IMPROVEMENT OF PROPAGATION EFFICIENCY OF *Anthurium andreanum* Andre.

By

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THESIS

Submitted in partial fulfilment of the
requirement for the degree of

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Department of Pomology and Floriculture
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1997

DECLARATION

I hereby declare that the thesis entitled **"Improvement of propagation efficiency of *Anthurium andreanum* Andre."** 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, fellowship, associateship or other similar title, of any other University or society.

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We, the undersigned members of the Advisory Committee of **Miss. Anu G. Krishnan**, a candidate for the Degree of **Master of Science in Horticulture**, with major in Pomology and Floriculture, agree that the thesis entitled **"Improvement of propagation efficiency of *Anthurium andreanum* Andre."** may be submitted by Miss. Anu G. Krishnan in partial fulfilment of the requirements for the Degree.

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ANU G. KRISHNAN

Dedicated to

my

Loving Parents

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Abbreviations used in the thesis

BA	Benzyl adenine
BAP	6-benzyl amino purine
°C	degree celcius
Ca	Calcium
CaCl2	Calcium chloride
Cm	centimeter
CW	Coconut water
°	degree
2,4-D	2,4-dichlorophenoxy acetic acid
GA_3	Gibberellic acid
g	gram(s)
HCl	Hydrochloric acid
IAA	Indole-3-acetic acid
2iP	2-isopentenyl adenine
K	Potassium
Mg	Magnesium
mg	milligram(s)
mg l^{-1}	milligram(s) per litre
ml	millilitre
mM	milliMolar
µM	microMolar
MS	Murrushige and Skoog's (1962) medium
N	Normality
NaoH	Sodium hydroxide
NAA	Naphthalene acetic acid
PBA	6-(Benzylamino)-9-(2-tetrahydropyranyl)-9H-purine
Psi	Pounds per square inch
%	per cent
TIBA	2,3,5-triiodo benzoic acid

Introduction

INTRODUCTION

Anthuriums are tropical plants of great beauty and grown either for their showy cut flower or for their unusually attractive foliage. They are gaining popularity as one of the most important commercial ornamental crops of the modern world. They are very popular with flower arrangers because of the bold effect and lasting qualities of flower when cut.

The name anthurium is derived from the Greek words, `anthus' which means flower and `aura' means tail, referring to the spadix. Anthuriums are perennial plants with creeping, climbing or arborescent stems. Leaves are variable and evergreen. The hermaphrodite flowers, which are small and insignificant, are densely packed on a cylindrical spadix subtended in large-heart shaped spathe. The spathe may either be flat or slightly undulating, ending with a pointed tip. Spathe and in many cases spadix are brilliantly coloured ranging from scarlet, red, salmon, orange, pink to white. Varieties having bicoloured spathe and spadix below spathe are also gaining importance as novel types.

The genus Anthurium belongs to the family Araceae. It consists of 600-700 known species, though there are

probably not more than fifty in cultivation and perhaps not more than ten or fifteen known to the trade (Bailey, 1963). Among the different species two most important ones are *Anthurium andreanum* and *A. scherzerianum*. *A. andreanum* is a native of Colombia and is grown almost exclusively for cut flower production. Growing of anthuriums on a commercial scale has recently been started in different countries. Hawaii is the largest producer of anthuriums in the U.S.A. Other leading producers of anthuriums are the Netherlands, Jamaica, Mauritius and Sri Lanka. Anthurium was introduced to India via England by tea and coffee planters.

Anthurium cut flower production in India is still in its infancy due to lack of good elite planting material and standard cultural practices. Anthurium occupies a very prominent place in the commercial floriculture industry of Kerala. Availability of planting material and marketing of flowers have been viewed as the major problems of commercial cultivation of anthurium in Kerala. The planting materials are priced very high ranging from 50 to 250 rupees per plant and it goes up to 700 rupees in case of good hybrid varieties.

Anthuriums are commonly propagated by seed and vegetatively by suckers and cuttings. Sucker production has been found to be a varietal character and most of the

hybrids are reported to be shy suckering. Increasing production of lateral shoots in anthurium by using growth regulators could be an effective method to improve suckering ability. *In vitro* propagation of anthurium hybrids is also widely used commercially, but has shortcomings in being relatively slow or unreliable with occasional somaclonal variation.

Artificial seeds or synseeds, consisting of tissue culture derived somatic embryos encased in a protective coating have been suggested as a powerful tool for mass production of elite plant species. Advantages of artificial seeds over somatic embryogenesis for propagation include ease of handling and potential long term storage. In view of the high volume, low cost of production and subsequent propagation, this technique is considered as an alternative method of propagation (Redenbaugh *et al.*, 1987). Micropropagation via synthetic seeds or synseeds holds tremendous potential in the anthurium production industry, because of the feasibility of rapid, large scale propagation of desired genotypes at a reduced cost of production (Redenbaugh *et al.*, 1991; Marsolais *et al.*, 1991).

The present study was thus undertaken to improve the propagation efficiency of anthurium with the following objectives.

1. To study the effect of growth substances on lateral shoot production.

2. To attempt somatic embryogenesis to produce synseeds through encapsulation of somatic embryos.

Review of literature

REVIEW OF LITERATURE

The effect of growth regulators on lateral shoot production and somatic embryogenesis in ornamental plants with particular reference to anthurium, has been reviewed in this chapter.

2.1 Effect of growth regulators on lateral shoot production

2.1.1 Effect of growth regulators on anthurium

Anthuriums are vegetatively propagated to ensure uniformity of cultivars. Conventional methods of propagation involve sprouting of vegetative buds positioned opposite to each leaf, at alternate nodes (Higaki, 1976). Topping of mature plants induces development of one or more lateral shoots, which are planted as cuttings once roots have developed.

Higaki and Rasmussen (1979) used various growth regulators to increase shoot development on mature anthurium plants. Foliar treatment with BAP at 1000 mg l^{-1} induced more shoot formation than ethephon or PBA at 100,500,1000 and 1500 mg l^{-1}. In a study on GA_3 effects on anthurium flowering, increased side shoot production was observed in mature plants treated with GA_3 concentrations of 250 to 1000 mgl^{-1} (Higaki and Rasmussen, 1979).

Use of juvenile plants, which have more nodes per unit stem length than do mature plants, appear to be a potentially rapid and inexpensive method of propagating anthuriums, as topped juvenile anthurium plants were found to produce more suckers than untopped plants (Higaki and Imamura, 1987).

Imamura and Higaki (1988) reported that when juvenile anthurium plants were treated by topping and/or with foliar sprays of GA_3 and BA, with increasing concentration (0 to 500 mg l^{-1}) of GA_3, topped plants showed an increase in lateral shoots and with increasing concentration (0 to 1000 mg l^{-1}) of BA, the number of lateral shoots increased on both topped and intact plants. Topping alone also increased lateral shoots.

A study conducted by Salvi (1997) in *A. andreanum* revealed that GA_3 750 mg l^{-1} produced maximum number (2.67) of lateral shoots and the suckers produced per plant was maximum (1.35) when BA 750 mg l^{-1} was used.

2.1.2 Effect of growth regulators on other ornamental aroids

Lateral bud break on *Dieffenbachia maculata* was increased significantly with a single foliar application of BAP at 500, 1000 and 2000 mg l^{-1}, but ethephon applied at the same rates was ineffective (Wilson and Nell, 1983). Henny and Fooshee (1985) found that application of 500 or

1000 mg l^{-1} BAP as a soil drench to spathiphyllum was shown to be more effective in increasing basal shoot production than as a foliar spray.

Foliar sprays of BAP were used successfully to increase lateral shoot production on a non branching dieffenbachia hybrid (Henny, 1986). In this study 3 foliar sprays of 250 mg l^{-1} BA, applied on consecutive days, were more effective than 1 or 2 sprays; 3 sprays at 500 or 750 mg l^{-1} BAP yielded more shoots than 250 mg l^{-1}.

In a related study, Henny and Fooshee (1986) reported that BAP treatment significantly increased basal shoot production on spathyphyllum 'Bennet' whereas other cytokinins did not. Wang and Boogher (1987) showed that foliar sprays of BAP on *Syngonium podophyllum* at 3 and 5 leaf stages induces earlier bud development of basal lateral shoots at increasing concentrations.

Foliar application of 500 to 1000 mg l^{-1} BAP or pyranyl benzyladenine to stock plants of *Epipremnum aureum* induced axillary bud elongation (Wang, 1990).

2.1.3 Effect of growth regulators on other ornamental plants

Growth substances have been extensively used to improve branching of other ornamental plants also. BAP and adenine induced bud break and shoot development on both pruned and unpruned greenhouse roses (Parups, 1971).

Milbocker (1972) observed that kinetin at 1 mg l^{-1} applied to poinsettia buds stimulated axillary bud development and shoot growth. Carpenter and Beck (1972) also reported that sprays of PBA and BAP effectively increased branching on poinsettia stock plants.

Foliar sprays of ethephon, 6-benzyl amino purine (BAP) and PBA significantly increased branching of pinched geraniums (Carpenter and Carlson, 1972a). BAP at 400 mg l^{-1} and PBA at 200 mg l^{-1} significantly increased branching in Chrysanthemum (Carpenter and Carlson, 1972b).

Kunisaki (1975) suggested that axillary shoots can be induced in orchids using PBA. In *Hedera helix* branching was enhanced and secondary lateral shoots were produced on pruned plants when GA_3 was applied as foliar spray (Lewns and Moser, 1976). GA_3 spray applications stimulated lateral budbreak of *Skimmia japonica,* with effects only slightly modified by sprays of BAP and TIBA (Whalley and Loach, 1978).

Outgrowth of axillary buds of *Pinus sylvestris* could be achieved with 225 mg l^{-1} BAP applied biweekly during a few months (Kossuth, 1978). Cohen (1978) showed that a single application of 500-1000 mg l^{-1} BAP resulted in axillary bud development in *Pinus strobus* after 8 weeks. In coleus, BAP at 800 mgl^{-1} increased the number of lateral branches (Khosh-Khui *et al.*, 1978).

Criley (1980) reported that foliar BAP sprays can be used to stimulate lateral branching in dracaena. Multiple axillary shoots were obtained from *Cordyline terminalis*, treated once weekly for 8 weeks, with 6 benzyl amino purine (BAP) from 100 to 500 mg l^{-1} (Maene and Debergh, 1982).

Injections of GA_3 into *Musa* lateral buds, before floral initiation, stimulated sucker production (Swennen *et al.*, 1984). Henny (1985) reported that BAP induced lateral branching of peperomia. Rudnicki and Rejman (1985) observed that branching was best in *Piltosporum tobira* after treatment with BAP at 200 mg l^{-1} and in *Chrysobalania icaco* with BAP at 100-800 mg l^{-1}. Branching of poinsettia and gerbera stock plants were increased after five repeated spraying with N^6 benzyladenine and N^6 (m-hydroxybenzyl) adenosine at 10^{-4} mg l^{-1} (Kaminek *et al.*, 1987).

Application of BAP at 100-400 mg l^{-1} to defoliated *Gerbera jamesonii* increased production of lateral shoots compared with untreated defoliated plants (Zieslin *et al.*, 1988). Michalak (1989) found that application of GA_3 at 50 mgl^{-1} stimulated new axillary bud development in *Ficus benjamina* and *Codiaeum variegatum*. The number of new axillary buds increased as GA_3 concentration was increased up to 200 mg l^{-1}.

In cryptanthus the number of offshoots produced was increased with four foliar sprays of BAP at two weeks interval and the most effective concentration was 800 mg l^{-1} BAP (Pytlewski, 1989). Al-Juboory and Williams (1990) reported that in *Hedera helix* sub sp. *canariensis* branch number, branch length and number of cuttings available increased significantly with a single spray application of BAP plus GA_{4+7} compared with untreated control plants.

Foliar application of BAP at 10 or 30 mg l^{-1} increased lateral bud elongation of verbena (Svenson, 1991). Foley and Keever (1992) found that foliar applications of Promalin (BA + GA_{4+7}) and Acel (PBA) at 75 mg l^{-1} or Florel (Ethephon) at 500 mg l^{-1} increased the axillary shoot development in geranium stock plants. Spraying of BAP at 25 mg l^{-1} three times weekly for sixteen weeks increased lateral shoot formation on *Tillandsia aeranthos* (Bessler and Zimmer, 1993).

Promalin at 250, 500 or 1000 mgl^{-1} increased the number of primary and secondary runners in *Vinca minor* cv. Alba (Foley and Keever, 1993). Bessler (1995) observed that BAP application increased lateral shoot production in *Physostegia virginiana* and *Coreopsis grandiflora* even at lower concentrations.

Ackerman and Hamernik (1995), in a nursery trial, found that GA_3 spraying increased lateral shoots significantly, in heuchera and scabiosa.

2.1.4 Effect of growth regulators on flowering

In *Dieffenbachia maculata,* number of inflorescence per plant was increased significantly with GA_3 100 mg l^{-1} (Henny, 1980). Promalin was found to be useful for flower induction in *D. maculata* (Gronborg, 1987). Biswas *et al.* (1983) reported that in tuberose number of flower spikes per plant was increased by GA_3 100 mg l^{-1}. Mukhopadhyay and Banker, 1986 stated that GA_3 advanced flowering and length of the flower spike in gladiolus. In marigold GA_3 200 mg l^{-1} increased the number and size of flowers (Lal and Mishra, 1986).

In spathiphyllum, GA_3 at 1250 mg l^{-1} was found to be inducing flowering (Blacquiere and De, 1990). Ohno and Kako (1991) observed that GA_3 (40 mg l^{-1}) stimulated stalk elongation and flower development in cymbidium. Flower production increased significantly by GA_3 500 mg l^{-1} in *Anthurium scherzerianum* (Henny and Hamilton, 1992). In gladiolus, greatest flower spike length, rachis length and number of florets per spike were obtained with the 50 mg l^{-1} GA_3 treatment (Ravidas *et al.* 1992).

Henny (1993) reported that GA_3 400 mg l^{-1} application increased flowering in *Agalonema commutatum.* GA_3 150 mg l^{-1} produced the heaviest and largest flowers in chrysanthemum with the longest vase life in distilled water (Deotale *et al.* 1995).

2.2 Somatic embryogenesis

Somatic embryogenesis is the process by which haploid or diploid somatic cells develop into differentiated plants through characteristic embryological stages without fusion of gametes. The first report of somatic embryogenesis was given by Reinert (1959) in carrot cultures. General pattern of *in vitro* embryogenesis includes direct initiation from differentiated tissue and indirect initiation via callus intermediary. Direct embryogenesis proceeds from embryogenically determined cells (Kato and Kateuchi, 1963). Indirect embryogenesis requires differentiation of the cells, callus proliferation and differentiation of embryogenic cells (Sharp *et al.*, 1980). The positive results are limited to a few species, but is a more rapid mode of plant regeneration (Evans *et al.*, 1981). It requires auxin medium for the induction of embryos and a medium devoid of growth regulators for its maturation (Ammirato, 1983). Hussey (1986) also reported that the level of growth regulators in the culture medium, particularly when the auxin level was lowered, there was the chance for the embryo formation.

