In vitro STUDIES ON *Anthurium andreanum* Lind. AND *A. crystallinum* Hort.

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BANGALORE
1997

In vitro STUDIES ON *Anthurium andreanum* Lind. AND *A. crystallinum* Hort.

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Thesis submitted to the
University of Agricultural Sciences, Bangalore
in partial fulfilment of the requirements
for the award of the Degree of

Master of Science (AGRICULTURE)

in

HORTICULTURE

BANGALORE FEBRUARY 1997

AFFECTIONATELY DIDECATED

TO

MY BELOVED PARENTS

SMT. SHIVAMMA ANGADI

&

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CERTIFICATE

"This is to certify that the thesis entitled "**IN VITRO STUDIES ON *Anthurium andreanum* Lind. AND *A. crystallinum* Hort.** " submitted by **Mr. MRITYUNJAY. B. ANGADI,** in partial fulfilment of the requirements for the degree of **MASTER OF SCIENCE (AGRICULTURE)** in **HORTICULTURE** to the University of Agricultural Sciences, Bangalore, is a record of research work carried out by him under my guidance and supervision, and that no part of the thesis has been submitted for the award of any other degree, diploma, associateship, fellowship or other similar titles.

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ACKNOWLEDGEMENTS

I wish to express, with great pleasure, my deep sense of gratitude and sincere thanks to ***Dr. (Mrs.) Devinder Prakash****, Senior Scientist, Division of Ornamental Crops, Indian Institute of Horticultural Research, Hessaraghatta, Bangalore and Chairman of my Advisory Committee for her valuable guidance, close counsel, constant supervision, sustained interest and encouragement throughout the period of my investigation and during the preparation of this thesis.*

I am immensely delighted to express my heartfelt gratitude and sincere thanks to ***Dr. P. Narayanaswamy****, Associate Professor, Division of Horticulture, University of Agricultural Sciences, Bangalore and Co-Chairman of my Advisory Committee, for his valuable suggestions, constant calm endurance and inspiring encouragement for critical processing of the manuscript of this thesis apart from advise given during the course of study.*

I also extend my heartfelt gratitude and sincere thanks to members of my Advisory Committee, ***Dr. Suresh N. Sondur****, Associate Professor, Division of Horticulture, University of Agricultural Sciences, Bangalore,* ***Dr. (Mrs.) Sangama****, Senior Scientist, Division of PHT, Indian Institute of Horticultural Research, Hessaraghatta, Bangalore and* ***Dr. (Mrs.) Leela Sahijram****, Senior Scientist, Division of Biotechnology, Indian Institute of Horticultural Research, Hessaraghatta, Bangalore, for their valuable advice and suggestions for carrying out this research work and for critical evaluation of this manuscript.*

I also express my sincere thanks to ***Dr. H. V. Nanjappa****, Associate Professor of Agronomy, UAS, Bangalore, for his close counselling, critical comments and valuable suggestions rendered during the UG studies. I wish to express my special thanks to* ***Dr. J. Venkatesh****, Associate Professor, Division of Horticulture, UAS, Bangalore for his valuable advise, suggestions and encouragement during both UG and PG programmes.*

I am indebted to my parents, brothers and sisters-in-law for their infinite affection and constant encouragement during my study.

I thank the ***Director****, IIHR, Hessaraghatta, Bangalore for providing the required facilities for conducting this research work. I also extend my sincere thanks to*

Dr.K.V.Prasad *and* ***Dr.C.Ashwath****, Scientists, Division of Ornamental Crops, IIHR, Bangalore for their valuable suggestions and kind co-operation during the course of my investigation.*

I can never forget the infinite affection, constant encouragement and help (directly/indirectly) apart from kind co-operations of ***Mr.S.U.Kamati****, AAO,* ***Jagadeesh,T.S.****and* ***Mr.Vannur,B.B****. I take this opportunity to thank* ***Mr. Lakshminarasimhaiah*** *for his timely help, kind co-operation and encouragement during my PG programme. I also extend my warm thanks to* ***Mr.Huliyappa*** *and* ***Mr.Panduranga*** *for thier kind co-operation during my research.*

I also owe my undiluted thanks and gratefulness to all the family members of ***Hiremath (Rapati), Vannur, Danappanavar, Madanabhavi, Kuratti, Meti, Mokashi (Byali), Ningappa Kamathi, Tikkundi, Ilagi, A.B.Kamati*** *and all the relatives, uncles and relatives of* ***Angadi*** *family for their affection, help, co-operation and constant encouragement during period of my study.*

I take this occasion for saying sincere thanks to my friends - ***Muthukumar,A., Rajashekar Malagond, Mokashi,G.G., Smt. Hemavathi,M., Amarananjundeswar., Shivareddy,N., Nagaraj, Jagannath,J., Thippeswamy,S., Nagaraj,M.S., Surendra,S.T., Ravi,M.V., Srinivasamurthy,S.K., Sreerama, Dhananjaya, Basavaprabhu Jirale, Veeresh G.Kadalli, S.U.Patil, Thippeswamy, Basavarajaiah,D., Ajith Mashaldi, Manju Ganiger, Neeralagi,N.S., Honakeri,S.H. and Honakeri,C.V., Manju Ghodase, Ravi Donappanavar, Halesh,D.P., Hanumantharaya,L., Sathish,K.M, Lokesh, Ranjan,M.P., Mallikarjun,K., Krishnakumar,B.G., Anjinappa,M., Shivalingappa*** *and* ***Sathish,N.C.*** *for their help and encouragement during my study period.*

Thanks are due to ***JINDAL EDUCATION TRUST*** *for providing financial assistance in the form of scholarship during PG., thanks due to* ***Thotadappa Hostel*** *for providing lodging and boarding during Junior M.Sc. and* ***Mrs. Radha*** *for neat typing. Any deletion in brief acknowledgement does not mean lack of gratitude.*

February, 1997. **(MRITYUNJAY.B.ANGADI)**

Bangalore

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INTRODUCTION

I. INTRODUCTION

World trade in anthurium is second to orchids among tropical flowers. In Asia in particular, their popularity is increasing. They are cultivated either for their showy, long lasting cut flowers or for their unusually attractive, colourful and handsome foliage.

The genus *Anthurium* consists of about 500-600 known species and belongs to the family 'Araceae' (Bailey, 1963). Anthuriums naturally fall into two groups, viz., foliage and flowering. Among the various species, *Anthurium andreanum* Lind. and *A. scherzerianum* Schott. are cultivated extensively for the production of flowers, whereas *A. crystallinum, A. magnificum, A. veitchii, A. warocqueanum* and *A. digitatum* are grown for their attractive, colourful and handsome foliage as indoor plants in the modern world. It is a native of tropical zones of Central and South America.

Anthurium andreanum was first brought to Hawaii from England in 1889 by Mr. S.M.Damon (Higaki and Watson, 1973). Anthurium planting material mainly imported from Netherlands, are grown commercially in the Caribbean. The Netherlands is still the world leader in anthurium production and trade with about 30 million stems from 69 hectares grown under cover. It is also grown on a large scale in Mauritius.

Anthurium is an epiphytic with some what vining habit of growth, anchoring itself by means of aerial roots to its support. The sequence of leaf, flower and new leaf is maintained throughout the entire life of the plant and lengthened with changes in the environmental conditions (Singh, 1992).

Production is generally highest from May to October because of higher light intensity during these months. Orange and red are the most commonly grown colours, followed by white and pink. Colour preference for anthurium varies throughout the Europe. Italy absorbs a great quantity of orange and orange-red coloured flowers mainly produced in Mauritius. The rest of European consumers prefer red, scarlet and a whole

array of pastels, from coral pink to creamy white, produced by the Dutch breeders. Although lot of breeding work is going on in different parts of the world, creation of yellow and blue anthuriums is still a challenge for the breeders. Dutch growers breed anthuriums for shape, stem length, disease resistance, new colours, productivity and long vase life (Laws and Galinsky, 1996).

Anthuriums are generally propagated through seed, division and cuttings. Plants derived from seeds show large variation with respect to colour, quality, annual yield and time of first flowering. The propagation of anthurium by seed is very slow and due to lower seed viability and poor germination. Hence the application of *in vitro* techniques will enable the breeders to multiply rapidly the elite clones having high yield, quality and disease resistance qualities.

Anthurium production in traditional producing countries has declined by 25 per cent since 1986, due to bacterial blight (Laws and Galinsky, 1996). The new production centres in other geographical locations are now contributing markedly by increasing the production of anthurium cut flowers considerably. So, there is a great potential to grow anthuriums in India commercially. At present anthuriums are grown only in home gardens and small nurseries. There are however, a few growers based at Bangalore, Coorg and Salem growing anthuriums commercially for cut flowers and attractive foliage purposes. However, in India the anthuriums cultivation is still at infancy. The major constraint is shortage of planting material. However, there are no organised efforts to grow anthuriums on commercial scale.

Hence there is a need for standardising the methods for quick and higher germination of anthurium seeds through *in vitro* techniques both for foliage and flowering anthuriums. The *in vitro* technique has opened new possibilities in conservation and commercialization of anthuriums, apart from ensuring rapid and round the year multiplication, in addition to the economy of time and space. Therefore the present project was initiated with the following specific objectives:

1. Selection of appropriate media for seed germination
2. Study the effect of growth adjuvants on seed germination
3. Initiation of the callus using plant growth regulators
4. Differentiation of callus and
5. Acclimatization.

REVIEW OF LITERATURE

II. REVIEW OF LITERATURE

The literature so far published, reveals that there is little information on *in vitro* propagation of flowering and particularly foliage anthuriums. However, the relevant literature available on *in vitro* propagation of anthuriums is presented under the following headings.

2.1 Botany

The flower of *Anthurium andreanum* Lind. is hermaphrodite with two carpelled ovary and four anthers. Plants maintain the sequence of leaf, flower and new-leaf production (Kamemoto and Nakasone, 1963).

The morphology of growth and flower formation of *A. andreanum* and *A. scherzerianum* Schott. was studied by Christiensen (1971). He described that during juvenile phase, plants produce vegetative bud in the leaf axil whereas, flower bud is produced during the generative phase. *A. scherzerianum* plants normally remain vegetative for longer period than others, branch profusely and are known as bush plants in commercial production.

Ali (1979) reported the natural triploidy in *A. crystallinum*. The presence of several varieties and hybrids are reported in anthuriums due to the outcrossing ability of different species (Richard and Thomas, 1983). The chromosome number of anthuriums is normally 2n = 30 (Sengupta and Chettri 1989), however, it ranged from 24 to 66 in the species reported from North, Central and South America. Aneuploidy, polyploidy and presence of B-chromosomes are the basic features of the genus (Sheffer and Croat, 1983). The presence of B-chomosomes was also reported in *A. warocqueanum* by Mari and Kamemoto (1983). Their analysis indicated that the presence of 1-3B chromosomes in the complement. Chromosomes with 4-fairly large, 22 medium and 4 small chromosomes were also reported (Sengupta and Chettri, 1989). However, no phenotypic

effect was observed due to the presence of B-chromosomes (Sheffer and Croat, 1983).

Tarasevich (1989) investigated the pollen grain ultrastructure of 34 species of anthurium in relation to the systematics. He categorised them into 4-morphological and 7-subtypes, on the basis of surface sculpturing. The different sections represented by 34 species were heterogeneous for pollen grain morphology. Anthurium is 'unique in 'Araceae' to possess pore aperture.

Leeuwan (1980) evaluated several anthurium cultivars, viz., Avo-Nette, Avo-Tineke, Favorite and Germa (Orange); Avo-Claudia, Avo-Ingrid and Novo-Aurara (Red); Avo-Jose, Cuba and Jamaica (White); Honette and Sarina (Pearl) and Avo-Anneke (Pink) for different characters.

Criley (1986) described the leaf blade of *A. andreanum* as drooping and cordate, spathe as cordate-ovate, thick in texture, 15-25 cm long and widely open-spreading. The length of spadix was 7.5 to 10 cm. The colour was yellowish with white band marking. Stigmas showed protogyny and were receptive in spadix zone.

Bose and Yadav (1989) have categorised the important anthurium species into flowering and foliage groups. The flowering group includes *A. andreanum, A. bakeri, A. brownii, A.* X *ferrierense, A. ornatum, A. regale, A. regenellianum, A. robustum* and *A. scherzerianum*. The members under foliage group are *A. clarinervium, A. carrugatum, A. crystallinum, A. haltonianum, A. leuconerum, A. magnificum, A. panduratum, A. papilionensis, A. splendidum, A. vetchii* and *A. warocqueanum*. Classification and description of 'Araceae' including *Anthurium* genus was attempted by Ray (1987).

Among the various species, *A. andreanum* and *A. scherzerianum* are cultivated extensively for the production of flowers. *A. andreanum* is an erect plant with oblong heart-shaped leaves measuring 20-25 cm long and 15-20 cm wide. The spathe is also heart-shaped, lacquered reddish orange or scarlet, 10-15 cm long with a yellow and white

Most of the potted and cut flower anthurium species are hybrids of *A. andreanum* and *A. scherzerianum* (Bhatt and Desai, 1989). The data on important *A. andreanum* varieties of Hawaii have been maintained by Criley (1989) which are suitable for cut flower.

Donselman and Broschat (1989) have described the characteristics of 16 selected anthurium species and their interspecific hybrids, including *A. superbum, A. magnificum* and *A.salvinae* with their culture details.

Dai and Paull (1990) studied the growth and development of anthurium cultivar 'Kaumana' flowers (spathe, spadix and peduncle) before and after emergence, the flower was about 0.3 cm long, enclosed by two tightly rolled stipules at the base of the subtending leaf petiole. During the rapid elongation stage of the leaf petiole the flower (0.8-1.0 cm long) growth was slow. Flower growth was resumed after the subtending leaf blade unfurled and had a positive photosynthetic rate. He recorded the complete spathe colour development by 28 days before emergence when flowers reached 50 per cent of final length. At flower emergence, about 80 per cent of the spathe was red. The lobes turned red completely by 7-8 days of emergence. Peduncle growth was sigmoid with the maximum growth rate covering 21 days after emergence. Spathe growth was double sigmoidal. The young growing subtending leaf blade had a negative net-photosynthetic rate. Removal of this leaf blade advanced flower emergence by 11 to 18 days. The soft green leaf had a slightly positive net photosynthetic rate. A mature subtending leaf had highest measured net photosynthetic rate and its removal had little effect on flower emergence, because it acted as a source of nutrients required for the developing flower.

Flowering behaviour of 30 Anthurium species was studied by Croat (1980;1991). He reported that Pachyneurium is one of 19 anthurium sections, consisting of 114 species representing 126 taxa in two series Pachyneurium and the new series Multineurium, with

110 and 16 taxa, respectively. Recently, 48 taxa including 9 sub-species or varieties are described as new.

2.2 Breeding

Kamemoto and Nakasone (1963) evaluated 113 clones and recommended 13 for commercial cut flower production. Among them 'Haga White', the orange variety 'Nitta' and the red varieties 'Kaumana', 'Ozaki', 'Konasko No.1' and 'Hirose' are more popular.

Two high yielding seedlings 'Uniwai' (White) and 'Marian Seefurth'(Pink) were introduced into the trade. Three bicoloured clones, namely, White Green (UH 8), Rose Opal Green (UH 16) and Coral Green (UH 39) are also popular (Kamemato *et al.*, 1968; 1969). Further they also released three new cultivars and two seedling selections, viz., 'Avenue' and 'Chameleon' which are suitable for cut flower production.

Gajek and Schwarz (1980) described and recommended several cultivars like mid sized 'Iga gold' with a shining red spathe and a white spadix with a yellow tip and the compact 'Ellrina' with a vermillon light salmon spathe and a sulphur yellow spadix.

Schmidt and Lavterbch (1985) described two cultivar groups, viz., a) Miniature cultivars - generally under 20 cm tall with narrow leaves and short petioles, including cultivars 'Oud Orange', 'Renata' and 'Amazone' and b) large plants with broad leaves and long petioles including cultivars 'Lachs', 'Flamenco' and K26.

Henny *et al.*(1988) have described and illustrated the new anthurium cultivars obtained from interspecific hybridization of a large pink *A. andreanum* cultivars with *A. amnicola* a dwarf species from Costa Rica. 'Southern Blush' an intermediate in size between its parents had 70 mm long and 50 mm wide spathe and medium pink with a slight lavender tint. The leaves are lanceolate and about 25 cm long.

Wannakarairoj and Komemoto (1990a and 1990b) proposed the scheme for the genetic control of purple spathe in anthurium. According to them the recessive allele 'p' modifies the colour of anthocyanins controlled by the M and O-loci. A spathe is purple when the genotype is M-O-pp. If the 'P' locus is dominant, M-O is red, while mm-O is orange. The 'p' allele has no effect on the -oo (White) genotype. They also described the distribution of anthocyanins in spathes of 17 anthurium species.

Arandt (1991) developed *A. scherzerianum* Variety 'Arabella' which is more uniform, dark green with short leaves. The red spathe is broad with free lobes, a shallow sinus and recuring spadix.

Schaper and Zimmer (1991) obtained ten genotypes by *in vitro* cloning of (*A. scherzerianum*) single seeds from two crosses and two selfings. They reported that the juvenile phase ended in all the cases with the development of the sixth leaf. At this point, a flower bud was initiated in each axil.

2.3 Propagation

Higaki and Rassmussen (1979) induced adventitious buds in 'Ozaki Red' plants by spraying with PBA, BA or Ethephon (100, 500, 1000 or 1500 mg/l). They obtained maximum shooting with BA, PBA and Ethephon at 1000, 1500 and 1000 mg/l, respectively.

Hata *et al.*(1994) enhanced rooting of *A. andreanum* cv. 'Marian Seefurth' cuttings by treating with hot water + IBA (0.8%) which increased the number of shoots per cutting.

2.4 Seeds

Singh (1987) reported that the growth of the spadix continued and resulted into a warty appearence after pollination and fertilization. After six to eight weeks, two to

three seeded berries were formed. Campbell (1905) reported that the presence of acicular crystals of calcium oxalate in the embryos of anthurium. These crystals were formed in compact bundles in special cells distinguished by more granular contents than those of the neighbouring cells. He also told that the inner-tissue of the ovary wall becomes mucilaginous and the ripened seeds are embedded in a pulpy adhesive mass.

Natesh and Rau (1984) reported that the presence of bipolar embryo in anthurium seeds. However, they were small, reduced and lacked proper differentiation at the time of dispersal.

2.5 Storage and *in vivo* seed germination

A. scherzerianum seeds germinated best when berries harvested at orange red ripe stage and fermented in water at 22°C to separate the seeds from pulp. Storage of seeds in water for five days reduced germination from 99 to 80 per cent and by ten days it was further reduced to 53 per cent. The media used were peat and perlite (Beele, 1971).

Bachthaler (1977; 1978; 1979; 1980 and 1993) reported that drying of spadix followed by storage of fresh seeds of *A. scherzerianum* at 20°C for 24 hours either at dark or at light did not result in the loss of seed viability. Cent per cent seed germination was noticed when seeds were extracted from green/unripe/half-ripe or reddish/full-ripe berries. However, seeds extracted from reddish-brown or over-ripen berries showed only 42 per cent germination. Further, incidence of cold injury and fungal infection were observed at 3°C. When the seeds were treated with thiram prior to seed storage helped to retain 95 per cent viability until 12 weeks, however it reduced to 60 per cent by 16 weeks. At 10°C conditions seeds without thiram treatment showed 90 per cent seed germination in four days. Further, his studies revealed that seeds treated with thiram (70%) followed by storage at lower pressure (700 and 400 h.Pa) decreased the germination percentage (67 and 50%, respectively), whereas, the seeds lost their viability completely after storing for 20 weeks at 100 h.Pa. pressure.

Maurer and Brandes (1979a; 1979b) suggested that soaking of mature fruits of *A. scherzerianum* hybrids in 13 per cent crystalline sodium carbonate at 20°C for 2.5 hours or in six per cent pectinose solution at 26-30°C for five hours was the best for extraction of seeds. The seeds left in spadices or berries were either dried or rotted. Germination percentage was reduced considerably when seeds were cleaned, dried and stored at 5°C, while they obtained good seed germination when they were stored at 8°C and above for 10 weeks.

A. andreanum seeds germinated better at 28°C in high peat substrate having 4.0-5.0 pH under continuous lighting, whereas highest germination of *A. scherzerianum* seeds was observed with 4.0 pH (Szendel *et al.*,1981). Hand pulping was the best method for seed extraction as it resulted in highest seed germination and subsequent growth of seedlings. The time taken for the harvest of berries varied from 120-130 days from the point of pollination in different cultivars of *A. andreanum* (Nirmala, 1989).

2.6 *In vitro* seed germination

Swaminathan (1986) reported that the Nitsch's medium was found to be the best for seed germination (*A. andreanum* cv.'Red'). NAA (0.1, 1.0 and 5.0 mg/l) did not had any effect but, IBA (0.1, 1.0 and 5.0 mg/l) delayed seed germination and promoted the subsequent growth of seedlings. GA_3 also did not influence seed germination but enhanced the subsequent growth of seedlings. The media containing thiamine and banana pulp showed good seed germination and subsequent seedling growth, whereas colchicine was inhibitory and lethal excepting 0.5 per cent concentration.

Zens and Zimmer (1988) obtained callus mediated multiple shoots from seeds of *A. scherzerianum*, on Nitsch's media, while the report of Randhawa (1990) showed the early seed germination and development of first leaf stage in Nitsch medium, whereas the subsequent seedling growth was completely inhibitory on Morel medium.

2.7 *In vitro* propagation through tissues

2.7.1 Explants

For *in vitro* propagation of anthuriums, the segments of leaf, petiole, spathe, spadix, pedicel, vegetative buds, shoot tips and roots are used as the source of explants.

Leaf segments have been widely used as explants by many research workers (Pierik, 1976; Novak and Nepustil, 1980; Geier, 1982; Eapen and Rao, 1985; Geier, 1986a; Keller *et al.*, 1986; Geier, 1987; Kuehnle and Sugii, 1991; Singh and Sangama, 1991; Kuehnle *et al.*, 1992; Nirmala and Singh, 1993 and Matsumoto *et al.*, 1996) in different species of anthurium. Callus mediated plantlets were obtained from leaf explants by Novak and Nepustil (1980) and Geier (1982).

