surface sterilization of the leaf explants (Table 9) as 88.8 per cent of sterile cultures were obtained by this treatment. Surface sterilization with sodium hypochlorite 1.0 per cent for 20 minutes (S_9) gave 100 per cent sterile cultures. But this treatment caused browning of the tissues and further response was prevented. Sodium hypochlorite in all the treatments caused more browning of tissues compared to mercuric chloride treatments. Hence mercuric chloride (0.1 per cent) for eight minutes was selected for surface sterilization in all further studies. The treatment S_{10} (mercuric chloride 0.1 percent

Table 9. Effect of surface sterilization treatments on microbial contamination in explants of A.andreanum var: Dragon's Tongue.

Treatment*	Explant	Cultures free from microbial contamination (%)
S_1	Leaf	83.30
S_2	Leaf	84.80
S_3	Leaf	86.00
S_4	Leaf	88.80
S_5	Leaf	88.80
S_6	Leaf	41.67
S_7	Leaf	66.67
S_8	Leaf	83.30
S_9	Leaf	100.00
S_{10}	Petiole	50.00
S_{11}	Spadix	30.00

*Composition of treatments given in Table 1.

The data represents the mean of 12 replications.

for ten minutes) gave 50 per cent sterile cultures of the petiole, whereas S_{11} (mercuric chloride 0.1 per cent for 15 minutes) gave only 30 per cent sterile spadix cultures.

4.2 Somatic organogenesis

4.2.1 Preliminary investigations were carried out using the five A.andreanum varieties, namely, Dragon's Tongue, Flaking, Pompon Red, Honeymoon Red and Nitta. The influence of nine different treatments on the initiation of callus in these varieties was studied.

Callusing was obtained in all the five varieties as shown in Table 10. The biggest size callus was obtained in the variety Dragon's Tongue (Plate 3a). The maximum number of cultures initiating callus in this variety was obtained in the treatment T_2. This treatment (MMS with quarter strength ammonium nitrate [200 mg/l] supplemented with BA 0.5 mg/l, 2,4-D 0.5 mg/l, sucrose 30.0 g/l, agar 6.0 g/l) recorded a response of 50 per cent. The treatments T_1, T_3, T_4, T_5 T_6 and T_7 showed a response ranging from 12.0 per cent to 42.8 per cent. No response was obtained in the treatments T_8 and T_9.

Callusing percentage was maximum in the variety

Table 10. Response of leaf explants of five varieties of A. andreanum on callus initiation

Treatment*	Cultures initiating callus (%)				
	D	F	P	H	N
T_1	13.33	0.00	0.00	0.00	50
T_2	50.00	0.00	0.00	0.00	75
T_3	25.00	0.00	0.00	0.00	20
T_4	13.33	6.67	6.67	5.46	50
T_5	14.28	0.00	0.00	0.00	0
T_6	42.80	0.00	0.00	0.00	0.
T_7	12.00	0.00	0.00	0.00	0
T_8	0.00	0.00	0.00	0.00	0
T_9	0.00	0.00	0.00	0.00	0

*Compositon of treatments given in Table 2.

D - Dragon's Tongue

F - Flaking

P - Pompon Red

H - Honeymoon Red

N - Nitta

The data represents the mean value of 30 replications.

Nitta. However the callusing was very little and restricted to the margin's of the explant (Plate 3b). The treatment T_2 recorded 75 per cent callusing in the variety Nitta. The same variety exhibited a response of 50 per cent in T_1 and T_4 whereas a lower response of 20 per cent was obtained in T_3. Response was not obtained for the variety Nitta in T_5, T_6, T_7, T_8 and T_9.

The varieties Flaking, Pompon Red and Honeymoon Red showed response only in the treatment T_4. This treatment (MMS [NH_4NO_3 200 mg/l] + BA 1.0 mg/l + 2,4-D 0.08 mg/l + sucrose 30.0 g/l + agar 6.0 g/l) gave a response of 6.67 per cent in both the varieties Flaking (Plate 3c) and Pompon Red (Plate 3d). The variety Honeymoon Red (Plate 3e) exhibited a lower response of 5.46 per cent in this medium.

The calli obtained in all the varieties were subcultured for callus multiplication twice in the same medium within a period of two months. The cultures on subculturing showed very little callus multiplication.

4.2.2 They were then transferred to the regeneration medium (MS + BA 0.5 mg/l + IAA 2.0 mg/l + sucrose 30.0 g/l + agar 6.0 g/l). The calli obtained in the varieties Flaking, Pompon Red, Honeymoon Red and Nitta did not show any

Plate 3a

Callus initiation fromleaf explants of A.andreanum var.Dragon's Tongue

Plate 3b

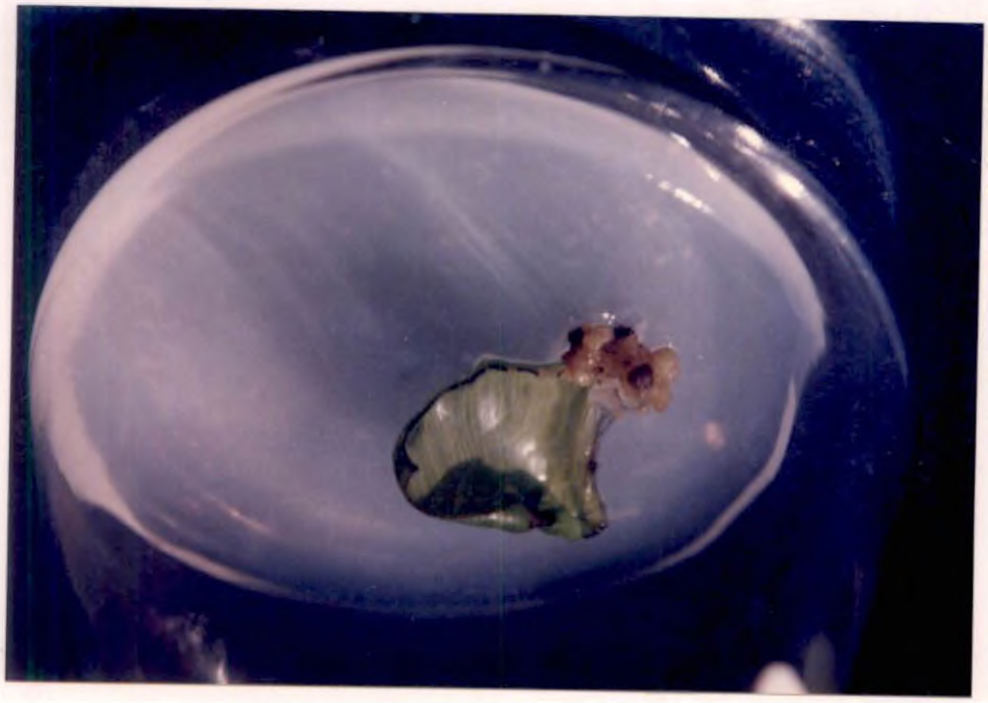
Callus initiation from leaf explants of A.andreanum var. Nitta.

Plate 3c

Callus initiation in leaf explants of A.andreanum var. Flaking.

Plate 3d

Callus initiation in leaf explants of A.andreanum var Pompon Red.



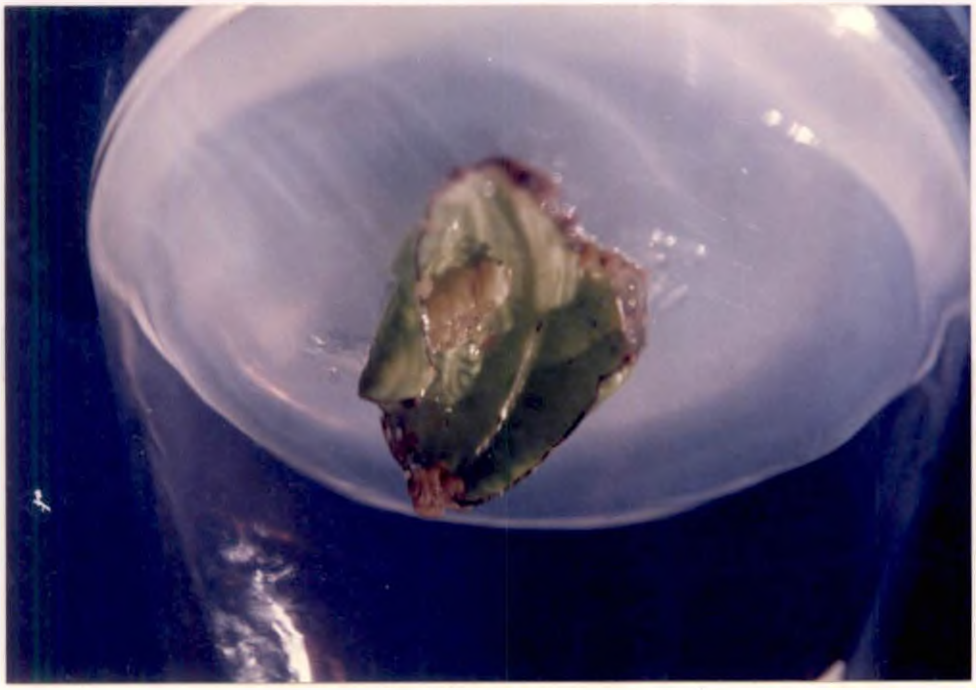


Plate 3e

Callus initiation in A.andreanum var Honeymoon Red.

response. Regeneration was obtained only in the variety Dragon's Tongue and hence further studies were carried out in this variety. The variety Dragon's Tongue was subjected to various treatments involving culture components and plant growth substances for the refinement of callus initiation and shoot proliferation.