Successful somatic embryogenesis had been reported in various crops (Murashige, 1978; Sharp *et al.*, 1979; Vasil and Vasil, 1980). Cheng and Raghavan (1985) could obtain somatic embryos in hyosyamus where pattern of development was similar to that of zygotic embryos. The somatic

embryos were formed from friable calli produced from petiole and ovary explants in medium containing 2,4-D. Embryogenesis was initiated when the friable calli were cultured in a medium lacking auxin. In *Nardostachys jatamansi,* it was found that embryogenesis could be initiated from callus upon subculture to a medium containing lesser auxin (NAA) and more cytokinin (kinetin) while the concentrations of NAA was to be more and kinetin less for callus initiation as reported by Mathur (1993). Induction of somatic embryogenesis by different growth regulators was observed in leaf disc cultures of *Nicotiana tabaccum* L. by Gill and Saxena (1993). Successful somatic embryogenesis has also been reported in crops like *Coffea arabica* (Sondahl *et al.*, 1979), *Carica papaya* (Litz and Connover, 1982), *Malus pumila* (James *et al.*, 1984), *Oryza sativa* (Ram and Nabors, 1984), *Citrus limon* (Carini *et al.*, 1994) and *Elaeis guinensis* (Teixeira *et al.*, 1994).

Somatic embryogenesis and successful plant regeneration was also reported recently in many ornamental plants like begonia (Zhang *et al.*,1988), rose (Rout *et al.*, 1989), carnations (Frey *et al.*,1992), camellia (Pedroso and Pais,1993), chrysanthemum (Pavingerova *et al.*, 1994) and cyclamen (Kreuger, *et al.*,1995).

2.2.1 Somatic embryogenesis in *Anthurium*

In vitro propagation of anthurium hybrids is widely used commercially, but has shortcomings in being either relatively slow or unreliable with occasional somaclonal variation. Somatic embryogenesis is an alternative micro-propagation method. Recently, an embryogenic-like callus of *Anthurium andraeanum* cultured on medium containing 2,4-D and BAP was described by Kuehnle and Sugii (1991). Leaf explants produced callus most successfully after 2-3 months on a modified Pierik medium and petiole explant callused best on Pierik, modified Pierik and Finnie and Van Staden media. Plants were readily obtained from callus but regeneration appeared to be organogenic than embryogenic and it varied among the genotypes tested.

Geier (1982) observed some embryoid formation from spadix derived callus of *A. scherzerianum*, but plants were not recovered. Kuehnle *et al.* (1992) described a method for the production of somatic embryos and subsequent plant regeneration for *A. andraeanum* hybrids. Whole leaf blade explants derived from plantlets grown *in vitro* formed translucent embryogenic calli at their basal ends within one month of culture in the dark. Embryogenesis was induced on modified half-strength Murashige and Skoog (MS) medium supplemented with 1.0 to 4.0 mg l^{-1} 2, 4-D and 0.33 to 1.0 mgl^{-1} kinetin.

Leaf discs of *A. andreanum* produced somatic embryos on modified Nitsch and Nitsch medium containing BAP, kinetin and 2,4-D. The embryos were germinated into plantlets on a modified MS medium containing BAP (Rajasekaran and Kumar, 1994).

Histological analysis of somatic embryos derived from *in vitro* cultured laminas of *A. andreanum* showed bipolarity with the presence of shoot and root poles connected by procambium (Matsumoto *et al.*, 1996). The origin of somatic embryos was from a proembryonic cell complex or possibly from a single cell by direct embryogenesis.

2.2.2 Somatic embryogenesis in other ornamental monocots

Radojevic *et al.* (1987) obtained somatic embryogenesis and plant regeneration from zygotic embryo-derived callus cultures of *Iris pumila*. Embryogenic callus was successfully induced on Murashige and Skoog medium containing 2,4-D 1.0 mg l^{-1}. Somatic embryos were differentiated in the same medium. Further development of embryos was achieved in liquid medium. Havel and Novak (1988) reported somatic embryogenesis in *Allium carinatum* from callus cultures derived from roots on BDS medium without 2,4-D.

In *Freesia refracta* epidermal cells of young inflorescence segments cultured on modified N_6 medium

containing IAA 2 mg l^{-1} and BAP 3 mgl^{-1} produced embryoids directly which could develop into new plants (Wang *et al.*, 1994). Somatic embryogenesis was also achieved by transferring pale yellow translucent callus induced on surface of similar explants cultured on MS medium containing IAA 2 mg l^{-1}, BAP 0.5 mgl^{-1} and or NAA 0.5 mgl^{-1} on to a modified N_6 medium containing IAA 2 mgl^{-1} and BAP 3 mgl^{-1} (Wang *et al.*, 1990).

Laublin *et al.* (1991) reported somatic embryos in *Iris pseudocorus, I. versicolor* and *I. setosa.* Callus was induced on root cultures on induction media containing 2, 4-D (45 or 22.5 *μM*), NAA (5.4 *μM*) and kinetin (0.5 *μM*). Somatic embryos were developed after transfer of callus on to regeneration media containing 9 or 22 *μM* BAP and 2*μM* TIBA or 9 *μM* BAP and 4 *μM* TIBA.

Lilien-Kipnis *et al.* (1992) obtained somatic embryogenesis and plant regeneration from inflorescence explants of *Nerine manselli* in liquid culture. Compact but friable meristematic callus was obtained in liquid medium containing 0.25 *μM* NAA, 10 *μM* BAP and paclobutrazol 2.5 mg l^{-1}. Exclusion of paclobutrazol from proliferation medium resulted in the development of embryonic masses. Differentiation of somatic embryos was enhanced by 2iP and these germinated in growth regulator free medium.

Plant regeneration of *Iris setosa* through somatic embryogenesis was reported by Radojevic and Subotic (1992). Embryogenic calluses were induced from mature zygotic embryos on Murashige and Skoog (MS) medium supplemented with 2,4-D 5 mg l^{-1}, kinetin 1 mg l^{-1}, α-proline 250 mgl^{-1} and casein hydrolysate 250 mg l^{-1}. Somatic embryos were regenerated to plantlets in MS medium with 1 per cent sucrose and 1 mg IAA + 3 mg kinetin + 1 mg GA_3 per litre.

Embryogenic callus was initiated from inner tissues of protocorm like bodies of cymbidium cultured on Murashige and Skoog (MS) medium containing 2 mg l^{-1} NAA + 0.5 mg l^{-1} 2,4-D. These embryogenic calli produced proembryoid like structures after 10 days of culture, which become globular embryos differentiated into protocorms and plantlet after being transferred to the hormone free MS medium (Begum *et al.*, 1994).

Stefaniak (1994) produced friable embryogenic callus and somatic embryos of four gladiolus cultivars on Murashige and Skoog (MS) medium with various concentrations of 2,4-D or NAA from corm slices, young leaf bases and whole, intact plantlets. Somatic embryos transferred on MS hormone-free medium regenerated into plantlets. Jehan *et al.* (1994) developed a method of rapid multiplication of *Iris pallida* and *I. germanica* by somatic embryogenesis. The optimum medium for embryogenic callus on leaf bases, ovaries, petals, sepals or rhizome apices

was Murashige and Skoog (MS) medium supplemented with 2.9 g proline/litre. Following transfer of callus to embryogenic expression medium, somatic embryos appeared on the callus surface which developed to plantlets on the same medium.

Somatic embryogenesis and plantlet regeneration were achieved from an ornamental bamboo, *Otatea acuminata azetocorum* by culturing zygotic embryo explants on both Murashige and Skoog (MS) medium and Gamborg medium supplemented with 2,4-D, benzyl amino purine and 2 per cent sucrose. Over 95 per cent of these embryoids were germinated to plantlets (Woods *et al.*, 1994).

Rout and Das (1994) reported somatic embryogenesis in 3 species of ornamental bamboo. Embryogenic callus was obtained following the culture of nodal explants from *in vitro* grown seedlings and excised mature zygotic embryos of *Bambusa vulgaris*, *Dendrocalamus giganteus* and *D. strictus* on Murashige and Skoog (MS) medium supplemented with 0.5 mg l^{-1} kinetin, 2.0 mg l^{-1} 2,4-D, 10 mg l^{-1} adenine sulfate and 3 per cent sucrose in the light or dark. Somatic embryos germinated to normal plant and were transferred to soil with 95 per cent success.

Dias *et al.* (1994) observed somatic embryogenesis and plant regeneration in the tissue cultures of *Geonoma gamiova*. Immature zygotic embryos from young fruits were

inoculated on semi-solid medium containing Murashige and Skoog (MS) salts, Morel vitamins, 1.5 g l^{-1} activated charcoal, 100 mg l^{-1} 2,4-D and 2 mg l^{-1} 2iP. One month after inoculation embryogenic masses were formed which later regenerated to embryoids on medium with 20 mgl^{-1} 2,4-D. These embryoids germinated on growth regulator free medium.

Somatic embryogenesis was obtained from suspension callus of gladiolus. The suspension callus was induced from sprouted cormel in Murashige and Skoog (MS) medium containing various concentrations of NAA. Somatic *embryogenesis* was obtained when callus was placed on growth regulator free medium or medium containing 0.1 mg l^{-1} BAP (Tomotsune, *et al.* 1994).

Atta-Alla and Van (1996) observed indirect somatic embryogenesis from leaves of *Yucca aloifolia*. The embryogenesis occurred on half strength Murashige and Skoog (MS) medium containing 2,4-D 2 mg l^{-1} and BAP 1.0 mg l^{-1}. Direct embryogenesis also occurred to a lesser extent. Plantlets were regenerated on media without growth regulators.

2.2.3 Encapsulation of somatic embryos

The term artificial seed was first coined by Murashige (1977) and in simple terms it means a somatic embryo entrapped in a biodegradable synthetic polymer coating that acts as an artificial seed coat. Kamada (1985) broadened

the scope of artificial seed technology by defining this somatic propagule as a capsule prepared by coating a cultured matter such as a piece of tissue or an organ which can grow in to a complete plant body along with nutrients with an artificial covering.

Redenbaugh *et al.* (1986) encapsulated somatic embryos of alfalfa, celery and cauliflower as single beads to produce individual somatic artificial seeds. The somatic embryos were mixed in 3.2 per cent sodium alginate, dropped individually into a 50 mM solution of calcium chloride and allowed to undergo complexation for 30 minutes to form an alginate bead.

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

Synthetic seeds were developed also for mulberry (Bapat *et al.*, 1987), sandal wood (Bapat and Rao, 1988) and *Valeriana wallichii* (Mathur *et al.*, 1989). In pineapple a 2.5 per cent solution of sodium alginate upon complexation with 75 mM of $CaCl_2 . 2H_2O$ solution gave

optimal, firm and round beads within an ion exchange duration of 30 minutes. The encapsulated beads stored at room temperature (30°C) retained their regeneration capacity for 30 days when put in cotton wool moistened with MS basal salts (Prabha, 1993).

Keshavachandran *et al.* (1993) reported that differentiating calli obtained from immature inflorescence of *Vetiveria zizanioides* (L) Nash, were successfully encapsulated into beads using sodium alginate and calcium chloride at suitable concentrations to produce firm, round beads. The beads started differentiating into shoots when put on regeneration medium in 4 days. The encapsulated explants could be successfully stored at 4°C for 45 days without loosing their capacity to regenerate. The encapsulated explants differentiated and regenerated shoots earlier than non-encapsulated explants. Root initiation was also slightly precocious. Encapsulating the differentiating calli resulted in economizing on the medium, space and time.

Somatic embryos of *Asparagus cooperi* were encapsulated as single embryos approximately 4-6 mm in diameter to produce individual synthetic seeds. The highest plant regeneration (32.2%) was obtained with 3.5 per cent sodium alginate and 50 mM calcium chloride. After storage for 90 days at 2°C, the conversion frequency of artificial seed was 8.3 per cent (Ghosh and Sen, 1994). Dias *et al.* (1994)

produced synthetic seed of *Geonoma gamiova* by encapsulating somatic embryos in sodium alginate.

Artificial seeds of *Pelargonium hortorum* were developed by encapsulating somatic embryos using 3 per cent sodium alginate and 50 mM calcium chloride. The resultant synthetic seeds germinated normally and produced flowering plants within 12-14 weeks (Gill *et al.*, 1994).

Materials and methods

MATERIALS AND METHODS

The present study on improvement of propagation efficiency of *Anthurium andreanum* Andre., was carried out at the All India Co-ordinated Floriculture Improvement Project (AICFIP), College of Horticulture, Kerala Agricultural University, Vellanikkara during the period 1995 to 1997. The materials used and methodology adopted for the study are described in this chapter.

Plant material

Anthurium is an important ornamental plant belonging to the family Araceae. This genus consists of about 500 species. Among these *Anthurium andreanum* is the most important. The present study was conducted on 7-8 months old red-coloured anthurium plants (Plate 1). The experimental plants were grown in pots with the potting mixture containing coarse sand, well rotten cowdung clumps, coconut husk, wood shavings, charcoal, earthen crocks and brick pieces 2:1:2:1:0.5:0.5:1) and under 75 per cent shade.

3.1 Effect of growth substances on lateral shoot production

Under this experiment, two types of plants were used: intact (T_1) and topped plants (T_2). In topped plants top portion is removed retaining two leaves. The following different concentrations of Benzyl Amino Purine (BAP) and

Plate 1 *Anthurium andreanum* (Red) grown in pot

Gibberellic acid (GA_3) were tried to study the effect of growth regulators on lateral shoot production. Three replications were maintained under each treatment.

Growth regulators were applied as foliar spray to run off with 0.05 per cent teepol without falling on other plants. The growth regulators were applied at monthly intervals from October to December. Aqueous spray was given to control plants.

Growth regulators and their concentrations tried were :

Growth regulator	Concentration (mg l^{-1})
Benzyl Amino Purine (BAP)	250 (B_1)
	500 (B_2)
	750 (B_3)
	1000 (B_4)
Gibberellic acid (GA_3)	250 (G_1)
	500 (G_2)
	750 (G_3)
	1000 (G_4)

Preparation of growth regulators

Chemically pure salts of BAP and GA_3 were used for preparation of growth regulators. GA_3 and BAP were first dissolved in minimum quantity of 70 per cent ethanol and

NaOH respectively and then volume of each was made upto one litre by slowly adding distilled water. Treatment solutions of the required concentrations were prepared from stock solution by proper dilution with distilled water. These solutions were used on the same day of preparation.

Observations recorded

The following observations were recorded six and eight months after the commencement of treatments.

1. Number of lateral shoots
2. Size of shoot measured as length of shoot
3. Number of leaves per shoot
4. Length of leaf
5. Breadth of leaf
6. Leaf area

   Leaf area was calculated using the formulae, -4.27 + 0.729 LB, where L is length of leaf from leaf base to the leaf tip and B is width of leaf at centre, measured in centimetre (Salvi, 1997).
7. number of flowers per plant
8. Length of stalk
9. Length of spathe
10. Breadth of spathe
11. Length of spadix
12. Angle between spathe and spadix

    The angle between spathe and spadix was measured using a protractor.

13. Biochemical compositon of spadix

Spadix was dried and powdered and analysed for calcium (Ca), magnesium (Mg) and potassium (K) using Atomic Absorption Spectrophotometer (Jackson, 1958).

Statistical analysis

The data collected on different characters were analysed by applying the technique of analysis of variance (ANOVA) for completely randomised design (CRD) following Panse and Sukhatme (1985).

3.2 Development of protocol for somatic embryogenesis and production of synthetic seeds

Studies were carried out in the Tissue Culture Laboratory of AICFIP, College of Horticulture, Vellanikkara. The main aspects of the study consisted of induction of embryogenic calli and production of synthetic seeds by encapsulation of somatic embryos.

3.2.1 Collection of explants

The explants were collected from the anthurium plants maintained in the net house attached to Department of Pomology and Floriculture. In order to control the microbial contamination, the plants were regularly sprayed with contact fungicide Indofil-M-45 0.1 per cent at weekly interval.