The presence of the midrib in the leaf segments has been reported to have a greater influence on callusing in *A. andreanum* in both liquid and solid media (Pierik, 1976). The intensity and frequency of callus was found to be highest in leaf sections cultured with midrib veins in *A. patulum* (Eapen and Rao, 1985). Geier (1986a) found that the presence or absence of midrib had no effect on callusing in leaf explants of *A. scherzerianum*. On the contrary, according to Nirmala and Singh (1993) leaf sections with midrib responded better to BA and 2,4-D applications while producing callus. Position of the leaf from where the explant is excised and the surface (abaxial) touching the media is also important for better callus production (Geier, 1986a). Leaf explants of *A. andreanum* formed callus in 1.5-2.0 months on MS medium supplemented with 2 mg/l Kinetin (Keller *et al.*, 1986). Regenerative callus was obtained from leaf explants of *A. andreanum* cultivars (Kuehnle and Sugii, 1991). Callus derived from leaf segments on modified Nitsch medium found to be poorly regenerative (Singh and Sangama, 1991). Kuehnle *et al.*(1992) obtained transluscent embryonic calluses at the basal ends of the leaf blade explants, obtained from *in vitro* grown plants, with in a month of culture under dark conditions in four cultivars of *A. andreanum*, viz., UH 780, UH 965, UH 1060 and UH 1003. Nirmala and Singh (1993) also obtained regenerative callus on Nitsch medium supplemented with BA and 2,4-D from leaf sections. Matsumoto *et al.*(1996) derived

somatic embryos from *in vitro* cultured leaf lamina of *A. andreanum* cvs. 'Anuenue' and 'Toyama Peach'. Mamet (1980) obtained the complete plantlets from culture of meristem tissues in *A. andreanum*.

Petiole segments of *A. scherzerianum* showed low regeneration capacity as compared to that of spadix segments (Geier, 1982). However, Eapen and Rao (1985) obtained good regeneration in *A. patulum* and Kuehnle and Sugii (1991) in *A. andreanum* using the same explant.

Geier (1982) reported that spadix fragments of *A. scherzerianum* cultivated *in vitro* showed high capacity for regeneration than segments of leaf petiole and spathe under darkness on modified Nitsch medium. Similar results were obtained by Singh and Sangama (1991) and, Nirmala and Singh (1993) in *A. andreanum*.

Zens and Zimmer (1986) reported that formation of callus and adventitious shoots by shoot tip explants was increased significantly by increasing NH_4-N:NO_3-N ratio from 1:1 to 1:5. Regenerative callus was obtained by Soczeck and Hampel (1989) from single node fragments of *in vitro* grown shoots of *A. andreanum* when cultured on half-strength MS medium containing various concentrations of different cytokinins. Cen *et al.*(1993) reported the superiority of light and darkness in induction and growth of callus as well as the further growth of the plantlets derived from adventitious buds. Similar results were also reported by Nirmala and Singh (1993) on liquid Vacin and Went medium.

2.7.2 Nutrient medium

The success of plant tissue culture as a means of plant propagation is greatly influenced by the nature of the culture medium used. Plant tissue culture media provide major and minor nutrient elements and carbohydrates. Improved results were obtained by providing trace amounts of organic compounds, notably vitamins, amino acids and plant growth regulators (George and Sherrington, 1984).

Most of the reports on anthuriums are based on MS and Nitsch media. Pierik *et al.*(1974) cultured embryos on modified MS medium containing half strength macro elements, micro elements, sucrose (3%) and organic constituents (except adenine, IAA and Kinetin) and difco-agar (0.07%). Further, he modified the macro elements of MS medium for callus induction, callus subculture, sprout regeneration and rooting of sprouts, but micro elements and other organic constituents were remained without change. He also found that lower concentration of NH_4NO_3 was an essential factor in the induction of adventitious shoots in callus tissues of almost all genotypes. Fersing and Lutz (1977) reported that medium supplemented with yeast extract stimulated shoot growth in *A. scherzerianum* but restricted in *A. andreanum*. Whereas, according to the report of Eapen and Rao (1985) half strength MS except Fe-EDTA, along with vitamins and 0.6 per cent agar was found to be most suitable for the production of callus mediated plant regeneration from leaf sections with midrib and petiole segments in *A. patulum*.

For the culture of spadix segments of *A. scherzerianum*, Geier (1982) employed modified Nitsch medium (Nitsch, 1969) which contained low levels of ammonium nitrate (100 mg/l). Further, he also made standard modifications to suit the culturing of leaf segments. He also indicated that 200 mg/l NH_4NO_3 was most suited for callus and shoot formation, whereas, 720 mg/l was effective for root formation and changing NH_4-N:NO_3-N ratio from 1:1 to 1:5 increased the formation of callus and adventitious shoot from shoot tip explants in liquid medium (Zens and Zimmer, 1986). Same workers (1988) also reported that MS solid medium supplemented with BA and NAA was found to be good for the production of callus mediated multiple shoots from seeds of *A. scherzerianum*. Root formation was not affected by varying concentrations of ammonium nitrate in MS medium (Lightbourn and Prasad, 1990). Good and regenerative calli were obtained from leaf explants of *A. andreanum* on modified Pierik medium containing 2,4-D and BA after 2-3 months. Petiole explants callused best on Pierik, modified Pierik and Finnie and Van Staden media, while multiple plantlets derived from callus was on Kunisaki medium (Kuhnle and Sugii, 1991).

Modified Nitsch medium was found to be good for the regeneration of plantlets from spadix segments (Singh and Sangama, 1991). Leaf explants when cultured on media containing the combination of 2 per cent sucrose and one per cent gelrite produced more somatic embryos than on half-strength MS medium with 0.7 per cent Bacto agar (Kuehnle *et al.*, 1992). MS medium supplemented with 3 per cent glucose had the greatest inductive effect on callus formation as compared to that of sucrose (Cen *et al.*, 1993). Leaf sections formed good callus on Nitsch medium containing BA and 2,4-D, spadix segments on MS medium with BA and 2,4-D, whereas vegetative buds callused in Vacin and Went liquid medium (Nirmala and Singh, 1993). Reduced concentration of MS major nutrient elememts and sucrose did not significantly influence the production of multiple shoots (Sreelatha *et al.*, 1994).

2.7.3 Growth regulators

Growth and morphogenesis in *in vitro* cultures are regulated by the interaction and balance between the growth regulators supplied with the medium and the growth substances produced endogenously by cultured cells. Besides, many synthetic regulators may in fact modify the level of endogenous growth substances, some times in a fashion which is heritable over many cell generations (George and Sherrington, 1984). The break through made in tissue culture is the discovery that root and shoot initiation is basically regulated by interaction between two hormonal substances namely auxins and cytokinins by Skoog and Miller (1957).

2.7.4 Callus induction and subculture

Generally a high concentration of auxin and a low concentration of cytokinin in the basal medium promotes cell proliferation and callus formation. Pierik *et al.*(1974) reported that in *A. andreanum* different organs of adult plants were capable of forming callus at 1-5 mg/l PBA added to modified MS medium. Callus produced from leaf, spathe, petiole and pedicel could be maintained on the basal medium supplied with PBA 1 mg/l and NAA 0.1 mg/l. Modified MS liquid medium was found to be good for callus

growth. Presence of NAA in the solid medium induced root formation (Pierik, 1975). Further, Pierik (1976) reported that callus induction can be achieved with PBA (1 mg/l), 2,4-D (0.08 mg/l) and can be subcultured with PBA (1 mg/l) in the medium. With BA (1 mg/l) and 2,4-D (0.1 mg/l) in Nitsch medium can induce callus from spadix segments (Geier, 1982) and leaf segments (Geier, 1986a) of *A. scherzerianum*. Leaf, pedicel, spathe and petiole segments produced pink coloured callus in *A. patulum* (Eapen and Rao, 1985).

The leaf explants of *A. andreanum* formed callus in 1.5-2.0 months on MS medium supplemented with 2 mg/l kinetin (Keller *et al.*, 1986). Variation in ploidy level was observed in the case of callus derived from shoot (Geier, 1988). Seeds of *A. scherzerianum* produced caulogenic callus or callus with new shoots and productivity depended on genotype, and it decreased with increased NH_4:NO_3 ratio in the medium (Zens and Zimmer, 1988). Callus production increased as cytokinins concentration was increased (0.125 to 2.0 mg/l) in *A. andreanum* (Soczek and Hempel, 1989). Best callusing was seen on medium supplemented with 0.5 mg/l, 2,4-D in the case of *A.andreanum* cv. 'Tulip' whereas in the case of cv. 'Tropical Pink' 0.05-0.5 mg/l 2,4-D was found to be effective (Lightbourn and Prasad, 1990). Embryogenic callus was obtained from basal ends of cut leaf blade explants within one month of culture in the dark on MS medium supplemented with 1-4 mg/l 2,4-D and 0.33-1.0 mg/l kinetin (Kuehnle *et al.*, 1992). Medium supplemented with 3 per cent glucose was found to be good for callus induction as compared to that of sucrose (Cen *et al.*, 1993). MS medium supplemented with NH_4NO_3, BA and 2,4-D induced callus from leaf midrib and spadix under dark, but, vegetative buds needed light (Nirmala and Singh, 1993). MS medium containing 1 mg/l each of 2ip and BA induced callus (Shreelatha *et al.*, 1994). Regenerative callus was obtained on the media supplemented with 2ip in *A. andreanum* cvs. 'Hazarija' and 'Ingrid' and *A. scherzerianum* cv. 'Belinda' (Yu and Paek, 1995).

2.7.5 Shoot bud differentiation

Low auxin and high cytokinin levels in the medium is a general requirement for shoot bud differentiation, though, Pierik *et al.*(1974) reported that sprout formation occurs in callus spontaneously on transferring the culture to light. Pierik (1976) obtained good plant regeneration from callus on solid MS medium as compared to that of liquid medium supplemented with yeast extract stimulated shoot growth of *A. scherzerianum* but restricted it in *A. andreanum* (Fersing and Lutz, 1977). Similarly, medium containing kinetin (3 mg/l) was found to be more effective in enhancing shoot differentiation as compared to that of BA and 2ip (Lefring and Soede, 1979). Subculturing of callus (3-6 times) on hormone free modified Nitsch medium resulted in the production of true to type multiple shoots at 2000 lux light for 14 hours/day (Geier, 1982).

Eapen and Rao (1985) reported that best response for shoot differentiation was obtained with BA (1.0 mg/l) and 2,4-D (0.1 mg/l). Kinetin and 2 ip stimulated shoot differentiation to a lesser degree, while zeatin was ineffective in the case of *A. patulum*.

In *A. scherzerianum* shoot regeneration was encouraged from the callus of leaf explants when medium was supplmented with BA (0.5 mg/l) and reduced level of NH_4NO_3 (200mg/l)(Geier, 1986a).

Maximum shoot multiplication was observed on MS medium supplemented with NAA and BA from seeds of *A. scherzerianum* (Zens and Zimmer, 1988). Same results obatianed by Lightbourn and Prasad (1990) in *A. andreanum* cvs. 'Tulip' and 'Tropical Pink' at 0.2-0.8 mg/l BA. Sreelatha *et al.* (1994) reported that kinetin (2 mg/l) and BA (1 mg/l) were effective in the production of shoots. Treatments with kinetin did not produced callus, whereas BA and 2 ip induced callus. Similar results were reported by Yu and Paek (1995) in *A. andreanum* cvs. 'Hazrija' and 'Ingrid' and *A. scherzerianum* cv. Belinda.

2.7.6 Rooting

Geier (1982) reported highest rooting of shootlets on hormone free Nitsch medium at 200 lux light duration for 14 hours per day. Rooting occurred after four weeks on cytokinin free medium in the case of *A. andreanum* (Kraft *et al.*, 1983). Hormone free medium and 720 mg/l NH_4NO_3 accelerated root formation (Geier, 1986a). Root formation was not influenced by varying concentrations of ammonium nitrate (Lightbourn and Prasad, 1980).

2.7.7 Response of genotypes

Pierik (1975) showed that growth rates were strongly dependent on the genotypes in *A. andreanum*. Most of the genotypes showed good response to the reduced level of NH_4NO_3 for sprout regeneration (Pierik, 1976). In *A. scherzerianum* also, genotypes strongly determined the regeneration ability. When leaf segments of 18 genotypes were incubated on low NH_4NO_3 (200 mg/l) containing medium, three genotypes did not show any regeneration, five produced only callus, whereas 10 produced caulogenic callus in the last group. The average number of shoots per explant was less than one in five genotypes, 1-10 in three genotypes and more than 10 in two genotypes (Geier, 1986a; 1986b).

MATERIAL AND METHODS

III. MATERIAL AND METHODS

The present investigations were carried out at the Orchid laboratory of the Indian Institute of Horticultural Research, Hessaraghatta, Bangalore. The mother plants of anthurium were maintained in the humidity controlled glasshouse attached to the orchid laboratory. The cultures were maintained at the temperature of 25 $\pm$ 2 $^{\circ}$C and under artificial fluorescent light of 2000 lux for 16 hours a day. Standard cultural practices were followed for growing the plants.

3.1 Plant material and seeds

The two species of anthurium used for the investigation are *Anthurium andreanum* Lind. Var. 'Agnihotri'and and *A. cystallinum* Hort. The plants of Agnihotri are perennial, erect and leaves are evergreen, heart shaped and medium in size. Spathe is flattened and slightly undulated, ending with a pointed tip and attractive deep red colour. The spadix is pendent, slightly slant and white with yellowish tinged tip (Plate 2).

A. crystallinum a very beautiful foliage anthurium. Plants are perennial with velvetty, deep green leaves having silvery deep embedded veination and is a very highly placed species originated form Central America and is most commonly used as an indoor foliage plant (Plate 1).

3.2 Floral biology

The anthurium flowers are very small, borne in hundreds on the candle like protrusion (spadix) arising from the base of the spathe. The flowers are hermaphrodite and protogynous. The flowers have four tepals and stamens are opposite to the tepals. Stigmas are sessile, on maturity they become sticky and shiny and ready for pollination. Artificial pollination is followed by transferring the pollen from the desired pollen parent to the receptive stigma with the help of a camel hair brush. Fully matured and ripe fruits

Plate 1. ***Anthurium crystallinum*** **specimen plant**

Plate 2. ***A. andreanum*** **spathe and spadix**

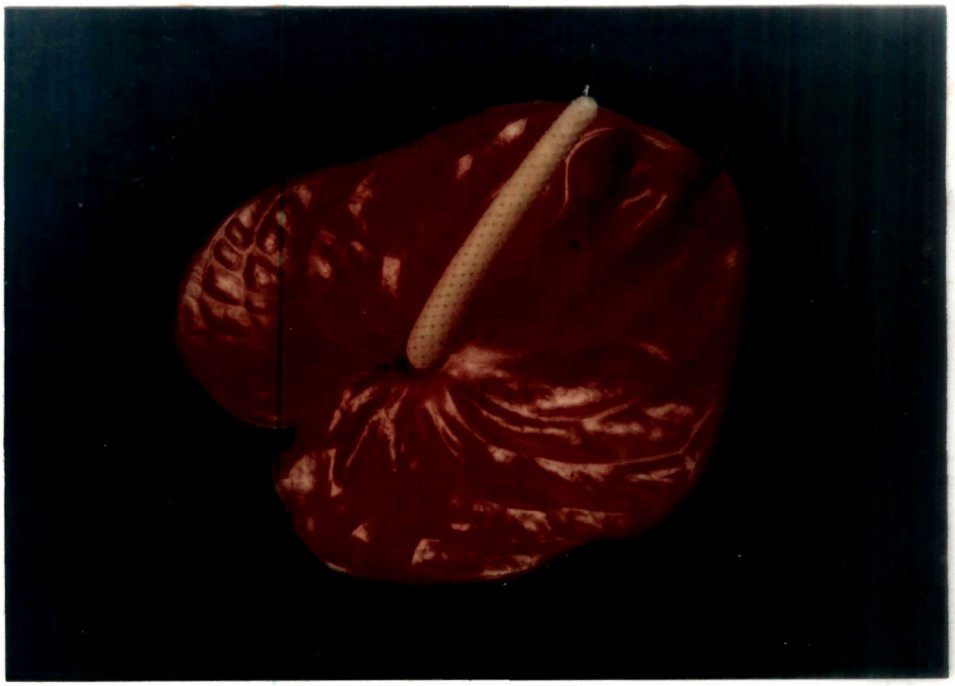
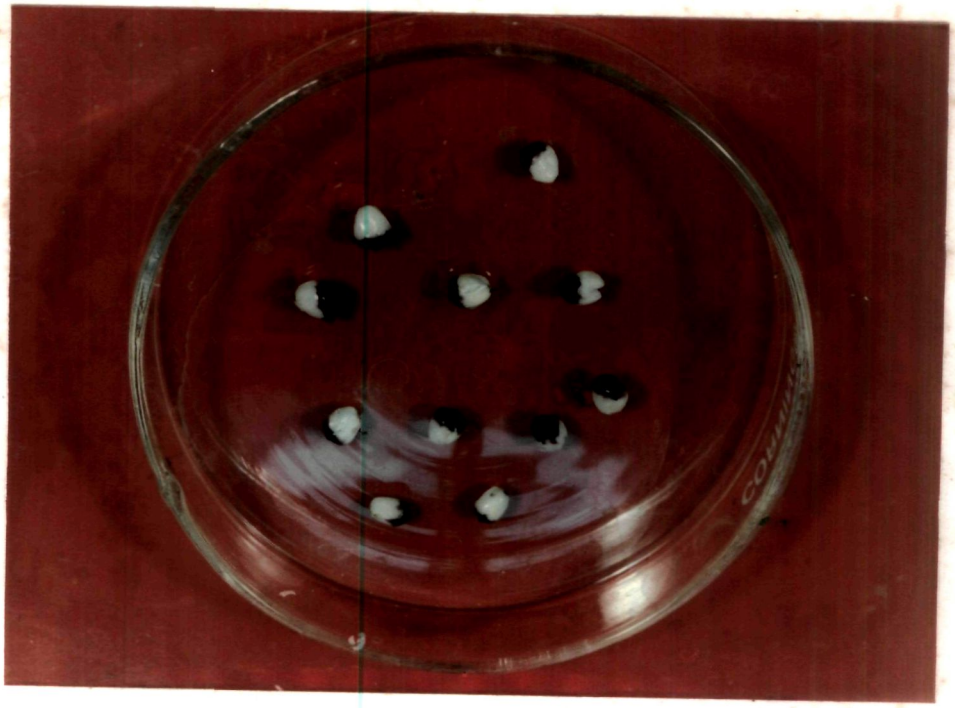
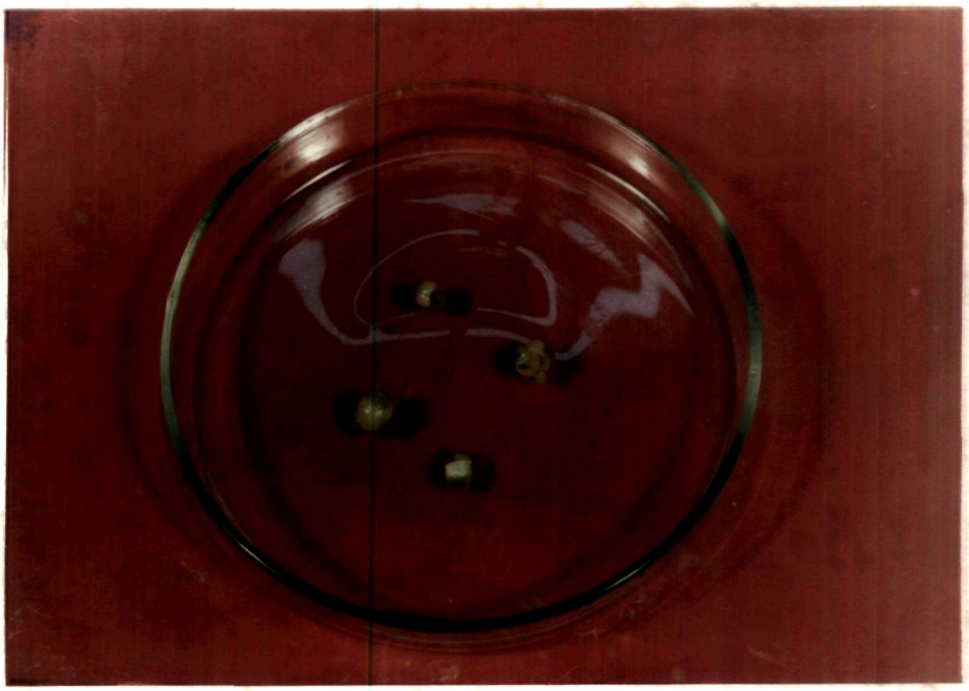


Plate 3. Close view of *A. crystallinum* berries

Plate 4 : Close view of *A. andreanum* berries





(berries) were harvested when they were yellow-orange or red in colour (Plates 3 and 4).

3.3 Extraction of seeds

The fresh red or fully ripen berries were used for the extraction of seeds immediately after harvest, as they loose viability with in a short period. The pulp of the berries was removed manually by hand crushing in wet muslin cloth after soaking in 13 per cent sodium carbonate or one per cent pectinase solution for about five hours and then washed in running water (Maurer and Brandis, 1979a).

3.4 Sterilization of seeds

The extracted seeds were thoroughly washed and sterilized using 4 per cent sodium hypochlorite solution for half an hour before sowing (Nirmala, 1989) and also sterilized the seeds by soaking in 70 per cent alcohol for 1-2 minutes followed by soaking in 0.1 per cent $HgCl_2$ solution for five minutes. The seeds were then thoroughly washed with sterile water for three times.

3.5 Media

The following six media were used in the current studies and the composition of each medium is presented in Appendices I to V.

1. MS medium (Murashige and Skoog, 1962)
2. Vacin and Went medium (Vacin and Went, 1949)
3. Full strength Nitsch medium (Nitsch, 1969)
4. Half strength Nitsch medium (Nitsch, 1969)
5. Knudson C medium (Knudson, 1951)
6. Morel's medium (Morel, 1948).

The stock solutions of macronutrients, micronutrients, vitamins and growth regulators required were prepared by dissolving known quantities of each chemical separately in known volume of distilled water (10X, 20X, 40X or 100X in one litre distilled water and 100 ml, 50 ml, 25 ml or 10 ml/l, respectively were drawn) and stored in brown coloured bottles in the refrigerator.

During the preparation of stock solutions, BAP was dissolved first in minimum quantity of 0.1 N NaOH, whereas, 2,4-D and NAA were dissolved in minimum quantity of ethanol and the required volume was made up with distilled water.