4.2.2 Callus initiation

4.2.2.1 Basal media

The response of six different basal media was studied with respect to callus initiation in the variety Dragon's Tongue. Leaf, petiole and spadix explants were tested in the different basal media. Only leaf explants initiated callus, whereas petiole and spadix explants showed only swelling (Table 11). Among leaf explants,the pieces containing midrib showed a higher response than sections without it.

The maximum number of cultures initiating callus (10.0 per cent) were recorded in the MMS medium as shown as Table 11. Callusing was observed in these cultures within 70 days. Treatment using Nitsch's as basal medium recorded six per cent callusing. In this case initiation of callus was

Table 11. Effect of basal media on callus initiation in A.andreanum var: Dragon's Tongue

Basal medium supplemented with BA 0.5 mg/l + 2,4-D 0.5 mg/l + sucrose 30.0 g/l + agar 6.0 g/l

Basal medium	Cultures initiating callus (%)		
	L	P	S
MS	0	0	0
MMS	10	0	0
Nitsch's	6	0	0
SH	0	0	0
LS	0	0	0
B5	0	0	0

Explants: L - Leaf

P - Petiole

S - Spadix

The data represents the mean value of 30 replications

obtained 60 days after culture. No callusing was observed in the MS, SH, LS and B5 basal media. A low callusing reponse was obtained in MMS and Nitsch's basal media. So these two media were further modified to obtain a higher callusing response.

4.2.2.2 Ammonium nitrate

An increase in the callusing response was observed on lowering the ammonium nitrate content to 200 mg/l as shown in Table 12. MMS (NH_4NO_3 200 mg/l) + BA 0.5mg/l + 2,4-D 0.5 mg/l + sucrose 30.0 g/l + agar 6.0 g/l was found to be the best treatment for initiating callus (50.0%). Further lowering the ammonium nitrate content to 100 mg/l was found to give a lower response of 15.0 per cent (Table 12). Reducing the NH_4NO_3 level to 200 mg/l in the Nitsch's medium gave an increased response of 20.0 per cent over the full strength of NH_4NO_3 in Nitsch's medium (6.0%).

4.2.2.3 Plant growth substances incorporated into the MMS medium

Various combinations of plant growth substances supplementing the MMS (NH_4NO_3 200 mg/l) medium were used to study their response on callus initiation.

Table 12. Effect of Ammonium nitrate concentration on callus initiation in A. andreanum var: Dragon's Tongue

Culture medium supplemented with BA 0.5 mg/l + 2.4-D 0.5 mg/l + sucrose 30.0 g/l + agar 6.0 g/l

Explant : Leaf

Basal medium	Ammonium nitrate (mg/l)	Cultures initiating callus (%)
MMS	800	10
MMS	200	50
MMS	100	15
Nitsch's	720	6
Nitsch's	200	20

The data represents the mean value of 30 replications

The most promising treatment for the variety Dragon's Tongue was found to be CP_6 (Table 13). Callusing in this treatment (MMS [NH_4NO_3 200 mg/l] + BA 0.5 mg/l + 2,4-D 0.5 mg/l + sucrose 30.0 g/l + agar 6.0 g/l) was obtained within 60 days. 50 per cent of the cultures initiated callus in this treatment. The treatment CP_{13} recorded 42.8 per cent response. Both the treatments CP_7 and CP_{14} recorded 25 per cent response. The treatments CP_1, CP_8 and CP_{17} recorded 13.33 per cent response. Callusing in all these treatments were obtained within 50-60 days. However, there was no response in the leaf explants treated with CP_2, CP_3, CP_4, CP_5, CP_9, CP_{10}, CP_{11}, CP_{15}, CP_{16}, CP_{19}, and CP_{20}.

4.2.2.4 Plant growth substances incorporated into the Nitsch's medium

The effect of different combinations of plant growth substances supplementing the Nitsch medium (NH_4NO_3 200 mg/l) on callus initiation in the variety Dragon's Tongue was studied. The maximum number of cultures initiating callus was observed in the treatment CP_{21} (Table 14). The treatment (Nitsch's [NH_4NO_3 200 mg/l] + BA 0.5 mg/l + 2,4-D 0.5 mg/l + sucrose 30.0 g/l + agar 6.0 g/l) gave a response of 20 per cent within 60 days. In the treatments

Table 13. Effect of plant growth substances incorporated into MMS medium on callus initiation in A.andreanum var: Dragon's Tongue

Basal medium : MMS* Explant : Leaf

Plant growth substances (mg/l)	Code No.	Cultures initiating callus (%)
BA 0.5 + 2,4-D 0.06	CP_1	13.33
BA 0.5 + 2,4-D 0.08	CP_2	0.00
BA 0.5 + 2,4-D 0.10	CP_3	0.00
BA 0.5 + 2,4-D 0.15	CP_4	0.00
BA 0.5 + 2,4-D 0.20	CP_5	0.00
BA 0.5 + 2,4-D 0.50	CP_6	50.00
BA 1.0 + 2,4-D 0.06	CP_7	25.00
BA 1.0 + 2,4-D 0.08	CP_8	13.33
BA 1.0 + 2,4-D 0.10	CP_9	0.00
BA 1.0 + 2,4-D 0.15	CP_{10}	0.00
BA 1.0 + 2,4-D 0.20	CP_{11}	0.00
BA 1.0 + 2,4-D 0.50	CP_{12}	14.28
BA 1.5 + 2,4-D 0.06	CP_{13}	42.80
BA 1.5 + 2,4-D 0.08	CP_{14}	25.00
BA 1.5 + 2,4-D 0.10	CP_{15}	0.00
BA 1.5 + 2,4-D 0.15	CP_{16}	0.00
BA 1.5 + 2,4-D 0.20	CP_{17}	13.33
BA 1.5 + 2,4-D 0.50	CP_{18}	12.00
BA 0.1 + kinetinl.0 + 2,4-D 0.1	CP_{19}	0.00
BA 1.0 + NAA 1.0 + 2, 4-D 0.05	CP_{20}	0.00

MMS* - modified MS basal medium with quarter strength NH_4NO_3

The data represents the mean of 30 replicatiions.

Table 14. Effect of plant growth substances incorporated into Nistch's medium on callus intiation in A.andreanum var: Dragon's Tongue.

Basal medium : Nitsch's* Explant : Leaf

Plant growth substances (mg/l)	Code No.	Cultures initiating callus (%)
BA 0.5 + 2,4-D 0.05	CP_{21}	20.0
Kinetin 0.5 + 2,4-D 0.5	CP_{22}	0.0
BA 0.5	CP_{23}	17.5
BA 0.7	CP_{24}	15.0
BA 0.9	CP_{25}	0.0
BA 1.1	CP_{26}	0.0
BA 1.5	CP_{27}	16.0

Nitsch's* - Nitsch's basal medium with NH_4NO_3 200 mg/l

The data represents the mean value of 30 replications.

involving BA as the sole hormone supplement, treatment CP_{23} gave the maximum response (Table 14). This treatment (Nitsch's [NH_4NO_3 200 mg/l] + BA 0.5 mg/l + sucrose 30.0 g/l + agar 6.0 g/l) recorded 17.5 per cent cultures initiating callus. Treatments CP_{27} and CP_{24} gave 16.0 per cent and 15.0 per cent response, respectively. No callusing was observed in the treatments CP_{22}, CP_{25} and CP_{26}.