3.2.2 Source of explants

Leaf, petiole, spathe and spadix were used as explant source. Young leaves about quarter the size of fully opened one before unfolding were used for taking leaf explants. Petiole from the same leaf was used as explant. Spadix used was taken before opening of spathe. Spathe of same spike was used as explant.

3.2.3 Surface sterilization

The explants were first washed with tap water containing few drops of Extran and then treated with 0.1 per cent Bavistin for 10 minutes. They were then washed with distilled water, blotted and wiped with cotton dipped in 70 per cent alcohol. Further sterilization procedure was carried out under the perfect aseptic conditions maintained in a laminar air flow cabinet. The explants were surface sterilized in beakers using different surface sterilants at various concentrations and duration as detailed in Table 1.

In all the treatments, the explants were submerged in the sterilant for the required period with frequent agitation. After surface sterilization the solution was drained off and the explants were washed four times using sterile water.

Table 1 Surface sterilants, their concentrations and duration of treatment for *in vitro* culture of different explants of *A. andreanum*.

Surface sterilants	Concentration (%)	Duration (minutes)
Domestos	5.00	5 10
Domestos	10.00	5 10
Mercuric chloride	0.05	5 7 10
Mercuric chloride	0.10	5 7 10
Alcohol	50.0	5 10
Alcohol	70.0	5 10

3.2.4 Culture medium

Half-strength MS medium (Murashige and Skoog, 1962) and Nitsch medium (Nitsch and Nitsch, 1969) were used for the study. Half strength MS medium with a basal composition of half strength macro nutrients and full strength MS micronutrients was used. The composition of different basal media tried are given in Appendix I.

3.2.5 Medium preparation

The various chemicals used for preparation of the medium were of analytical grade from SISCO Research Lab (SRL), British Drug House (BDH), Merck and Sigma. Standard procedures were followed for preparation of the medium. Stock solutions of major and minor elements were prepared first and were stored under refrigerated condition in amber coloured bottles. The growth regulators and vitamin stocks were prepared separately and fresh stocks were prepared at six week interval.

An aliquot of different stock solutions was pipetted out into a clean vessel which was rinsed with distilled water. Sucrose and inositol were added fresh and dissolved. Required quantities of growth regulators were also added and the solution was made upto the required volume. The pH of the solution was adjusted between 5.5 and 5.8 using 1N NaOH or 1N HCl. Agar was then added to the medium and stirred thoroughly.

The solutions were then melted by keeping in a water bath, maintained at a temperature of 92-95°C, until the medium became clear. About 15 ml of the medium was poured hot to oven sterilized culture tubes which were previously rinsed thoroughly twice with distilled water. The containers with the medium were then tightly plugged with nonabsorbent cotton wool plugs. Borosilicate test tubes of

size of 10.0 x 2.5 cm and 15.0 x 2.5 cm were used as the containers.

The medium was autoclaved by applying 15 psi pressure for 20 minutes. After sterilization the culture tubes were stored in an air conditioned culture room for further use.

3.2.6 Inoculation process

Inoculation was carried out under strict aseptic conditions in a laminar air flow cabinet. Sterilized forceps, petridishes, surgical blades and blotting paper were used.

In the case of leaf and spathe explants, after the surface sterilization, discs of about $1cm^2$ size were separated using sterile knife from the intact leaves and in case of petiole and spadix 1cm long bits were separated and cultured.

3.2.7 Culture conditions

The cultures were incubated in a culture room under dark. The temperature was maintained at 27 ± 2°C.

3.2.8 Induction of embryogenic calli

For inducing embryogenic calli the explants were cultured in half strength MS and Nitsch medium containing different concentrations of 2,4-D (0.06 - 15.0 mg l^{-1}),

Kinetin (0.3 - 3.0 mg l^{-1}), BAP (0.1 - 1.0 mg l^{-1}) and NAA (0.1 - 0.5 mg l^{-1}). The following treatment combinations were tried for induction of embryogenic calli:

1 ½ MS + 2,4-D 1 mg l^{-1}

2 ½ MS + 2,4-D 2 mg l^{-1}

3 ½ MS + 2,4-D 3 mg l^{-1}

4 ½ MS + 2,4-D 4 mg l^{-1}

5 ½ MS + 2,4-D 1 mg l^{-1} + Kinetin 0.3 mg l^{-1}

6 ½ MS + 2,4-D 2 mg l^{-1} + Kinetin 0.3 mg l^{-1}

7 ½ MS + 2,4-D 2 mg l^{-1} + Kinetin 0.4 mg l^{-1}

8 ½ MS + 2,4-D 3 mg l^{-1} + Kinetin 0.5 mg l^{-1}

9 ½ MS + 2,4-D 4 mg l^{-1} + Kinetin 0.3 mg l^{-1}

10 ½ MS + 2,4-D 4 mg l^{-1} + Kinetin 0.5 mg l^{-1}

11 ½ MS + 2,4-D 5 mg l^{-1} + Kinetin 1 mg l^{-1}

12 ½ MS + 2,4-D 6 mg l^{-1} + Kinetin 2 mg l^{-1}

13 ½ MS + 2,4-D 8 mg l^{-1} + Kinetin 3 mg l^{-1}

14 ½ MS + 2,4-D 9 mg l^{-1} + Kinetin 2 mg l^{-1}

15 ½ MS + 2,4-D 10 mg l^{-1} + Kinetin 2 mg l^{-1}

16 ½ MS + 2,4-D 11 mg l^{-1} + Kinetin 2 mg l^{-1}

17 ½ MS + 2,4-D 12 mg l^{-1} + Kinetin 2 mg l^{-1}

18 ½ MS + 2,4-D 15 mg l^{-1} + Kinetin 2 mg l^{-1}

19 ½ MS + 2,4-D 2 mgl^{-1} + BAP 1mg l^{-1} + Kinetin 0.3 mg l^{-1}

20 ½ MS + 2,4-D 2 mg l^{-1} + Kinetin 0.3 mg l^{-1} + Casein hydrolysate 100 mg l^{-1} + Glutamine 400 mg l^{-1}

21 ½ MS + 2,4-D 2 mg l^{-1} + Kinetin 0.3 mg l^{-1} + Casein hydrolysate 100 mg l^{-1} + 6% sucrose+Charcoal 50 mg l^{-1}

22 ½ MS + 2,4-D 2 mg l^{-1} + Kinetin 0.3 mg l^{-1} + Casein hydrolysate 200 mg l^{-1}

23 ½ MS + 2,4-D 2 mg l^{-1} + Kinetin 0.3 mg l^{-1} + Casein hydrolysate 300 mg l^{-1}

24 ½ MS + 2,4-D 2 mg l^{-1} + Kinetin 0.3 mg l^{-1} + Casein hydrolysate 400 mg l^{-1}

25 ½ MS + 2,4-D 2 mg l^{-1} + Kinetin 1 mg l^{-1} + Casein hydrolysate 250 mg l^{-1}

26 ½ MS + 2,4-D 2 mg l^{-1} + Kinetin 0.3 mg l^{-1} + BAP 1 mg l^{-1}

27 ½ MS + 2,4-D 2 mg l^{-1} + Kinetin 0.3 mg l^{-1} + CW 10%

28 ½ MS + 2,4-D 2 mg l^{-1} + Kinetin 0.3 mg l^{-1} + CW 15%

29 ½ MS + 2,4-D 2 mg l^{-1} + Kinetin 0.3 mg l^{-1} + CW 20%

30 ½ MS + 2,4-D 2 mg l^{-1} + Kinetin 0.3 mg l^{-1} + CW 25%

31 ½ MS + 2,4-D 2 mg l^{-1} + Kinetin 0.3 mg l^{-1} + CW 30%

32 ½ MS + 2,4-D 2 mg l^{-1} + BAP 1 mg l^{-1}

33 ½ MS + 2,4-D 2 mg l^{-1} + BAP 0.1 mg l^{-1}

34 ½ MS + 2,4-D 2 mg l^{-1} + BAP 0.3 mg l^{-1}

35 ½ MS + 2,4-D 2 mg l^{-1} + BAP 0.5 mg l^{-1}

36 ½ MS + 2,4-D 0.06 mg l^{-1} + BAP 1 mg l^{-1}

37 ½ MS + 2,4-D 0.08 mg l^{-1} + BAP 1 mg l^{-1}

38 ½ MS + 2,4-D 0.1 mg l^{-1} + BAP 1 mg l^{-1}

39 ½ MS + 2,4-D 2 mg l^{-1} + BAP 0.7 mg l^{-1}

40 ½ MS + 2,4-D 2 mg l^{-1} + BAP 0.9 mg l^{-1}

41 ½ MS + 2,4-D 2 mg l^{-1} + BAP 1.1 mg l^{-1}

42 ½ MS + 2,4-D 2 mg l^{-1} + BAP 1.3 mg l^{-1}

43 ½ MS + 2,4-D 2 mg l^{-1} + BAP 1.5 mg l^{-1}

44 ½ MS + NAA 0.1 mg l^{-1} + BAP 0.1 mg l^{-1}

45 ½ MS + NAA 0.2 mg l^{-1} + BAP 0.1 mg l^{-1}

46 ½ MS + NAA 0.3 mg l^{-1} + BAP 0.1 mg l^{-1}

47 ½ MS + NAA 0.4 mg l^{-1} + BAP 0.1 mg l^{-1}

48 ½ MS + NAA 0.5 mg l^{-1} + BAP 0.1 mg l^{-1}

49 ½ MS + NAA 0.1 mg l^{-1} + BAP 0.2 mg l^{-1}

50 ½ MS + NAA 0.2 mg l^{-1} + BAP 0.2 mg l^{-1}

51 ½ MS + NAA 0.3 mg l^{-1} + BAP 0.2 mg l^{-1}

52 ½ MS + NAA 0.4 mg l^{-1} + BAP 0.2 mg l^{-1}

53 ½ MS + NAA 0.5 mg l^{-1} + BAP 0.2 mg l^{-1}

54 ½ MS + NAA 0.1 mg l^{-1} + BAP 0.3 mg l^{-1}

55 ½ MS + NAA 0.2 mg l^{-1} + BAP 0.3 mg l^{-1}

56 ½ MS + NAA 0.3 mg l^{-1} + BAP 0.3 mg l^{-1}

57 ½ MS + NAA 0.4 mg l^{-1} + BAP 0.3 mg l^{-1}

58 ½ MS + NAA 0.5 mg l^{-1} + BAP 0.3 mg l^{-1}

59 ½ MS + NAA 2 mg l^{-1} + BAP 1 mg l^{-1}

60 Nitsch + 2,4-D 1 mg l^{-1}

61 Nitsch + 2,4-D 2 mg l^{-1}

62 Nitsch + 2,4-D 3 mg l^{-1}

63 Nitsch + 2,4-D 4 mg l^{-1}

64 Nitsch + 2,4-D 1 mg l^{-1} + Kinetin 0.3 mg l^{-1}

65 Nitsch + 2,4-D 1.5 mg l^{-1} + Kinetin 0.3 mg l^{-1}

66 Nitsch + 2,4-D 2 mg l^{-1} + Kinetin 0.3 mg l^{-1}

67 Nitsch + 2,4-D 2.5 mg l^{-1} + Kinetin 0.3 mg l^{-1}

68 Nitsch + 2,4-D 3 mg l^{-1} + Kinetin 0.3 mg l^{-1}

69 Nitsch + 2,4-D 3.5 mg l^{-1} + Kinetin 0.3 mg l^{-1}

70 Nitsch + 2,4-D 4 mg l^{-1} + Kinetin 0.3 mg l^{-1}

71 Nitsch + 2,4-D 2 mg l^{-1} + Kinetin 0.3 mg l^{-1} + BAP 1 mg l^{-1}

72 Nitsch + 2,4-D 2.5 mg l^{-1} + Kinetin 0.3 mg l^{-1} + BAP 1 mg l^{-1}

Results

RESULTS

The present investigation was carried out to study the effect of growth substances on lateral shoot production and to attempt production of artificial seeds through encapsulation of somatic embryos in anthurium. The experiment was conducted at AICFIP, Department of Pomology and Floriculture, College of Horticulture, Vellanikkara, Thrissur during 1995-1997. The results of the study are presented below.

4.1 Effect of growth regulators on lateral shoot production

4.1.1 Number of lateral shoots

The data on the effect of growth regulators on lateral shoot production during different months after their first application are presented in Table 2a and 2b. They revealed that one month after the first spray of growth regulators, the lateral shoots were produced only in topped plants treated with BAP. In the second month, lateral shoots began to appear on intact plants sprayed with different levels of BAP also. There was not much increase in the number of lateral shoots among various treatments during third, fourth, fifth and sixth months. After eight months of the first spray with GA_3 at different levels, intact plants also produced lateral shoots, and topped plants produced more shoots. In case of BAP treatments, even after eight months there was not much increase in lateral shoot production for both intact and topped plants.

Table 2a The effect of growth regulators on lateral shoot production during different months after the first application **(In intact plants)**

Treatments	Ist month	2nd month	3rd month	4th month	5th month	6th month	8th month
Benzyl amino purine (BAP)							
250 mg l^{-1}	0.00	1.67	2.67	2.67	3.67	3.67	3.67
500 mg l^{-1}	0.00	1.67	1.67	1.67	2.33	2.33	2.33
750 mg l^{-1}	0.00	1.33	1.33	1.33	1.33	1.33	1.67
1000 mg l^{-1}	1.00	1.00	1.00	1.67	2.00	2.33	3.00
Gibberellic acid (GA_3)							
250 mg l^{-1}	0.00	0.00	0.00	0.00	0.00	0.00	2.00
500 mg l^{-1}	0.00	0.00	0.00	0.00	0.00	0.00	1.33
750 mg l^{-1}	0.00	0.00	0.00	0.00	0.00	0.00	1.33
1000 mg l^{-1}	0.00	0.00	0.00	0.00	0.00	0.00	1.33
Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table 2b The effect of growth regulators on lateral shoot production during different months after the first application **(In topped plants)**

Treatments	Ist month	2nd month	3rd month	4th month	5th month	6th month	8th month
Benzyl amino purine (BAP)							
250 mg l^{-1}	1.33	1.33	1.33	2.33	2.33	2.33	2.33
500 mg l^{-1}	1.33	1.33	2.33	2.33	2.33	2.33	2.33
750 mg l^{-1}	2.00	2.67	2.67	2.67	2.67	2.67	2.67
1000 mg l^{-1}	1.00	1.00	1.00	1.00	1.00	1.00	1.67
Gibberellic acid (GA_3)							
250 mg l^{-1}	0.00	1.33	1.50	1.50	1.50	1.50	3.00
500 mg l^{-1}	0.00	1.33	1.33	2.00	2.33	2.67	4.33
750 mg l^{-1}	1.33	1.33	1.67	1.67	1.67	1.67	4.67
1000 mg l^{-1}	0.00	0.33	1.00	1.00	1.00	1.00	3.00
Control	1.33	1.33	1.33	1.67	1.67	1.67	1.67

The effect of topping on lateral shoot production is evident from Table 3, which compare topped and intact control plants. It was seen that topping alone increase lateral shoot production significantly (Plate 2). There were no side shoots in any of the intact control where as topped control produced a mean number of 1.67 lateral shoots.