For the preparation of MS and Nitsch media, Na_2.EDTA stock was prepared from Na_2.EDTA and $FeSO_4.7H_2O$. Required quantities were taken and separately dissolved in distilled water, Na_2 EDTA solution was heated to about 60°C temperature, when both the solutions were poured into another flask simultaneously and the required volume was made up with distilled water. For Vacin and Went medium, tricalcium phosphate was dissolved in minimum quantity of 0.1 N HCl and the volume was made up with distilled water.

While preparing the media, required quantity of solutions of macronutrients, micronutrients, vitamins and growth regulators were taken in suitable boiling flask (500 ml or 1000 ml). To the mixture 2.5 to 3.0 per cent sucrose was added and dissolved completely and pH was adjusted to 5.6-5.7 using 0.1 N HCl. In the case of solid media either agar (9 g/l) or gelrite (3.0 g/l) were added and heated slowly till the agar or gelrite was dissolved. Care was taken not to overheat the agar to prevent charring. When the media was supplemented with adjuvants (except coconut water) it was not supplemented with either agar or gelrite. About 50 ml of the prepared medium was dispensed into 250 ml bottles and were closed with lids. They were covered with butter paper or brown paper before autoclaving for 121°C and 15 psi for 20 minutes. Autoclaved bottles were cooled to room temperature before inoculation.

3.6 Inoculation of seeds and subculture of callus

Sterilised seeds and callus were inoculated on to the media 4-5 days after media preparation to make sure there was no contamination of media at storage. In order to provide absolute sterilised conditions laminar air flow chamber was completely swabbed with 70 per cent alcohol. UV and fluorescent lights were switched 'on' before switching 'on' the laminar air flow. The tools and hands were also disinfected using alcohol and the tools were rested at steribead having 200°C for about about 30 seconds. They were cooled before using. The sterilised seeds/cut pieces (5 seeds/flask) were transferred on to the media and the culture bottles were placed in the growth chambers having $25 \pm 2^{\circ}C$ temperature and 2000 lux light intensity for 16 hours photoperiod.

3.7 Adjuvants

Coconut water, banana pulp, wheat malt and ragi malt were supplemented individually to study their effect on seed germination and subsequent seedling growth.

Tender coconuts were used to obtain coconut water (150 ml/l), banana pulp (100 g/l) of 'Yelakki Bale' was obtained from unripe fruits and homogenised. Wheat and ragi flour was sieved at 0.2 mm sieve and 100 g/l was utilised.

3.8 Experimental details

The experimental material included two cultivars and all the experiments conducted during the investigations were laid out and analysed according to the Factorial Completely Randomised Design. Treatments in all the experiments included ten replications.

3.8.1 Media, seed germination and subsequent seedling growth

The treatments of included the following media

T1. MS medium

T2. Vacin and Went medium

T3. Full strength Nitsch medium

T4. Half strength Nitsch medium

T5. Knudson C medium

T6. Morel's medium

Observations were recorded on number of days taken for seed germination, number of seeds germinated and subsequent seedling growth, fresh weight, dry weight (mg), shoot and root length (cm) and shoot to root ratio.

3.8.2 Media adjuvants, seed germination and subsequent seedling growth

The treatments included the Nitsch medium supplemented with different adjuvants

T1. Basal Nitsch medium

T2. Basal (Nitsch) + Coconut water (150 ml/l)

T3. Basal medium + Banana pulp (100 g/l)

T4. Basal medium + Wheat malt (100 g/l)

T5. Basal medium + Ragi malt (100 g/l)

Observations were recorded on number of days taken for seed germination, number of seeds germinated and subsequent seedling growth, fresh weight, dry weight (mg), shoot and root length (cm) and shoot to root ratio.

3.8.3 Plant growth regulators and callus initiation

The treatments included the medium supplemented with BAP and different concentrations of 2,4-D

T1. Nitsch medium without plant growth regulators

T2. Nitsch medium + BAP (1 mg/l)

T3. Nitsch medium + BAP (1 mg/l) + 2,4-D (1 mg/l)

T4. Nitsch medium + BAP (1 mg/l) + 2,4-D (2 mg/l)

T5. Nitsch medium + BAP (1 mg/l) + 2,4-D (4 mg/l)

T6. Nitsch medium + BAP (1 mg/l) + 2,4-D (6 mg/l)

Observations were recorded on number of days taken for callus initiation, type of callus produced, colour and amount of callus produced.

3.8.4 BAP and regeneration from callus

The treatments included the medium supplemented with different concentrations of BAP

T1. Control (only Nitsch medium)

T2. Nitsch medium + BAP (1 mg/l)

T3. Nitsch medium + BAP (2.5 mg/l)

T4. Nitsch medium + BAP (5.0 mg/l)

Observations were recorded on number of days taken for shoot initiation, mean number of shoots per flask and length of the shoot, root length and number of roots.

3.8.5 NAA on rooting of *in vitro* microshoots

The treatments included the medium supplemented with different concentrations of NAA

T1. Control (only Nitsch medium)

T2. Nitsch medium + NAA (0.1 mg/l)

T3. Nitsch medium + NAA (0.5 mg/l)

T4. Nitsch medium + NAA (1.0 mg/l)

Observations were recorded on number of days taken for root initiation, mean number of roots and root length (cm).

3.8.6 Acclimatisation of seedlings

The treatments included different environmental conditions

T1. Greenhouse

T2. Mist chamber

T3. Room conditions

Observations were recorded on survivability, mean number of leaves per seedling, leaf length and breadth, shoot length, mean number of roots and root length.

3.9 Weaning procedure

Young seedlings of 140 days old were taken from the bottles or flasks at fourth leaf stage and the media adhered to the plantlets were removed using running tap water. Then seedlings were dipped in one per cent bavistin (for 10 minutes) followed by 0.1 per cent ridomil (for 5 minutes) and washed thoroughly to remove all the traces. Plantlets were transplanted into community pots having 1:1:1 mixture of compost, sand and leaf mould.

3.10 Statistical analysis

The experiments were laid out and the data obtained was analysed according to Factorial Completely Randomised Design (CRD) as suggested by Sunderraj *et al.*(1972).

EXPERIMENTAL RESULTS

IV. EXPERIMENTAL RESULTS

Results of the experiments conducted on *in vitro* seed germination, callusing differentiation and acclimatization of *in vitro* propagated plants of two *Anthurium* species are presented hereunder.

4.1 Effects of different Media

4.1.1 Seed germination

Data on seed germination as influenced by different *Anthurium* species, media and their interactions are presented in Table-1 and Fig.1.

Seed germination was initiated three days after sowing (DAS) in both the species and continued upto 14 DAS. Seed germination did not differ significantly with respect to species. Maximum number of seeds germinated in the case of *A. andreanum* (4.22), followed by *A. crystallinum* (4.12). However, there was no differences in the time taken for seed germination in both the species (13 DAS).

Seed germination rates differed significantly at different DAS, with respect to different media. Highest seed germination was observed on both Nitsch and MS media (4.60), followed by Morel's and Vacin and Went (VW) media (4.15) which were at par. Significantly lowest seed germination (3.60) was observed with Knudson C (KC) medium which was on par with 1/2 strength Nitsch medium (3.90). Early seed germination (7 DAS) was observed with Morel's medium, followed by 1/2 strength Nitsch, Nitsch, MS and VW media (9, 10, 13 and 13 DAS, respectively). Seed germination was late (14 DAS) on KC medium.

The interaction effects for seed germination for different species and different media were significant only during initial stages (9 DAS). But the results were non significant after 10 DAS.

Table-1 : Effects of different media on germination of seeds of the two species of anthurium

Treatments	DAS											
	3	4	5	6	7	8	9	10	11	12	13	14
Ac	0.35	1.47	1.72	1.95	2.50	3.23	3.35	3.98	4.08	4.10	4.12	4.12
Aa	0.48	0.55	1.60	1.93	2.18	3.37	3.75	3.88	3.95	4.08	4.15	4.22
SEM±	0.08	0.10	0.13	0.11	0.10	0.13	0.12	0.13	0.13	0.12	0.12	0.12
C.D. at 5%	NS	0.28	NS	NS	0.29	NS	0.34	NS	NS	NS	NS	NS
Nitsch	0.00	1.80	3.70	3.90	4.20	4.45	4.50	4.60	4.60	4.60	4.60	4.60
½ Nitsch	0.00	1.20	2.40	2.95	3.25	3.70	3.90	3.90	3.90	3.90	3.90	3.90
MS	0.00	0.00	0.00	0.00	1.40	3.80	4.15	4.35	4.40	4.55	4.60	4.60
KC	0.00	0.00	0.00	0.00	0.00	0.90	1.15	2.90	3.20	3.30	3.40	3.60
V&W	0.00	0.00	0.00	0.75	1.05	2.80	3.45	3.70	3.85	4.05	4.15	4.15
Morel	2.50	3.05	3.85	2.05	4.15	4.15	4.15	4.15	4.15	4.15	4.15	4.15
SEM±	0.15	0.17	0.22	0.20	0.18	0.22	0.21	0.23	0.23	0.22	0.21	0.20
C.D. at 5%	0.40	0.49	0.60	0.54	0.51	0.61	0.58	0.64	0.64	0.60	0.59	0.57

A x B

Treatments	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa
Nitsch	0.00	0.00	3.60	0.00	3.90	3.50	4.10	3.70	4.10	4.30	4.20	4.70	4.30	4.70	4.50	4.70	4.50	4.70	9.50	4.70	4.50	4.70
½ Nitsch	0.00	0.00	2.40	0.00	2.70	2.10	3.50	2.40	3.80	2.70	3.90	3.50	4.00	3.80	4.00	3.80	4.00	3.80	4.00	3.80	4.00	3.80
MS	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.80	0.00	3.70	3.90	4.00	4.30	4.20	4.50	4.30	4.50	4.30	4.80	4.30	4.90	4.30	4.90
KC	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.80	0.00	2.30	3.10	2.70	3.40	3.00	3.50	3.10	3.60	3.20	3.60	3.60
V&W	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.50	0.00	2.10	3.30	2.30	3.50	3.40	3.80	3.60	4.00	3.70	4.00	4.10	4.00	4.30	4.00	4.30
Morel	2.10	0.00	2.80	3.30	3.70	4.00	4.10	4.00	4.30		4.30	4.00	4.30	4.00	4.30	4.00	4.30	4.00	4.30	4.00	4.30	4.00	4.30	4.00
SEM±	0.21		0.25		0.31		0.28		0.26		0.31		0.30		0.33		0.32		0.31		0.30		0.29	
C.D. at 5%	NS		0.69		NS		0.77		0.71		0.86		0.82		NS		NS		NS		NS		NS	

* Ac - Anthurium crystallinum
* Aa - Anthurium andreanum

Number of seeds sown = 5 per flask

Table-2 : Effects of different media on initiation of first leaf of the two species of anthurium

Treatments	DAS										
	20	22	24	26	28	30	32	34	36	38	40
Ac	0.82	1.26	1.88	2.52	2.88	3.28	3.48	3.66	3.70	3.78	3.84
Aa	0.96	1.66	2.16	2.64	3.18	3.46	3.74	3.98	4.08	4.08	4.08
SEM±	0.09	0.13	0.14	0.14	0.14	0.14	0.13	0.13	0.13	0.13	0.13
C.D. at 5%	NS	0.36	NS	NS	NS	NS	NS	NS	0.35	NS	NS
Nitsch	2.60	2.90	3.30	3.85	4.30	4.55	4.55	4.55	4.55	4.55	4.55
½ Nitsch	1.30	1.80	2.00	2.30	2.80	3.25	3.45	3.70	3.80	3.80	3.80
MS	0.00	0.30	1.15	1.60	2.05	2.55	3.30	3.55	3.75	3.95	4.10
KC	0.55	1.30	1.60	2.65	3.10	3.30	3.30	3.45	3.45	3.45	3.45
V&W	0.00	1.00	2.05	2.50	3.90	3.20	3.55	3.85	3.90	3.90	3.90
SEM±	0.14	0.21	0.23	0.25	0.23	0.23	0.21	0.20	0.20	0.20	0.20
C.D. at 5%	0.38	0.51	0.63	0.63	0.63	0.63	0.58	0.56	0.55	0.55	0.57

A x B

Treatments	20		22		24		26		28		30		32		34		36		38		40	
	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa
Nitsch	2.30	2.90	2.50	3.30	2.90	3.70	3.50	4.20	4.80	4.60	4.50	4.60	4.50	4.60	4.50	4.60	4.50	4.60	4.50	4.60	4.50	4.60
½ Nitsch	1.40	1.20	1.60	2.00	2.00	2.00	2.50	2.10	2.10	2.70	3.50	3.00	3.80	3.10	3.90	3.50	3.90	3.70	3.90	3.70	3.90	3.70
MS	0.00	0.00	0.00	0.60	0.80	1.50	1.30	1.90	1.90	2.30	2.10	3.00	2.70	3.70	3.00	4.10	3.10	4.40	3.50	4.40	3.80	4.40
KC	0.40	0.70	1.40	1.20	1.90	1.30	2.80	2.50	2.50	3.10	3.30	3.30	3.30	3.30	3.30	3.60	3.30	3.60	3.30	3.60	3.30	3.60
VQW	0.00	0.00	0.80	1.20	1.80	2.30	2.50	2.50	2.50	3.20	3.00	3.00	3.10	4.00	3.60	4.10	3.70	4.10	3.70	4.10	3.70	4.10
SEM±	0.19		0.29		0.32		0.32		0.32		0.23		0.30		0.29		0.28		0.28		0.29	
C.D. at 5%	NS		NS		NS		NS		NS		NS		0.82		NS		NS		NS		NS	

* Ac - Anthurium crystallinum
* Aa - Anthurium andreanum

Number of seeds sown = 5 per flask

4.1.2. First leaf initiation

First leaf initiation did not differ significantly with respect to *Anthurium* species, but differed significantly with respect to time taken for first leaf initiation. First leaf initiation was early (36 DAS) in the case of *A. andreanum* and was late (40 DAS) in the case of *A. crystallinum*.

First leaf initiation varied significantly for different DAS, with respect to different media. Maximum number of germinated seeds initiated first leaf on Nitsch medium (4.55) which was on par with MS medium (4.10). Significantly lowest number of germinated seeds initiated first leaves on KC medium (3.45), followed by 1/2 strength Nitsch (3.80) and VW (3.90), but they were at par. Significant differences were also observed with respect to the time. First leaf initiation was early on Nitsch medium (30 DAS) followed by KC 1/2 strength Nitsch and VW media (34, 36 and 36 DAS, respectively). First leaf initiation was late (40 DAS) on MS medium.

Interactions between different species of anthurium and media were not significant for first leaf initiation. But, time taken for first leaf initiation, was early (34 DAS) on Nitsch and was late (40 DAS) on MS medium. There was no shoot formation on Morels medium (Table-2).

4.1.3. Second leaf initiation

Second leaf initiation differed significantly with respect to species during 36 to 60 DAS, but there were no differences after 64 days and also during initial periods (28 and 32 DAS). Maximum number of seedlings initiated second leaf in the case of *A. andreanum* (3.96), followed by *A. crystallinum* (3.78). There were no differences in the time taken in both the species.

Significant differences were also observed between media and second leaf initiation. Maximum number of seedlings initiated second leaf on Nitsch medium (4.50)

Table-3 : Effects of different media on initiation of second leaf of the two species of anthurium

Treatments	DAS											
	28	32	36	40	44	48	52	56	60	64	68	72
Ac	0.58	1.06	1.56	2.00	2.48	2.88	3.18	3.44	3.50	3.66	3.76	3.78
Aa	0.78	1.32	1.96	2.52	3.08	3.44	3.74	3.84	3.90	3.96	3.86	3.96
SEM±	0.08	0.11	0.12	0.13	0.14	0.14	0.14	0.13	0.13	0.13	0.13	0.13
C.D. at 5%	NS	NS	0.33	0.36	0.38	0.39	0.38	0.35	0.36	NS	NS	NS
Hitsch	1.45	2.10	3.20	3.75	4.30	4.50	4.50	4.50	4.50	4.50	4.50	4.50
½ Hitsch	0.50	1.05	1.70	2.25	2.65	3.00	3.35	3.50	3.50	3.50	3.50	3.50
MS	0.40	0.60	1.05	1.30	1.90	2.45	2.95	3.25	3.50	3.85	4.10	4.15
KC	0.65	1.30	1.60	2.10	2.60	3.05	3.25	3.35	3.35	3.35	3.35	3.35
V&W	0.40	0.90	1.25	1.90	2.45	2.80	3.25	3.60	3.65	3.85	3.85	3.85
SEM±	0.13	0.17	0.19	0.20	0.22	0.22	0.22	0.20	0.20	0.20	0.21	0.21
C.D. at 5%	0.36	0.47	0.52	0.56	0.62	0.62	0.61	0.56	0.56	0.57	0.57	0.57

A x B

Treatments	28		32		36		40		44		48		52		56		60		64		68		72	
	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa
Hitsch	1.30	1.60	2.10	2.10	2.90	3.50	3.40	4.10	4.10	4.50	4.50	4.50	4.50	4.50	4.50	4.50	4.50	4.50	4.50	4.50	4.50	4.50	4.50	4.50
½ Hitsch	0.60	0.40	1.10	1.00	1.60	1.80	2.00	2.50	2.40	2.90	2.90	3.10	3.30	3.40	3.60	3.40	3.60	3.40	3.60	3.40	3.60	3.40	3.60	3.40
MS	0.30	0.50	0.60	0.60	1.00	1.10	1.30	1.30	1.70	2.10	2.00	2.90	2.40	3.50	2.80	3.70	3.00	4.00	3.40	4.30	3.90	4.30	4.00	4.30
KC	0.20	1.10	0.70	1.90	1.20	2.00	1.70	2.50	2.20	3.00	2.60	3.50	3.00	3.50	3.20	3.50	3.20	3.50	3.20	3.50	3.90	3.50	4.00	3.50
VQW	0.50	0.30	0.80	1.00	1.10	1.40	1.60	2.20	2.00	2.90	2.40	3.20	2.70	3.80	3.10	4.10	3.20	4.10	3.60	4.10	3.20	4.10	3.60	4.10
SEM±	0.19		0.24		0.26		0.29		0.31		0.32		0.31		0.29		0.29		0.29		0.29		0.29	
C.D. at 5%	0.51		NS		NS		NS		NS		NS		NS		NS		NS		NS		NS		NS	

* Ac - Anthurium crystallinum
* Aa - Anthurium andreanum

Number of seeds sown = 5 per flask

which was on par with MS medium (4.15). Minimum number of seedlings initiated second leaf on KC (3.35) which was on par with 1/2 strength Nitsch and VW (3.50 and 3.85, respectively) media. Early second leaf initiation was observed on Nitsch medium (48 DAS), followed by 1/2 strength, Nitsch, KC and VW media (56, 56 and 64 DAS respectively). Second leaf initiation was late (72 DAS) on MS medium. Interaction effect was non-significant between species and media. Both the species of anthurium initiated second leaf early on the Nitsch medium (48 DAS) and it was late (60 DAS) on both MS and VW media (Table-3).

4.1.4. Third leaf initiation

There were no significant differences among the two species with respect to initiation of third leaf during 52 to 64 DAS and during the periods of 88 to 104 DAS. But significant difference was observed among the species during 68 to 84 DAS. *A. andreanum* required 104 days for the third leaf initiation followed by *A. crystallinum* (100 DAS).

Significant differences were observed with respect to different media and third leaf initiation. Maximum number of seedlings initiated third leaf on Nitsch (4.25) followed by MS and VW (4.00 and 3.65, respectively) which were at par. Least number of seedlings initiated third leaf on 1/2 strength Nitsch medium (2.35), however, was on par with KC medium (3.05). Third leaf initiation was early on Nitsch (88 DAS), followed by 1/2 strength Nitsch, MS and KC media which recorded 92, 96 and 100 DAS, respectively. Third leaf initiation was delayed (104 DAS) on VW medium.

The interaction effects for third leaf initiation for different species and different media were observed to be non-significant. Early third leaf initiation was observed in the case of *A. andreanum* (4.20) on Nitsch medium (80 DAS), followed by *A. crystallinum* (4.30) on the same medium (88 DAS) and was late (104 DAS) on VW medium (3.40), followed by *A. crystallinum* (100 DAS) on the same medium (4.0) (Table-4, Plates 8a and 8b).