4.2.2.5 Carbon source

Studies were made to find out the effect of two carbon sources (sucrose and glucose) on initiation of callus. The treatment containing surcose 30.0 g/l recorded a better response (50.0 per cent) over glucose 30.0 g/l (25.0 per cent) as shown in Table 15.

4.2.2.6 Culture conditions

When the cultures (CP_6) were kept under light (3000 lux) none of the cultures initiated callus from leaf explants and browning of tissues was observed. When the cultures were kept in complete darkness, 50.0 per cent of the cultures were responsive (Table 16).

4.2.3 Regeneration of shoots and roots

The callus cultures of the variety Dragon's Tongue

Table 15. Effect of carbon source on callus initiation in A.andreanum var: Dragon's Tongue.

Culture medium : MMS* + BA 0.5 mg/l + 2,4-D 0.05 mg/l + agar 6.0 g/l

Explant : Leaf

Carbon source (g/l)	Cultures initiating callus (%)
Sucrose 30	50.0
Glucose 30	25.0

MMS* - modified MS basal media with quarter strength NH_4NO_3

The data represents the mean values of 30 replications.

Table 16. Influence of light on callus initiation in A.andreanum var: Dragon's Tongue.

Explant : Leaf

*Treatment	Cultures initiating callus (%)
Light (CP_6)	0
Dark (CP_6)	50

Composition of treatment given in Table 13.
The data represents the mean value of 30 replications.

(two months after callus initiation) were transferred to a regeneration medium (MS + BA 0.5 mg/l + IAA 2.0 mg/l + sucrose 30.0 g/l + agar 6.0 g/l). Regeneration was obtained within one month (Plate 4a and 4b) when these cultures were transferred to light conditions (3000 lux) under a 16 hr photoperiod. During subculture some of the cultures produced a greenish yellow friable callus (Plate 5) which failed to regenerate. On an average 4-8 shoots (Plate 6) were produced per culture after a period of three months. Shoot proliferation continued even after three months giving a maximum of 50-60 shoots (Plate 7) at the end of seven months.

However, the cultures kept under dark conditions failed to regenerate.

Studies were continued to increase the shoot proliferation rate. Results of the studies are presented below.

4.2.4 Shoot proliferation

4.2.4.1 Basal media

MS medium was found to be more responsive than

Plate 4a and 4 b

Shoot regeneration from leaf explants of *A.andreanum* var. Dragon's Tongue.

Plate 5

Non regenerative callus in A.andreanum var. Dragon's Tongue..

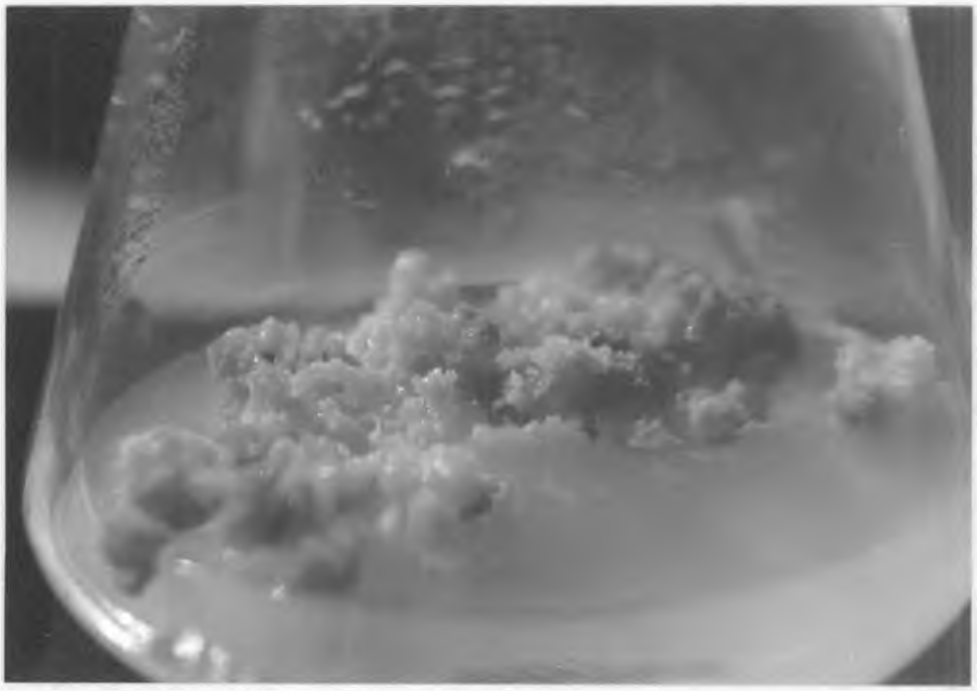
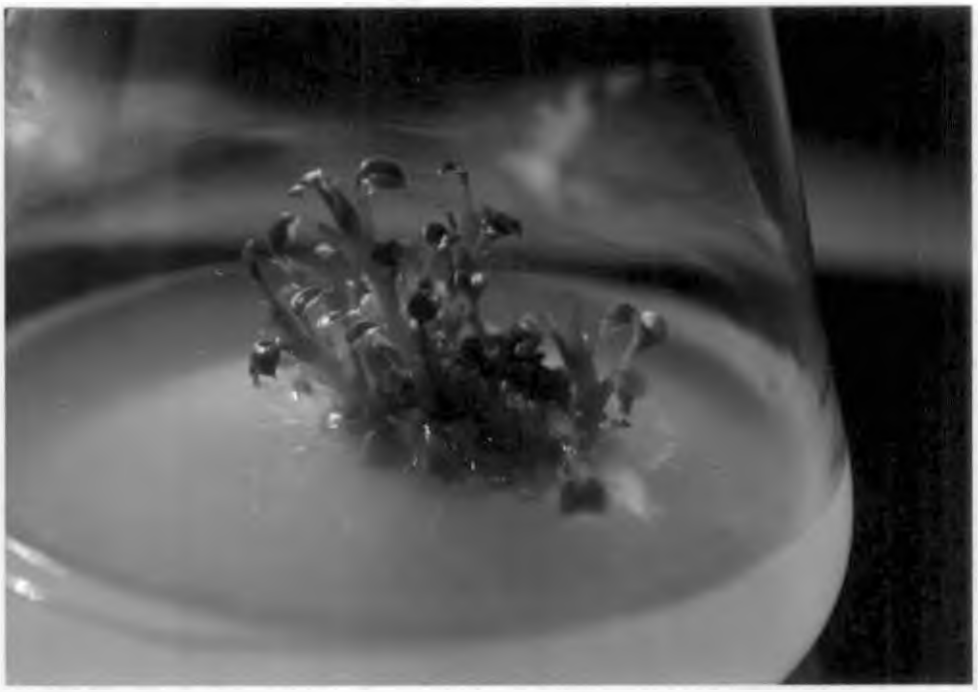


Plate 6 and 7

Shoot proliferation in regeneration medium.



Nitsch's medium (Table 17). The treatment (MS + BA 0.5 mg/l + IAA 2.0 mg/l + sucrose 30.0 g/l + agar 6.0 g/l) produced an average of 6.26 shoots. This treatment recorded a leaf width of 0.38cm and the longest shoot was 2.45 cm.

4.2.4.2 Ammonium nitrate

Comparing the different levels of ammonium nitrate, the normal ammonium nitrate level in the MS medium (1650 mg/l) was found to be optimum (Table 18). This level produced the maximum number of shoots (6.26), maximum width of leaf (0.38 cm) and longest shoot (2.45cm). The least response was recorded at the lowest concentration of ammonium nitrate (200mg/l).

4.2.4.3 Organics

Full level of organics in the MS medium was found to be most responsive (Table 19). Full strength of organics in the medium recorded an average of 6.26 shoots. A leaf width of 0.38 cm and shoot length of 2.45 cm was obtained. However quarter strength was found to be more responsive than half strength.

4.2.4.4 Carbon source

Among the two different carbon sources tried,

Table 17. Effect of basal media on shoot proliferation in A.andreanum var. Dragon's Tongue.

Culture medium supplemented with BA 0.5 mg/l + IAA 2.0 mg/l + sucrose 30.0 g/l + agar 6.0 g/l

Basal medium	No.of shoots (>0.5mm) per culture	No.of shoots (<0.5mm) per culture	Total no.of shoots per culture	Length of the longest shcot (cm)	Width of the big-gest leaf (cm)
MS	4.38	1.88	6.26	2.45	0.38
Nitsch's	2.00	1.67	3.67	2.00	0.33

The data represents the mean values of 3 replications.