A comparison of topped plants and intact plants on lateral shoot production is presented in Table 4. It revealed that six months after the first spray of growth regulators, no lateral shoots were produced in intact plants treated with GA_3 and intact control (Fig.1). All the other treatments produced lateral shoots, but the values were statistically *on par.* However, after eight months lateral shoots were formed on intact plants treated with different levels of GA_3 and the treatments were significantly different (Plate 3; Fig.2). GA_3 750 mg l^{-1} on topped plants recorded highest mean value (4.67) which was followed by GA_3 500 mg l^{-1} (4.33) on topped plants, BAP 250 mg l^{-1} (3.6) BAP 1000 mg l^{-1} (3.00), GA_3 250 mg l^{-1} (3.00) and GA_3 1000 mg l^{-1} on intact plants. All the above treatments were statistically *on par.* The lowest mean number of shoots (1.33) was recorded by GA_3 500 mg l^{-1}, GA_3 750 mg l^{-1} and GA_3 1000 mg l^{-1} on intact plants.

Data pertaining to the effect of growth regulators on lateral shoots in intact plant showed that six months after

Fig.1 Effect of growth regulators on lateral shoot production (after six months)

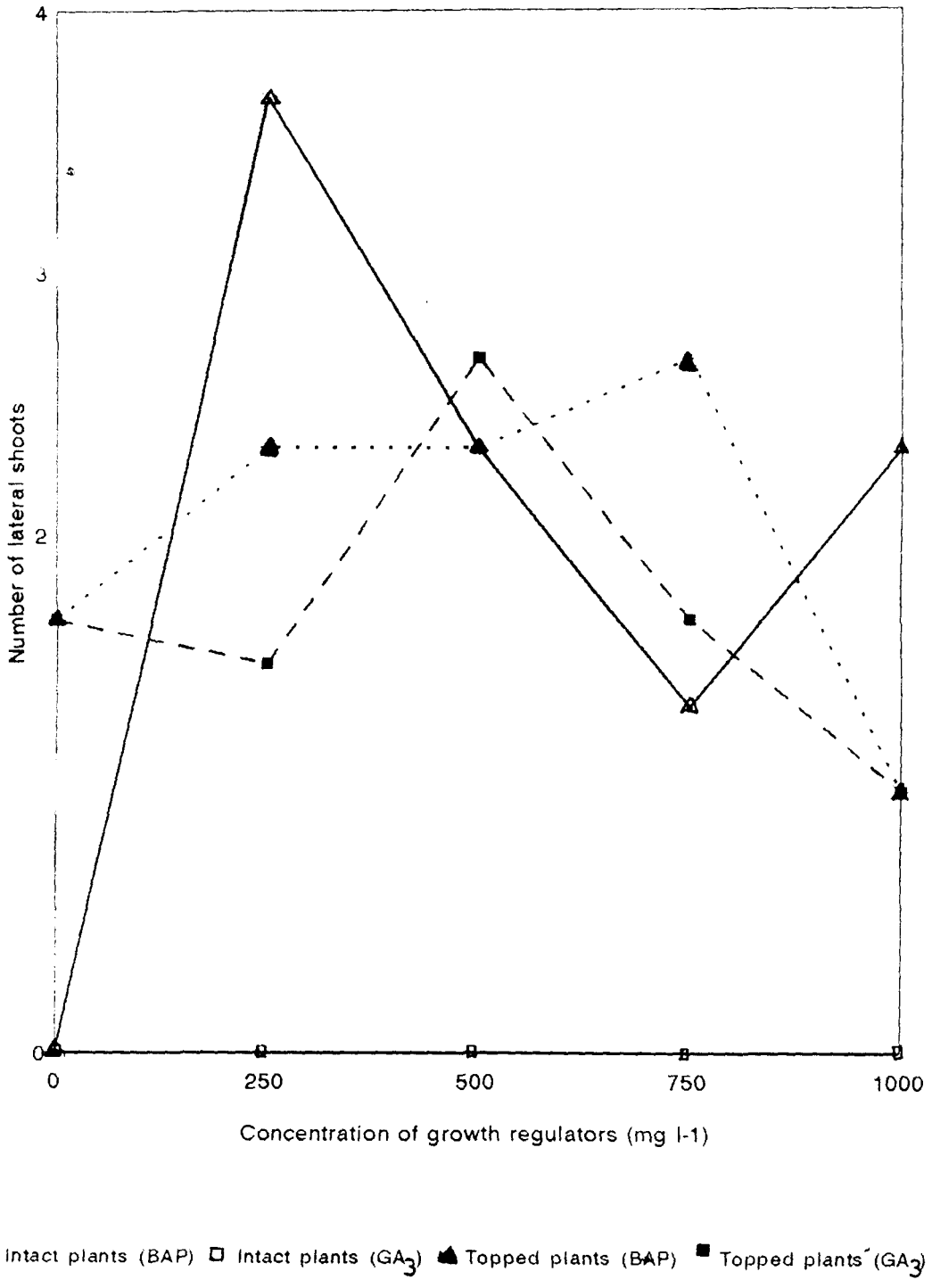


Fig.2 Effect of growth regulators on lateral shoot production (after eight months)

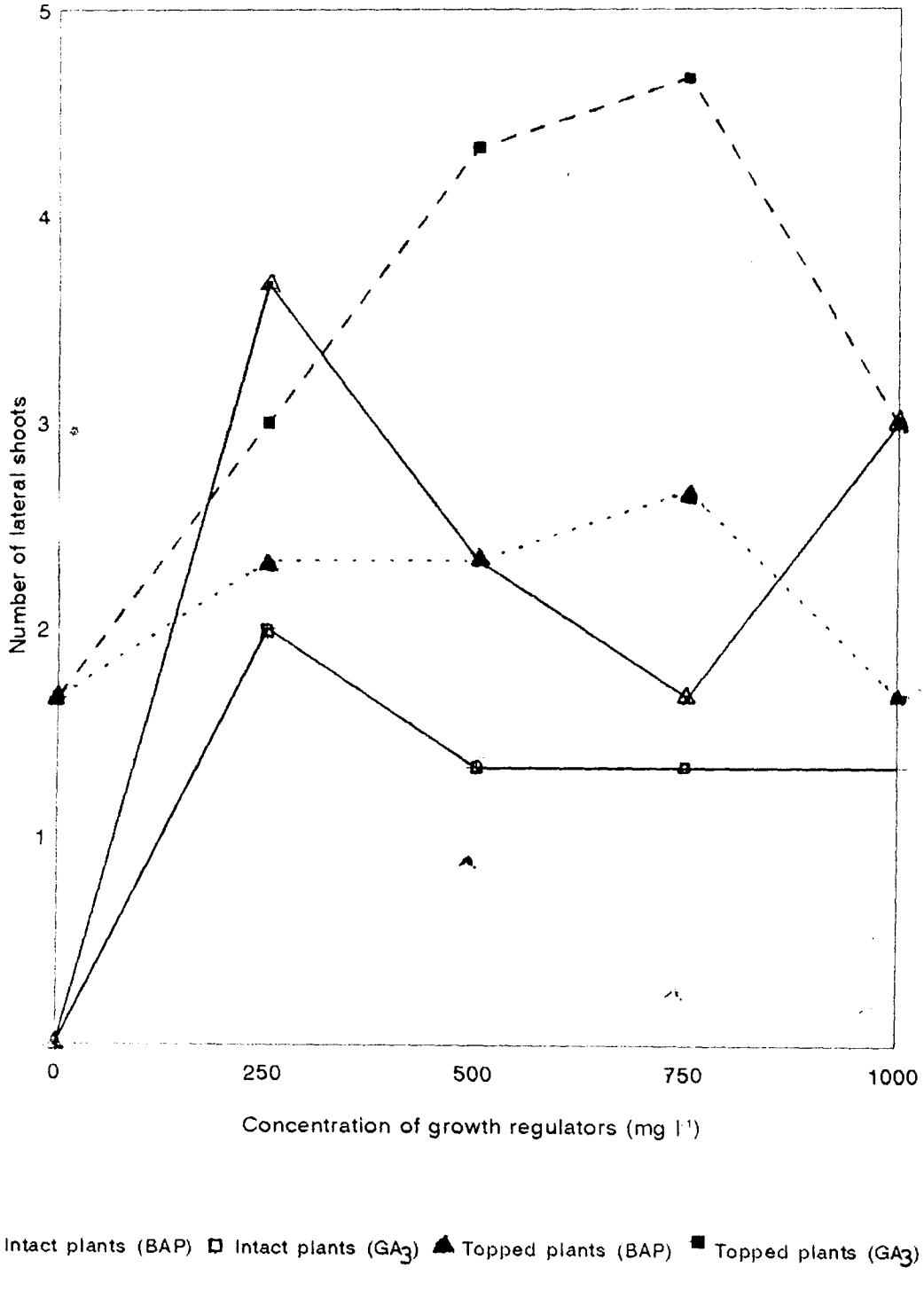


Plate 2 Effect of topping on lateral shoot production after eight months (T1 - intact, T2 - topped)

Plate 3 Effect of different levels of GA_3 on lateral shoot production in **intact plants** (T1) after eight months


CONTROL-T1
CONTROL-T2


CONTROL-T1
T1G1
T1G2
T1G3
T1G4

Table 3 Effect of topping on lateral shoot production in *A. andreanum*

Treatments	Mean number of lateral shoots
Intact control	0.00
Topped control	1.67

Table 4 Comparison of topped plants and intact plants on lateral shoot production

Treatments		6 months after		8 months after	
		Number of shoots per plant	Length of shoot (cm)	Number of shoots per plant	Length of shoot (cm)
Intact plants	Benzyl amino purine				
	250 mg l^{-1}	3.67	2.3^{a}	3.67^{bcd}	2.4^{a}
	500 mg l^{-1}	2.33	2.3^{a}	2.33^{ab}	3.5^{ab}
	750 mg l^{-1}	1.33	3.2^{ab}	1.67^{a}	4.1^{abc}
	1000 mg l^{-1}	2.33	1.4^{a}	3.00^{abcd}	2.7^{ab}
	Gibberellic acid				
	250 mg l^{-1}	0.00	0.00	2.00^{ab}	6.4^{abc}
	500 mg l^{-1}	0.00	0.00	1.33^{a}	6.1^{abc}
	750 mg l^{-1}	0.00	0.00	1.33^{a}	14.8^{cde}
	1000 mg l^{-1}	0.00	0.00	1.33^{a}	6.5^{abc}
	Control	0.00	0.00	0.00	0.00
Topped plants	Benzyl amino purine				
	250 mg l^{-1}	2.33	20.9^{cd}	2.33^{ab}	24.2^{ef}
	500 mg l^{-1}	2.33	25.6^{d}	2.33^{ab}	31.4^{f}
	750 mg l^{-1}	2.67	17.0^{bcd}	2.67^{abc}	20.9^{def}
	1000 mg l^{-1}	1.00	10.5^{abc}	1.67^{a}	13.0^{bcd}
	Gibberellic acid				
	250 mg l^{-1}	1.50	22.1^{cd}	3.00^{abcd}	18.5^{de}
	500 mg l^{-1}	2.67	28.3^{de}	4.33^{cd}	22.9^{def}
	750 mg l^{-1}	1.67	42.3^{e}	4.67^{d}	20.7^{def}
	1000 mg l^{-1}	1.00	29.5^{de}	3.00^{abcd}	13.5^{bcde}
	Control	1.67	27.3^{d}	1.67^{a}	29.5^{f}
	CD(0.05)	NS	14.56	1.88	10.815

* Treatment means in a column with same letters do not differ significantly

the first spray of growth regulators, BAP at different levels was superior to control (Table 5; Plate 4). But various BAP levels were statistically *on par*. However, BAP 250 mg l^{-1} gave maximum mean value for number of shoots (3.67) (Plate 5) followed by BAP 500 mg l^{-1} and BAP 1000 mg l^{-1} which recorded a mean value of 2.33.

Eight months after first spray there was significant difference among treatments in the number of lateral shoots (Table 6). BAP 250 mg l^{-1} showed highest average value (3.67) (Plate 9) as in the case of six month and it was followed by BAP 1000 mg l^{-1} (3.00) and 500 mg l^{-1} (2.33). However, BAP 250 mg l^{-1}, BAP 500 mg l^{-1}, BAP 750 mg l^{-1}, BAP 1000 mg l^{-1} and GA_3 250 mg l^{-1} were statistically *on par*. Lowest values for number of lateral shoots were recorded by GA_3 500 mg l^{-1}, GA_3 750 mg l^{-1} and GA_3 1000 mg l^{-1} which gave a mean value of 1.33. Among various GA_3 levels, GA_3 250 mg l^{-1} recorded maximum mean value (2.00) (Plate 5).

The observations on topped plants revealed that there was no significant difference between various treatments after 6 months and 8 months (Tables 7 & 8; Plates 6 & 7). However, BAP 750 mg l^{-1} and GA_3 500 mg l^{-1} showed highest mean value (2.67) after six months. Lowest mean value (1.00) was recorded by BAP 1000 mg l^{-1} and GA_3 1000 mg l^{-1}. After eight months GA_3 750 mg l^{-1} recorded highest mean value (4.67) (Plates 8, 10 & 11) followed by GA_3 500 mg l^{-1}. BAP 750 mg l^{-1} recorded highest mean value (2.67) among

Table 5 Effect of benzyl amino purine on lateral shoot production in **intact plants** (after a period of six months)

Concentrations	Number of lateral shoots per plant	Length of lateral shoots (cm)
250 mg l^{-1}	3.67	2.3
500 mg l^{-1}	2.33	2.3
750 mg l^{-1}	1.33	3.2
1000 mg l^{-1}	2.33	1.4
Control	0.00	-
CD	NS	NS

Note: No lateral shoots were produced after a period of six months in GA_3 treatments

Plate 4 Effect of different levels of BAP on lateral shoot production in intact plants (T_1)

Plate 5 Effect of BAP 250 mg l^{-1} and GA_3 250 mg l^{-1} on lateral shoot production in intact plants (T_1)


CONTROL-T1
T1B1
T1B2
T1B3
T1B4


CONTROL-T1
T1B1
T1G1

Table 6 Effect of growth regulators on lateral shoot production in intact plants (after a period of eight months)

Treatments	Number of lateral shoots per plant	Length of shoots (cm)
Benzyl amino purine (BAP)		
250 mg l^{-1}	3.67^{b}	2.4^{a}
500 mg l^{-1}	2.33^{ab}	3.5^{a}
750 mg l^{-1}	1.67^{ab}	4.1^{a}
1000 mg l^{-1}	3.00^{b}	2.7^{a}
Gibberellic acid (GA_3)		
250 mg l^{-1}	2.00^{ab}	6.4^{a}
500 mg l^{-1}	1.33^{a}	6.1^{a}
750 mg l^{-1}	1.33^{a}	14.8^{b}
1000 mg l^{-1}	1.33^{a}	6.5^{a}
Control	0.00	-
CD (0.05)	1.49	6.3

* Treatment means in a column with same letters do not differ significantly

Plate 6 Effect of different levels of BAP on lateral shoot production in topped plants (T_2)

Plate 7 Effect of different levels of GA_3 on lateral shoot production in topped plants (T_2)