Plate 8a. Effects of different media on thrid leaf initiation in *A. crystallinum*

1. 1/2 Nitsch 2. Nitsch 3. MS 4. KC 5. VW

Plate 8b. Effects of different media on thrid leaf initiation in *A. andreanum*

1. Nitsch 2. 1/2 Nitsch 3. MS 4. KC 5. VW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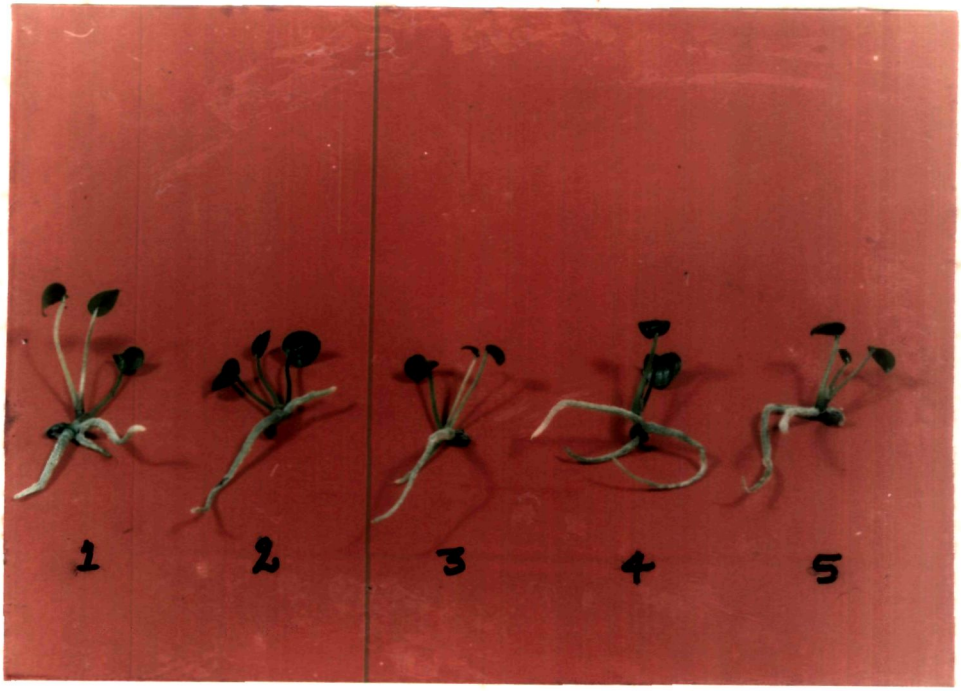
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Table-4 : Effects of different media on initiation of third leaf of the two species of anthurium

Treatments	DAS													
	52	56	60	64	68	72	76	80	84	88	92	96	100	104
Ac	0.34	0.36	0.07	0.96	1.16	1.44	1.86	2.16	2.52	2.80	4.14	3.32	3.46	3.46
Aa	0.24	0.48	0.90	1.20	1.66	2.14	2.42	2.70	2.92	3.12	3.30	3.36	3.42	3.48
SEM±	0.07	0.07	0.08	0.10	0.12	0.14	0.14	0.14	0.13	0.13	0.14	0.14	0.14	0.14
C.D. at 5%	NS	NS	NS	NS	0.32	0.38	0.38	0.38	0.35	NS	NS	NS	NS	NS
Hitsch	0.25	0.00	1.45	1.90	2.35	3.00	3.50	3.75	4.05	4.25	4.25	4.25	4.25	4.25
½ Hitsch	0.05	0.55	0.45	0.50	0.75	1.15	1.45	1.60	1.80	2.00	2.35	2.35	2.35	2.35
MS	0.05	0.35	0.85	1.25	1.85	2.25	2.55	2.95	3.40	3.70	3.85	4.00	4.00	4.00
KC	0.65	0.15	0.70	1.00	1.25	1.35	1.75	2.05	2.30	2.45	2.85	3.00	3.05	3.05
V&W	0.45	1.05	0.55	0.75	0.85	1.20	1.45	1.80	2.05	2.40	2.80	3.10	3.55	3.65
SEM±	0.11	0.11	0.10	0.17	0.18	0.22	0.22	0.22	0.20	0.21	0.22	0.22	0.22	0.22
C.D. at 5%	0.30	0.29	0.27	0.48	0.51	0.60	0.60	0.60	0.55	0.58	0.60	0.61	0.61	0.60

A x B

Treatments	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa
Hitsch	0.20	0.30	0.00	0.00	1.20	1.70	1.70	2.10	2.00	2.70	2.50	3.50	3.00	4.00	3.30	4.20	3.90	4.80	4.30	4.20	4.30	4.20	4.30	4.20	4.30	4.20
½ Hitsch	0.10	0.00	0.50	0.50	0.40	0.50	0.40	0.60	0.50	1.00	0.80	1.50	1.00	1.90	1.30	1.90	1.60	2.00	1.90	2.10	2.20	2.50	2.20	2.50	2.20	2.50
MS	0.00	0.10	0.40	0.40	0.70	1.00	1.00	1.50	1.30	2.40	1.60	2.90	2.10	3.00	2.40	3.50	2.90	3.90	3.30	4.10	3.60	4.10	3.90	4.10	3.90	4.10
KC	0.60	0.70	0.00	0.30	0.50	0.90	0.70	1.30	1.00	1.50	1.00	1.70	1.50	2.00	1.70	2.40	1.90	2.70	1.90	3.00	2.50	3.20	2.80	3.80	2.90	3.20
V&W	0.80	0.10	0.90	1.20	0.77	0.40	1.00	0.50	1.00	0.70	1.30	1.10	1.70	1.20	2.10	1.50	2.30	1.80	2.60	2.20	3.10	2.50	3.40	2.80	4.00	3.10	4.00	3.40
SEM±	0.15		0.15		0.22		0.24		0.26		0.31		0.31		0.31		0.28		0.29		0.31		0.31		0.31		0.30	
C.D. at 5%	0.42		NS		NS		NS		NS		NS		NS		NS		NS		NS		NS		NS		NS		NS	

* Ac - Anthurium crystallinum
* Aa - Anthurium andreanum

Number of seeds sown = 5 per flask

Table-5 : Effects of different media on initiation of [illegible]

Treatments	DAS														
	84	88	92	96	100	104	108	112	116	120	124	128	132	136	140
Ac	0.08	0.14	0.48	0.74	1.04	1.30	1.52	1.84	2.12	2.28	2.38	2.50	2.54	2.62	2.66
Aa	0.52	0.74	1.12	1.46	1.84	0.14	2.36	2.66	2.74	2.88	2.94	3.00	3.00	3.00	3.00
SEM±	0.05	0.08	0.10	0.10	0.12	0.13	0.13	0.13	0.13	0.14	0.13	0.14	0.14	0.14	0.14
C.D. at 5%	0.14	0.22	0.28	0.27	0.33	0.37	0.37	0.37	0.37	0.38	0.37	0.38	0.38	NS	NS
Hitsch	0.80	1.30	2.00	2.40	2.90	3.25	3.40	3.55	3.70	3.70	3.70	3.70	3.70	3.70	3.70
½ Hitsch	0.15	0.15	0.20	0.25	0.40	0.50	0.75	1.15	1.25	1.35	1.45	1.50	1.50	1.50	1.50
MS	0.30	0.50	0.95	1.40	1.95	2.50	2.70	3.10	3.25	3.45	3.50	3.50	3.50	3.50	3.50
KC	0.85	0.25	0.50	0.80	1.05	1.20	1.55	1.90	2.20	2.35	2.50	2.50	2.55	2.55	2.55
V&W	0.00	0.00	0.35	0.65	0.90	1.15	1.30	1.55	1.75	2.05	2.50	2.55	2.60	2.80	2.90
SEM±	0.08	0.12	0.16	0.16	0.19	0.21	0.21	0.21	0.20	0.22	0.21	0.22	0.21	0.22	0.22
C.D. at 5%	0.22	0.34	0.44	0.43	0.52	0.59	0.58	0.58	0.55	0.60	0.59	0.60	0.59	0.61	0.61

A x B

Treatments	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa
Hitsch	0.40	1.20	0.70	1.90	1.50	2.50	1.70	3.10	2.10	3.70	2.60	3.90	2.90	3.90	3.20	3.50	3.50	3.90	3.50	3.90	3.50	3.90	3.50	3.90	3.70	3.90	3.50	3.90	3.50	3.90
½ Hitsch	0.00	0.30	0.00	0.30	0.00	0.40	0.00	0.50	0.20	0.60	0.30	0.70	0.50	1.00	0.90	1.00	1.00	1.50	1.00	1.70	1.70	1.70	1.30	1.70	1.30	1.70	1.30	1.70	1.30	1.70
MS	0.00	0.60	0.00	1.00	0.60	1.30	1.10	1.70	1.50	2.40	2.00	3.00	2.10	3.30	2.60	2.90	2.90	3.60	3.30	3.60	3.40	3.60	3.40	3.60	3.40	3.60	3.40	3.60	3.40	3.60
KC	0.00	0.50	0.00	0.50	0.00	1.00	0.50	1.10	0.70	1.40	0.70	1.70	1.10	2.00	1.30	1.70	1.70	2.70	2.00	2.70	2.10	2.70	2.30	2.70	2.40	2.70	2.40	2.70	2.40	2.70
V&W	0.00	0.00	0.00	0.00	0.30	0.40	0.40	0.90	0.70	1.10	0.90	1.40	1.00	1.60	1.20	1.50	1.50	2.00	1.60	2.50	1.70	2.80	2.00	3.10	2.10	3.10	2.50	3.10	2.70	3.10
SEM±	0.11		0.18		0.22		0.22		0.27		0.30		0.30		0.30		0.30		0.31		0.30		0.30		0.31		0.31		0.31	
C.D. at 5%	0.31		0.49		NS		NS		NS		NS		NS		NS		NS		NS		NS		NS		NS		NS		NS	

* Ac - Anthurium crystallinum
* Aa - Anthurium andreanum

Number of seeds sown = 5 per flask

4.1.5. Fourth leaf initiation

Significant differences were observed with respect to fourth leaf initiation of anthurium species at 84 and 132 DAS, but, were non-significant after 136 days. Maximum number of seedlings initiated fourth leaf in the case of *A. andreanum* (3.00) and was on par with *A. crystallinum* (2.66).

Fourth leaf initiation differed significantly at different DAS, with respect to different media. Maximum number of seedlings reached fourth leaf stage on Nitsch medium (3.70) which was on par with MS medium (3.50). Similarly, VW medium (2.90) was on par with KC medium (2.55). Least number of seedlings initiated fourth leaf on 1/2 strength Nitsch medium (1.50). Early fourth leaf initiation was observed on Nitsch medium at 116 DAS. Followed by MS, 1/2 strength Nitsch and KC medium, respectively, (124, 128 and 132 DAS, respectively). Fourth leaf initiation was late (140 DAS) on VW medium.

The interactions for fourth leaf initiation for different species and different media were non-significant (Table-5 and Plate 9a and b).

4.1.6. Seedling growth

Data on root length, number of roots per seedling, shoot length, root to shoot ratio, fresh weight, dry weight and (FW/DW x 100) index are presented in Table-6 and Fig.2.

4.1.6.1. Longest root length (cm 1) of the seedling

Higher root length was recorded by *A. crystallinum* (6.36 cm) and was lower in *A. andreanum* (4.24 cm). Higher root length was recorded on Nitsch medium (7.80 cm). Root length was 6.00 cm on VW medium which was on par with MS medium (5.78 cms). Similarly, root length in the case of 1/2 strength Nitsch medium (5.04 cms) was

Plate9a. Effects of different media on fourth leaf initiation in *A. crystallinum*

1. Nitsch 2. 1/2 Nitsch 3. MS 4. KC 5. VW

Plate 9b. Effects of different media on thrid leaf initiation in *A. andreanum*

1. Nitsch 2. 1/2 Nitsch 3. MS 4. KC 5. VW

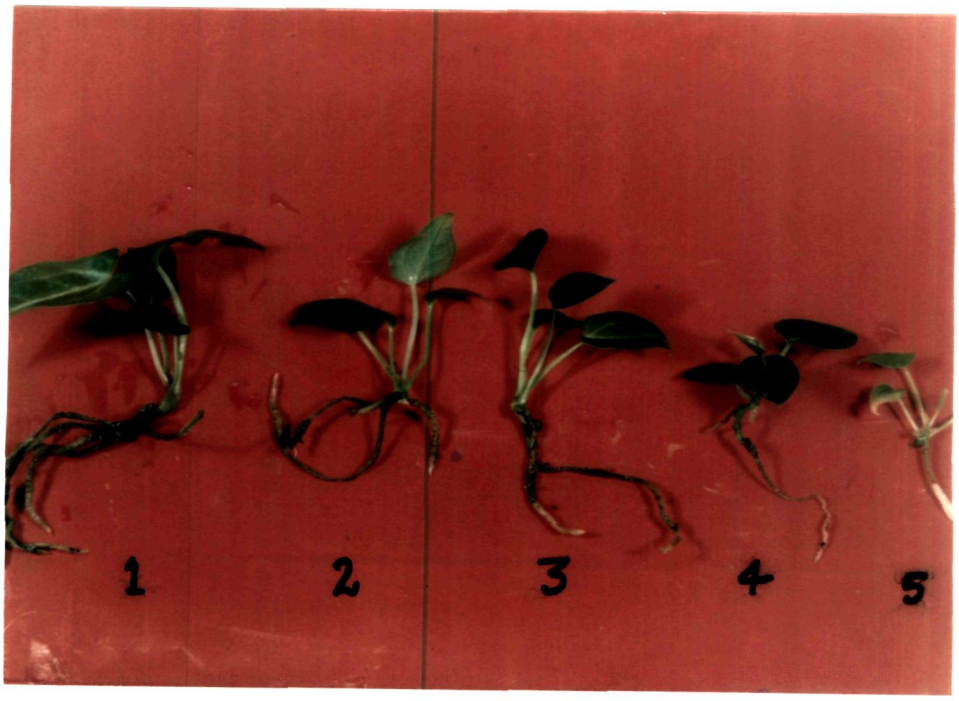
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Table-6 : Effects of different media on the seedling growth parameters of the two species of anthurium

Treatments	Root length (cm)	Number of roots per seedling	Shoot lenght (cm)	Root to shoot ratio	Fresh weight (mg)	Dry weight (mg)	FW / DW x 100 Index
Ac	6.36	2.73	3.04	0.45	333.20	35.72	925.98
Aa	4.24	2.33	2.48	0.54	219.98	23.58	930.32
SEM±	0.09	0.08	0.06	0.01	4.61	0.45	3.25
C.D. at 5%	0.25	0.21	0.17	0.04	12.79	1.25	NS
Nitsch	7.80	3.30	3.24	0.43	374.70	38.10	979.55
½ Nitsch	5.04	2.35	2.49	0.51	222.00	23.80	930.15
MS	5.78	2.95	2.88	0.52	278.00	31.30	885.45
KC	4.67	2.60	2.24	0.48	236.20	26.20	903.75
V&W	6.00	2.75	2.98	0.51	272.05	28.85	941.85
Morel	2.53	1.25	0.00	0.00	0.00	0.00	0.00
SEM±	0.16	0.13	0.10	0.02	7.29	0.72	5.13
C.D. at 5%	0.44	0.36	0.26	NS	20.21	1.99	14.23

A x B

Treatments	Root length (cm)		Number of roots per seedling		Shoot lenght (cm)		Root to shoot ratio		Fresh weight (mg)		Dry weight (mg)		FW / DW x 100 Index	
	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa
Nitsch	9.70	5.90	3.40	3.20	3.58	2.89	0.37	0.50	498.70	250.70	50.60	25.60	985.20	973.90
½ Nitsch	6.23	3.85	2.50	2.20	2.68	2.29	0.44	0.59	263.10	180.90	28.20	19.40	933.20	927.10
MS	6.95	4.61	3.10	2.80	3.10	2.65	0.46	0.58	321.70	234.30	36.30	26.30	880.80	890.10
KC	5.38	3.96	2.90	2.30	2.55	1.93	0.48	0.49	269.00	203.40	30.30	22.10	887.70	919.80
V&W	6.86	5.14	3.10	2.40	3.30	0.00	0.49	0.54	313.50	230.60	33.20	24.50	943.00	940.70
Morel	3.06	2.00	1.40	1.10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
SEM±	0.23		0.18		0.13		0.04		10.32		1.02		7.26	
C.D. at 5%	0.63		NS		NS		NS		28.59		2.82		20.12	

* Ac = Anthurium crystallinum
* Aa = Anthurium andreanum

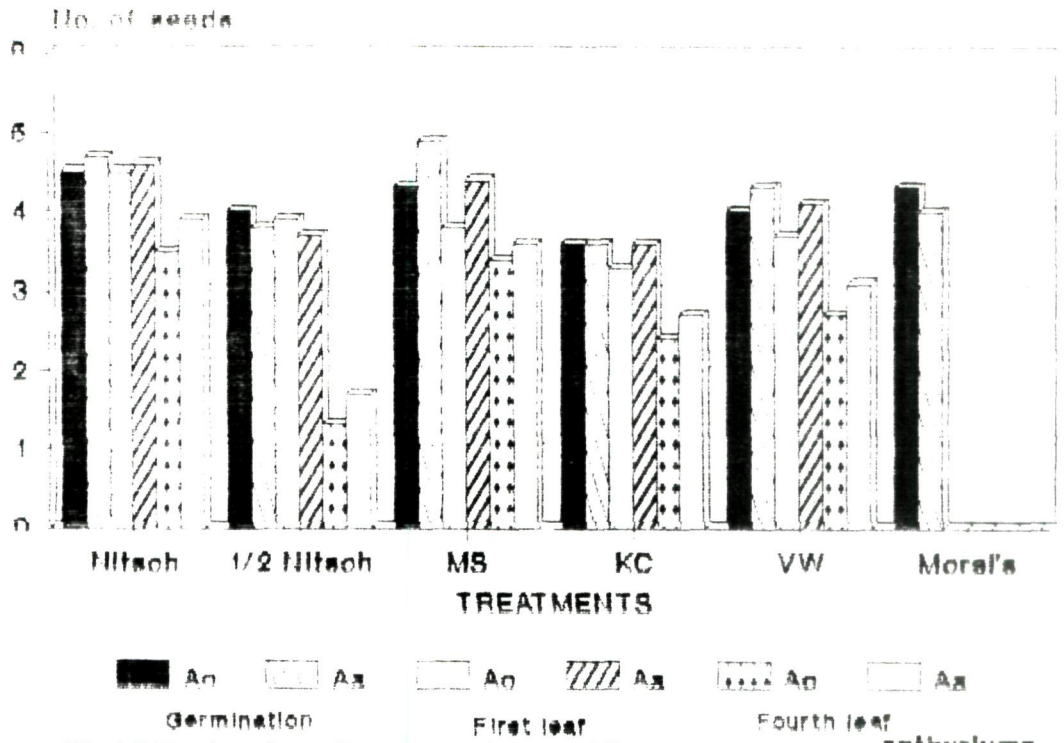

Fig.1:Effects of media on seed germition and growth of anthurlums

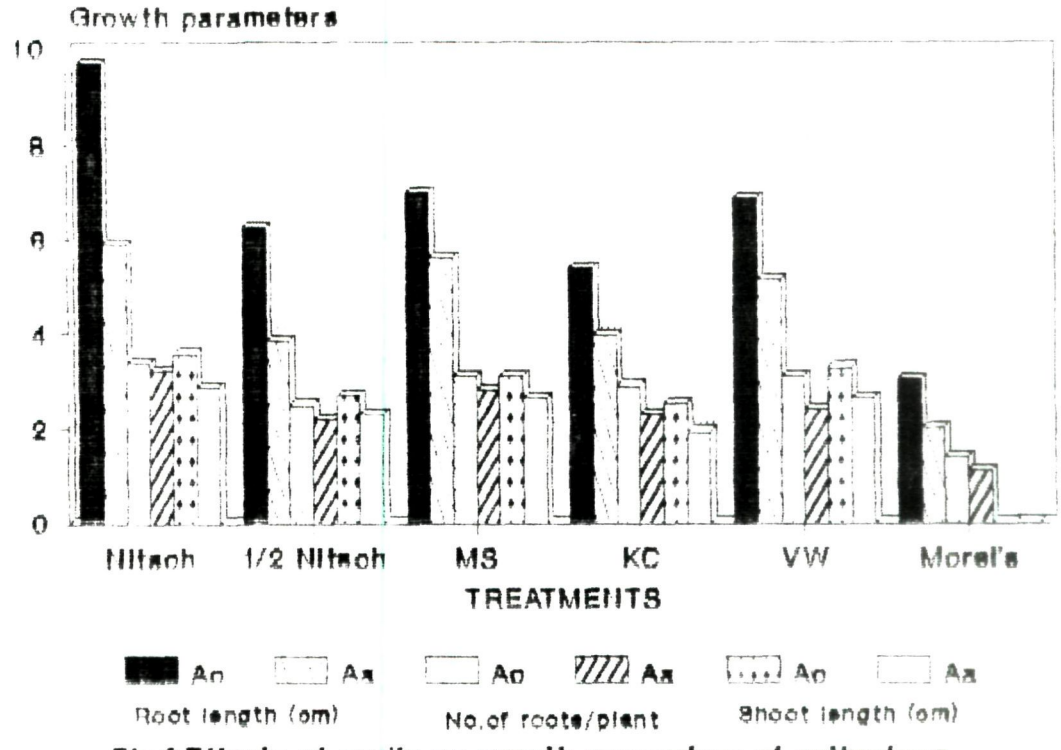

Fig.2:Effects of media on growth parameters of anthurlums

on par with KC medium (4.67 cm). Shortest root length was recorded on Morel's medium (2.53 cm).

The interaction effects for root length for different species and media were significant. Longest root length was recorded on Nitsch medium (9.70 cm) and was least on Morel's medium (3.06 cm), where as it was on par with MS (6.95 cm) and VW media (6.86 cm). Similarity, VW medium (6.86 cm), 1/2 strength Nitsch and KC media (5.38 cm) were also on par in the case of *A. crystallinum*. And in the case of *A. andreanum* maximum root length was recorded on Nitsch medium (5.90 cm) and was minimum on Morel's medium (2.00 cm). Root length was 5.14 cm on VW medium which was on par with MS medium (4.61 cm). Similarly, root length on KC medium (3.96 cm) was on par with 1/2 strength Nitsch medium.

4.1.6.2. Number of roots per seedling

Anthurium species differed significantly with respect to number of roots per seedling. It was higher in the case of *A. crystallinum* (2.73) and was lower in *A. andreanum* (2.33).

Similarly, number of roots per seedling differed significantly with respect to different media. Highest number of roots per seedling was recorded on Nitsch medium (3.30) which was on par with MS medium (2.95). Where as MS medium (2.95) was also on par with VW (2.75) and KC (2.60) media. Similarly, KC (2.60) was on par with 1/2 strength Nitsch medium (2.35). Least number of roots per seedling was recorded on Morel's medium (1.25). Interaction effects were not significant between anthurium species and different media with respect to number of roots per seedling.

4.1.6.3. Shoot length (cm)

Shoot length differed significantly with different species. Highest shoot length was recorded in the case of *A. crystallinum* (3.04 cm) and was least in *A. andreanum*

(2.48 cm).

Media effect was also significant with respect to shoot length in different media. Highest shoot length was recorded on Nitsch medium (3.24 cm), followed by VW (2.98 cm) and MS medium (2.88 cm) which were at par. Similarly, shoot length on 1/2 strength Nitsch medium (2.49 cm) was on par with KC medium (2.24 cm) and differed significantly with other media. There was no significant difference among the interactions for species and different media.

4.1.6.4. Root to shoot ratio

Root to shoot ratio differed significantly between two species. It was higher in the case of *A. andreanum* (0.54) and was lower in *A. crystallinum* (0.45). Interaction effects were not significant for root to shoot ratio between species and media.

4.1.6.5. Fresh weight (mg)

Significantly higher fresh weight of seedlings was recorded in the case of *A. crystallinum* (333.20 mg) and was lower in *A. andreanum* (219.98 mg). Similarly, highest fresh weight was recorded by Nitsch medium grown seedling (374.70 mg) followed by MS and VW (278.0 and 272.05 mg, respectively) were at par. Lowest fresh weight was recorded on 1/2 strength Nitsch medium (222.0 mg) which was on par with KC medium (236.0 mg).