Table 18. Effect of ammonium nitrate concentration on shoot proliferation in A.andreanum var: Dragon's Tongue.

Culture medium : MS + BA 0.5 mg/l + IAA 2.0 mg/l + sucrose 30.0 g/l + agar 6.0 g/l

Ammonium nitrate (mg/l)	No.of shoots (>0.5mm) per culture	No.of shoots (<0.5mm) per culture	Total no.of shoots per culture	Length of the longest shoot (cm)	Width of the biggest leaf (cm)
1650	4.38	1.88	6.26	2.45	0.38
800	2.66	1.00	3.66	3.00	0.27
200	2.67	0.67	3.34	2.83	0.27

The data represents the mean values of 3 replications.

sucrose at 30.0 g/l (RC_3) was found to be most effective (Table 20). This treatment recorded an average of 4.38 and 1.88 long and short shoots respectively. Maximum number of shorter shoots was obtained in RC_6 (glucose 10.0 g/l). No long shoots were produced in the treatments RC_5, RC_6 and RC_7.

Maximum width of the leaf (0.38 cm) was obtained in RC_3. The longest shoot (3.66 cm) was obtained in RC_2 (sucrose 20.0 g/l) whereas RC_3 produced only 2.45cm long shoot.

4.2.4.5 Media Supplements

Among the different levels of casein hydrolysate tried, 150 mg/l (Plate 8) was found to be most promising (Table 21). RS_3 produced an average of 7.33 shoots and a leaf width of 0.33cm and shoot length of 4.3cm. All the results indicate a better response than the control.

Glutamine at 150mg/l was found to be the best among various levels tried for regeneration (Table 21). However, this treatment produced only an average of 3.67 shoots, 0.2cm wide leaf and 2.33 cm long shoot. The results obtained showed much lower values compared to control.

Table 19. Effect of organic compounds on shoot proliferation in A.andreanum var: Dragon's Tongue

Culture medium: MS medium + BA 0.5 mg/l + IAA 2.0 mg/l + sucrose 30.0 g/l + agar 6.0 g/l.

Organics in MS medium	No.of shoots (>0.5mm) per culture	No.of shoots (<0.5mm) per culture	Total no.of shoots per culture	Length of the longest shoot (cm)	Width of the biggest leaf (cm)
Full	4.38	1.88	6.26	2.45	0.38
Half	1.67	0.67	2.34	3.23	0.17
Quarter	2.67	0.33	3.00	3.97	0.33

The data represents the mean values of 3 replications.

Table 20. Effect of carbon source on shoot proliferation in A.andreanum var: Dragon's Tongue

*Treatment	No.of shoots (>0.5mm) per culture	No.of shoots (<0.5mm) per culture	Total no.of shoots per culture	Length of the longest shoot (cm)	Width of the biggest leaf (cm)
RC_1	2.16	1.33	3.49	2.50	0.30
RC_2	1.50	1.66	3.16	3.66	0.33
RC_3	4.38	1.88	6.26	2.45	0.38
RC_4	3.00	1.00	4.00	0.85	0.20
RC_5	0.00	1.00	1.00	2.00	0.26
RC_6	0.00	4.33	4.33	2.16	0.13
RC_7	0.00	1.33	1.33	1.93	0.13
RC_8	1.11	3.00	4.11	2.17	0.17
RC_9	1.80	3.00	4.80	2.00	0.25
RC_{10}	1.67	0.67	2.34	2.50	0.30

*Composition of treatments given in Table 5.
The data represents the mean values of 3 replications

Table 21. Effect of media supplements on shoot proliferation in A.andreanum var: Dragon's Tongue.

Culture medium: MS + BA 0.5 mg/l + IAA 2.0 mg/l + sucrose 30.0 g/l + agar 6.0 g/l

Treatment	Code No.	No.of shoots (>0.5mm) per culture	No.of shoots (<0.5mm) per culture	Total no.of shoots per culture	Length of the longest shoot (cm)	Width of the biggest leaf (cm)
Control		4.38	1.88	6.26	2.45	0.38
Casein hydrolysate (mg/l)						
50	RS_1	1.33	3.56	4.89	2.63	0.20
100	RS_2	2.00	3.67	5.67	3.23	0.18
150	RS_3	4.33	3.00	7.33	4.30	0.30
Glutamine (mg/l)						
50	RS_4	1.00	0.00	1.00	1.76	0.13
100	RS_5	1.00	2.00	3.00	1.75	0.40
150	RS_6	1.00	2.67	3.67	2.33	0.20
Coconut water (ml/l)						
100	RS_7	1.50	3.50	5.00	3.33	0.23
150	RS_8	1.00	3.38	4.38	2.50	0.33
200	RS_9	1.00	0.00	1.00	2.73	0.62
250	RS_{10}	1.67	1.33	3.00	2.73	0.47

The data represents the mean values of 3 replications.

Comparing the different levels of coconut water used, the lowest concentration of 100 ml/l (RS_7) produced maximum number of shoots (5.0) and also the longest shoot (3.33cm) as shown in Table 21. Greater leaf width was observed in RS_9 (CW 200 ml/l). However, the results show that the control was found superior over the treatments containing coconut water.

4.2.4.6 Plant growth substances

Among the different combinations of plant growth substances, combinations of kinetin and IAA were found best for shoot proliferation. The treatment RP_{17} (Plate 9) was found to be most promising (Table 22). The maximum number of shoots having length greater than 0.5 mm (8.16) as well as less than 0.5 mm (5.33) was obtained in MS + kinetin 1.5 mg/l + IAA 3.0 mg/l + sucrose 30.0 g/l + agar 6.0 g/l. An average of 13.49 shoots was recorded in this treatment.RP_{10} (MS + kinetin 0.5 mg/l + IAA 2.0 mg/l + sucrose 30.0 g/l + agar 6.0 g/l) also gave a good response. This treatment could produce an average of 7.5 long shoots and 3.5 short shoots. The next best response was obtained in RP_{16}. Thus treatment produced an average of 5.66 and 4.33 long and short shoots respectively. Treatments RP_8 and RP_{14} recorded

Table 22. Effect of plant growth substances on shoot proliferation in A.andreanum var: Dragon's Tongue

*Treatment	No.of shoots (>0.5mm) per culture	No.of shoots (<0.5mm) per culture	Total no.of shoots per culture	Length of the longest shoot(cm)	Width of the biggest leaf(cm)
RP_1	4.38	1.88	6.26	2.45	0.38
RP_2	1.75	1.33	3.08	2.37	0.23
RP_3	2.25	1.00	2.35	2.10	0.40
RP_4	3.44	2.00	5.44	3.07	0.30
RP_5	2.67	1.67	4.33	2.67	0.23
RP_6	4.25	4.50	8.75	1.90	0.20
RP_7	2.72	1.50	4.22	1.93	0.30
RP_8	5.33	4.00	9.33	3.66	0.35
RP_9	7.00	2.00	9.00	5.00	0.17
RP_{10}	7.50	3.50	11.00	5.35	0.36
RP_{11}	1.91	3.66	5.57	3.93	0.36
RP_{12}	3.33	2.33	5.66	3.00	0.30
RP_{13}	3.66	2.00	5.66	3.66	0.40
RP_{14}	3.00	6.33	9.33	3.10	0.23
RP_{15}	2.86	0.66	3.52	3.43	0.46
RP_{16}	5.66	4.33	9.99	4.33	0.60
RP_{17}	8.16	5.33	13.49	3.00	0.36
RP_{18}	3.00	2.66	5.66	3.23	0.36
RP_{19}	1.00	0.00	1.00	1.66	0.18
RP_{20}	0.50	0.66	1.16	1.66	0.27
RP_{21}	1.20	0.00	1.20	1.16	0.36
RP_{22}	1.00	0.00	1.00	1.30	1.00

*Composition of treatments given in Table 7 (i).
The data represents the mean value of 3 replications.

Plate 8

Shoot proliferation in medium supplimented with casein hydrolysate (150 mg/l).

Plate 9

Shoot proliferation in medium supplemented with kinetin 1.5 mg/l + IAA 3.0 mg/l.

the same number of total shoots (9.33). However, RP_8 produced more number of longer shoots (5.33) than RP_{14} (3.0). RP_{14} recorded more number of shorter shoots (6.33) than RP_8 (4.0).