CONTROL-T2
T2B1
T2B2
T2B3

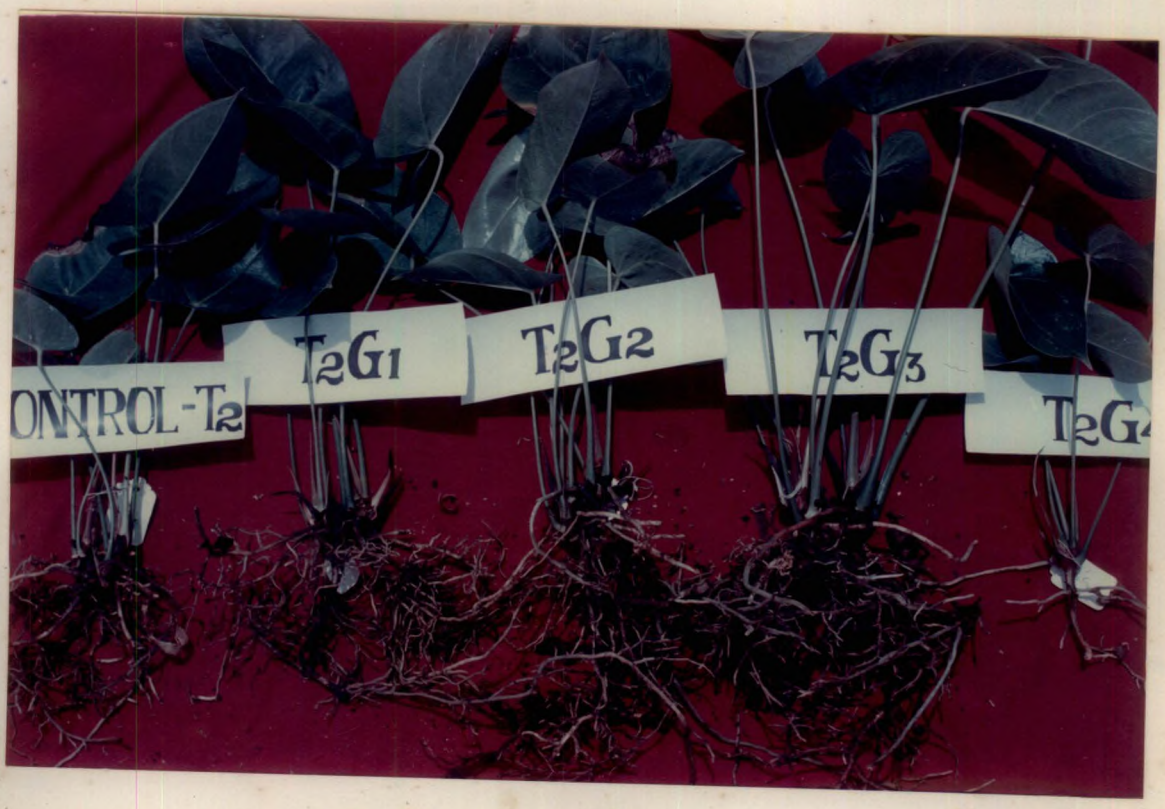

T2G1
T2G2
T2G3

Table 7 Effect of growth regulators on lateral shoot production in topped plants (after a period of six months)

Treatments	NUmber of lateral shoots/ plants	Length of shoot (cm)	Number of leaves	Mean leaf length (cm)	Mean leaf breadth (cm)	Mean individual leaf area (cm^2)
Benzyl amino purine (BAP)						
250 mg l^{-1}	2.33	20.9	1.43	12.83^{cd}	8.23^{c}	72.59 (8.52^{cde})
500 g l^{-1}	2.33	25.6	2.50	10.64^{bcd}	6.63^{bc}	45.02 (6.71^{abc})
750 mg l^{-1}	2.67	17.0	2.27	7.23^{ab}	5.33^{ab}	23.33 (4.83^{a})
1000 mg l^{-1}	1.00	10.5	1.27	4.23^{a}	3.67^{a}	18.75 (4.33^{a}0
Gibberellic acid (GA_3)						
250 mg l^{-1}	1.50	22.1	2.76	12.87^{cd}	7.45^{bc}	65.61 (8.10^{bcde})
500 mg l^{-1}	2.67	28.60	2.60	13.68^{cd}	8.20^{c}	77.44 (8.80^{de})
750 mg l^{-1}	1.67	42.3	2.83	14.47^{d}	8.40^{c}	84.27 (9.18^{e})
1000 mg l^{-1}	1.00	29.5	3.00	9.53^{bc}	5.97^{b}	36.48 (6.04^{ab})
Control	1.67	27.3	2.00	11.03^{bcd}	6.63^{bc}	48.86 (6.99^{abcd})
CD (0.05)	NS	NS	NS	4.75	2.64	2.08

* Treatment means in a column with same letters do not differ significantly

Plate 8 A comparison of GA_3 treatments on lateral shoot production in **topped plants** (T2) and in **intact plants** (T1)

Plate 9 A comparison of BAP treatments on lateral shoot production in **topped plants** (T2) and in **intact plants** (T1)


CONTROL-T2
T1G1
T2G3
CONTROL-T1


CONTROL-T1
CONTROL-T2
T1B1
T2B3

Table 8 Effect of growth regulators on lateral shoot production in topped plants (after a period of eight months)

Treatments	Number of lateral shoots/ plants	Size of shoot (cm)	Number of leaves	Mean leaf length (cm)	Mean leaf breadth (cm)	Mean individual leaf area (cm^2)
Benzyl amino purine (BAP)						
250 mg l^{-1}	2.33	24.2	2.33	10.5	5.7	39.06 (6.25^{cd})
500 mg l^{-1}	2.33	31.4	3.08	11.8	5.4	38.85 (6.23^{cd})
750 mg l^{-1}	2.67	20.9	2.70	7.5	5.8	27.04 (3.94^{abc})
1000 mg l^{-1}	1.67	13.0	2.00	5.8	3.9	21.53 (21.53^{abc})
Gibberellic acid (GA_3)						
250 mg l^{-1}	3.00	18.5	1.60	9.7	4.9	29.05 (5.39^{abcd})
500 mg l^{-1}	4.33	22.9	1.86	10.6	5.1	34.54 (5.88^{bcd})
750 mg l^{-1}	4.67	20.7	1.96	7.0	3.5	13.32 (3.65^{ab})
1000 mg l^{-1}	3.00	13.5	1.73	5.2	3.0	6.93 (2.63^{a})
Control	1.67	29.4	3.17	12.4	6.7	55.70 (7.46^{d})
CD (0.05)	NS	NS	NS	NS	NS	2.52

* Treatment means in a column with same letters do not differ significantly

Plate 10 Effect of GA_3 750 mg l^{-1} and BAP 750 mg l^{-1} on lateral shoot production in topped plants (T_2)

Plate 11 Effect of GA_3 750 mg l^{-1} on lateral shoot production in topped plants (T_2)


CONTROL-T2
T2G3
T2B3


CONTROL-T1
CONTROL-T2
T2G3

different levels of BAP (Plates 9 & 10). Plants treated with BAP 1000 mg l^{-1} and untreated control produced the minimum number (16.7) of shoots.

4.1.2 Size of shoots

There was significant difference between topped and intact plants on size of lateral shoots during 6 months and 8 months after first spray of growth regulators (Table 4). GA_3 750 mg l^{-1} on topped plants recorded maximum size (42.3 cm) in sixth month. GA_3 1000 mg l^{-1}(29.5 cm) and GA_3 500 mg l^{-1} (28.3) recorded values which were *on par* with each other. BAP 1000 mg l^{-1} on intact plants gave lowest mean value (1.4) for size of lateral shoot. BAP 500 mg l^{-1} on topped plant showed highest mean value (31.4 cm) during eight month. It was followed by topped control which recorded a mean value of 29.5 cm, both being statistically homogenous.

However, growth regulators sprayed on intact plants after 6 months recorded size of shoots which were statistically not significant (Table 5). But they differed significantly after eighth month (Table 6). Among the growth regulators, and their concentrations, BAP 750 mg l^{-1} showed maximum size of lateral shoots (4.1 cm). It was followed by BAP 500 mg l^{-1} which recorded

a mean value of 3.5. After eight months GA_3 750 mg l^{-1} recorded highest mean value (14.8 cm) which was followed by GA_3 1000 mg l^{-1} (6.5 cm).

Table 6,7 and 8 showed that there was no significant difference among treatments for size of lateral shoots in topped plants. GA_3 750 mg l^{-1} showed maximum size (42.3 cm) 6 months after. It was followed by GA_3 1000 mg l^{-1} which recorded 29.5 cm. After 8 months BAP 500 mg l^{-1} showed the highest mean value (31.4 cm) for size of lateral shoots.

4.1.3 Leaf characters

4.1.3.1 Number of leaves

Lateral shoots formed on the untopped plants did not produce any leaf even eight months after first spray of growth regulators (Table 6). In topped plants, number of leaves did not differ significantly even after eight months as shown in Tables 7 and 8. However, plants treated with GA_3 1000 mg l^{-1} showed highest mean value (3.00) for number of leaves after six months of first spray. BAP 1000 mg l^{-1} recorded the lowest mean value (1.27). After eight months, control plants recorded maximum mean value (3.17) for number of leaves which was followed by BAP 500 mg l^{-1} (3.08).

4.1.3.2 Leaf length

Results showed that there was significant difference between treatments for leaf length during six months after first spray (Table 7). However, no significant difference was observed after 8 months (Table 8). After six months GA_3 750 mg l^{-1} recorded maximum leaf length (14.47 cm) followed by GA_3 500 mg l^{-1} (13.68 cm); both being statistically *on par*. Control plants showed maximum mean value for leaflength (12.4 cm) after eight months, though not statistically significant.

4.1.3.3 Leaf breadth

Data on leaf breadth differed significantly six months after first spray (Table 7). GA_3 750 mg l^{-1} showed maximum mean value (8.40 cm) followed by BAP 250 mg l^{-1} and GA_3 500 mg l^{-1} (8.20 cm) which were statistically *on par*. After eight months there was no significant difference for leaf breadth (Table 8). For leaf breadth also control plants recorded maximum mean values (6.7 cm) as in the case of leaf length.

4.1.3.4 Leaf area

From Tables 7 and 8 it can be seen that leaf area varied significantly six months and eight months after first spray of growth regulators. GA_3 sprays showed superiority in leaf area and 750 mg l^{-1} (84.27 cm^2) and 500 mg l^{-1} (77.44 cm^2) treatments showed maximum values after

six months. After eight months maximum leaf area was recorded by control plants (55.70 cm^2). It was followed by BAP 250 mg l^{-1} (39.06 cm^2) which was statistically *on par* with control plants.

4.2 Effect of growth regulators on flower production

Results presented in Table 9 revealed that flowers were produced only on intact plants and there was no significant difference among the treatments on number of flowers per plant, length of stalk, length of spathe, breadth of spathe and length of spadix.

However, mean value of number of flowers per plant was highest (1.33) in control, BAP 250 mg l^{-1} gave highest mean value for stalk length (43.9 cm), length of spathe (8.1 cm) and length of spadix (5.9 cm). Breadth of spathe was highest for BAP 250 mg l^{-1} (7.1 cm).

Data on angle between spadix and spathe showed significant difference among the treatments (Table 9; Plates 12a & 12b). They revealed that growth regulators increased the angle and GA_3 500 mg l^{-1} was superior to all treatments (58.3°) and it was followed by GA_3 750 mg l^{-1} (46.6°) and GA_3 1000 mg l^{-1} (45°). Flower spikes from control plants recorded the lowest angle (16.6°) between

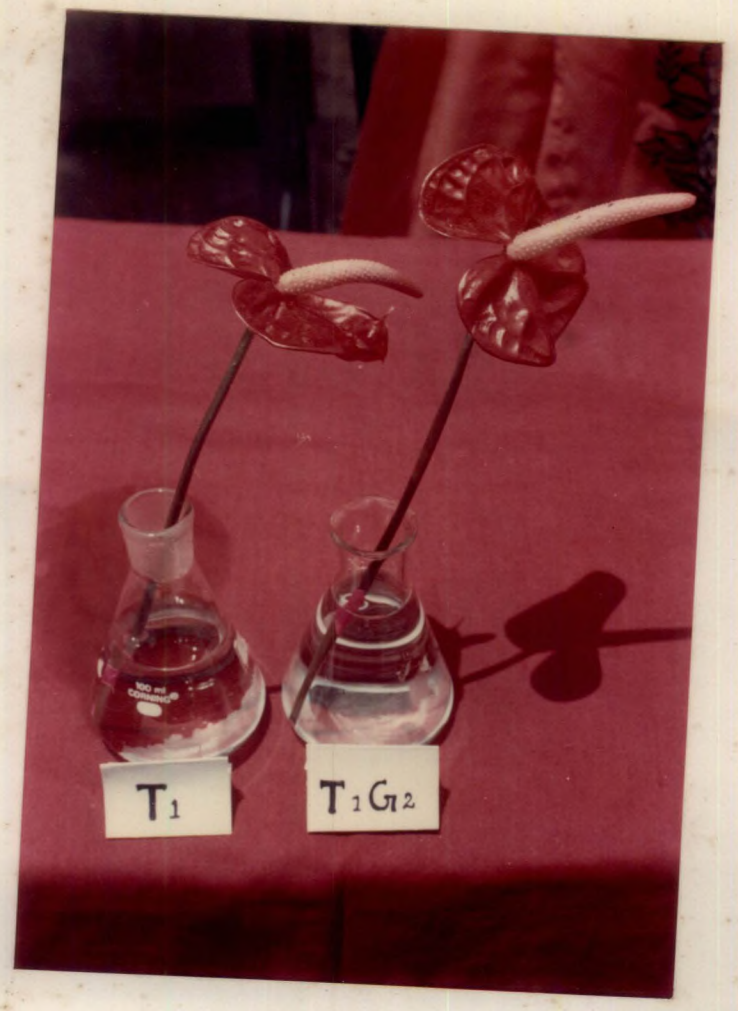
Table 9 Effect of growth regulators on flower production and floral characters

Treatments	Number of flowers per plant	Length of stalk (cm)	Length of spathe (cm)	Breadth of spathe (cm)	Length of spadix (cm)	Angle between spathe and spadix
Benzyl amino purine (BAPP)						
250 mg l^{-1}	1.33	40.3	6.6	5.9	4.5	25.0^{ab}
500 mg l^{-1}	0.67	39.1	5.7	5.6	5.0	27.5^{abc}
750 mg l^{-1}	1.00	43.9	8.1	6.2	5.9	25.0^{ab}
1000 mg l^{-1}	0.67	34.6	6.7	5.6	5.2	28.0^{bc}
Gibberellic acid (GA_3)						
250 mg l^{-1}	0.67	41.5	7.0	7.1	5.9	26.7^{ab}
500 mg l^{-1}	1.00	36.0	7.1	6.0	5.2	58.3^{e}
750 mg l^{-1}	1.33	39.4	7.0	5.6	5.2	46.7^{d}
1000 mg l^{-1}	1.33	43.5	7.2	5.6	5.7	45.0^{d}
Control	1.33	39.4	7.5	5.8	5.5	16.7^{a}
CD (0.05)	NS	NS	NS	NS	NS	19.5

* Treatment means in a column with same letters do not differ significantly

Plate 12a Effect of GA_3 500 mg l^{-1} on angle between spathe and spadix

Plate 12b Effect of GA_3 500 mg l^{-1} on angle between spathe and spadix


T1
T1G2


CONTROL-T1
T1G2

spathe and spadix which was statistically *on par* with BAP 250 mg l^{-1} (25°), BAP 750 mg l^{-1} (25°), BAP 500 mg l^{-1} (27.5°) GA_3 250 mg l^{-1} (26.6°) and BAP 1000 mg l^{-1} (28°).