Significant differences were also recorded with respect interactions of different species and media. Highest fresh weight was recorded on Nitsch medium (498.70 mg) and was least on 1/2 strength Nitsch medium (263.10 mg), which was on par with KC medium (269.00 mg). Similarly, fresh weight of seedlings grown on MS medium (321.70 mg) was on par with VW medium (313.50 mg) in the case of *A. crystallinum*. While in *A. andreanum* highest fresh weight was recorded on Nitsch medium (250.70 mg) which was on par with MS and VW media (234.30 and 230.60 mg, respectively). Similaly, VM

medium (230.60 mg) was on par with KC medium (203.40 mg). Least fresh weight was recorded on 1/2 strength Nitsch medium (180.90 mg) but was also on par with KC medium (203.40 mg).

4.1.6.6. Dry weight (mg) of seedling

Anthurium species showed significant differences with respect to dry weight of seedlings. Higher dry weight was recorded in the case of *A. crystallinum* (35.72 mg) and was lower in *A. andreanum* (23.58 mg).

Significant differences were also recorded with respect to dry weight of seedling in different media. Highest dry weight was recorded on Nitsch medium (38.10 mg), followed by MS, VW and KC media (31.30, 28.85 and 26.20 mg, respectively). Lowest dry weight was recorded on 1/2 strength Nitsch medium (23.80 mg).

Interaction effects between anthurium species and different media with respect to dry weight were also significant. Highest dry weight of seedlings was recorded on Nitsch medium (50.60 mg) and was least on 1/2 strength Nitsch medium (28.20 mg) which was on par with KC medium (30.30 mg) in the case of *A. crystallinum*. Where as in the case of *A. andreanum*, highest dry weight was recorded on MS medium (26.30 mg) followed by Nitsch and VW media (25.60 and 24.50 mg, respectively) but were at par. Lowest dry weight was recorded on 1/2 strength Nitsch medium (19.40 mg) which was on par with KC medium (22.10 mg).

4.1.6.7. Index between fresh weight and dry weight

Anthurium species did not show any significant differences with respect to FW/DW X 100 index. But significant differences were observed with respect to media. Highest index was recorded on Nitsch medium (979.55) and was least on MS medium (885.45). Where as VW and 1/2 strength Nitsch media were at par (941.85 and 930.15, respectively).

The interaction effects for FW/DW X 100 index with respect to different species and media were also differed significantly. Highest index was recorded on Nitsch medium (985.20), followed by VW medium (943.00) which was on par with 1/2 strength Nitsch (933.20). Least index was recorded on MS medium (880.80) and was on par with KC medium (887.70) in *A. crystallinum*. While in the case of *A. andreanum*, highest index was recorded on Nitsch medium (973.90) followed by VW medium (940.70) which was on par with 1/2 strength Nitsch medium (927.10). Similarly, 1/2 strength Nitsch medium (927.10) was on par with KC medium (919.80). Least index was recorded on MS medium (890.10).

4.2. Adjuvants

4.2.1. Seed germination

The data on seed germination as influenced by different anthurium species and different adjuvants are furnished in Table-7, Fig.3 and Plate 5.

Seed germination was initiated at three DAS in both the species and extended upto 13 DAS. Seed germination rate differed significantly with respect to species. Highest number of seeds germinated in the case of *A. andreanum* (3.96) and was least in *A. crystallinum* (3.44). Both the species have taken same number of days (13 DAS) for seed germination.

Seed germination rate differed significantly with respect to different media also. Highest number of seeds germinated on control (4.45), followed by medium supplemented with coconut water and wheat malt (4.30 and 3.75, respectively) which were at par. Lowest number of seeds germinated on medium supplemented with ragi malt (2.65) which was on par with banana pulp (3.35). Early seed germination was recorded on medium supplemented with coconut water (10 DAS), followed by control and banana pulp (11 and 12 DAS, respectively). Seed germination was late on medium supplemented with ragi malt and wheat malt (13 DAS).

Plate 5. Effects of adjuvants on germination of *A. crystallinum* seeds

1. Banana pulp 2. Ragi malt 3. Wheat malt 4. Coconut water 5. Control

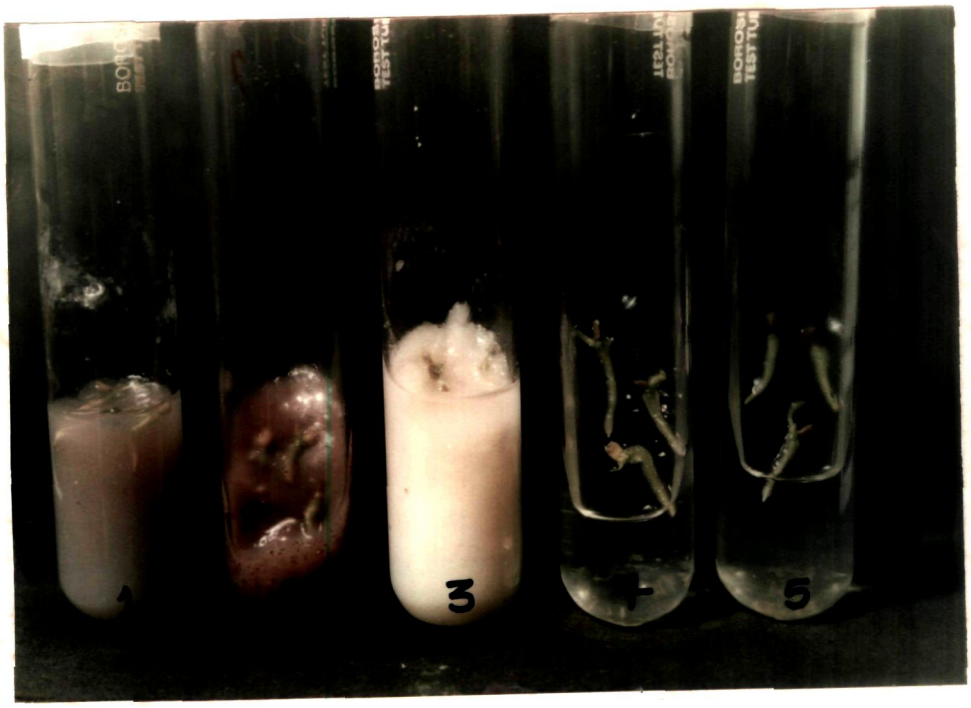
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Table-7 : Effects of Mitsch medium supplemented with different adjuvants on germinaton of seeds of the two species of anthurium

Treatments	DAS										
	3	4	5	6	7	8	9	10	11	12	13
Ac	2.06	2.12	2.34	2.54	2.72	2.82	3.00	3.28	3.28	3.40	3.44
Aa	2.66	2.78	3.04	3.18	3.32	3.46	3.56	3.66	3.84	3.94	3.96
SEM±	0.13	0.13	0.15	0.14	0.15	0.16	0.16	0.16	0.17	0.17	0.17
C.D. at 5%	0.37	0.37	0.40	0.40	0.41	0.43	0.45	NS	0.46	0.47	0.47
Adjuvants											
Mitsch	4.00	4.00	4.05	4.05	4.15	4.15	4.25	4.25	4.45	4.45	4.45
Ragi Malt	1.10	1.30	1.55	1.75	1.80	1.95	2.05	8.30	2.55	2.60	2.65
Banana Pulp	1.60	1.65	2.10	2.30	2.65	2.85	2.85	3.10	3.10	3.35	3.35
Coconut water	3.05	3.20	3.55	3.75	3.90	3.90	4.15	4.30	4.30	4.30	4.30
Wheat Malt	2.05	2.10	2.20	2.45	2.60	2.85	3.10	3.25	3.40	3.65	3.75
SEM±	0.21	0.21	0.23	0.23	0.23	0.25	0.26	0.26	0.26	0.27	0.27
C.D. at 5%	0.58	0.59	0.64	0.63	0.65	0.72	0.71	0.72	0.72	0.74	0.74

A x B

Treatments	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa
Mitsch	3.80	4.20	3.80	4.20	3.90	4.20	3.90	4.20	4.10	4.20	4.10	4.20	4.30	4.20	4.30	4.20	4.30	4.60	4.30	4.60	4.30	4.60
Ragi Malt	1.10	1.10	1.40	1.20	1.60	1.50	1.80	1.70	1.80	1.80	1.80	2.10	2.00	2.10	2.20	2.40	2.50	2.60	2.50	2.70	2.50	2.80
Banana Pulp	1.30	1.90	1.30	2.00	1.40	2.80	1.70	2.90	2.10	3.20	2.20	3.50	2.20	3.50	2.50	3.70	2.50	3.70	2.90	3.80	2.90	3.80
Coconut water	2.00	4.10	2.00	4.40	2.60	4.50	2.80	4.10	3.10	4.70	3.10	4.70	3.60	4.70	3.90	4.70	3.90	4.70	3.90	4.70	3.90	4.70
Wheat Malt	2.10	2.00	2.10	2.10	2.20	2.20	2.50	2.40	2.50	2.70	2.90	2.80	2.90	3.30	3.20	3.30	3.20	3.60	3.40	3.90	3.60	3.90
SEM±	0.29		0.30		0.32		0.32		0.33		0.35		0.36		0.36		0.37		0.38		0.38	
C.D. at 5%	0.83		0.83		0.90		0.90		NS		NS		NS		NS		NS		NS		NS	

* Ac - Anthurium crystallinum
* Aa - Anthurium andreanum

Number of seeds sown = 5 per flask

The interactions for seed germination with respect to different species and media supplemented with different adjuvants differed significantly during initial period (3 to 6 DAS) but there were no significant differences after 7 DAS.

4.2.2. First leaf initiation

Significant difference was observed with respect to first leaf initiation among different species. Higher number of germinated seeds initiated first leaf in the case of *A. andreanum* (3.96) and was lower in *A. crystallinum* (3.48). There was no difference with respect to time taken for first leaf initiation in both the species (36 DAS).

Different media showed significant differences with respect to first leaf initiation. Highest number of germinated seeds initiated first leaf on control (4.45), which was on par with medium supplemented with coconut water and wheat malt (4.40 and 3.75, respectively). Similarly, first leaf initiation on medium supplementd with wheat malt (3.75) was also on par with banana-pulp (3.35). Lowest number of germinated seeds initiated first leaf on medium supplemented with ragi malt (2.65) which was on par with banana pulp (3.35). First leaf initiation was early on control and medium supplemented with wheat malt (32 DAS) followed by ragi malt and banana pulp (34 DAS). First leaf initiation was delayed in medium supplemented with coconut water (36 DAS).

Interactions for first leaf initiation with respect to different species and medium supplemented with different adjuvants were non significant (Table-8 and Plates 6 and 7).

4.2.3. Second leaf initiation

Results were not significant with second leaf initiation in different species. The interaction effects between species and adjuvants were also non significant (Table-9).

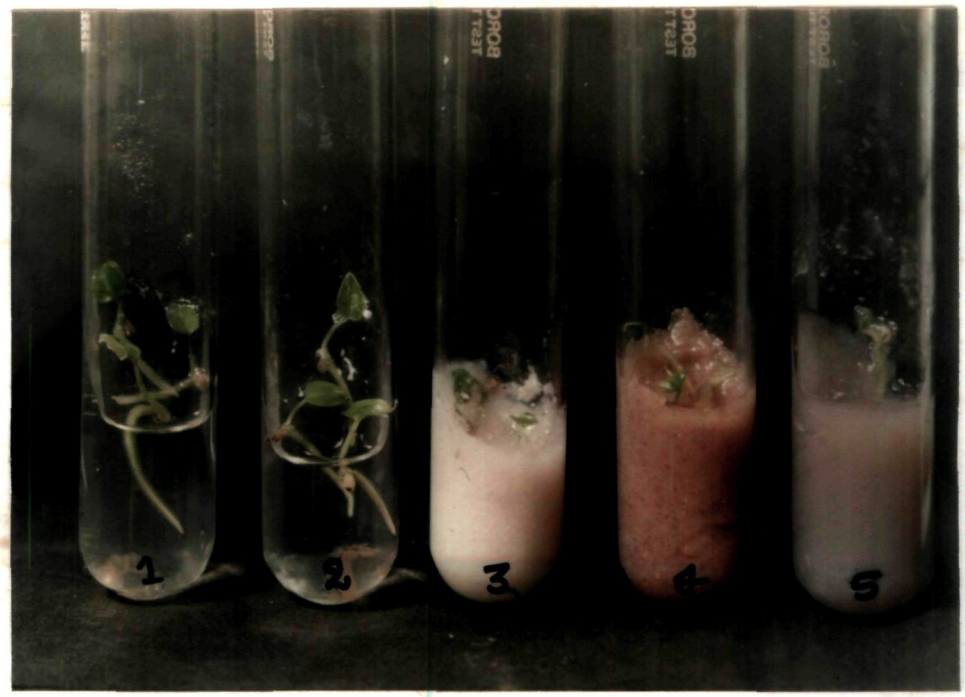
Significant differences were observed with respect to medium supplementd with different adjuvants for second leaf initiation. Highest number of seedlings initiated second

Plate 6 : Effects of adjuvants on first leaf initiation in *A. crystallinum*

1. Control 2. Coconut water 3. Wheat malt 4. Ragi malt 5. Banana pulp

Plate 7 : Effects of adjuvants on first leaf initiation in *A. andreanum*

1. Control 2. Coconut water 3. Wheat malt 4. Ragi malt 5. Banana pulp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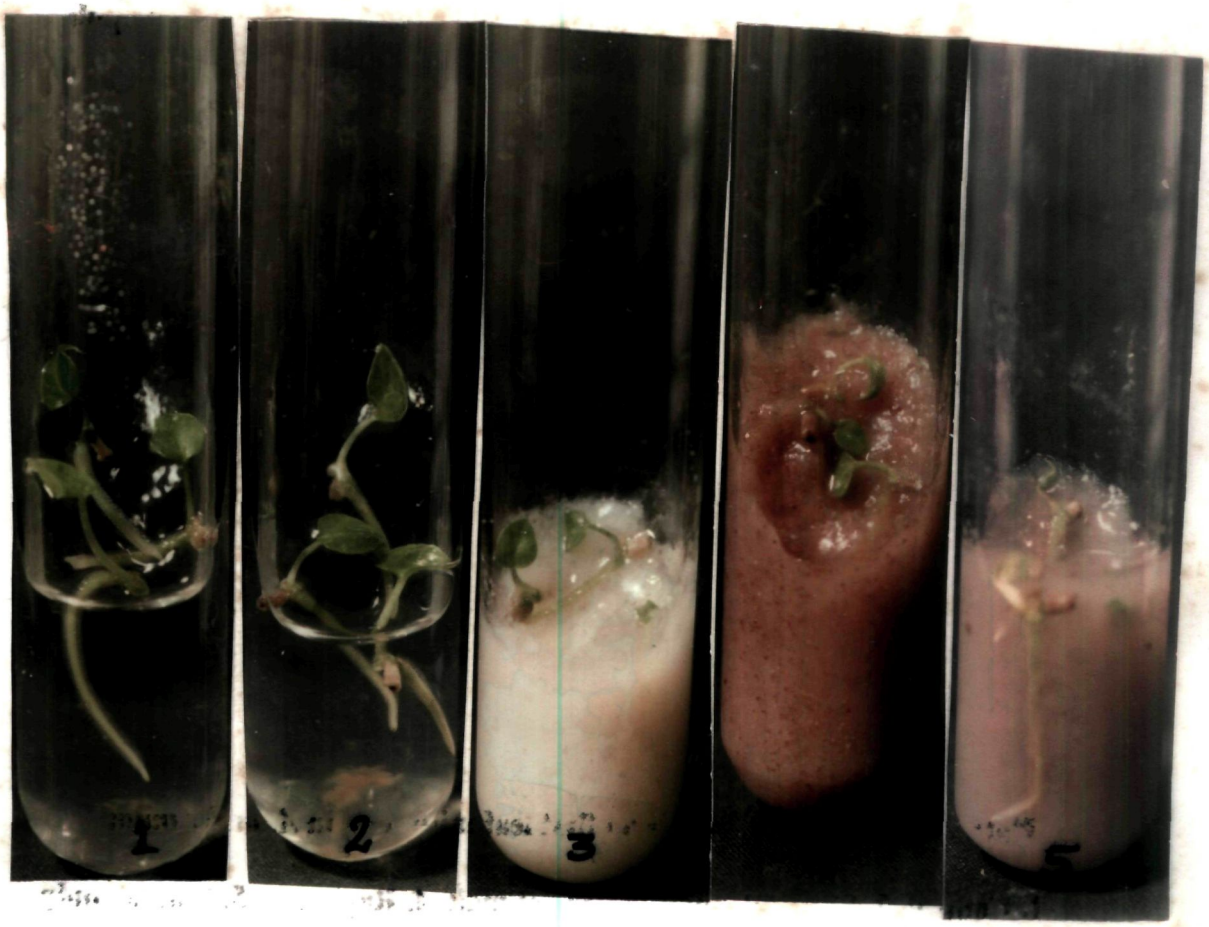
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Table-8 : Effects of Nitsch medium supplemented with different adjuvants on initiation of first leaf of the two species of anthurium

Treatments	DAS								
	20	22	24	26	28	30	32	34	36
Ac	0.54	1.30	1.90	2.30	2.62	2.90	3.26	3.40	3.40
Aa	1.36	1.66	2.30	2.78	3.30	3.56	3.86	3.96	3.96
SEM±	0.11	0.15	0.16	0.16	0.16	0.17	0.18	0.17	0.17
C.D. at 5%	0.32	NS	NS	0.44	0.44	0.48	0.49	0.48	0.47
Control	1.95	2.75	3.05	3.60	4.00	4.30	4.45	4.45	4.45
Ragi Malt	0.55	0.80	1.15	1.55	1.80	2.10	2.55	2.65	2.65
Banana Pulp	0.60	0.95	1.70	2.25	2.50	2.75	3.15	3.35	3.35
Coconut water	0.90	1.70	2.30	2.70	3.30	3.55	3.90	4.20	4.40
Wheat Malt	0.75	1.20	2.30	2.60	3.20	3.45	3.75	3.75	3.75
SEM±	0.18	0.26	0.25	0.25	0.25	0.27	0.28	0.27	0.27
C.D. at 5%	0.50	0.65	0.70	0.69	0.70	0.75	0.77	0.76	0.74

A x B

Treatments	20		22		24		26		28		30		32		34		36	
	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa
Control	1.50	2.40	2.50	3.00	2.90	3.20	3.20	4.00	3.60	4.40	4.00	4.60	4.30	4.60	4.30	4.60	4.30	4.60
Ragi Malt	0.50	0.60	0.80	0.80	1.00	1.30	1.30	1.80	1.80	2.00	1.80	2.40	2.30	2.80	2.50	2.80	2.50	2.80
Banana Pulp	0.10	1.10	0.80	1.10	1.40	2.00	2.00	2.50	2.10	2.90	2.40	3.10	2.70	3.60	2.90	3.80	2.90	3.80
Coconut water	0.20	1.60	1.40	2.00	1.90	2.70	2.40	3.00	2.70	3.90	2.90	4.20	3.40	4.40	3.70	4.70	4.10	4.70
Wheat Malt	0.40	1.10	1.00	1.40	2.30	2.30	2.60	2.60	3.10	3.30	3.40	3.50	3.60	3.90	3.60	3.90	3.60	3.90
SEM±	0.26		0.33		0.36		0.35		0.36		0.39		0.39		0.39		0.38	
C.D. at 5%	NS		NS		NS		NS		NS		NS		NS		NS		NS	

* Ac - Anthurium crystallinum
* Aa - Anthurium andreanum

Number of seeds sown = 5 per flask

Table-9 : Effects of Nitsch medium supplemented with different adjuvants on initiation of second leaf of the two species of anthurium

Treatments	DAS											
	30	32	34	36	38	40	42	44	46	48	50	52
Ac	0.28	0.46	1.08	1.34	1.52	1.94	2.28	2.82	2.96	3.30	3.44	3.48
Aa	0.36	0.46	0.82	1.14	1.30	1.86	2.32	2.80	3.36	3.66	3.80	3.82
SEM±	0.08	0.09	0.10	0.11	0.12	0.16	0.19	0.21	0.21	0.18	0.18	0.18
C.D. at 5%	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Control	0.00	0.40	1.40	2.00	2.20	2.80	3.20	3.55	3.80	4.15	4.35	4.34
Ragi Malt	0.00	0.15	0.50	0.70	0.80	0.95	1.15	1.75	2.20	2.55	2.60	2.60
Banana Pulp	0.90	0.05	0.40	0.55	0.60	1.50	1.90	2.65	3.20	3.20	3.20	3.20
Coconut water	0.50	1.15	1.70	1.80	2.15	2.30	2.95	3.35	3.50	3.95	3.20	4.35
Wheat Malt	0.20	0.55	0.75	1.15	1.30	1.95	2.35	2.75	3.10	3.55	3.75	3.75
SEM±	0.12	0.14	0.16	0.18	0.18	0.25	0.30	0.33	0.33	0.29	0.28	0.28
C.D. at 5%	0.34	0.39	0.45	0.50	0.51	0.71	0.83	0.91	0.92	0.81	0.78	0.78

A x B

Treatments	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa
Control	0.00	0.00	0.40	0.40	1.80	1.00	2.40	1.60	2.60	1.80	3.30	2.30	3.80	2.60	4.10	3.00	4.10	3.50	4.30	4.00	4.30	4.40	4.30	4.40
Ragi Malt	0.00	0.00	0.20	0.10	1.80	0.20	0.80	0.60	1.00	0.60	1.20	0.70	1.20	1.10	1.90	1.60	2.00	2.40	2.40	2.70	2.50	2.70	2.50	2.70
Banana Pulp	0.80	1.00	0.10	0.00	0.60	0.20	0.80	0.30	0.90	0.30	1.50	1.50	1.80	2.00	2.60	2.70	2.90	3.50	3.50	3.50	2.90	3.50	2.90	3.50
Coconut water	0.40	0.60	1.20	1.10	1.50	1.90	1.60	2.00	1.90	2.40	1.90	2.70	2.60	3.30	3.20	3.50	3.20	3.80	4.30	4.30	3.90	4.50	4.10	4.60
Wheat Malt	0.20	0.20	0.40	0.70	0.70	0.80	1.10	1.20	1.20	1.40	1.80	2.10	2.00	2.60	2.30	3.20	2.60	3.60	3.30	3.80	3.60	3.90	3.60	3.90
SEM±	0.18		0.20		0.23		0.25		0.26		0.36		0.43		0.46		0.47		0.41		0.40		0.40	
C.D. at 5%	NS		NS		NS		NS		NS		NS		NS		NS		NS		NS		NS		NS	

* Ac - Anthurium crystallinum
* Aa - Anthurium andreanum

Number of seeds sown = 5 per flask

Plate 10.a,b : Effects of different media on fourth leaf initiation in *A. crystallinum*

1. Control 2. Banana pulp 3. Coconut water 4. Wheat malt 5. Ragi malt

10 a

10 b

leaf on control and medium supplemented with coconut water (4.35), which was on par with wheat malt (3.75). Least number of seedlings initiated second leaf on medium supplemented with ragi malt (2.60), followed by banana pulp (3.75) which were at par. Similarly, medium supplemented with wheat malt (3.75) and banana pulp (3.20) were on par.