The least response was recorded by all combinations of BA and NAA. Among this group, the best response was obtained in RP_{24} (MS + BA 1.0 mg/l + NAA 4.0 mg/l + sucrose 30.0 g/l + agar 6.0 g/l). This treatment could produce only 1.20 long shoots. No short shoots were produced. All the treatments in this combination with the exception of RP_{20} produced only long shoots.

With respect to leaf size, the best response was recorded by RP_{16} (Table 22). This treatment (MS + kinetin 1.5 mg/l + IAA 2.0 mg/l + sucrose 30.0 g/l + agar 6.0 g/l) recorded a leaf width of 0.6 cm (largest leaf) whereas, RP_{17} recorded a leaf width of 0.36 cm.

The best response for length of the shoot was obtained in RP_{10} (Table 22). The length of the longest shoot was 5.35 cm in this treatment (MS + kinetin 0.5 mg/l + IAA 2.0 mg/l sucrose 30.0 mg/l + agar 6.0 g/l) whereas RP_{17} recorded a value of 3.0 cm.

4.2.4.7 Activated charcoal

Since the above treatments produced a high rate of

shoot proliferation with reduced leaf development, some of cultures were transferred to a hormone-free MS medium supplemented with activated charoal (AC) at 1.0 gm/l. The cultures transferred to an intermediary medium containing activated charcoal recorded satisfactory growth, both in leaf development and shoot growth (Plate 10)

4.2.4.8 Plant growth substances tested on regenerated shoots pre-cultured in medium containing AC

The effect of increased levels of IAA on growth of shoots precultured in medium containing AC for one month, was studied (Table 23).

The treatment RP_{24} containing kinetin 0.5 mg/l + IAA 8.0 mg/l showed the maximum increase in number of shoots (two), whereas the best response for increase in number of leaves (eight) was shown by RP_{25} (kinetin 0.5 mg/l + IAA 16.0 mg/l) [Plate 11].

4.2.4.9 Gelling agent

Agar was found to be superior to agarose (Table 24) Agar at 6.0 g/l (RG_3) produced the maximum number of shoots (13.49) and largest leaf width (0.36 cm). However longest shoot (4.67 cm) was obtained when concentration of agar was

Table 23. Effect of plant growth substances on shoot proliferation from regenerated shoots pre-cultured on medium containing AC in A.andreanum var: Dragon's Tongue.

Basal Medium : MS

Plant growth substances (mg/l)	Code No.	Increase in no.of shoots per culture	Increase in no.of leaves per culture
Kinetin 0.5 + IAA 4	RP_{23}	1	4.0
Kinetin 0.5 + IAA 8	RP_{24}	2	4.5
Kinetin 0.5 + IAA 16	RP_{25}	1	8.0
Kinetin 1.0 + IAA 4	RP_{26}	0	7.0
Kinetin 1.0 + IAA 8	RP_{27}	0	7.0
Kinetin 1.0 + IAA 16	RP_{28}	1	4.0
Kinetin 0 + IAA 8	RP_{29}	0	2.5

The data represents the mean values of 3 replicaitons.

Plate 10

Shoot growth in medium supplemented with activated charcoal (0.1%).

Plate 11

Shoot growth in medium supplemented with kinetin 0.5 mg/l + IAA 16.0 mg/l.


250 ml
BOROSIL

Table 24. Effect of different gelling agents on shoot proliferation in A.andreanum var: Dragon's Tongue.

Culture medium : MS + kinetin 1.5 mg/l + IAA 3.0 mg/l + sucrose 30.0 g/l 6.0 g/l

*Treatment	No of shoots (<0.5mm) per culture	No.of shoots (<0.5mm) per culture	Total no.of shoots per culture	Length of the longest shoot (cm)	Width of the biggest leaf (cm)
RG_1	2.50	4.50	7.00	2.00	0.25
RG_2	5.17	1.83	7.00	2.20	0.20
RG_3	8.16	5.33	13.49	3.00	0.36
RG_4	6.80	0.00	6.80	4.67	0.30
RG_5	5.33	1.87	7.20	3.83	0.13
RG_6	7.00	0.00	7.00	2.60	0.30
RG_7	5.33	3.00	8.33	2.50	0.20

* Composition of treatments given in Table 8.

The data represents the mean values of 3 replications

increased to 7.0 g/l.

4.2.5.9 Rooting

All the treatments tried for shoot proliferation produced satisfactory number of roots (1-10) and hence a seperate rooting phase was not necessary.

4.3 Planting out

Rooted plantlets (Plate 11) were found superior to shoots without any roots during Planting out establishement (Plate 13). The rooted plantlets had a higher survival rate (60 per cent) as compared to the shoots without roots (10 per cent) after a period of 30 days as shown in table 25.

Table 25 : Planting out

	No. of plantlets transferred	No. of plantlets	% survival
Rooted plantlets	20	12	60
Microshoots	20	2	10

Plate 12

Rooted plantlets

Plate 13

Planting out

Discussion

5. Discussion

Anthurium is gaining popularity as one the most important commercial ornamental crops of the modern world. The cultivation of anthurium is relatively simple. The colourful cut flowers are highly prized for their long shelf life and they can be marketed to distant places and even overseas without damage.

The humid warm climate of Kerala is highly conducive for the cultivation of anthuriums. The Government of India has identified Kerala as the Product Specific Intensive Floriculture Zone for anthurium and orchids. In view of its economic prospects, farmers of Kerala have started commercial cultivation of anthuriums. It gives an employment potential to the unemployed educated youth and an additional source of income for the housewives.

There is an increasing demand for anthurium flowers both in the domestic and international markets. The cut flowers find an important place in decorating homes, offices, hotels and commercial institutions. There is a great demand for new hybrids and new varieties in the highly competitive international market. Consumer preference with regard to flower colour, shape and size is highly dynamic

and changes within a short time span.

Anthuriums are conventionally propagated by seeds. Since they are cross pollinated, the seed propagated progenies are heterozyous and not uniform in performance. Long periods of seed maturity (6-7 months) as well as juvenile phase and a short viable period of the seeds are other disadvantages of this method. There may be great variation in the number, colour and shape of flowers as well as in overall growth characteristics among the progeny of a single plant. In many cases one-third of the seedlings may have to be discarded before flowering (Geier, 1990). Vegetative propagation methods are available. They include division of old plant suckers, topping and nodal stem cuttings. However, the rate of multiplication by these methods is low and insufficient to meet the increasing demand for propagules. In this context, an *in vitro* system of propagation becomes relevant as it ensures rapid clonal multiplication.

There are reports on *in vitro* propagation of anthurium *via* enhanced release of axillary buds, somatic embryogenesis and somatic organogenesis.

Somatic organogenesis has proved to be the most

successful route of *in vitro* propagation in *Anthurium andreanum* (Pierik, 1976; Pierik *et al.*, 1979; Sreelatha, 1992; Sreekumar *et al.*, 1992; Nirmala and Singh, 1993; Satheeshkumar and Seeni, 1994).

The *in vitro* response of plants is influenced by genotype in many cases. This influence is highly pronounced in the case of *A. andreanum*. Protocols for *in vitro* propagation have been standardised for a few varieties of *A. andreanum*.

The present study was taken up with the objective of standardisation of media and culture conditions for *A. andreanum* varieties not having protocols for *in vitro* propagation. Five varieties namely, Dragon's Tongue, Flaking, Pompon Red, Honeymoon Red and Nitta were selected for the study. The salient results are discussed in the following pages.

Microbial contamination has long been a major problem in the culture establishment of explants. Since plant parts are exposed in the field for a long time, they harbour various microorganisms and many of which penetrate into the plant tissue resulting in systemic infection. Cultures from such tissues are hence easily contaminated (Chen and Evans, 1990).

In the present study also, high rate of fungal contamination was observed since the explants were collected from field grown plants. In order to minimise the rate of contamination, the explants were subjected to various surface sterilization treatments. Mercuric chloride was found to be a better sterilant than sodium hypochlorite. Mercuric chloride 0.1 per cent was found to be an effective surface sterilant (Table 9). Sodium hypochlorite treatments caused browning of the explants which affected the later *in vitro* response. In humid tropical countries, where there is microbial contamination throughout the year, milder sterilants such as sodium hypochlorite will not always be effective. Satheeshkumar and Seeni (1994) reported surface sterilization of anthurium leaf explants with five per cent sodium hypochlorite for 15-20 minutes followed by 0.5 per cent mercuric chloride for four minutes.