4.2.1 Biochemical composition of spadix

Application of growth regulators BAP and GA_3 manifested profound variation in the potassium (K), calcium (Ca) and magnesium (Mg) content in the spadix (Table 10). Benzyl amino purine increased the calcium content by 403 per cent and magnesium by 242 per cent while GA_3 increased the Ca and Mg content by 50 per cent. The potassium uptake was best affected and an increase by 64 per cent was confined only to GA_3. No significant trend in the variation of the content of Ca and Mg could be observed with increasing levels of growth regulators.

Data on floral characteristics (Table 9) showed that the angle between spathe and spadix increased with an increase in the content of Ca and Mg in the spadix. Progressive increase in level of application of BAP failed to register a corresponding change in Ca and Mg contents or angle of the spadix. GA_3, however, showed specifically significant increase in the angle of the spadix when it was sprayed at 500 mg l^{-1}.

Table 10 Biochemical composition of spadix as influenced by the application of growth regulators

Treatments	Ca (%)	Mg (%)	K (%)
Benzyl amino purine (BAPP)			
250 mg l^{-1}	2.474^{d}	0.805^{bc}	0.432
500 mg l^{-1}	2.47^{d}	1.343^{d}	0.609
750 mg l^{-1}	1.239^{b}	0.404^{a}	0.406
1000 mg l^{-1}	3.308^{e}	1.27^{d}	0.521
Gibberellic acid (GA_3)			
250 mg l^{-1}	1.051^{ab}	0.548^{ab}	0.57
500 mg l^{-1}	0.619^{a}	0.85^{c}	0.75
750 mg l^{-1}	1.512^{c}	0.505^{a}	0.49
1000 mg l^{-1}	1.442^{bc}	0.44^{a}	0.451
Control	0.588^{a}	0.395^{a}	0.458
CD (0.05)	0.546	0.272	NS

* Treatment means in a column with same letters do not differ significantly

4.3 Somatic embryogenesis

The results of the various experiments carried out to standardise protocol for somatic embryogenesis and production of synthetic seeds are presented here under.

4.3.1 Surface sterilisation of explant

The data of the trial on surface sterilisation of explants are presented on Table 11. Of the different surface sterilants tried, mercuric chloride 0.1 per cent for 10 minutes was found to be effective for all the explants. For spadix segments bacterial contamination was found to be a major problem. This was overcome by the addition of streptomycin 200 mg l^{-1} to the culture media.

4.3.1.1 Contamination rate as influenced by season of collection of explants

The rate of contamination varied with the season of collection of explants (Table 12). Better survival of cultures and least contamination was noticed with explants collected during the period from January to May whereas June to December was found to be the conducive season for the growth of microorganisms and the consequent microbial load on the explants.

Table 11 Effect of different surface sterilants and duration of surface sterilization on survival rate of different explants of *A. andreanum*

Surface sterilants	Concentration (%)	Duration of surface sterilization (minutes)	% survival after 2 weeks		
			Leaf discs	Petiole	Spadix
Domestos	5	5	Nil	Nil	Nil
Domestos	5	10	Nil	Nil	Nil
Domestos	10	5	Nil	Nil	Nil
Domestos	10	10	Nil	Nil	Nil
Mercuric chloride	0.05	5	25	13	5.8
Mercuric chloride	0.05	7	33.5	22.3	15.5
Mercuric chloride	0.05	10	48.5	27.5	23
Mercuric chloride	0.1	5	42.5	20.1	13.5
Mercuric chloride	0.1	7	68.3	38	23
Mercuric chloride	0.1	10	95	75	40.5
Alcohol	50	5	Nil	Nil	Nil
Alcohol	50	10	Nil	Nil	Nil
Alcohol	70	5	Nil	Nil	Nil
Alcohol	70	10	Nil	Nil	Nil

Table 12 Seasonal variations in the rate of contamination in *A. andreanum*

Month	Contaminated (%)			Uncontaminated (%)		
	Leaf	Petiole	Spadix	Leaf	Petiole	Spadix
January	18.0	35.0	75.0	82.0	65.0	25.0
February	10.0	30.0	65.0	90.0	70.0	35.0
March	8.3	30.0	64.5	91.7	70.0	35.5
April	6.5	28.0	62.0	93.5	72.0	38.0
May	5.0	25.0	59.5	95.0	75.0	40.5
June	85.0	98.0	100.0	15.0	2.0	Nil
July	90.0	100.0	100.0	10.0	Nil	Nil
August	80.5	91.5	100.0	19.5	8.5	Nil
September	76.2	90.0	100.0	23.8	10.0	Nil
October	72.5	88.5	95.0	27.5	11.5	5.0
November	65.5	85.0	95.0	34.5	15.0	5.0
December	60.0	79.5	90.0	40.0	20.5	10.0

4.3.2 Culture media

Two different basal media, viz., half-strength MS medium and Nitsch medium, were tried for embryogenesis. Half-strength MS medium was found to be good for the growth of cultures and callus induction (Table 13).

4.3.3 Callus induction

Of the different explants tried, spadix segments produced more healthy friable nodular callus having embryogenic potential (Table 14). Leaf explants and petiole explants produced callus only at cut ends and were white and compact with poor multiplication ability. Spathe explants did not exhibit swelling or callusing.

The positioning of the leaf explants on the medium was found to influence callus initiation. Those leaf explants with midrib and the upper epidermis (abaxial) in contact with the culture medium only produced callus while those with opposite orientation dried after a few days (Table 15).

Leaf discs having central vein showed better response than other parts. Spadix segments from all the portions responded equally. Callusing was observed only in those explants kept under complete darkness. When explants were kept under light they dried within 15 days.

Table 13 Effect of different basal media on callus induction in *A. andreanum*

Basal media	Callus growth	Colour of callus
MS	+	Cream
Nitsch	-	-

The basal media was supplemented with 2, 4-D 2 mg l^{-1} + Kinetin 0.3 mg l^{-1}
+ Callus formed - No response

Table 14 Effect of different types of explant on callus formation

Explant	Callus growth	Days taken for callus initiation	Nature of callus
Leaf	+	90	White and compact
Petiole	+	60	White and compact
Spadix	++	60	Cream, friable and nodular
Spathe	-	-	-

++ Good multiplication rate
+ Poor multiplication rate
- No response

Table 15 Effect of positioning of leaves on callus formation in *A. andreanum*

Positioning of the leaves on the medium	Response
Abaxial surface	Callus formation
Adaxial surface	Leaves dried

4.3.3.1 Response of leaf explants to somatic embryogenesis

Data presented in Table 16 shows that the best medium for callusing is 1/2 MS + 2,4-D 0.08 mg l^{-1} + BAP 1 mg l^{-1} as it produced callusing within 15 days of inoculation (Plate 13). After 2 months of inoculation callusing was noted in medium containing 1/2 MS + NAA 0.3 mg l^{-1} + BA 0.1 mg l^{-1} , 1/2 MS + NAA 0.1 mg l^{-1} + BA 0.3 mg l^{-1} and 1/2 MS + 2,4-D 2 mg l^{-1} + kinetin 0.3 mg l^{-1} + casein hydrolysate 200 mg l^{-1} whereas callusing was observed in leaf explants cultured on to half strength MS medium supplemented with 2.4-D 3 mg l^{-1}, 2,4-D 4 mg l^{-1}, 2,4-D 1 mg l^{-1} + kinetin 0.3 mg l^{-1}, 2,4-D 2 mg l^{-1} + kinetin 0.3 mg l^{-1} and 2,4-D 3 mg l^{-1} + kinetin 0.5 mg l^{-1} only after 3 months.

The callus produced from leaf explants was white coloured and friable. But the quantum of callus produced was less and was confined only to the cut ends. These calli were not found to be embryogenic. Puckering and expansion of cut ends and veins only were noted in some cases. Explants in those medium which showed no response dried 3 to 5 months after inoculation without any change. Coconut water was also not found to be beneficial for embryogenic callus initiation in leaf explants (Table 17). When casein hydrolysate was added to the medium for embryogenic callus induction, 200 mg l^{-1} produced white compact calli. However, the calli produced were not embryogenic in nature (Table 18).

Table 16 Response of leaf explants to different growth regulators

Sl. No	Medium	Response
	(1)	(2)
1	½ MS + 2,4-D 1 mg l^{-1}	No response
2	½ MS + 2,4-D 2 mg l^{-1}	No response
3	½ MS + 2,4-D 3 mg l^{-1}	Callusing at cut ends after 3 months. Only 13% showed callusing
4	½ MS + 2,4-D 4 mg l^{-1}	6.7% showed callusing at cut ends after 3 months
5	½ MS + 2,4-D 1 mg l^{-1} + Kinetin 0.3 mg l^{-1}	20% showed callusing and 35% showed puckering after 3 months
6	½ MS + 2,4-D 2 mg l^{-1} + Kinetin 0.3 mg l^{-1}	13% showed callusing 3 months after and 100% showed little puckering 15 days after inoculation
7	½ MS + 2,4-D 2 mg l^{-1} + Kinetin 0.4 mg l^{-1}	Dried after 5 months
8	½ MS + 2,4-D 3 mg l^{-1} + Kinetin 0.5 mg l^{-1}	24% showed callusing after 3 months
9	½ MS + 2,4-D 4 mg l^{-1} + Kinetin 0.3 mg l^{-1}	No response
10	½ MS + 2,4-D 4 mg l^{-1} + Kinetin 0.5 mg l^{-1}	No response
11	½ MS + 2,4-D 5 mg l^{-1} + Kinetin 1 mg l^{-1}	No response
12	½ MS + 2,4-D 6 mg l^{-1} + Kinetin 2 mg l^{-1}	No response
13	½ MS + 2,4-D 8 mg l^{-1} + Kinetin 3 mg l^{-1}	No response
14	½ MS + 2,4-D 9 mg l^{-1} + Kinetin 2 mg l^{-1}	No response
15	½ MS + 2,4-D 10 mg l^{-1} + Kinetin 2 mg l^{-1}	No response
16	½ MS + 2,4-D 11 mg l^{-1} + Kinetin 2 mg l^{-1}	No response

Contd...

Table 16 contd...

	(1)	(2)
17	½ MS + 2,4-D 12 mg l^{-1} + Kinetin 2 mg l^{-1}	No response
18	½ MS + 2,4-D 15 mg l^{-1} + Kinetin 2 mg l^{-1}	Dried after 15 days
19	½ MS + 2,4-D 2 mg l^{-1} + BAP 1mg l^{-1} + Kinetin 0.3 mg l^{-1}	No response
20	½ MS + 2,4-D 2 mg l^{-1} + Kinetin 0.3 mg l^{-1} + Casein hydrolysate 100 mg l^{-1} + Glutamine 400 mg l^{-1}	No response
21	½ MS + 2,4-D 2 mg l^{-1} + Kinetin 0.3 mg l^{-1} + Casein hydrolysate 100 mg l^{-1} + 6% sucrose + Charcoal 50 mg l^{-1}	No response
22	½ MS + 2,4-D 2 mg l^{-1} + Kinetin 0.3 mg l^{-1} + Casein hydrolysate 200 mg l^{-1}	Little puckering 1 week after and callusing 2 months after at cut ends
23	½ MS + 2,4-D 2 mg l^{-1} + Kinetin 0.3 mg l^{-1} + Casein hydrolysate 300 mg l^{-1}	Cut ends expanded 15 days after in 40% explants
24	½ MS + 2,4-D 2 mg l^{-1} + Kinetin 0.3 mg l^{-1} + Casein hydrolysate 400 mg l^{-1}	No response
25	½ MS + 2,4-D 2 mg l^{-1} + Kinetin 1 mg l^{-1} + Casein hydrolysate 250 mg l^{-1}	No response
26	½ MS + 2,4-D 2 mg l^{-1} + Kinetin 0.3 mg l^{-1} + BAP 1 mg l^{-1}	No response
27	½ MS + 2,4-D 2 mg l^{-1} + Kinetin 0.3 mg l^{-1} + CW 10%	No response
28	½ MS + 2,4-D 2 mg l^{-1} + Kinetin 0.3 mg l^{-1} + CW 15%	No response
29	½ MS + 2,4-D 2 mg l^{-1} + Kinetin 0.3 mg l^{-1} + CW 20%	No response
30	½ MS + 2,4-D 2 mg l^{-1} + Kinetin 0.3 mg l^{-1} + CW 25%	No response

Contd...

Table 16 contd...

	(1)	(2)
31	½ MS + 2,4-D 2 mg l^{-1} + Kinetin 0.3 mg l^{-1} + CW 30%	Veins expanded 2 weeks after in 20% explants
32	½ MS + 2,4-D 2 mg l^{-1} + BAP 1 mg l^{-1}	No response
33	½ MS + 2,4-D 2 mg l^{-1} + BAP 0.1 mg l^{-1}	No response
34	½ MS + 2,4-D 2 mg l^{-1} + BAP 0.3 mg l^{-1}	No response
35	½ MS + 2,4-D 2 mg l^{-1} + BAP 0.5 mg l^{-1}	No response
36	½ MS + 2,4-D 0.06 mg l^{-1} + BAP 1 mg l^{-1}	No response
37	½ MS + 2,4-D 0.08 mg l^{-1} + BAP 1 mg l^{-1}	White callus formed at cut ends 15 days after.
38	½ MS + 2,4-D 0.1 mg l^{-1} + BAP 1 mg l^{-1}	No response
39	½ MS + 2,4-D 2 mg l^{-1} + BAP 0.7 mg l^{-1}	No response
40	½ MS + 2,4-D 2 mg l^{-1} + BAP 0.9 mg l^{-1}	No response
41	½ MS + 2,4-D 2 mg l^{-1} + BAP 1.1 mg l^{-1}	No response
42	½ MS + 2,4-D 2 mg l^{-1} + BAP 1.3 mg l^{-1}	No response
43	½ MS + 2,4-D 2 mg l^{-1} + BAP 1.5 mg l^{-1}	No response
44	½ MS + NAA 0.1 mg l^{-1} + BAP 0.1 mg l^{-1}	Veins expanded and showed puckering 1 month after
45	½ MS + NAA 0.2 mg l^{-1} + BAP 0.1 mg l^{-1}	Puckering 1 month after.
46	½ MS + NAA 0.3 mg l^{-1} + BAP 0.1 mg l^{-1}	Little puckering 1 week after and 2 months after little callusing at cut ends
47	½ MS + NAA 0.4 mg l^{-1} + BAP 0.1 mg l^{-1}	No response

Contd...

Table 16 contd...

	(1)	(2)
48	½ MS + NAA 0.5 mg l^{-1} + BAP 0.1 mg l^{-1}	No response
49	½ MS + NAA 0.1 mg l^{-1} + BAP 0.2 mg l^{-1}	Cut ends and veins enlarged one week after in 30% explants
50	½ MS + NAA 0.2 mg l^{-1} + BAP 0.2 mg l^{-1}	One week after inoculation cut end expanded in 30% of explants. Puckering and expansion of veins were showed after 45 days in 57% explants.
51	½ MS + NAA 0.3 mg l^{-1} + BAP 0.2 mg l^{-1}	Puckering and expansion of cut ends in 46% explants after 45 days and little callusing after 2 months
52	½ MS + NAA 0.4 mg l^{-1} + BAP 0.2 mg l^{-1}	Veins and cut end expanded 45 days after
53	½ MS + NAA 0.5 mg l^{-1} + BAP 0.2 mg l^{-1}	Little puckering in 30% explants after 15 days
54	½ MS + NAA 0.1 mg l^{-1} + BAP 0.3 mg l^{-1}	Veins expanded 15 days after and callusing at cut ends 2 months after
55	½ MS + NAA 0.2 mg l^{-1} + BAP 0.3 mg l^{-1}	Cut end expanded in 30% of explants after 15 days
56	½ MS + NAA 0.3 mg l^{-1} + BAP 0.3 mg l^{-1}	No response
57	½ MS + NAA 0.4 mg l^{-1} + BAP 0.3 mg l^{-1}	No response
58	½ MS + NAA 0.5 mg l^{-1} + BAP 0.3 mg l^{-1}	No response
59	½ MS + NAA 2 mg l^{-1} + BAP 1 mg l^{-1}	No response
60	Nitsch + 2,4-D 1 mg l^{-1}	No response
61	Nitsch + 2,4-D 2 mg l^{-1}	No response
62	Nitsch + 2,4-D 3 mg l^{-1}	No response
63	Nitsch + 2,4-D 4 mg l^{-1}	No response

Contd...