4.2.4. Third leaf initiation

Results were significant for third leaf initiation and species. Interactions between species and medium supplemented with different adjuvants were significant during initial stages (56 and 64 DAS, respectively). But there were no significant differences after 60 and 68 DAS, respectively.

Significant differences were observed among the media supplemented with different adjuvants with respect to third leaf initiation. Highest number of seedlings initiated third leaf on medium supplemented with coconut water (4.25), followed by control (4.10), but were at par. Similarly, medium supplemented with banana pulp (3.20) was on par with wheat malt (3.00). Least number of seedlings initiated third leaf on medium supplemented with ragi malt (1.55). Third leaf initiation was early with medium supplemented with ragi malt (68 DAS), followed by banana pulp, wheat malt and coconut water (76, 76 and 80 DAS, respectively). Third leaf initiation was late (84 DAS) in the case of control (Table-10, Plates 10a and 10b).

Interactions were also significant with species and media supplemented with different adjuvants during 56 to 64 DAS, but differences were not significant after 68 DAS.

4.2.5. Fourth leaf initiation

Higher number of seedlings initiated fourth leaf in the case of *A. andreanum* (2.32) and was lower in *A. crystallinum* (1.99). There was no difference with respect to

Table-10: Effects of Nitsch medium supplemented with different adjuvants on initiation of third leaf of the two species of anthurium

Treatments	DAS										
	48	52	56	60	64	68	72	76	80	84	88
Ac	0.54	0.90	1.92	2.30	2.58	2.86	3.10	3.16	3.16	3.16	3.16
Aa	0.12	0.54	1.26	1.90	2.46	2.60	2.90	3.16	3.24	3.28	3.30
SEm±	0.07	0.09	0.13	0.15	0.15	0.17	0.18	0.17	0.17	0.17	0.17
C.D. at 5%	0.19	0.25	0.35	NS	NS	NS	NS	NS	NS	NS	NS
Control	0.55	1.45	2.25	2.40	2.70	2.95	3.55	3.85	4.00	4.10	4.10
Ragi Malt	0.10	0.25	0.60	1.15	1.55	1.55	1.55	1.55	1.55	1.55	1.55
Banana Pulp	0.10	0.20	1.35	2.00	2.65	2.85	3.10	3.20	3.20	3.20	3.20
Coconut water	0.70	1.35	2.30	2.85	3.40	3.65	3.95	4.20	4.25	4.25	4.25
Wheat Malt	0.20	0.35	1.45	2.10	2.30	2.65	2.85	3.00	3.00	3.00	3.00
SEm±	0.11	0.14	0.20	0.24	0.24	0.26	0.28	0.27	0.27	0.27	0.27
C.D. at 5%	0.30	0.40	0.56	0.66	0.66	0.73	0.78	0.76	0.76	0.76	0.76

A x B

Treatments	48		52		56		60		64		68		72		76		80		84		88	
	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa
Control	0.90	0.20	1.90	1.00	3.00	1.50	3.30	1.50	3.50	1.90	3.70	2.20	4.00	3.10	4.00	3.70	4.00	4.00	4.00	4.20	4.00	4.30
Ragi Malt	0.20	0.00	0.30	0.20	1.00	0.20	1.40	0.90	1.40	1.70	1.40	1.70	1.40	1.70	1.40	1.70	1.40	1.70	1.40	1.70	1.40	1.70
Banana Pulp	0.20	0.00	0.30	0.10	1.70	1.00	2.50	1.50	2.60	2.70	2.70	3.00	2.90	3.30	2.90	3.50	2.90	3.50	2.90	3.50	2.90	3.50
Coconut water	1.10	0.30	1.40	1.30	2.60	2.00	2.70	3.00	3.40	3.40	3.80	3.50	4.10	3.80	4.10	4.30	4.10	4.40	4.10	4.40	4.10	4.40
Wheat Malt	0.30	0.10	0.60	0.10	1.30	1.60	1.60	2.60	2.00	2.60	2.70	2.60	3.10	2.60	3.40	2.60	3.40	2.60	3.40	2.60	3.40	2.60
SEm±	0.16		0.20		0.28		0.34		0.34		0.37		0.40		0.39		0.39		0.38		0.38	
C.D. at 5%	NS		NS		0.79		0.93		0.93		NS		NS		NS		NS		NS		NS	

* Ac - Anthurium crystallinum
* Aa - Anthurium andreanum

Number of seeds sown = 5 per flask

Table-11: Effects of Nitsch medium supplemented with different adjuvants on initiation of fourth leaf of the two species of anthurium

Treatments	DAS									
	80	84	88	92	96	100	104	108	112	116
Ac	0.18	0.28	0.88	0.94	1.40	1.68	1.80	1.86	1.90	1.96
Aa	0.48	0.98	1.28	1.74	2.00	2.22	2.32	2.32	2.32	2.32
SEM±	0.08	0.10	0.11	0.11	0.13	0.13	0.14	0.14	0.13	0.13
C.D. at 5%	0.21	0.28	0.31	0.31	0.35	0.37	0.38	0.38	0.37	0.37
Control	0.40	0.80	1.41	1.90	2.35	3.00	3.50	3.50	3.60	3.70
Ragi Malt	0.00	0.00	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Banana Pulp	0.40	0.10	1.60	2.25	2.80	3.00	3.00	3.00	3.00	3.00
Coconut water	0.75	1.10	1.75	2.55	3.35	3.75	3.95	3.95	3.95	3.95
Wheat Malt	0.10	0.35	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55
SEM±	0.12	0.16	0.18	0.18	0.20	0.21	0.22	0.22	0.21	0.21
C.D. at 5%	0.34	0.44	0.49	0.49	0.56	0.59	0.60	0.60	0.59	0.60

A x B

Treatments	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa
Control	0.30	0.50	0.40	1.20	1.00	1.80	1.60	2.20	2.00	2.70	2.40	3.60	2.70	4.00	3.00	4.00	3.20	4.00	3.40	4.00
Ragi Malt	0.00	0.00	0.00	0.00	0.20	0.00	0.20	0.00	0.20	0.00	0.20	0.00	0.20	0.00	0.20	0.00	0.20	0.00	0.20	0.00
Banana Pulp	0.00	0.80	0.00	1.80	0.80	2.40	1.40	3.10	2.10	3.50	2.50	3.50	2.50	3.50	2.50	3.50	2.50	3.50	2.50	3.50
Coconut water	0.50	1.00	0.70	1.50	1.30	2.20	1.70	3.40	2.90	3.80	3.50	4.00	3.80	4.10	3.80	4.10	3.80	4.10	3.80	4.10
Wheat Malt	0.10	0.10	0.30	0.40	1.10	0.40	1.10	0.40	1.10	0.40	1.10	0.40	1.10	0.40	1.10	0.40	1.10	0.40	1.10	0.40
SEM±	0.17		0.22		0.25		0.25		0.29		0.30		0.30		0.30		0.30		0.29	
C.D. at 5%	0.48		0.62		0.69		0.69		NS		NS		NS		NS		NS		NS	

* Ac = Anthurium crystallinum
* Aa = Anthurium andreanum

Number of seeds sown = 5 per flask

time taken for fourth leaf initiation and it was maximum at 116 DAS.

Significant differences were observed among different adjuvants for fourth leaf initiation. Highest number of seedlings initiated fourth leaf on medium supplemented with coconut water (3.95) and was on par with control (3.70), followed by banana pulp (3.00). Very less number of seedlings initiated fourth leaf on medium supplemented with ragi malt (0.10) which was on par with wheat malt (0.55).

Interactions were also significant with species and the medium supplemented with different adjuvants during 84 to 92 DAS, but there were no significant differences after 96 DAS (Table-11).

4.2.6. Seedling growth

The data on root length, number of roots per seedling, shoot length, root to shoot ratio, fresh weight, dry weight and the index between fresh weight and dry weight as influenced by different anthurium species, different adjuvants and their interactions are presented in Table-12 and Fig.4.

4.2.6.1. Root length (cm)

Root length differed significantly with anthurium species. Root length was higher in the case of *A. crystallinum* (5.65 cm) and was lower in *A. andreanum* (4.15 cm). Maximum root length was recorded on control (7.43 cm), followed by medium supplementd with coconut water (7.02 cm), however, they were at par. The medium supplemented with banana pulp recorded 6.07 cm root length and was 3.13 cm in wheat malt. It was minimum in the case of medium supplemented with ragi malt (0.84 cm). Interactions between root length, anthurium species and different adjuvants, differed significantly. Root length was highest in the case of control (9.28 cm) and was on par with medium supplemented with banana pulp (8.15 cm) in the case of *A. crystallinum*. This was followed by medium supplemented with coconut water (7.04 cm) and lowest

Table-12: Effects of Nitsch medium supplemented with different adjuvants on the seedling growth parameters in of the two species of anthurium

Treatments	Root length (cm)	Number of roots per seedling	Shoot lenght (cm)	Root to shoot ratio	Fresh weight (mg)	Dry weight (mg)	FW --- x 100 Index DW
Ac	5.65	2.96	3.40	1.08	531.94	52.76	958.1
Aa	4.15	2.48	2.83	0.97	216.76	21.70	993.4
SEM±	0.19	0.11	0.06	0.08	5.04	0.64	8.2
C.D. at 5%	0.52	0.29	0.17	NS	13.95	1.78	22.8
Control	7.43	2.95	3.00	0.42	354.25	33.70	1035.7
Ragi Malt	0.84	1.65	2.03	2.48	125.30	15.60	865.5
Banana Pulp	6.07	3.70	3.83	0.74	496.30	47.90	1028.3
Coconut water	7.02	3.05	4.19	0.63	701.15	66.95	1030.8
Wheat Malt	3.13	2.25	2.52	0.88	194.75	22.00	918.3
SEM±	0.30	0.17	0.10	0.12	7.96	1.01	13.0
C.D. at 5%	0.83	0.46	0.27	0.33	22.06	2.81	36.0

Treatments	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa
Control	9.28	5.57	3.10	2.80	3.37	2.64	0.37	0.48	464.00	244.50	43.40	24.00	1053.5	1017.8
Ragi Malt	0.89	0.79	2.10	1.20	2.25	1.80	2.75	2.22	166.70	83.90	22.70	8.50	744.8	986.2
Banana Pulp	8.15	4.00	4.10	3.30	4.75	2.91	0.64	0.84	818.80	173.80	78.50	17.30	1052.3	1004.2
Coconut water	7.04	7.00	3.20	2.90	3.88	4.49	0.61	0.65	973.30	429.00	91.30	42.60	1054.0	1007.7
Wheat Malt	2.87	3.39	2.30	2.20	2.77	2.30	1.05	0.71	236.90	152.60	27.90	16.10	885.7	950.9
SEM±	0.42		0.24		0.14		0.17		11.26		1.43		18.4	
C.D. at 5%	1.17		0.66		0.38		0.47		31.20		3.97		50.9	

* Ac - Anthurium crystallinum
* Aa - Anthurium andreanum

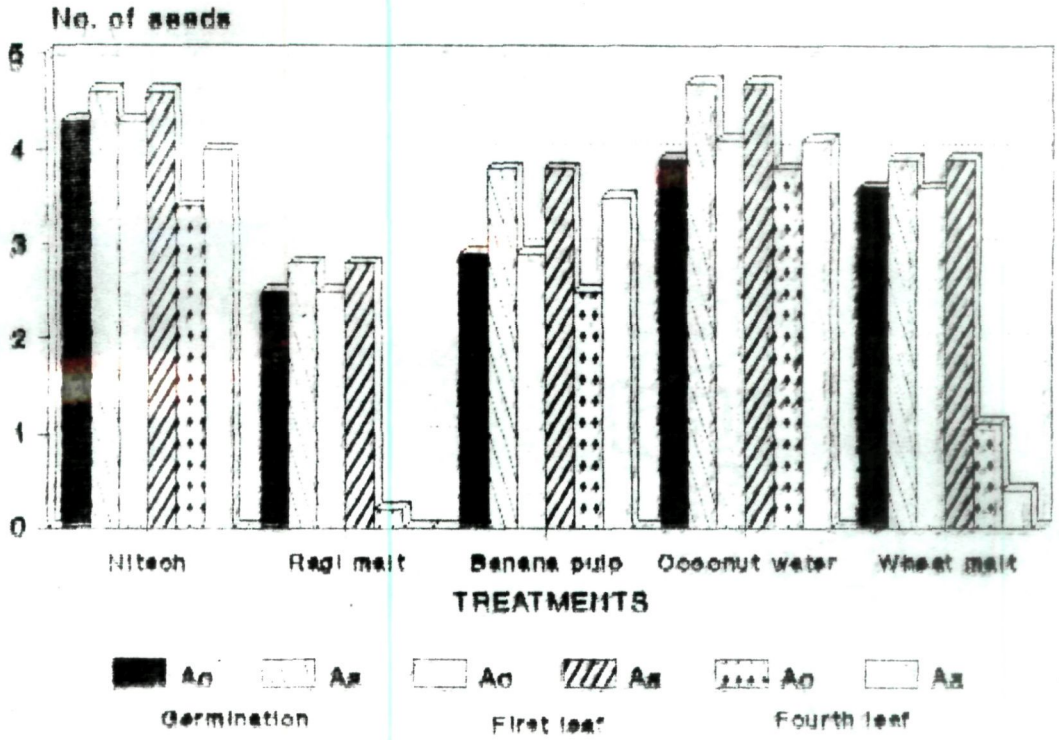


Fig.3:Effects of adjuants on seed germination and growth of anthuriums

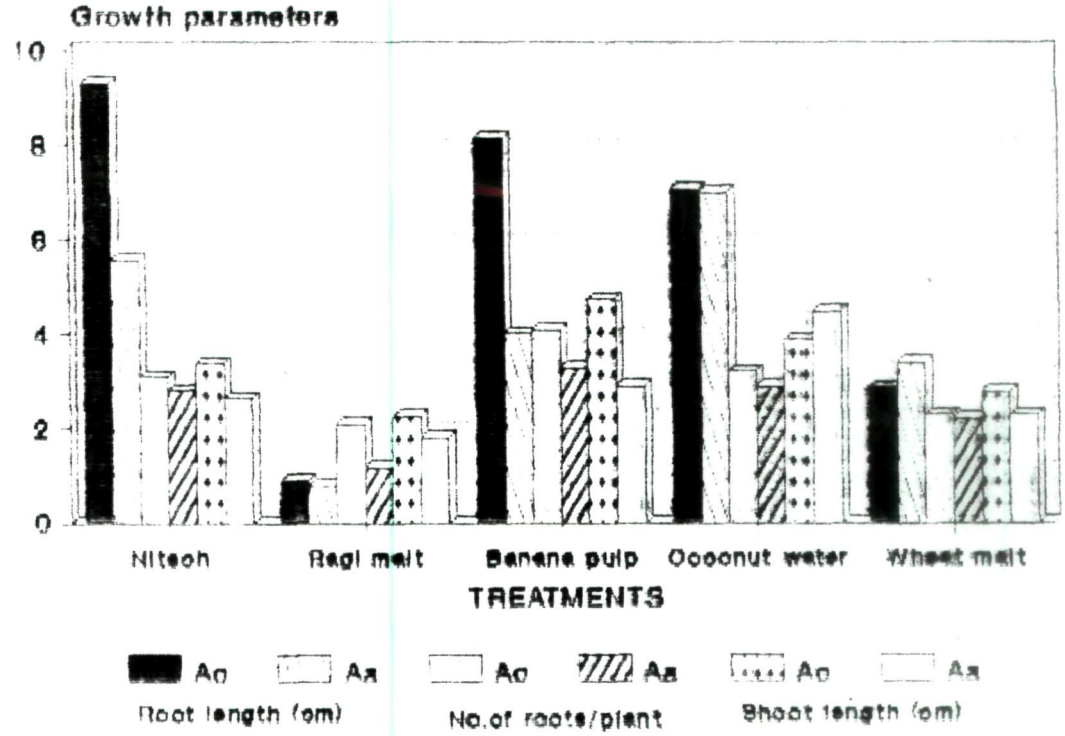


Fig.4:Effects of adjuvants on growth parameters of anthurium

root length was recorded in ragi malt (0.89 cm). While in the case of *A. andreanum* longest root length was recorded with coconut water (7.00 cm), followed by control (5.57 cm). It was on par with medium supplemented with banana pulp (4.00 cm) and wheat malt (3.39 cm). Root length was lowest in the case of medium supplemented with ragi malt (0.79 cm).

4.2.6.2. Number of roots per seedling

Significantly more number of roots per seedling was recorded in *A. crystallinum* (2.96) and was less in *A. andreanum* (2.48). Among the different adjuvants, more number of roots per seedling was recorded in the case of medium supplemented with banana pulp (3.70). The medium supplemented with coconut water (3.05) was on par with control (2.95), followed by wheat malt (2.25) and ragi malt (1.65) in producing number of roots per seedling.

Significant differences were not observed in the interactions for anthurium species and media supplemented with different adjuvants.

4.2.6.3. Shoot length (cm)

Among the two anthurium species significantly higher shoot length was recorded in the case of *A. crystallinum* (3.40 cm) and was lower in *A. andreanum* (2.83 cm).

Similarly, shoot length differed significantly with respect to medium supplementd with different adjuvants. Highest shoot length was recorded in the case of medium supplemented with coconut water (4.19 cm), followed by banana pulp (3.83 cm). Least shoot length was recorded in medium supplemented with ragi malt (2.03 cm).

Shoot length differed significantly with respect to interactions between different species and media supplemented with adjuvants. Highest shoot length was recorded in the case of medium supplemented with banana pulp (4.75 cm) and was lowest with ragi malt

(2.03 cm) in *A. crystallinum*. Where as in the case of *A. andreanum*, highest shoot length was recorded with coconut water (4.49 cm). Medium supplemented with banana pulp (2.91 cm) was on par with Nitsch medium without adjuvants (2.64 cm). Similarly, Nitsch medium without adjuvants (2.64 cm) was also on par with medium supplemented with wheat malt (2.30 cm). Least shoot length was recorded in the case of medium supplemented with ragi malt (1.80 cm).

4.2.6.4. Root to shoot ratio

Root to shoot ratio did not differ significantly among the anthurium species. Interactions were also non significant between anthurium species and media supplemented with different adjuvants.

Root to shoot ratio differed significantly with respect to media supplemented with different adjuvants. Highest ratio was recorded in the case of medium supplemented with ragi malt (2.48) and lowest root to shoot ratio was recorded on control (0.42). Where as medium supplemented with wheat malt, banana pulp and coconut water were at par (0.88, 0.74 and 0.63, respectively). Similarly, media supplemented with banana pulp, coconut water and Nitsch medium were also at par (0.74, 0.63 and 0.42, respectively).

4.2.6.5. Fresh weight (mg) of the seedling

Significant differences were observed with respect to fresh weight in different species. Higher fresh weight was recorded in the case of *A. crystallinum* (531.94 mg) and was lower in *A. andreanum* (216.76 mg).

The media supplemented with different adjuvants differed significantly with respect to fresh weight of seedlings. Highest fresh weight was recorded in the case of medium supplemented with coconut water (701.15 mg), followed by banana pulp (496.30 mg), control (354.25 mg) and wheat malt (194.75 mg). Least fresh weight of the seedling was recorded in the case of medium supplemented with ragi malt (125.30 mg).

The interaction effects for fresh weight of the seedling for different species and media supplemented with different adjuvants differed significantly. Highest fresh weight of seedlings was recorded in the case of medium supplemented with coconut water (973.30 mg) and was least with ragi malt (166.70 mg) in *A. crystallinum*. Where as *A. andreanum* significantly registered highest fresh weight of seedlings with medium supplemented with coconut water (429.00 mg), followed by Nitsch medium without adjuvants (244.50 mg). The medium supplemented with banana pulp (173.80 mg) was on par with wheat malt (152.60 mg). Least dry weight of seedlings was recorded in the case of medium supplemented with ragi malt (83.90 mg).

4.2.6.6. Dry weight (mg) of the seedlings

Significant differences were recorded among the two species, with respect to dry weight of the seedlings. Higher dry weight was recorded in the case of *A. crystallinum* (52.76 mg) and it was least in *A. andreanum* (21.70 mg).

Dry weight of the seedlings also differed significantly with respect to media supplemented with different adjuvants. Highest dry weight was recorded on medium supplemented with coconut water (66.95 mg); followed by banana pulp, Nitsch medium (without adjuvants) and wheat malt (47.10, 33.70 and 22.00 mg, respectively). Least dry weight was recorded in ragi malt containing medium (15.60 mg).

Similarly, interaction effects for dry weight of the seedling for different species and media supplemented with different adjuvants were found to be significant. Highest dry weight of the seedling was recorded in the case of medium supplemented with coconut water (91.30 mg), followed by banana pulp, Nitsch medium without adjuvants and wheat malt (78.50, 43.40 and 27.90 mg, respectively). Least dry weight of seedlings was recorded by *A. crystallinum* on media supplemented with ragi malt (22.70 mg), while it was highest (42.60 mg) in the case of *A. andreanum* when coconut water was used as the adjuvant, followed by Nitsch medium without adjuvants (24.00 mg). Dry weight of the seedlings was at par (17.30 and 16.10 mg, respectively) in the medium supplemented

with banana pulp and wheat malt. It was least in the case of medium supplemented with ragi malt (8.50 mg).

4.2.6.7. Index between fresh weight and dry weight

FW/DW X 100 index of the seedling differed significantly with respect to species. Higher index was recorded in the case of *A. andreanum* (993.4) and was lower in *A. crystallinum* (958.1).

Differences were significant with respect to media supplemented with different adjuvants for FW/DW X 100 index of the seedling. Highest index was recorded in the case of Nitsch medium without adjuvants (1035.70), which was on par with coconut water and banana pulp (1030.80 and 1028.30, respectively), followed by wheat malt (918.3). Least index was recorded in the case of medium supplemented with ragi malt (865.3).