Among the explants, the highest number of sterile cultures (88.8 per cent) was observed in leaf sections using mercuric chloride 0.1 per cent for eight minutes. A much lower percentage of sterile cultures was obtained for petiole (50.0) and spadix (30.0) even at a prolonged treatment time of 10 and 15 minutes, respectively (Table 9). A higher time of surface sterilization for the spadix

explants was given because the spadix surface is not smooth but irregular due to the presence of thickly packed flowers in its surface in slanting serial rows as compared to the smooth surface of the petiole. A comparison of the size of the vascular bundles (of the different explants) and incidence of microbial contamination was attempted by Sreelatha (1992). Studies showed that vascular bundles of the leaf were the smallest, compared to that of other explants. It was observed that there may be less translocation of microorganisms along smaller conducting vessels. Thus the reduced microbial contamination on the leaf explants may be due to smaller vascular bundles.

In the preliminary studies calli of varying degree (Table 10) was obtained in all the five varieties (Dragon's Tongue, Flaking, Pompon Red, Honeymoon Red and Nitta). Good callusing was obtained only in the variety Dragon's Tongue. Although the variety Nitta showed a high percentage of callusing, the callus was scanty and there was no growth in subcultures. The callusing percentage in Flaking, Pompon Red and Honeymoon Red was very poor.The most critical factor in Anthurium tissue culture is the genotype. Pierik *et al.* (1975) studied 38 genotypes of *A. andreanum* and obtained moderate to strong callus formation from leaf segments in 31

types, very poor callus in 4 types and no response in the remaining 3 genotypes. Leffring et al. (1976a) found that in most genotypes callus growth was too slow and inconsistent to be exploited for large scale propagation.

The calli were further subcultured for regeneration. However, regeneration was obtained only in the variety Dragon's Tongue. Leffring and Soede (1979a) after extensive studies were able to induce callusing in 22 out of 23 genotypes, but only 15 genotypes could be induced to form shoots. Similar varietal difference in the morphogenetic response in callus cultures of A. scherzerianum has been reported by Geier (1986). There are intervarietal differences in the morphogenetic capacities of callus tissues (George and Sherrington, 1984). In many instances callus obtained from one kind of explant of several different plant varieties on a single medium, when treated in the same way showed differences in the organogenetic potential with respect to the varieties (Ohki et al., 1978). According to the genetic control of morphogenesis, some genes exert their influence by regulating the effective levels of plant growth substances. Endogenous levels of plant growth substances may vary in different genotypes and thus account for the difference in

spadix explants produced only swelling. Sreelatha (1992) also obtained callus induction only in leaf explants in the pink variety. For any given species or variety, a particular explant may be necessary for successful plant regeneration (Flick et al., 1983). The kind of explant chosen, its size, age and the manner in which it is cultured can all influence the in vitro success. According to Sreelatha (1992) morphological and physiological status of the explant can account for the difference in the response. The less lignified tissues of the leaf may facilitate easy de-differentiation process than the tissues of other plant parts. There are reports of callusing in petiole and spadix explants by Singh and Sangama (1990), Sreekumar et al. (1992) and Singh (1994). This difference in response may be due to the influence of genotype.

Variation in the regenerative capacity can be found within the same organ. Different regenerative capacities are often found in leaf tissues. In the present studies, leaf sections with midrib or veins gave a better callusing response than sections without it. Kato (1974) opined that it was often beneficial to include a portion of midrib or leaf vein in leaf sections to obtain callus formation and organogenesis. This may be due to the higher meristematic

response.

The choice of the basal medium depends on the plant species and the intended use of culture. Among the six basal media tried, the best callusing response was obtained in MMS medium (Table 11). Callusing was not supported by the MS, B5, LS and SH media. Pierik et al. (1975) and Pierik (1976) reported that MS medium with macronutrients at half strength or even greatly reduced strength and MS micro elements at full strength was best for in vitro culture in anthurium. The MS medium characterised by high concentrations of mineral salts has been widely used for general plant tissue culture (Murashige, 1974). Although MS major nutrients are a good starting point for medium development, more dilute solutions may prove to be better in few circumstances. Adjustment of the ionic concentration becomes necessary when one or more ions at the normal level are inhibitory to a species.

Nitsch's medium was found to support callusing next to MMS (Table 11). Sreekumar et al. (1992) found Nitsch's as the best medium for callusing. The basal medium requirement is highly tissue dependent and varies from one variety to another.

In the present investigations, sections of tender leaf, petiole and spadix were used as explants for the induction of somatic organogenesis. Callusing was observed only in the leaf explants (Table 11), whereas petiole and

activity of the tissues of the veins. Similar results were obtained by Leffring (1976b) and Sreekumar et al. (1992). However, Nirmala and Singh (1993) reported similar response from leaf sections with and without the midrib.

Different concentrations of ammonium nitrate were tried to determine the optimum level. The best response was obtained at a reduced ammonium nitrate level of 200 mg/l (Table 12). The nitrogen requirement in the basal medium is met by three forms namely, nitrate, ammoniacal and organic. The concentration of ammonium nitrate was found to influence callus initiation. The level of ammonium ions in the medium is highly tissue specific and if found in supraoptimal levels it can lead to toxicity. The positive effect of lowering the ammonium nitrate concentration in anthurium was proved by many workers. (Pierik et al., 1975; Pierik, 1976; Geier, 1986; Sreelatha, 1992 and Nirmala and Singh, 1993). According to Pierik et al. (1979) this effect is caused by the ammonium ion and not by the nitrate ion.

The nature and concentration of growth hormones in the culture medium are critical for in vitro growth and morphogenesis of plants (Skoog and Miller, 1957). In the present investigation, in order to standardise a suitable hormone combination for better culture establishment,

studies were carried out using BA, kinetin, 2,4-D and NAA at various concentrations. When a combination of BA 0.5 mg1/1 and 2,4-D 0.5 mg/1 was added to the medium, significantly superior results were obtained in the precentage of cultures initiating callus (50.0) as shown in Table 13. In anthurium, a low content of auxin and a high content of cytokinin has been reported to be suitable for callus formation (Pierik et al., 1975; Pierik, 1979; Finnie and Van staden, 1986; Geier, 1986). Hcwever in the present instance maximum callusing was obtained at equal concentrations of auxin and cytokinin in the medium. This may be due to variation in the endogenous level of auxins and cytokinins among the different varieties.

Normally a sugar is incorporated into the medium as a carbon and energy sources as well as an osmoregulatory factor. In the present studies, a comparison of the two carbon sources showed sucrose to be superior over glucose for callus initiation as shown in Table 15. Sucrose is almost universally used for micropropagation as it is generally utilisable by tissue cultures (George and Sherrington, 1984).

Culture conditions influence the response of explants for initiation of callus. In the present instance, when the cultures were kept under darkness 50.0 per cent of the cultures initiated callus, whereas none of the cultures responded when under light (Table 16). Most workers observed callus formation and subsequent growth in continuous

darkness (Pierik et al., 1974a and b; Pierik, 1976; Sreekumar et al., 1992 and Sreelatha, 1992). According to Sreelatha (1992), the beneficial effect of darkness in the initiation of callus may be attributed to etiolation effect. Reid (1972) reported that the etiolated tissues may be less lignified than the light grown tissues, which facilitate easy de-differentiation. Herman and Hess (1963) proposed an increased content of auxin cofactors in the etiolated tissues which increased the tissue's specificity to exogenously applied auxin. Browning of the explants occured when the explants were exposed to light for 16 hours. This may be due to oxidation of phenolic compounds under light (Sreelatha, 1992). Inhibitory effect of light on callusing and further growth, has been reported in cyclamen (Lowenberg, 1969), in freesia (Bajaj and Pierik, 1974; Pierik and Steegmans, 1975) and in fuchsia (Bouharmont and Dabin, 1986).

Against callusing, organogenesis was found to be promoted by light. Limited organogenetic response of the callus subcultured in the dark indicated the positive influence of pigment and photosynthetic systems (Sreekumar et al., 1992). The dark grown callus cultures contain plastids lacking chlorophyll. When they are transferred to

light, the plastids develop the green pigment chlorophyll and become chloroplasts (George and Sherrington, 1984). Chlorophyll is required for photosynthesis which in turn promotes shoot regeneration.

In most studies of *in vitro* culture of anthuriums, MS medium has been used. In the present study also MS medium was found superior to Nitsch's medium for shoot proliferation (Table 17). MS medium is especially suitable for morphogenesis, meristem culture and regeneration. The basal medium preferred for shoot proliferation may vary from that required for callusing.