Table 16 contd...

	(1)	(2)
64	Nitsch + 2,4-D 1 mgl^{-1} Kinetin 0.3 mg l^{-1}	No response
65	Nitsch + 2,4-D 1.5 mgl^{-1} Kinetin 0.3 mg l^{-1}	No response
66	Nitsch + 2,4-D 2 mgl^{-1} Kinetin 0.3 mg l^{-1}	No response
67	Nitsch + 2,4-D 2.5 mgl^{-1} Kinetin 0.3 mg l^{-1}	No response
68	Nitsch + 2,4-D 3 mgl^{-1} Kinetin 0.3 mg l^{-1}	No response
69	Nitsch + 2,4-D 3.5 mgl^{-1} Kinetin 0.3 mg l^{-1}	No response
70	Nitsch + 2,4-D 4 mgl^{-1} Kinetin 0.3 mg l^{-1}	No response
71	Nitsch + 2,4-D 2 mgl^{-1} Kinetin 0.3 mg l^{-1} + BAP 1 mg l^{-1}	Veins expanded in 20% explants after 2 weeks
72	Nitsch + 2,4-D 2.5 mgl^{-1} Kinetin 0.3 mg l^{-1} + BAP 1 mgl^{-1}	No response

Table 17 Effect of coconut water on callus initiation in leaf explants of *A. andreanum*

Sl. No.	Medium	Response
1	½ MS + 2,4-D 2 mg l^{-1} + Kinetin 0.3 mg l^{-1} + CW 10%	No response
2	½ MS + 2,4-D 2 mg l^{-1} + Kinetin 0.3 mg l^{-1} + CW 15%	"
3	½ MS + 2,4-D 2 mg l^{-1} + Kinetin 0.3 mg l^{-1} + CW 20%	"
4	½ MS + 2,4-D 2 mg l^{-1} + Kinetin 0.3 mg l^{-1} + CW 25%	"
5	½ MS + 2,4-D 2 mg l^{-1} + Kinetin 0.3 mg l^{-1} + CW 30%	"

Table 18 Effect of media complements on somatic embryogenesis in *A. andreanum*

Medium	Concentration of growth regulators	Additives	Response
½ MS	2,4-D 2 mg l^{-1} + Kinetin 0.3 mg l^{-1}	Casein hydrolysate 100 mg l^{-1}	-
"	"	Casein hydrolysate 200 mg l^{-1}	Non-embryogenic callus
"	"	Casein hydrolysate 300 mg l^{-1}	-
"	"	Casein hydrolysate 400 mg l^{-1}	-
"	"	Charcoal 50 mg l^{-1}	-
"	"	Charcoal 100 mg l^{-1}	-
"	"	Glutamine 400 mg l^{-1}	-

- No response

Plate 13 Callus growth after 15 days of inoculation, in leaf explants in 1/2 strength MS medium supplemented with 2,4-D 0.08 mg l^{-1} + BAP 1 mg l^{-1}

Plate 14 Callus growth after one month of inoculation, in petiole explants in 1/2 strength MS medium supplemented with NAA 0.5 mg l^{-1} + BAP 0.01 mg l^{-1}

4.3.3.2 Response of petiole explants to somatic embryogenesis

The data on the response of petiole explants in different media compositions are presented in Table 19. Explants produced callus after 1 month in half strength MS medium supplemented with 2,4-D 3 mg l^{-1} + kinetin 0.5 mg l^{-1} and NAA o.5 mg l^{-1} + BAP 0.1 mg l^{-1} (Plate 14). Two months after inoculation callus was produced in 1/2 MS + 2,4-D 2 mg l^{-1} + BAP 1 mg l^{-1} + kinetin 0.3 mg l^{-1}. In Nitsch medium supplemented with 2,4-D 3.5 mg l^{-1} + kinetin 0.3 mg l^{-1} petiole explants produced callus after 3 months. Petiole explants inoculated into 1/2 MS + 2,4-D 1 mg l^{-1} and 1/2 MS + 2,4-D 1 mg l^{-1} + kinetin 0.3 mgl^{-1} took five months for callus production. In MS combinations viz., 1/2 MS + 2,4-D 2 mg l^{-1} + kinetin 0.3 mg l^{-1} + casein hydrolysate 200 mg l^{-1} and 1/2 MS + 2,4-D 2 mg l^{-1} + kinetin 0.3 mg l^{-1} + casein hydrolysate 100 mg l^{-1} + glutamine 400 mg l^{-1} cut ends of explants expanded after one month but there was no callusing. The petiole explants also produced callus at cut ends only and the quantum of calli produced was less. The callus formed was white, friable and non embryogenic.

4.3.3.3 Response of spadix explants to somatic embryogenesis

Bacterial contamination was found to be a major problem with spadix explants. The culture media was supplemented with streptomycin to overcome this. Persistence of bacterial contamination even after addition of streptomycin was a constraint to study the response of cultures to various media combination. Spadix produced

Table 19 Response of petiole explants to different growth regulators

Sl. No.	Medium*	Response
1	½ MS + 2,4-D 0.5 mg l^{-1} + Kinetin 0.5 mg l^{-1} +	29% showed little callusing at cut ends after one month
2	½ MS + 2,4-D 1 mg l^{-1}	Hardy callus (cream coloured) at cut ends after 5 months
3	½ MS + 2,4-D 0.3 mg l^{-1} + Kinetin 0.3 mg l^{-1} +	Little callus formed at cut ends after 5 months
4	½ MS + 2,4-D 2 mg l^{-1} + BAP 1 mgl^{-1} + Kinetin 0.3 mg l^{-1}	Callusing at cutends after 2 months in 20% explants
5	½ MS + 2,4-D 2 mg l^{-1} + Kinetin 0.3 mg l^{-1} + Casein hydrolysate 100 mg l^{-1} + Glutamine400 mg l^{-1}	Cut ends expanded one month after, but no callusing
6	½ MS + 2,4-D 2 mg l^{-1} + Kinetin 0.3 mg l^{-1} + Casein hydrolysate 200 mg l^{-1}	One month after cut end expanded
7	½ MS + NAA 0.5 mg l^{-1} + BAP 0.1 mg l^{-1}	Callusing at cut ends one month after.
8	½ MS + NAA 0.4 mg l^{-1} + BAP 0.2 mg l^{-1}	Cut ends expanded after 45 days
9	½ MS + NAA 0.3 mg l^{-1} + BAP 0.3 mgl^{-1}	Cut ends expanded after 2 months
10	Nitsch + 2,4-D 3.5 mg l^{-1} + Kinetin 0.3 mgl^{-1}	20% explants produced callus at cut ends after 3 months
11	Nitsch + 2,4-D 2 mg l^{-1} + Kinetin 0.3 mg l^{-1} + BAP 1 mgl^{-1}	Cut ends expanded 2 weeks after
12	Nitsch + 2,4-D 2.5 mg l^{-1} + Kinetin 0.3 mg l^{-1} + BAP 1 mg l^{-1}	Cut ends expanded 2 weeks after and no further response

*Note: Only twelve culture media out of 72 different media combinations showed response *in vitro*

callus at cut ends in media containing 1/2 MS + 2,4-D 1 mg l^{-1} + kinetin 0.3 mg l^{-1}, 1/2 MS + 2,4-D 2 mg l^{-1} + kinetin 0.3 mg l^{-1} and Nitsch + 2,4-D 2.5 mg l^{-1} + kinetin 0.3 mg l^{-1} + BAP 1 mgl^{-1} (Table 20). The callus produced in 1/2 MS + 2,4-D 2 mg l^{-1} + kinetin 0.3 mg l^{-1} was found to have high multiplication rate (Plate 15 & 16). It was white coloured, friable and nodular in nature. This calli was multiplied and transferred to different media combinations to convert to somatic embryos (Table 21). This callus was converted to green coloured organogenic calli in half strength MS medium supplemented with IAA 1 mg l^{-1} + kinetin 0.3 mg l^{-1}. (Plate 17). Embryo-like structures were produced when callus was transferred into half strength MS basal medium after keeping them on half strength MS basal medium (liquid) on shakers (Plate 18).

But these structures did not develop into plantlets. Rhizogenesis was observed in half strength MS medium supplemented with IAA 1 mg l^{-1} + BAP 1 mg l^{-1} (Plate 19). Globular and translucent calli with potential to produce embryos were produced in 1/2 MS + BAP 1 mg l^{-1} (Plate 20). But they were not converted to embryoids when kept in basal media. Hardy and compact callus was produced in 1/2 MS + BAP 2 mg l^{-1} (Plate 21). The organogenic calli produced plantlets in 1/2 MS + 2,4-D 2 mg l^{-1} + kinetin 0.3 mg l^{-1} after 2 months (Plate 22a & 22b).

Table 20 Response of spadix explants to different medium composition

Sl. No.	Medium*	Response
1	½ MS + 2,4-D 1 mg l^{-1} + Kinetin 0.3 mg l^{-1} +	Cut ends expanded 2 months after and callus formed at cut ends after 3 months
2	½ MS + 2,4-D 2 mg l^{-1} + Kinetin 0.3 mg l^{-1} +	White nodular callus formed at cut ends after 2 months which has high multiplication rate
3	Nitsch + 2,4-D 2.5 mg l^{-1} + Kinetin 0.3 mg l^{-1} + BAP 1 mg l^{-1}	Little callusing at cut ends after 2 months, but no further growth

*Note: Only three culture media out of 72 different media combinations showed response *in vitro*

Table 21 Effect of growth regulators on somatic embryogenesis from callus of *A. andreanum*

Sl. No.	Growth regulators	Response
1	½ MS + 2,4-D 2 mg l^{-1} + Kinetin 0.3 mg l^{-1} +	Organogenesis
2	½ MS + IAA 1 mg l^{-1} + BAP 1 mg l^{-1}	Rhizogenesis
3	½ MS + BAP 1 mg l^{-1}	Transluscent, globular callus
4	½ MS + BAP 2 mg l^{-1} + Kinetin 0.5 mg l^{-1}	Cream, compact callus
5	½ MS + Basal MS (liquid)	Protocorm like bodies were formed
6	½ MS + Basal MS (solid)	Organogenic callus

Plate 15 Callus growth after two months of inoculation in spadix explants in 1/2 strength MS medium supplemented with 2,4-D 2.0 mg l^{-1} + kinetin 0.3 mg l^{-1}

Plate 16 Callus multiplication in spadix explants in 1/2 strength MS medium supplemented with 2,4-D 2.0 mg l^{-1} + kinetin 0.3 mg l^{-1}

Plate 17 Organogenic calli produced on spadix explants in 1/2 strength MS medium supplemented with IAA 1 mg l^{-1} + kinetin 0.3 mg l^{-1}

Plate 18 Embryo like structures produced in MS basal medium

Plate 19 Roots produced from the callus in 1/2 strength MS medium supplemented with IAA 1 mg l^{-1} + BAP 1 mg l^{-1}

Plate 20 Globular and transluscent callus produced in 1/2 MS + BAP 1 mg l^{-1} + streptomycin 200 mg l^{-1}

Plate 21 Hardy compact callus produced in 1/2 MS+ BAP 2 mg l^{-1} + streptomycin 200 mg l^{-1}

Plate 22a Plantlets produced from organogenic calli after two months in 1/2 MS + 2,4-D 2 mg l^{-1} + kinetin 0.3 mg l^{-1}

Plate 22b Plantlets produced after 3 months from organogenic calli in 1/2 MS + 2,4-D 2 mg l^{-1} + kinetin 0.3 mg l^{-1}

Discussion

DISCUSSION

The results of the present investigations carried out to improve propagation efficiency of *A. andreanum* are discussed in this chapter.

5.1 Effect of growth regulators on lateral shoot production

Topping the plants significantly improved lateral shoot production in anthurium. One month after the growth regulator application lateral shoots were formed on topped plants only. This may be due to the effect of topping which reduces apical dominance. Apical dominance is due to the downward movement of an influence originating in apical part of shoots to the lateral shoots (Phillips, 1975). By topping the movement of this influence was removed and which induced lateral shoot production. BAP treatments produced lateral shoots two months after the first spray. This can be attributed to the effect of BAP which reduces the apical dominance and increases lateral bud break (Phillips, 1975). Apical dominance is due to correlative inhibition of lateral buds which is hormonal in nature. Cytokinins are known to release intact plants from correlative inhibition (Saches and Thimann , 1967). Active bud is a source of auxin and gibberellin. It was found that a primary function of cytokinins in releasing bud from apical dominance is to promote synthesis of auxin and other

growth regulators. There was a rise in extractable auxin in cytokinin treated buds one to few hours after treatment. At the same time auxin production in the terminal buds decreased gradually until both growth and auxin production were completely suppressed by laterals which developed into main shoots. The influence of cytokinin in promoting growth of lateral buds and branching may include effects on mobilization of nutrients and assimilation of products to growing points (Skoog and Schmitz, 1979). Skoog and Armstrong (1970) also reported that cytokinin remove apical dominance due to promotion of biosynthesis of a factor which is normally produced in adequate amounts, only in active meristems.

Lateral shoots were not formed on intact plants treated with different levels of GA_3 till eight months after the first spray. But in topped plants GA_3 treatments produced lateral shoots two months after the first spray. Gibberellins also play a primary regulatory role in correlative bud inhibition and because of this the treatment with gibberellin of the cut end of decapitated stem most commonly resulted in an increased rate of lateral bud outgrowth whereas intact plants showed enhanced apical dominance in response to GA_3 treatment. It was suggested previously that increased apical dominance in intact plants treated with GA_3 is an indirect effect of the enhancement of growth in main shoot, and increase in endogenous auxin levels. Transport of gibberellins through an elongating

internode was less than through a mature internode and thus only a small quantity of exogenous gillerellin applied to a young internode would reach the lateral bud situated below which also resulted in apical dominance in intact plants (Phillips, 1975).

The results revealed that topping alone increased lateral shoot production. This is in confirmity with the earlier report of Higaki and Imamura (1987) in Authurium. Improvement in lateral shoot production in topped plants in the present investigation may be due to the removal of apical dominance.

Six months after the first spray of growth regulators the lateral shoots were formed by all the treatments except GA_3 treatments in intact plants and intact control. But the treatments did not differ significantly. After eight months lateral shoots were produced on intact plants treated with GA_3 also. The treatments were statistically significant also. GA_3 750 mg l^{-1} on topped plants produced highest mean value. This revealed that more time is required to obtain the effect of GA_3. But effect of BAP is obtained from fifth month after first spray.