The interactions of different species and the media supplemented with different adjuvants also differed significantly. Although, *A. crystallinum* showed higher FW/DW X 100 index with the medium containing coconut water, the results were at par with Nitsch medium without adjuvants and banana pulp (1054.0, 1053.5 and 1052.3, respectively), followed by medium wheat malt (885.7). Index was least in the case of medium supplemented with ragi malt (744.8). *A. andreanum* registered lesser index as compared to that of *A. crystallinum*. Among different adjuvants, in the case of *A. andreanum* highest index was recorded with Nitsch medium without adjuvants (1017.8), which was on par with media supplemented with coconut water and banana pulp (1007.7 and 1004.2, respectively). Lesser index was recorded with wheat malt (950.9) which was on par with ragi malt (986.2). Similarly, media supplemented with coconut water, banana pulp and ragi malt were also at par (1007.7, 1004.2 and 986.2, respectively).

4.3. Effects of BAP and 2,4-D on callus induction

Data on number of seeds initiating callus, callus index, callus colour and texture of the callus as influenced by BAP in combination with various concentrations of 2,4-D are presented in Table-13, Fig.5, Plates 11a, b, c and d.

4.3.1. Number of seeds initiating callus on Nitsch media

Results were not significant for number of seeds producing callus and different species at different weeks after inoculation (WAI). Similarly results were also not significant between the time taken for callus initiation and species (7 WAI).

Callus induction differed significantly with respect to medium supplemented with BAP and various concentrations of 2,4-D. Highest number of seeds induced callus in the case of Nitsch medium supplemented with 1.0 ppm each of BAP and 2,4-D (3.15), which was on par with 1.0 ppm BAP (2.70) alone. This (2.70) was also on par with 1.0 ppm BAP and 2.0 ppm 2,4-D (2.40). Least number of seeds induced callus with 1.0 ppm BAP and 6 ppm 2,4-D (2.05), but there was no callus induction in control where no BAP and 2,4-D were present. Results were non significant with respect to time taken for callus induction in all the treatments (7 WAI). The interactions of different species and treatments with BAP and 2, 4-D were non significant throughout the experimental period, except for six WAI.

4.3.1.6. Number of seeds initiating callus on MS medium

There was no significant difference with respect to number of seeds initiating callus among the two species. Similarly, time taken for callus induction in both the species was also non significant.

Number of seeds initiating callus differed significantly with respect to MS medium supplemented with BAP and various concentrations of 2,4-D. Highest number of seeds

Plate 11a : Effects of BAP and 2,4-D levels on callus initiation in *A. crystallinum* (Nitsch)

1. 1+0(ppm) 2. 1+1(ppm) 3. 1+2(ppm) 4. 1+4(ppm) 5. 1+6(ppm)

Plate 11b : Effects of BAP and 2,4-D levels on callus initiation in *A. andreanum* (Nitsch)

1. 1+0(ppm) 2. 1+1(ppm) 3. 1+2(ppm) 4. 1+4(ppm) 5. 1+6(ppm)


1
2
3
4
5


100 ml
BOROSIL
100 ml
VYLINE
100 ml
VYLINE
5
4
3
2
1

Plate 11c : Effects of BAP and 2,4-D levels on callus initiation in *A. crystallinum* (MS)

1. 1+0(ppm) 2. 1+1(ppm) 3. 1+2(ppm) 4. 1+4(ppm) [redacted]

Plate 11d : Effects of BAP and 2,4-D levels on callus initiation in *A. andreanum* (MS)

1. 1+0(ppm) 2. 1+1(ppm) 3. 1+2(ppm) 4. 1+4(ppm) 5. 1+6(ppm)


1
2
3
4

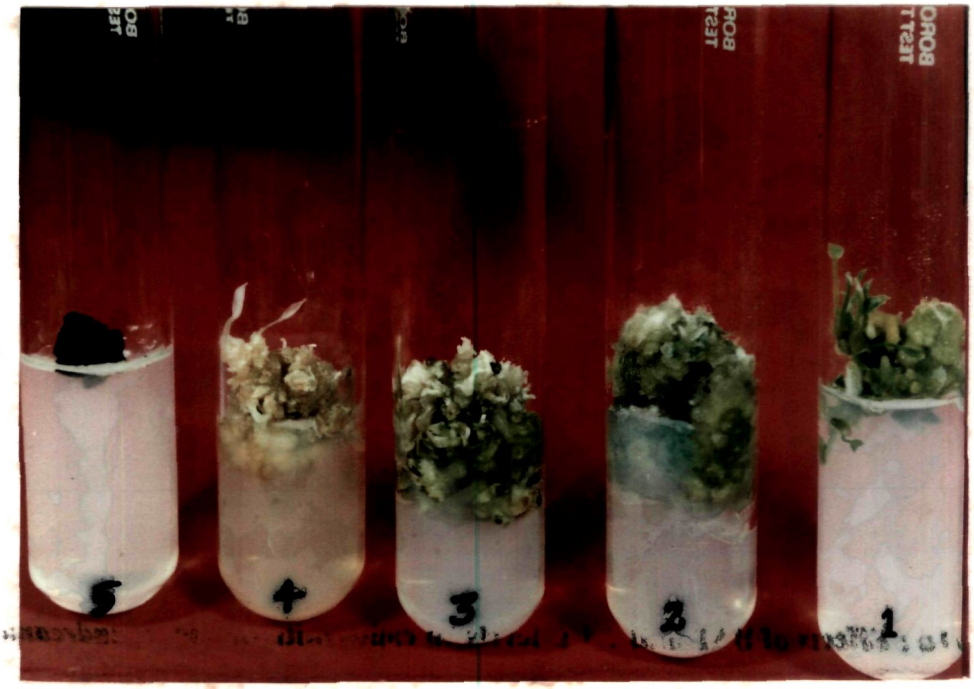

5
4
3
2
1

Table-13: Effects of BAP and different concentrations of 2, 4-D on callus induction of the two species of anthurium

Treatments (N + BAP + 2,4-D) ppm	WAI 3	4	5	6	7	Treatments (MS+BAP+2,4-D) ppm	8	9
Ac	1.48	1.63	1.80	1.85	2.02	Ac	2.13	2.23
Aa	1.57	1.68	1.78	1.92	2.00	Aa	2.05	1.10
SEM±	0.09	0.10	0.10	0.10	0.10	SEM±	0.09	0.09
C.D. at 5%	NS	NS	NS	NS	NS	D.Dat 5%	NS	NS
Control	0.00	0.00	0.00	0.00	0.00	1 + 0	0.00	0.00
1 + 0	1.85	2.05	2.35	2.55	2.70	1 + 0	3.00	3.05
1 + 1	2.00	2.40	2.60	2.85	3.15	1 + 1	3.35	3.60
1 + 2	2.15	2.25	2.35	2.35	2.40	1 + 2	2.40	2.50
1 + 4	1.70	1.80	1.90	1.90	2.05	1 + 4	2.05	2.05
1 + 6	1.45	1.45	1.55	1.65	1.75	1 + 6	1.75	1.80
SEM±	0.16	0.17	0.18	0.18	0.17	SEM±	0.16	0.16
C.D. at 5%	0.45	0.47	0.50	0.50	0.46	C.Dat 5%	0.44	0.44

A x B

Treatments (N + BAP + 2,4-D) ppm	3 Ac	3 Aa	4 Ac	4 Aa	5 Ac	5 Aa	6 Ac	6 Aa	7 Ac	7 Aa	Treatments (MS+BAP+2,4-D) ppm	8 Ac	8 Aa	9 Ac	9 Aa
Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	MS + 0 + 0	0.00	0.00	0.00	0.00
1 + 0	1.70	2.00	1.90	2.20	2.30	2.40	2.40	2.70	2.50	2.90	MS + 1 + 0	2.80	3.20	2.90	3.20
1 + 1	1.80	2.20	2.30	2.50	2.30	2.90	2.30	3.40	2.60	3.70	MS + 1 + 1	3.00	3.70	3.50	3.70
1 + 2	2.20	2.10	2.40	2.10	2.60	2.10	2.60	2.10	2.70	2.10	MS + 1 + 2	2.70	2.10	2.70	2.30
1 + 4	1.80	1.60	1.80	1.80	2.00	1.80	2.00	1.80	2.30	1.80	MS + 1 + 4	2.30	1.80	2.30	1.80
1 + 6	1.40	1.50	1.40	1.50	1.60	1.50	1.80	1.50	2.00	1.50	MS + 1 + 6	2.00	1.50	2.00	1.60
SEM±	0.23		0.24		0.25		0.25		0.23		SEM±	0.22		0.22	
C.D. at 5%	NS		0.67		NS		0.71		NS		C.Dat 5%	0.62		0.62	

* N - Nitsch medium
* MS - Murashige and Skoog's medium
* Ac - Anthurium crystallinum
* Aa - Anthurium andreanum

Number of seeds sown = 5 per flask

induced callusing in the case of MS medium supplemented with 1.0 ppm each of BAP and 2,4-D (3.60), followed by 1.0 ppm BAP alone (3.05) and 1.0 ppm BAP and 2.0 ppm 2,4-D (2.50). Least number of seeds initiated callus in the case of MS medium supplemented with 1.0 ppm BAP + 6.0 ppm 2,4-D (1.80). This treatment was also on par with 1.0 ppm BAP + 4.0 ppm 2,4-D (2.05). There was no callus induction in the case of MS medium supplemented with no BAP and 2,4-D. There was no difference in time taken for callus induction in different treatments.

Interaction effects for number of seeds initiating callus with respect to different species and MS medium supplemented with BAP and various concentrations of 2,4-D were significant at eight WAI. But, results were not significant after nine WAI.

4.3.2. Callus index, colour and texture

Callus index, colour and texture of callus did not vary significantly with respect to different species. But varied significantly with respect to Nitsch and MS medium and also media supplemented with BAP and 2,4-D.

In the case of Nitsch medium supplemented with 1.0 ppm BAP alone, 1.0 ppm BAP + 1.0 ppm 2,4-D and 1.0 ppm BAP + 2.0 ppm 2,4-D showed similar response with respect to callus index (++), colour (green) and texture (granular). Where as Nitsch medium supplemented with 1.0 ppm BAP + 4.0 ppm 2,4-D and 1.0 ppm BAP + 6.0 ppm 2,4-D responded similarly with respect to callus index (+), colour (green to grey) and texture (granular).

Callus obtained from Nitsch medium was inoculated on to MS medium containing same growth regulators. MS medium enhanced the growth of callus as compared to that of Nitsch. However, the callus characters such as callus index (++++), colour (green/greyish green/pale green to pinkish) and texture (granular/friable) were similar in both the species.

MS medium supplemented with 1.0 ppm BAP alone and 1.0 ppm each of BAP and 2,4-D recorded similar callus index (++++), callus colour (pale green) and texture (granular) but differed with respect to production of multiple shoots and roots. MS medium containing 1.0 ppm BAP alone induced more number of shoots and root, but medium supplemented with 1.0 ppm each of both BAP and 2, 4-D showed less number of shoots and there was no rooting. Similarly, MS medium supplemented with 1.0 ppm BAP + 2.0 ppm 2,4-D and 1.0 ppm BAP + 4.00 ppm 2,4-D responded in the same fashion with respect to callus index (++), callus colour (pale green and pale green to greyish, respectively) and texture (granular). Where as MS medium supplemented with 1.0 ppm BAP + 6.0 ppm 2,4-D, did not increase the callus growth (+), instead they turned grey to brown and resulted in drying.

4.4. Effects of different concentrations of BAP on shoot regeneration

Data on number of shoots per flask at different WAI shoot length, root length, number of roots per shooted callus clump, leaf length and leaf breadth as influenced by different concentrations of BAP are presented in Table-14, Fig.6, Plates 12 a and b.

4.4.1. Number of shoots per flask

Shoot regeneration in both the species initiated seven WAI and remained constant until 13 WAI. The number of shoots per flask differed significantly with respect to species. Higher number of shoots per flask was recorded in *A. andreanum* (22.28) and was lower in *A. crystallinum* (15.25). There was no difference in the time taken with respect to highest number of shoots per flask in both the species (13 WAI).

Number of shoots per flask differed significantly, at different WAI, with respect to different concentrations of BAP. Maximum number of shoots per flask was recorded in the case of medium supplemented with different concentrations of BAP 1.0, 2.5 and 5.0 ppm (30.00, 25.00 and 20.00, respectively). There was no shoot formation in the case of control (medium supplemented with no BAP). But there was no difference among

Plate 12a. : Effects of BAP on shoot regeneration in *A. crystallinum*

1. 1.0(ppm) 2. 5.0(ppm)

Plate 12b. : Effects of BAP on shoot regeneration in *A. andreanum*

1. 5.0(ppm) 2. 2.5(ppm) 3. 1.0(ppm)


1
2
3

Table-14: Effects of different concentration of BAP on shoot regeneration of the two species of anthurium

Treatments (MS+BAP) ppm	(No. of shoots/flask) WAI							Shoot Length (cms)	Root length (cms)	No.of roots per shooted callus clump	Leaf length (cm)	Leaf breadth (cm)
	7	8	9	10	11	12	13					
Ac	4.25	7.25	10.50	12.25	13.50	14.75	15.25	6.37	6.52	6.25	1.34	0.86
Aa	6.23	11.00	16.50	18.50	20.00	20.75	22.28	4.02	4.75	5.15	0.96	0.59
SEM±	0.24	0.35	0.45	0.49	0.47	0.42	0.39	0.19	0.16	0.19	0.02	0.02
C.D. at 5%	0.66	0.97	1.23	1.35	1.30	1.15	1.08	0.54	0.44	0.52	0.07	0.06
Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1.0	8.90	16.00	22.00	25.00	26.50	29.00	30.00	6.30	8.38	8.90	1.62	1.04
2.5	6.50	12.00	19.00	21.00	22.50	23.50	25.05	6.87	7.41	7.65	1.51	0.95
5.0	5.55	8.50	13.00	15.50	18.00	18.50	20.00	7.62	6.75	6.25	1.47	0.92
SEM±	0.34	0.49	0.63	0.69	0.66	0.59	0.55	0.29	0.22	0.26	0.03	0.03
C.D. at 5%	0.94	1.37	1.74	1.91	1.84	1.63	1.53	0.76	0.62	0.73	0.10	

A x B

Treatments (MS+BAP) ppm	7		8		9		10		11		12		13		Shoot Length (cms)		Root length (cms)		No.of roots per shooted callus clump		Leaf length (cm)		Leaf breadth (cm)	
	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa
Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	36.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1.0	8.00	9.80	12.00	20.00	15.00	29.00	18.00	32.00	20.00	33.00	23.00	35.00	24.00	30.00	7.77	4.82	9.98	6.78	9.80	8.00	1.84	1.40	1.22	0.85
2.5	5.00	8.00	10.00	14.00	15.00	23.00	17.00	25.00	18.00	27.00	20.00	27.00	20.00	23.00	8.53	5.21	8.42	6.39	8.50	6.80	1.79	1.22	1.13	0.77
5.0	4.00	7.10	7.00	10.00	18.00	14.00	14.00	17.00	16.00	20.00	16.00	21.00	17.00	23.00	9.19	6.04	7.66	5.83	6.70	5.80	1.73	1.21	1.09	0.75
SEM±	0.48		0.70		0.89		0.97		0.94		0.83		0.78		0.39		0.32		0.37		0.05		0.05	
C.D. at 5%	1.33		1.94		2.47		2.70		2.60		2.31		2.16		1.08		0.88		NS		0.15		0.14	

* Ac = Anthurium crystallinum
* Aa = Anthurium andreanum

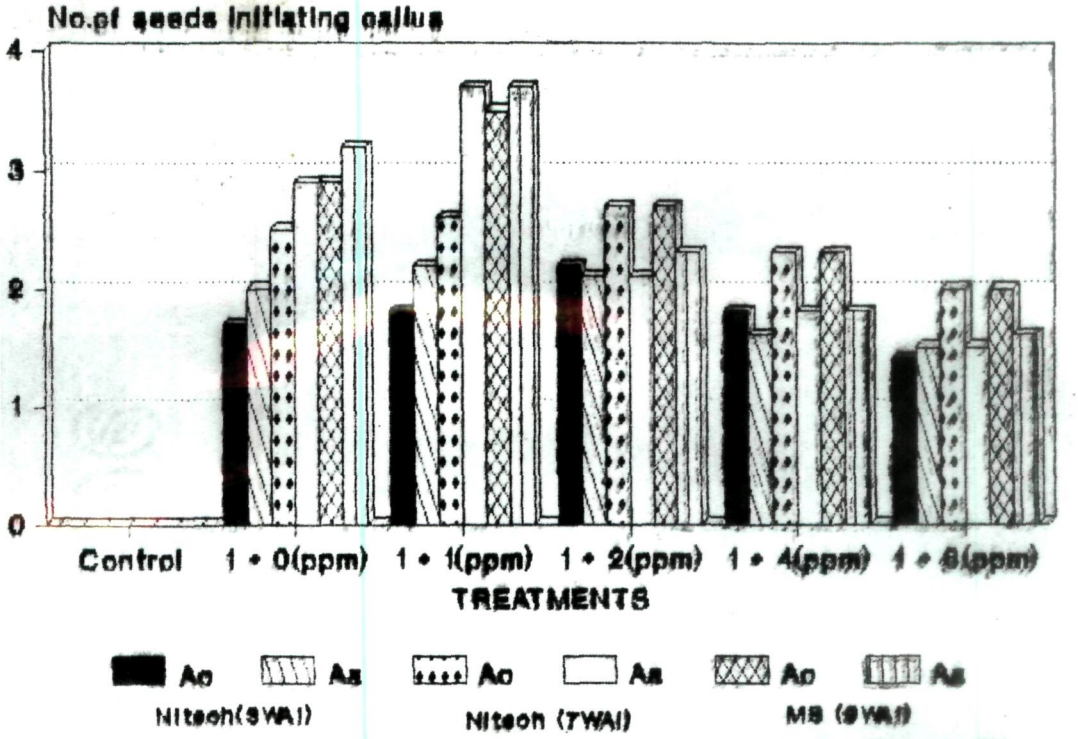


Fig.5:Effects of BAP & 2,4-D levels on callus induction in anthuriums

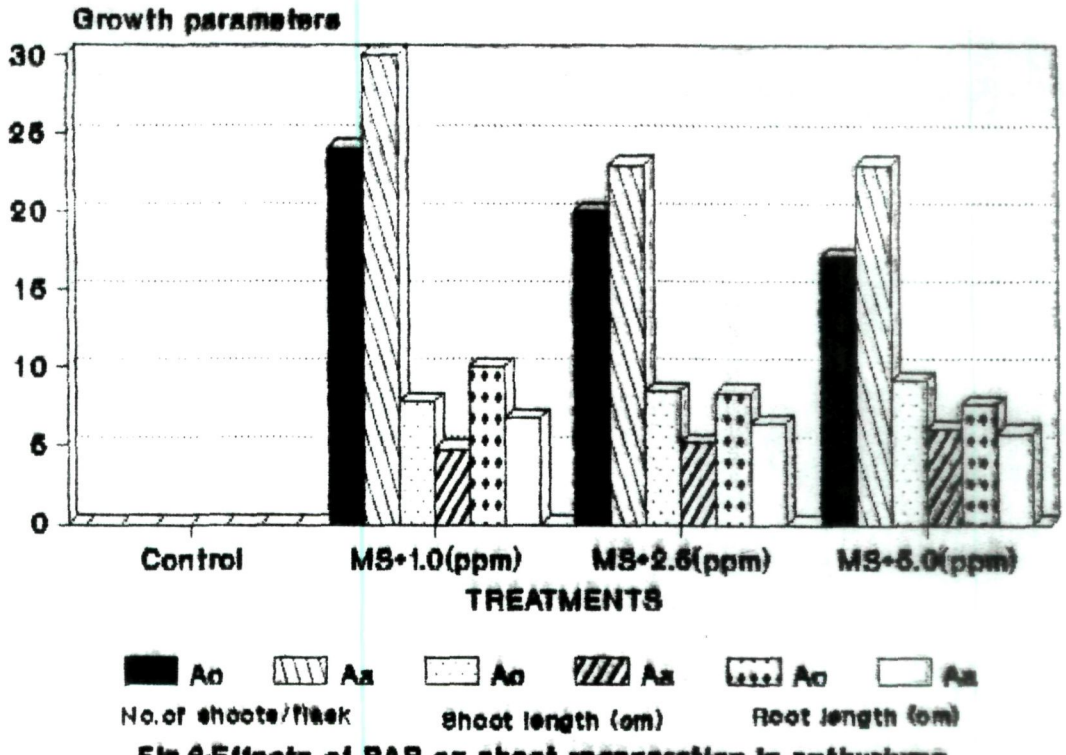


Fig.6:Effects of BAP on shoot regeneration in anthuriums

different concentrations of BAP with respect to time taken for shooting (7 to 13 WAI).

The interaction effects for number of shoots per flask for different concentrations of BAP differed significantly with different WAI. Highest number of shoots per flask was recorded in the case of medium supplemented with 1.0 ppm BAP (36.00) followed by 2.5 and 5.0 ppm of BAP (30 and 23, respectively) in the case of *A. andreanum*. Where as in *A. crystallinum*, highest number of shoots per flask was recorded in medium supplemented with 1.0 ppm BAP (23), followed by 2.5 and 5.0 ppm of BAP (20 and 17, respectively). But, the interactions were non significant for time taken for shoot regeneration and the species.

4.4.2. Shoot length (cm)

Anthurium species differed significantly with respect to shoot length. Higher shoot length was recorded in the case of *A. crystallinum* (6.37 cm) and was lower in *A. andreanum* (4.02 cm).

Different concentrations of BAP also showed significant differences with respect to shoot length. Highest shoot length was recorded in medium supplemented with 5.0 ppm BAP (7.62 cm) which was on par with 2.5 ppm BAP (6.87 cm), followed by 1.0 ppm BAP (6.30 cm). There was no shoot regeneration in the case of control.

The interactions between both the species and the concentrations of BAP differed significantly for shoot length. Highest shoot length was recorded in medium supplemented with 5.0 ppm BAP (9.19 cm), which was on par with 2.5 ppm BAP (8.53 cm). The treatment with BAP 2.5 ppm (8.53 cm) was also on par with 1.0 ppm BAP (7.77 cm) in the case of *A. crystallinum*. Similarly, in the case of *A. andreanum*, significantly highest shoot length was recorded with 5.0 ppm BAP (6.04 cms) which was on par with 2.5 ppm BAP (5.21 cm). Treatments 2.5 (5.21 cm) and 1.0 ppm BAP (4.82 cm) were also on par. There was no shoot regeneration in the case of control.