The present studies showed that full strength of ammonium nitrate in the MS medium was best for shoot proliferation (Table 18). As the callus cells developed into organized structures like shoots and roots, their tolerance to ammonium nitrate concentration might have increased.

The concentration of organic compounds in the basal medium influences the rate of shoot proliferation. In the present studies, different levels of organics were tried and the full level of organic compounds in the MS medium was

found to give the best results as shown in Table 19. The organic compounds are found in trace amounts in the culture media and so are usually needed in full strength to support growth.

Sucrose was found to be more promising than glucose for shoot proliferation. Sucrose three per cent was found optimum as carbon and energy sources as well as osmoregulatory factor (Table 20). Similar results were obtained by Sreelatha (1992). The general superiority of sucrose over glucose for the culture of organised plant tissues may be on account of the more effective translocation of sucrose to the apical meristems (Butcher and Street, 1964).

Inclusion of casein hydrolysate (CH) at a concentration of 150 mg/l in the shoot proliferation medium significantly enhanced the multiplication rate and also produced longer shoots (Table 21). CH is a non-specific organic nitrogen source and serves as an amino acid supplement (Skoog and Miller, 1957). CH has been known to increase the rate of *in vitro* proliferation in pineapple (Mathews and Rangan, 1981) and pomegranate (Mascarenhas *et al*., 1981).

The addition of glutamine to the culture medium was

found inhibitory for growth (Table 21). Certain specific amino acids added to the culture medium are found to inhibit growth. Coconut water contains several cell division factors (Shantz and Steward,1952; Letham, 1974) and a large number of free amino acids. In the present studies, addition of coconut water to the culture medium was found to have an inhibitory effect on shoot proliferation (Table 21). Coconut water has proved tobe inhibitory to growth in some instances (George and Sherrington, 1984).

Growth and morphogenesis in vitro are regulated by the interaction and balance between the plant growth substances supplied in the medium. Twenty-two treatments involving different combinations of auxins and cytokinins were tried tostudy their effect on shoot proliferation. The treatment containing kinetin 1.5 mg/l + IAA 3.0 mg/l was found to give the best response (Table 20). A higher level of auxin was used since IAA is thermolabile and a much lower concentration is only available after autoclaving the medium. Leffring and Soede (1979b), observed optimum branching in A. andreanum shoots in liquid medium containing kinetin. The cytokinins BA and 2ip caused less branching, but at the same time it promoted callus formation. George

and Sherrington (1984) opined that generally a high cytokinin to auxin ratio is needed for regeneration of shoots from callus. Cytokinins help in cell division and are needed for shoot proliferation. Auxins in the regeneration medium promote cell elongation and may be used to nullify the suppressive effect of cytokinins on shoot regeneration (Lundergan and Janick, 1980).

Activated Charcoal (AC) has the capacity to adsorb the toxic substances and residual cytokinin from the medium (Fridborg et al., 1978). A few cultures were transferred to a medium containing AC with a view of removing the toxic substances and residual cytokinins which might have been inhibitory for growth. As expected these cultures showed better shoot growth and leaf development. In general conditions that stimulate abundant shoot induction inhibit continued shoot growth (David, 1982; Amerson et al., 1985). Reduction in the concentration of the cytokinin BA for shoot elongation has been observed by Dantu and Bhojwani (1987) and Rajmohan (1985).

Agar was found better than agarose as a gelling agent. Agar is a cheaper source than agarose. Among the different concentrations tried, agar six per cent gave the best results (Table 24). This concentration of agar might

have created an osmotic potential favourable for the uptake of nutrients.

In anthurium tissue culture, no special rooting treatments were found necessary. Rooting of shoots occured spontaneously in the shoot proliferation medium itself. The high level of endogenous auxins and the prolonged exposure to light (for shoot proliferation) might have enhanced spontaneous rooting of shoots.

It is worthwhile to suggest some future prospects from the results of the present study. Caulogenic or regenerative callus was obtained only in the variety Dragon's Tongue. Further work has to be carried out to develop protocols which can be applicable to a larger number of varieties. Studies for *in vitro* crop improvement can also be attempted.

Summary

SUMMARY

Attempts were made to optimise the techniques for *in vitro* somatic organogenesis in selected varieties of *Anthurium andreanum*. Five varieties namely Dragon's Tongue, Flaking, Pompon Red, Honeymoon Red and Nitta were used. Responses of the varieties as well as explants were studied. Standardisation of surfacre sterilization as well as basal media, plant growth substances, culture medium components and conditions during the two stages of somatic organogenesis, namely, callus initiation and regeneration was attempted. The studies were conducted from January, 1994 to August 1995 at the Plant Tissue Culture, Laboratory, Department of Horticulture, College of Agriculture, Vellayani.

The salient findings of the studies are summarised below :

1. Among the different surface sterilization treatments tried on leaf explants, the highest per cent of sterile live cultures (88.8) was obtained with mercuric chloride 0.1 per cent for eight minutes. Even though sodium hypochlorite 1.0 per cent for twenty minutes gave 100 per cent sterile cultures, it

produced tissue browning. Among the three explants tried, the leaf explants with the smallest vascular bundles recorded the maximum percentage of sterile cultures compared to the petiole and spadix explants.

2. Variations were observed in the response of different varieties to callus initiation. Even though the maximum percentage of cultures initiating callus was obtained in the variety Nitta (75.0), the callusing was scanty and did not grow on subculturing. The variety Dragon's Tongue produced the biggest size callus and showed a good response of 50.0 per cent. The other varieties produced callusing only to a very small extent. The variety Dragon's Tongue only produced caulogenic callus capable of regeneration. All the other varieties produced non caulogenic calli which failed to regenerate. Hence the variety Dragon's Tongue was used for further refinement of callus initiation and shoot proliferation.

3. Three types of explants viz, sections of leaf and segments of petiole and spadix were tried. Callusing was observed only in leaf sections. Petiole and spadix segments produced only swelling.

4. Modified MS medium was the best basal medium for callus initiation (10.0 per cent). Nitsch's medium produced callus to a lesser extent (6.0 per cent) whereas MS, LS, SH and B5 basal media did not produce callus.

5. Lowering the ammonium nitrate concentration had a beneficial effect on callusing. The maximum number of cultures initiating callus (50.0 per cent) was recorded in modified MS basal medium with a reduced ammonium nitrate level of 200 mg/l. However a lower response (15.0 per cent) was observed on further lowering the ammonium nitrate level to 100 mg/l.

6. BA 0.5 mg/l + 2,4-D 0.5 mg/l was found to be best combination of plant growth substances for callus initiation.

7. Sucrose 30.0 g/l was found to be a better carbon source than glucose 30.0 g/l for callus initiation.

8. Callus formation took place when the cultures were kept under darkness. There was no response under light.

9. Regeneration was obtained for the variety Dragon's

Tongue in the MS basal medium supplemented with BA 0.5 mg/l, IAA 2.0 mg/l, sucrose 30.0 g/l and agar 6.0 g/l. Regeneration occured only under light.

10. MS basal medium was found ideal for shoot proliferation (6.26 shoots) compared to Nitsch's medium (3.67 shoots)

11. The full strength of ammonium nitrate in the MS medium was found to be best for shoot proliferation. Decreasing the concentration of ammonium nitrate was found to reduce the rate of shoot proliferation.

12. Full strength of organics in the MS medium was found to be the most responsive for shoot proliferation.

13. Sucrose 30.0 g/l was found to be the best carbon source for shoot proliferation.

14. Three different media supplements viz, casein hydrolysate, glutamine and coconut water were tried to find their effect on shoot proliferation. Casein hydrolysate (150 mg/l) when incorporated into the medium (7.33 shoots) was found superior to control (6.26 shoots). However treatments containing either glutamine or coconut water was found to be inferior

to the control.

15. Among the various combinations of plant growth substances tried for shoot proliferation, kinetin 1.5 mg/l + IAA 3.0 mg/l was found to be most promising (13.49 shoots).

16. Improvement in the growth of shoots was observed when cultured on MS medium supplemented with activated charcoal (1.0 g/l) followed by subculturing in MS medium supplemented with kinetin 0.5 mg/l and IAA 16.0 mg/l.