Among the various BAP treatments on intact plants, 250 mg l^{-1} gave highest mean value (3.67). With increase in concentration there was no progressive increase in number of lateral shoots (Fig. 1&2). This is contradictory to the

earlier reports by Higaki and Ramussen (1979) and Imamura and Higaki (1988) in anthurium. In topped plants of anthurium BAP 750 mg l^{-1} showed highest mean value (2.67) (Table 7). A slight increase in the number of shoots produced by topped plants occurred with increase in BAP concentrations.

Foliar sprays of BAP were used successfully to increase lateral shoot production on a non-branching dieffenbachia hybrid (Henny, 1986). Also BAP treatment induced axillary bud elongation in spathiphyllum (Henny and Fooshee, 1986), syngonium (Wang and Boogher, 1987) and epipremnun (Wang, 1990).

GA 250 mg l^{-1} recorded highest mean value (2.00) on intact plants among various concentrations of GA_3. A slight linear decrease in the number of shoots produced by untopped plants occurred with increased GA_3 concentration. However, a linear increase in number of shoots produced in topped plants was evident with increasing GA_3 concentration up to 750 mg l^{-1} (Fig.2). This response is consistent with observations by Immamura and Higaki (1988) in anthurium. The results revealed that increasing GA_3 concentration up to 1000 mg l^{-1} is not effective. GA_3 750 mg l^{-1} gave highest mean value (4.67) for number of lateral shoots on topped plants.

The conflicting results obtained in the experiment on the effect of BAP might have been due to the difference in cultivar as the tendency for sucker production in mature as well as in juvenile anthuriums has been noted to be highly cultivar dependent.

Size of the lateral shoots was more in topped plants. In intact plants BAP 750 mg l^{-1} produced lateral shoot having maximum size 6 months after first spray (3.17 cm). After eight months GA_3 750 mg l^{-1} recorded highest mean value (14.77 cm). Compared to BAP treatments, GA_3 at different levels, produced better sized lateral shoots. This may be due to the effect of gibberellins on cell enlargement and cell elongation. Stowe and Yamaki (1959) reported that stems of gibberellin sprayed plants become much longer than normal.

In topped plants GA_3 750 mg l^{-1} recorded maximum size (42.3 cm) 6 months after first spray. However, 8 months after, the highest mean value for size of the lateral shoots were given by BAP 500 mg l^{-1} . This difference in effect of GA_3 was due to the increased number of lateral shoots which were too small in size.

Lateral shoots formed on the intact plants did not produce any leaf even eight months after first spray of growth regulators (Table 5). The topped plants produced

lateral shoots having few leaves which can be readily used as good propagules. However, the number of leaves produced in different treatments did not differ significantly.

Leaf length, leaf breadth and leaf area differed significantly among treatments. GA_3 750 mg l^{-1} produced leaves having maximum length, breadth and area, six months after first spray. This may be due to increased cell division (Saches *et al.*, 1959) and cell enlargement (Haber and Luippoid, 1960) as a result of application of gibberellic acid. Stowe and Yamaki (1959) reported that leaf area also is increased by application of gibberellin due to its growth stimulating effect. After 8 months, leaf length, leaf breadth and leaf area were maximum in lateral shoots produced by control plants. This is because of the increased number of lateral shoots without leaves that were formed in growth regulator treated plants after eight months.

Growth regulators did not affect number of flowers per plant and flower size significantly. But the angle between spathe and spadix was found to be influenced by the growth regulators. Both BAP and GA_3 increased the angle between spathe and spadix. GA_3 500 mg l^{-1} produced flowers with maximum angle between spathe and spadix. This could be attributed to the increased calcium and magnesium content in the spadix of the GA_3 and BAP sprayed plants.

5.2 Somatic embryogenesis

5.2.1 Surface sterilization

To remove the microbes present on the explant, different surface sterilization treatments were given to various explants used. Explants from different parts of plant may vary in their sensitivity to bleach solutions (George and Sherrington, 1984). As with most plant species, the higher percentage of explant survival reported here depended on the duration of treatment, kind of explants and sterilants. Results of the present study have indicated 0.1 per cent mercuric chloride as an effective surface sterilization agent for *A. andreanum* and the duration of treatment was 10 minutes for all types of explants. Effective use of mercuric chloride as a surface sterilant at 0.1 per cent level has been reported in dendrobium (Devi, 1992).

Microbial contamination was a serious problem in the present study. So an initial treatment with Bavistin followed by wiping with alcohol was done before treating with mercuric chloride which reduced the rate of contamination. In spadix explants, bacterial contamination was a major problem. Geier (1986) reported that losses of spadix explants due to contamination was very high in *Anthurium scherzerianum*. In the present study, the antibiotic streptomycin added to the culture media controlled the bacterial contamination to a great extent.

Seasonal variation was observed for the microbial contamination of *in vitro* cultures. The cultures showed least contamination during the months of January to May whereas June to December was found to be favourable season for growth of microbes (Table 12). In general, during rainy season the contamination rate was high and in dry periods microbial population was less in cultures.

5.2.2 Culture initiation

Half strength MS medium was found to be a good basal medium for the initiation of cultures in anthurium. Kuehnle *et al.* (1992) reported that half strength MS medium was good as basal medium for somatic embryogenesis in *A. andreanum*. Sreelatha (1992) also found that modified MS with reduced major nutrient concentration was better for callus formation in anthurium.

5.2.3 Callus initiation

Results revealed that callusing was better with spadix explants (Table 14). They produced cream, friable and nodular callus within 60 days. It was reported that some genotypes of anthurium were more easily propagated from floral spikes than from leaf cuttings (Geier and Reuther, 1981). In *A. sherzerianum* somatic embryogenesis from spadix callus has been reported by Geier (1982).

Leaf and petiole explants produced white and compact calli at cut ends, but further growth was poor. Spathe did not exhibit any response. Sreelatha (1992) also reported that spathe did not exhibit any swelling or callusing. The positioning of the laminar explants on the medium was found to have an influence on callus initiation and when abaxial surface of the explants touched the medium, callus was formed (Table 14). Those discs, sliced with midrib and with upper epidermis in contact with the culture medium only produced embryoids in anthurium and those placed in the opposite orientation produced little callus or dried after few weeks (Rajasekaran and Kumar, 1994). This may be due to the better absorption of media through the midrib portion.

Callusing was observed only in cultures which are kept under dark and cultures under light turned yellow and died. This is in confirmity with the report of Sreelatha (1992). 2,4-D at high concentration (2 mg l^{-1}) and kinetin at lower concentration (0.3 mg l^{-1}) were found good for callus production (Table 20). This may be because a high concentration of auxin and low concentration of cytokinin in the medium promote abundant cell proliferation with the formation of callus (Skoog and Miller, 1957).

Calli produced on spadix segments was found to have a high multiplication rate. They produced, globular translucent calli which have the potential to become

embryoids. But in the present investigation, none of the media tried was able to convert them into embryoids. In most cases the calli turned organogenic and produced plantlets. Kuehnle and Sugii (1991) observed embryogenic callus of *A. andreanum* cultures on medium containing 2,4-D and BAP. But the regeneration appeared to be organogenic than embryogenic. Transfer of callus to medium containing IAA 1mg l^{-1} + BAP 1 mg l^{-1} resulted in rhizogenesis. Sreelatha (1992) also observed some embryogenic callus in anthurium but was not successful in converting them into embryoids. Somatic embryogenesis was reported in *A. andreanum* by Kuehnle *et al.* (1992) on modified half-strength MS medium supplemented with 1.0 to 4.0 mg l^{-1} 2,4-D and 0.33 to 1.0 mg l^{-1} kinetin. Rajasekaran and Kumar (1994) produced somatic embryos of anthurium on modified Nitsch and Nitsch medium containing BAP, kinetin and 2,4-D. However, in the present study somatic embryos could not be obtained with various combinations of auxins and cytokinins formulated based on earlier reports in anthurium and other ornamental plants. Further studies have to be conducted to find out the role of different types of auxins and cytokinins in somatic embryogenesis in anthurium.

The low success recorded for somatic embryogenesis in anthurium in the present study could be attributed to various factors. Among them, the microbial interference during the culture establishment stage was the main constraint in studying the response of cultures to various

media combinations. The red genotype used in this study may not have good potential for somatic embryogenesis. Red varieties of anthurium have been observed to be showing high degree of recalcitrance *in vitro*. Kuehnle *et al.* (1992) reported that response to somatic embryogenesis vary with the genotypes in anthurium. Geier (1986) also observed that genotype strongly determines regeneration ability in anthurium.

Further research work has to be carried out to study somatic embryogenesis, encapsulation of somatic embryos and germination and viability of somatic embryos in anthurium.

Summary

SUMMARY

The investigation on improvement of propagation efficiency of *Anthurium andreanum* Andre. was conducted at AICFIP, Department of Pomology and Floriculture, College of Horticulture, Vellanikkara, Thrissur during 1995-1997 in order to study the effect of growth substances on lateral shoot production and to attempt production of synseeds through encapsulation of somatic embryos. The salient findings of the investigations are summarised below:

1. Topping alone increased lateral shoot production.

2. The length of lateral shoots was more in topped plants. In intact plants lateral shoots formed did not have enough growth to produce any leaf.

3. The effect of GA_3 was obtained only 8 months after first spray in both topped and intact plants. But the effect of BAP is evident from fifth month itself.

4. Effective concentration of growth substances in terms of number of lateral shoots in topped plants is GA_3 750 mg l^{-1} whereas in intact plants BAP 250 mg l^{-1} was found to be an effective treatment.

5. GA_3 750 mg l^{-1} in topped plants produced highest number of lateral shoots per plant among all the treatments.

6. Size of lateral shoots formed was maximum with GA_3 750 mg l^{-1} in both topped plants and intact plants. Also all the GA_3 treatments were superior to BAP treatments for size of lateral shoot.

7. The number of leaves produced by the lateral shoots on topped plants did not differ significantly between different treatments. But leaf length, leaf breadth and leaf area was maximum with GA_3 750 mg l^{-1}.

8. Flowers were produced only on intact plants and growth substances did not affect number, stalk length and size of flowers significantly. However, the angle between spathe and spadix was affected by growth substances and GA_3 500 mg l^{-1} produced maximum angle.

9. Application of growth regulators, BAP and GA_3 manifested profound variation in the potassium (K), calcium (Ca) and magnesium (Mg) content in the spadix. It was found that the angle between spathe and spadix increased with an increase in the content of Ca and Mg in the spadix.

10. For micropropagation, the explants were effectively surface sterilized with 0.1 per cent mercuric

chloride. The optimum duration of surface sterilization was 10 minutes for all types of explants. An initial treatment with Bavistin 0.1 per cent followed by wiping with alcohol (70%) was given before subjecting to surface sterilization.

11. Seasonal variation was observed for the microbial contamination of *in vitro* cultures. The cultures showed least contamination during the months from January to April, whereas May to December was found to be favourable season for the growth of microbes.

12. Half strength MS medium was found to be a good basal medium for the initiation of cultures.

13. Among various explants used, spadix explants produced more healthy calli which were friable and nodular.

14. Leaf discs sliced with midrib and abaxial surface in contact with medium only produced callus.

15. Callusing was observed only in cultures kept under dark.

16. 2, 4-D at higher concentration (2 mg l^{-1}) and kinetin at lower concentration (0.3 mg l^{-1}) were found to be good for callus production.

17. Callus production was comparatively less in leaf explants and supplementing the medium with coconut water did not improve growth of callus.

18. Addition of casein hydrolysate in the medium improved callusing in leaf explants.

19. The regeneration from the callus was organogenic than embryogenic.

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* Originals not seen

Appendix

APPENDIX

Composition of different basal media tried for *in vitro* culture of *Anthurium andreanum*

Macronutrients	Concentration mg l^{-1}	Micronutrients	Concentration mg l^{-1}	Organic constitutents	Concentration mg l^{-1}
a) MS medium (Murashige and Skoog, 1962)					
NH_4NO_3	1650	$MnSO_4.4H_2O$	22.30	Myo-inositol	100
KNO_3	1900	$ZnSO_4.7H_2O$	8.60	Thiamine-Hcl	0.1
$CaCl_2.2H_2O$	440	H_3BO_3	6.20	Nicotinic acid	0.5
$MgSO_4.7H_2O$	370	KI	0.83	Pyridoxine-HCl	0.5
KH_2PO_4	170	$CuSO_4.5H_2O$	0.025	Glycine	20.0
		$Na_2MoO_4.2H_2O$	0.25	Sucrose	30,000
		$CoCl_2.6H_2O$	0.025		
		$FeSO_4.7H_2O$	27.80		
		$Na_2EDTA.2H_2O$	37.30		
b) Nitsch's media (Nitsch and Nitsch, 1969)					
NH_4NO_3	750	$MnSO_4.4H_2O$	25.00	Myo-inositol	100
KNO_3	950	$2nSO_4.7H_2O$	10.00	Thiamine.HCl	0.5
		$CuSO_4.5H_2O$	0.025	Nicotinic acid	5.0
$MgSO_4.7H_2O$	185	$Na_2MoO_4.2H_2O$	0.25	Pyridoxine-HCl	0.5
KH_2PO_4	68	$CoCl_2.6H_2O$	0.025	Glycine	2.0
		$FeSO_4.7H_2O$	27.80	Biotin	0.05
		Na_2EDTA	37.30	Sucrose	20,000

IMPROVEMENT OF PROPAGATION EFFICIENCY OF *Anthurium andreanum* Andre.

By

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ABSTRACT OF A THESIS

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ABSTRACT

The investigation was carried out at AICFIP, Department of Pomology and Floriculture, Vellanikkara, Thrissur during 1995-1997 to study the effect of growth regulators on lateral shoot production and to attempt production of synseeds through encapsulation of somatic embryos in anthurium.

Anthurium andreanum occupies a very prominent place in the commercial floriculture industry of Kerala. Availability of planting material is one of the major problems of its commercial cultivation in Kerala. Hence, this study on improvement of propagation efficiency of *Anthurium andreanum* Andre has great relevance.

In the present study two growth regulators, Gibberellic acid (GA_3) and Benzyl amino purine (BAP) at four levels (250 mg l^{-1}, 500 mg l^{-1}, 750 mg l^{-1} and 1000 mg l^{-1}) were tried on intact and topped plants.

Topping alone could induce lateral shoot production. Size of the lateral shoots was also high in topped plants. Effect of different growth regulators were expressed during different periods. Effect of BAP was evident from fifth month after first spray whereas GA_3 effect was expressed only after 8 months. GA_3 750 mg l^{-1} on topped plants produced highest number of lateral shoots per

plant among all the treatments. In intact plants BAP 250 mg l^{-1} was found to be more effective. GA_3 treatments produced larger sized shoots compared to BAP treatments. Growth regulators also changed the angle between spathe and spadix of the flower spike. Plants sprayed with GA_3 500 mg l^{-1} produced flowers with maximum angle between spathe and spadix.

Application of growth regulators, BAP and GA_3 manifested profound variation in the potassium (K), calcium (Ca) and magnesium (Mg) content in the spadix. It was found that the angle between spathe and spadix increased with an increase in the content of Ca and Mg in the spadix.

In micropropagation, callus was formed in explants from the leaf, petiole and spadix. The callus production was good in explants from spadix in ½ MS medium supplemented with 2,4-D 2 mg l^{-1} and kinetin 0.3 mgl^{-1}. Addition of casein hydrolysate in the medium improved callusing in leaf explants. However, the calli did not respond to somatic embryogenesis induction treatments. The regeneration from callus was organogenic than embryogenic. Further studies are needed to standardise a complete protocol for somatic embryogenesis and encapsulation of embryoids to produce synthetic seeds.