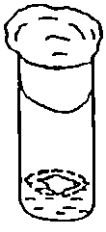
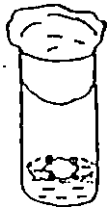
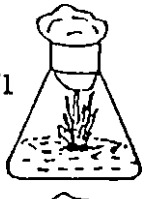
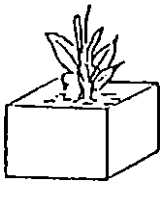
17. Agar was found superior to agarose as a gelling agent in the shoot proliferation studies. Agar at a concentration of 6.0 g/l was found most responsive.

18. Rooting of shoots occured simultaneously. Hence no seperate rooting treatments were necessary.

19. Plantlets survived better than microshoots when planted out.

PROTOCOL

Species : Anthurium andreanum var. Dragon's Tongue (Red)

Factors influencing		Stages & Duration
Explant-Leaf		Explant
Mercuric chloride 0.1% for 8 minutes		Surface-sterilization
MMS*+2,4-D 0.5 mg/l + BA 0.5 mg/l + sucrose 30.0g/l+ agar 6.0 g/l		Explanting 50 - 60 days
MMS* + 2,4D 0.5 mg/l + BA 0.5 mg/l + sucrose 30.0 g/l + agar 6.0 g/l		Callus initiation 60 days
MMS* + 2,4-D 0.5 mg/l + BA 0.5 mg/l + sucrose 30.0 g/l + agar 6.0 g/l		Callus multiplication 30 days
MS + BA 0.5 mg/l + IAA 2.0 mg/l + sucrose 30.0 g/l + agar 6.0 g/l		Sprout regeneration 72 - 90 days
MS + kinetin 1.5 mg/l + IAA 3.0 mg/l + CH 150 mg/l + sucrose 30.0 g/l + agar 6.0 g/l		Shoot proliferation, growth & rooting 30 days
Potting medium - sand		Planting out

MMS* - Modified MS basal medium with NH_4NO_3 200 mg/l

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* - Originals not seen.

APPENDIX - 1

Composition of MS and modified MS basal media

Nutrient	Quantify per litre (mg/l)	
	MS	Modified MS
Macronutrients		
NH_4NO_3	1650.00	825.00
KNO_3	1900.00	950.00
$MgSO_4\ 7H_2O$	370.00	370.00
KH_2PO_4	170.00	85.00
$CaCl_2.\ 2H_2O$	440.00	440.00
Micronutrients		
H_3BO_3	6.20	6.20
$MnSO_4.\ 4H_2O$	22.30	22.30
$ZnSO_4.\ 7H_2O$	8.60	8.60
KI	0.83	0.83
$Na_2MoO_4.\ 2H_2O$	0.25	0.25
$CuSO_4.\ 5H_2O$	0.025	0.025
$CoCl_2.\ 6H_2O$	0.025	0.025
$FeSO_4.\ 7H_2O$	27.85	27.85
$Na_2EDTA.\ 2H_2O$	37.25	37.25
Vitamins		
Thiamine. HCl	0.10	0.10

Pyridoxine. HCl	0.50	0.50
Nicotinic acid	0.50	0.50
Amino acid		
Glycine	2.00	2.00
Others		
Inositol	100.00	100.00
Sucrose*	30.00	30.00
Agar*	6.00	6.00

* g/l.

APPENDIX - II

Composition of Nitsch's, B5, LS and SH basal media

Nuttrient	Quantify per litre (mg/l)			
	Nitsch's	B5	LS	SH
Macronutrients				
NH_4NO_3	720	-	1650	-
$(NH_4)_2SO_4$	-	134	-	-
$NH_4H_2PO_4$	-	-	-	300
KNO_3	950	2500	1900	2500
$MgSO_4.7H_2O$	185	250	370	400
KH_2PO_4	68	-	170	-
$NaH_2PO_4.H_2O$	-	150	-	-
$CaCl_2.2H_2O$	166	150	440	200
Micronutrients				
H_3BO_3	10.00	3.00	6.20	5.00
$MnSO_4 . 4H_2O$	25.00	13.20	22.30	13.20
$ZnSO_4 . 7H_2O$	10.00	2.00	8.60	1.00
KI	-	0.75	0.83	1.00
$Na_2MoO_4 . 2H_2O$	0.25	0.25	0.25	0.10
$CuSO_4 . 5H_2O$	0.25	0.025	0.025	0.20
$CoCl_2 . 6H_2O$	-	0.025	0.025	0.10
$FeSO_4 . 7H_2O$	27.85	27.85	27.85	15.00

Na_2 EDTA . $2H_2O$	37.25	37.25	37.25	20.00
Vitamins				
Thiamine . HCl	0.50	10.00	0.40	5.00
Pyridoxine . HCl	0.50	1.00	-	0.50
Nicotinic acid	5.00	1.00	-	5.00
Biotin	0.05	-	-	-
Folic acid	0.50	-	-	-
Amino acid				
Glycine	2.00	-	-	-
Others				
Inositol	100	100	100	1000
Sucrose*	30	30	30	30
Agar*	6	6	6	6

* g/l

APPENDIX - III

* Description of varieties of Anthurium andreanum Lind.

Sl. No.	Name of variety	Plant height (cm)	Leaf length (cm)	Leaf width (cm)	Spathe length (cm)	Spathe width (cm)	Position of candle to spathe angle	Candle length (cm)	Candle spathe ratio	Spathe colour	Young leaf colour	Young petiole colour	Candle colour	Spathe texture	Suckering ability
1.	Dragon's Tongue	35	38	22.0	16.0	12.5	40°	6.7	1:4.2	Maroon red	Red-dish green	Red-dish green	Cream	Deeply blistered	-
2.	Pompon red	32	30	16.0	7.5	8.0	25°	5.5	1:2.8	Deep red	Red-dish brown	Red-dish brown	Light yellow	Lightly blistered	+
3.	Flaking	30	25	13.0	12.0	8.5	50°	7.0	1:2.9	Bright red	Light green	Red-dish green	Light yellow	Lightly blistered	+
4.	Honeymoon Red	46	43	26.0	16.0	12.0	80°	10.0	1:2.8	Red	Redi-dish green	Red-dish green	Light red	Smooth	+
5.	Nitta	28	29	14.0	14.0	12.0	40°	5.8	1:4.5	Dark Orange	Light green	Light green	Cream	Deeply blistered	-

* From Mercy, S.T. and Suma, M. Catalogueing of varieties of Anthurium andreanum Lind. (unpublished)

MICROPROPAGATION IN SELECTED VARIETIES OF *Anthurium andreanum* Lind.

BY

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ABSTRACT OF A THESIS

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ABSTRACT

Studies were conducted to optimise the *in vitro* propagation techniques *via* somatic organogenesis in *Anthuruim andreanum* varieties (Dragon's Tongue, Flaking, Pompon Red, Honeymoon Red and Nitta) during 1994-1995 at the Department of Horticulture, College of Agriculture, Vellayani.

All the five varieties responded to the callusing treatments in varying degrees. Regeneration was obtained only in the variety Dragon's Tongue and this variety was subjected to different treatments for refinement of callusing and shoot proliferation. The protocol for *in vitro* propagation of the variety Dragon's Tongue could be standardised.

Among the different explants tried only leaf explants were found responsive for callusing. Callus was best initiated (50.0 %) within 60 days when leaf explants were cultured in darkness on modified Murashige and Skoog basal medium (NH_4NO_3 200 mg/l) supplemented with 2,4-D 0.5 mg/l, BA 0.5 mg/l, sucrose 30.0 g/l and agar 6.0 g/l.

The callus cultures were subcultured in the same medium for two months for callus multiplication.

Regeneration was obtained within one month on Murashige and Skoog basal medium supplemented with BA 0.5 mg/l, IAA 2.0 mg/l, sucrose 30.0 g/l and agar 6.0 g/l. Light was essential for regeneration.

The maximum rate of shoot proliferation (13.49 shoots) was observed on Murashige and Skoog basal medium supplemented with kinetin 1.5 mg/l, IAA 3.0 mg/l, casein hydrolysate 150.0 mg/l, sucrose 30.0 g/l and agar 6.0 g/l after a period of six weeks.

Improvement in growth of shoots was obtained by culturing in Murashige and Skoog basal medium supplemented with activated charcoal (1.0 g/l) and further subculturing to Murashige and Skoog basal medium supplemented with kinetin 0.5 mg/l and IAA 16.0 mg/l.

A separate rooting phase was not necessary since satisfactory rooting was obtained in the shoot proliferation medium itself.

Rooted plantlets gave a survival rate of 60.0 per cent on planting out.