4.4.3. Root length (cm)

Significant difference was observed with respect to species and root length. Higher root length was recorded in the case of *A. crystallinum* (6.52 cm) and was lower in *A. andreanum* (4.75 cm).

Significant differences were also observed with respect to different concentrations of BAP for root length. Highest root length was recorded in the case of medium supplemented with 1.0 (8.38 cm), followed by 2.5 and 5.0 ppm BAP (7.41 and 6.75 cm, respectively). There was no root formation in the case of control.

Interactions differed significantly with respect to root length, anthurium species and different concentrations of BAP. Highest root length was recorded in the case of 1.0 ppm BAP (9.98 cm), followed by 2.5 ppm BAP (8.42 cm), which was on par with 5.0 ppm BAP (7.66 cm) in the case of *A. crystallinu*m. Similar trend was also observed in *A. andreanum*. Highest root length was recorded in the case of medium supplemented with 1.0 ppm BAP (6.78 cm), which was on par with 2.5 ppm BAP (6.39 cm). Similarly, BAP treatments 2.5 (6.39 cm) and 5.0 ppm (5.83 cm) were at par. Root regeneration was not observed in control treatment in both the species.

4.4.4. Number of roots per shooted callus clump

Number of roots per shooted callus clump differed significantly with respect to both the species. Higher number of roots per shooted callus clump was recorded in the case of *A. crystallinum* (6.25) and was lower in *A. andreanum* (5.15).

Differences were significant for number of roots per shooted callus clump and various concentrations of BAP. Highest number of roots were recorded in the case of medium supplemented with 1.0 ppm BAP (8.90), followed by 2.5 and 5.00 ppm BAP (7.65 and 6.25, respectively). There was no rhizogenesis in the case of control.
The interactions for number of roots per shooted callus clump with respect to species and

the medium supplemented with various concentrations of BAP were non significant.

4.4.5. Leaf length (cm)

Differences were significant for leaf length and different species. Higher leaf length was recorded in the case of *A. crystallinum* (1.34 cm) and was lower in *A. andreanum* (0.96 cm).

Leaf length differed significantly with respect to medium supplemented with various concentrations of BAP. Highest leaf length was recorded in medium supplemented with 1.0 ppm BAP (1.62 cm), followed by 2.5 ppm and was on par with 5.0 ppm (1.51 and 1.47 cm, respectively). There was no shoot regeneration in the case of control.

The interaction effects between different species and media containing various concentrations of BAP differed significantly. Highest leaf length was recorded in medium supplemented with 1.0 ppm BAP (1.84 cm), which was on par with 2.5 and 5.0 ppm BAP (1.79 and 1.73 cm, respectively) in the case of *A. crystallinum*. Where as *A. andreanum* registered highest leaf length with 1.0 ppm BAP (1.40 cm), followed by 2.5 and 5.0 ppm BAP (1.22 and 1.13 cm, respectively), which were also at par.

4.4.6. Leaf breadth (cm)

Leaf breadth differed significantly with respect to anthurium species. Highest leaf breadth was recorded in the case of *A. crystallinum* (0.86 cm) and was least in *A. andreanum* (0.59 cm).

Significant difference was observed with leaf breadth and different concentrations of BAP. Highest leaf breadth was recorded in the medium supplemented with 1.0 ppm BAP (1.04 cm), which was on par with 2.5 ppm BAP (0.95 cm). Similarly, 2.5 ppm BAP (0.95 cm) was also on par with 5.0 ppm (0.92 cm).

The interaction effects for leaf breadth, species, and the medium supplemented with various concentrations of BAP differed significantly. Highest leaf breadth was recorded in the medium containing 1.0 ppm BAP (1.22 cm) but was on par with 2.5 ppm (1.13 cm), which was also on par with 5.0 ppm BAP (1.09 cm) in the case of *A. crystallinum*. Where as *A. andreanum* recorded highest leaf breadth with 1.0 ppm BAP (0.85 cm) which was on par with both 2.5 and 5.0 ppm BAP (0.77 and 0.75 cm, respectively).

4.5. Effects of different concentrations of NAA on rhizogenesis

The data on root length and number of roots per shooted callus clump are presented in Table-15, Fig.7, Plates 13a and b.

4.5.1. Root length (cm) per shooted callus clump

Root length differed significantly with respect to different species. Higher root length was recorded in the case of *A. crystallinum* (11.40 cm) and was lower in *A. andreanum* (9.50 cm).

Similarly, significant differences were observed with respect to medium supplemented with various concentrations of NAA. The treatment with 1.0 ppm NAA recorded highest root length (13.45 cm), followed by 0.5 and 0.1 ppm NAA (11.64 and 10.31, respectively). Least root length was recorded in the case of control (6.41 cm).

The interaction effects for root length, different species and various concentrations of NAA were non significant.

4.5.2. Number of roots per shooted callus clump

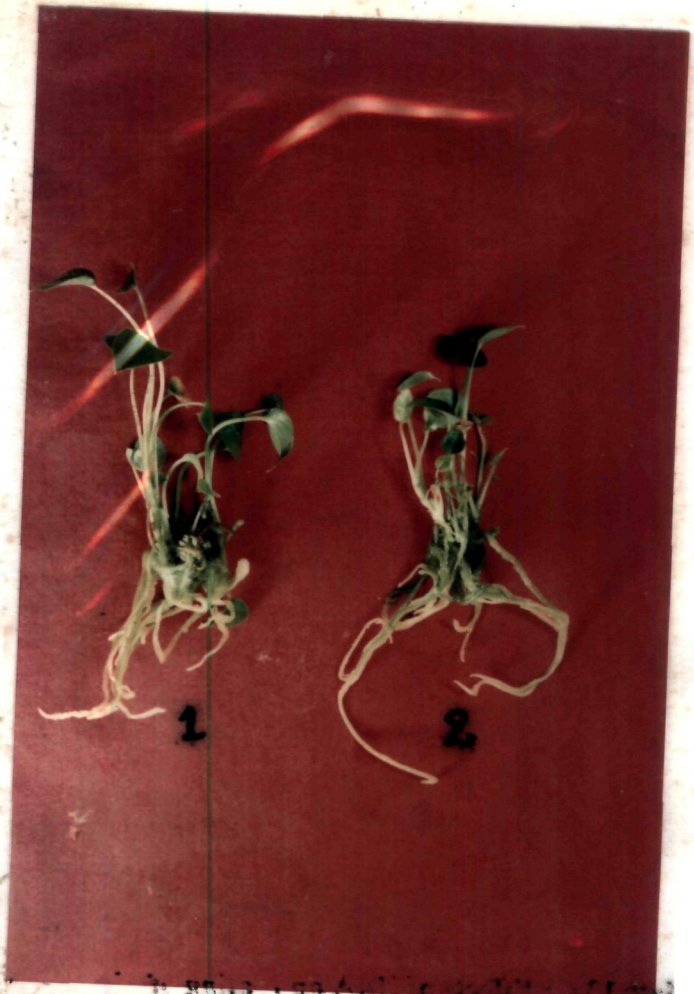
Anthurium species differed significantly with respect to number of roots per shooted callus clump. Higher number of roots were recorded by *A. crystallinum* (10.75)

Plate 13a. : Effects of NAA on rooting of shooted callus clump in *A. crystallinum*

1. 0.5(ppm) 2. 1.0(ppm)

Plate 13b. : Effects of NAA on rooting of shooted callus clump in *A. andreanum*

1. 0.5(ppm) 2. 1.0(ppm)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1
2

Table-15: Effects of different concentrations of NAA on rooting of shooted callus clump of the two species of anthurium

Treatments (MS +NAA) ppm	Root length (cm)	No.of roots per shotted callus clump
Ac	11.40	10.75
Aa	9.50	8.78
SEM±	0.17	0.29
C.D. at 5%	0.48	0.81
Control	6.41	5.35
0.1	10.31	9.90
0.5	11.64	11.45
1.0	13.45	12.55
SEM±	0.24	0.41
C.D. at 5%	0.68	1.14

A x B

Treatments (MS +NAA) ppm	Ac	Aa	Ac	Aa
Control	6.95	5.86	5.70	5.00
0.1	11.44	9.18	11.00	8.80
2.5	12.48	10.80	12.80	10.10
5.0	14.74	12.15	13.50	11.60
SEM±	0.35		0.58	
C.D. at 5%	NS		NS	

* Ac - Anthurium crystallinum
* Aa - Anthurium andreanum

and was lower in *A. andreanum* (8.88).

Differences were significant for number of roots per shooted callus clump and different concentrations of NAA. Highest number of roots was recorded in medium supplemented with 1.0 ppm NAA (12.55), which was on par 0.5 ppm NAA (11.45), followed by 0.1 ppm NAA and was lowest in the case of control (5.35).

Interactions between different anthurium species and various concentrations of NAA did not differ significantly.

4.6. Effects of different growing conditions on the acclimatisation of seedlings

Data on shoot length, root length, number of roots per seedling, number of leaves per seedling, leaf length and leaf breadth as influenced by different species and the different environmental conditions are presented in Table-16, Fig.8 and Plate 14.

4.6.1. Shoot length (cm)

Shoot length varied significantly with different environmental conditions. Maximum shoot length was recorded in the case of mist chamber (6.49 cm), followed by greenhouse conditions (5.91 cm). Shoot length was least at room conditions (4.43 cm).

Significant differences were observed with respect to different species for shoot length. Higher shoot length was recorded by *A. andreanum* (6.36 cms) and was lower in the case of *A. crystallinum* (4.86 cm). Interaction between shoot length, anthurium species and growing conditions did not differ significantly.

Plate 14. ***In vitro*** **raised plantlets at the stage of weaning.**

Table-16: Effects of different environmental conditions on seedling growth parameters in of the two species of anthurium

Treatments	Shoot length (cms)	Root length (cms)	Number of roots per seedling	Number of leaves per plant	Leaf length	Leaf breadth
Green house	5.91	8.85	5.95	5.40	3.43	1.96
Lab.	4.43	8.91	5.60	5.25	2.37	1.88
Mist Chamber	6.49	10.29	6.15	5.70	3.66	2.33
SEM±	0.20	0.35	0.24	0.20	0.16	0.07
C.D. at 5%	0.57	0.98	NS	NS	0.44	0.21
Ac	4.86	11.02	6.66	5.10	3.30	2.46
Aa	6.36	7.68	5.13	5.80	3.00	1.63
SEM±	0.17	0.29	0.19	0.16	0.13	0.06
C.D. at 5%	0.47	0.80	0.53	0.45	NS	0.17

A x B

Treatments	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa	Ac	Aa
Green house	4.94	6.88	10.17	7.52	6.50	5.40	5.00	5.80	3.60	3.25	2.29	1.63
Lab.	4.03	4.83	10.35	7.47	6.50	4.70	4.90	5.60	2.47	2.26	2.31	1.45
Mist Chamber	5.61	7.36	12.54	8.04	7.00	5.30	5.40	6.00	3.82	3.50	2.84	1.81
SEM±	0.29		0.50		0.33		0.28		0.23		0.10	
C.D. at 5%	NS		NS		NS		NS		NS		NS	

* Ac - Anthurium crystallinum
* Aa - Anthurium andreanum

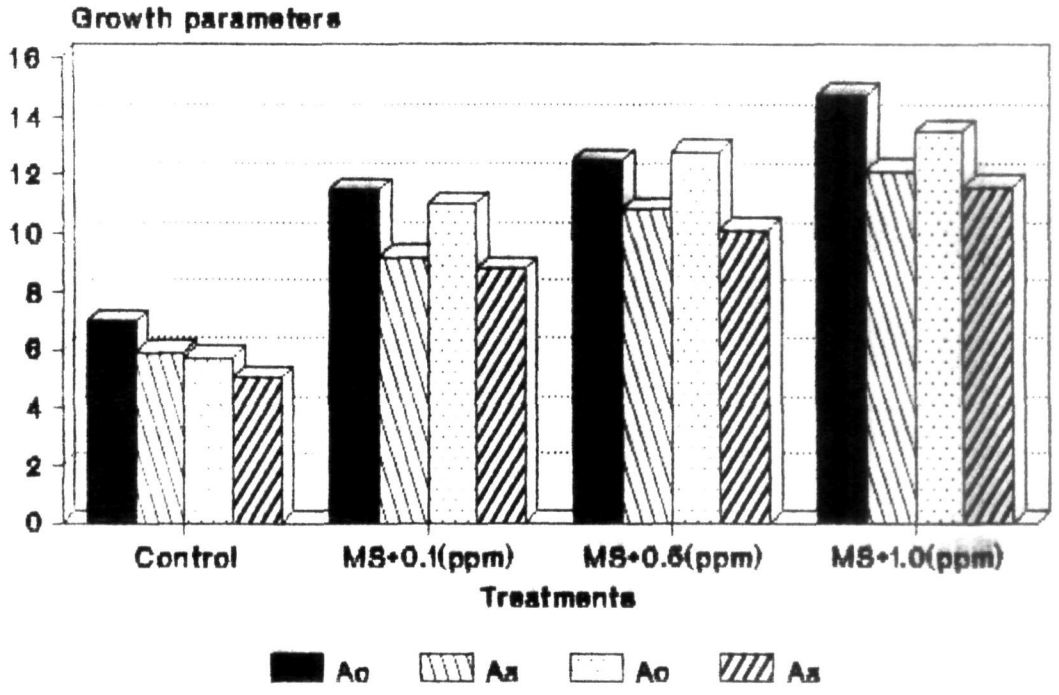

Fig.7:Effects of NAA on rhizogenesis in anthuriums

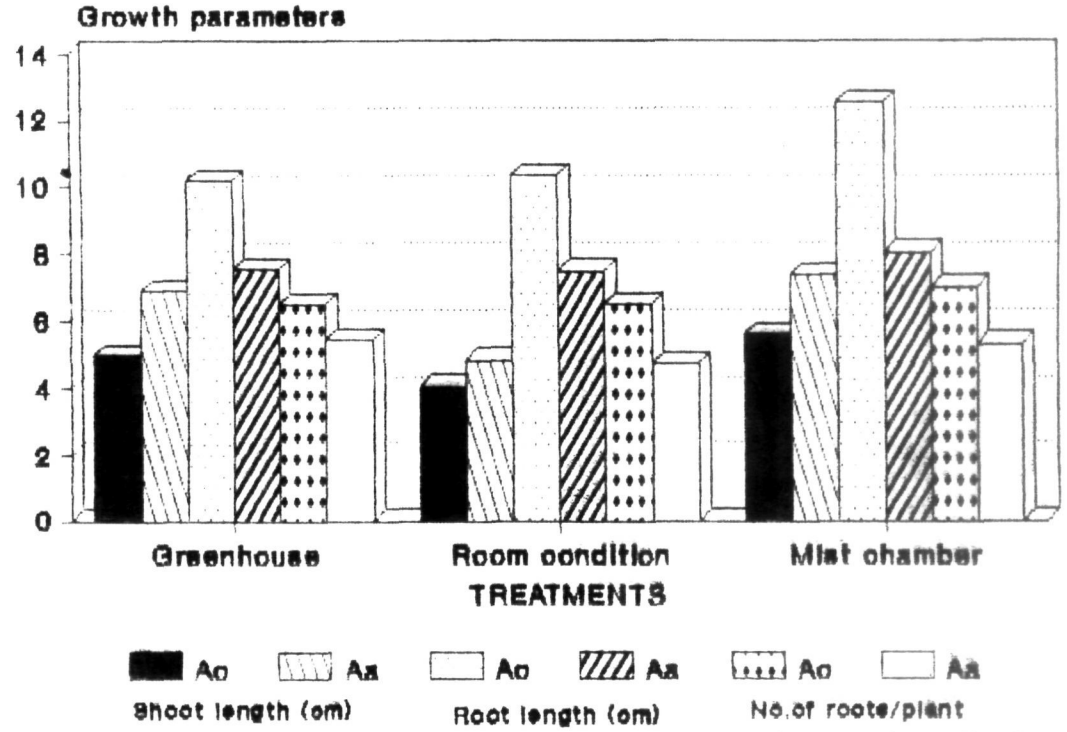

Fig.8:Effects of environmental conditions on acclimatisation in anthuriums

4.6.2. Root length (cm)

Root length differed significantly with respect to different environmental conditions. Maximum root length was recorded in the case of mist chamber (10.29 cm) and minimum root length was recorded in the case of green house (8.85 cm), which was on par with room conditions (8.91 cm).

Anthurium species showed significant difference with respect to root length. Higher root length was recorded by *A. crystallinum* (11.02 cms) and was lower in *A. crystallinum* (7.68 cm). Interactions were not significant for root length, environmental conditions and different species.

4.6.3. Number of roots per seedling

There was no significant differences with respect to number of roots per seedling, for different environmental conditions and also with respect to interactions between environmental conditions and different species.

More number of roots per seedling was recorded by *A. crystallinum* (6.66) and was less in the case of *A. andreanum* (5.13).

4.6.4. Number of leaves per plant

Number of leaves per plant, different environmental conditions, and interaction effects between different environmental conditions and different species did not differ significantly.

Number of leaves per plant differed significantly with respect to different species. More number of leaves per plant was recorded by *A. andreanum* (5.80) and was less in the case of *A. crystallinum* (5.10).

4.6.5. Leaf length (cm)

Leaf length differed significantly with respect to different environmental conditions. Highest leaf length was observed in the case of mist chamber (3.66 cm), which was on par with greenhouse (3.43 cm) and was least in room conditions (2.37 cm). Leaf length, different species and their interaction effects did not differ significantly.

4.6.6. Leaf breadth (cm)

Significant differences were observed with respect to different environmental conditions for leaf breadth was concerned. Significantly highest leaf breadth was recorded in the case of mist chamber (2.33 cm). Leaf breadth was lowest under room conditions (1.88 cm), but was on par with greenhouse conditions (1.96 cm).

Similarly, higher leaf breadth was recorded by *A. crystallinum* (2.48 cm) and was least in the case of *A. andreanum* (1.63 cm). The interactions between leaf breadth, different environmental conditions and different species were non significant.

DISCUSSION

V. DISCUSSION

The results of the experiments conducted on *in vitro* seed germination, callusing, differentiation and hardening of plantlets in *Anthurium andreanum* var.'*Agnihotri*' and *A. crystallinum* are discussed in this chapter.

5.1 Propagation

Anthuriums are generally propagated through seed, divisions and cuttings. But in these methods multiplication rate is very slow and viability of seed is also very poor. Due to these problems in recent years, *in vitro* propagation techniques are being employed to achieve rapid multiplication of disease free quality planting material.

5.2. Anthurium seeds

Anthurium berries mature by 4-5 months and each berry contains 2-3 seeds which are surrounded by pulp. In a majority of the angiosperms seeds at maturity contain a bipolar embryos. However, in a number of genera including anthurium the embryo remains small, reduced and lacks proper differentiation at the time of dispersal (Natesh and Rau, 1984). Intraseminal growth and after ripening are reported in different species (Raghavan, 1980). The improper development of embryo makes them heterotrophic and hence dependent on the metabolites present in surrounding cells or endosperm. This may be the main reason for the poor germination of seeds. This indicates that the nutrition requirement of anthurium seed is very complex and highly specific in nature.

5.3 Effect of different media

Among Nitsch, 1/2 strength Nitsch, MS, KC, VW and Morel's media tested, best seed germination (Fig.1) was observed on Nitsch medium, followed by Morel's and VW media. Morel's medium did not support subsequent seedling growth and there was no shoot elongation even though it supported early seed germination (7 DAS). Normally seed germination is not influenced by the extent of nutrients present

in the media. However, subsequent growth of seedlings essentially require the nutrients. From the present studies, it is clear that anthurium seeds germinated easily on Morel's medium utilising the stored nutrients present in the endosperm and cotyledons. It is also obvious that the inability of the seedlings to continue further growth on Morel's medium might be due to the unavailability of important components such as nitrogen, phosphorus and vitamins (see Appendix 5 for the composition of Morel's medium). These are available in the other media tested and hence the subsequent growth of *in vitro* seedlings was better in the other media tested. Similar opinion was also expressed by Swaminathan (1986) in *A. andreanum* cv. Red.

Similar analogy also applies to the early germination (7 DAS) favoured by Morel's medium. As compared to Morel's medium rest of the media had higher concentrations of nutrients and salts, which probably delayed the seed germination rate. This opinion is well supported by the reason given by Nirmala (1989) in anthurium, that higher concentrations of Ca and K were inhibitory for seed germination.

In the present studies, it was found that both the species of anthurium, seeds after germination, Nitsch, MS, VW, 1/2 strength Nitsch and KC media encouraged the plantlets to attain first and second leaves early. Although, similar trend was reported while producing third and fourth leaves, only 1/2 strength Nitsch was the last in the order. It was also found that Nitsch medium was found to be the best in attaining leaves early for all the four stages. This may be due to the fact that media compositions of Nitsch medium seems to be congenial as compared to any other media.

Higher root length was recorded in the case of *A. crystallinum* (6.36 cm) and it was least in *A. andreanum* (4.24 cm). Similarly, highest root length was recorded in Nitsch medium (7.80 cm), followed by MS, VW media and was least in the case of Morel's medium (2.53 cm). It indicated that root length was not only depended upon nutrients, but also on genotypes.

Similarly, number of roots per seedling did not vary singificantly with respect to species but also varied with respect to media. Higher number of roots per seedling was recorded by *A. crystallinum* (2.72 cm) and was lower in *A. andreanum* (2.33). Whereas with respect to different media, highest number of roots per seedling was observed in Nitsch medium (3.30), followed by MS and was least in Morel's medium (1.25). The results also revealed that growth rates were strongly dependent on the genotypes in anthurium. Pierik (1975) also reported the strong relation between growth rate and genotypes in anthurium.

Best shoot length was observed on Nitsch medium in both *A. crystallinum* (9.70 cm) and *A. andreanum* (5.90 cm), followed by VW, MS, 1/2 strength Nitsch and KC, while in the case of Morel's medium it was least in both the species (Fig.3). These results were on par with the earlier work of Swaminathan (1986), He also reported that highest root length was obtained on Nitsch medium as compared to other media used in his experiment.

Fresh weight of the seedling was highest on Nitsch medium in both *A. crystallinum* (498.70 mg) and *A. andreanum* (250.70 mg). And it was least on 1/2 strength Nitsch medium. While, the dry weight of seedling was highest on Nitsch medium in the case of *A. crystallinum* (50.60 mg) and that of *A. andreanum* was least on MS medium (26.30 mg). Least dry weight was recorded in the 1/2 strength Nitsch medium in both *A. crystallinum* (28.20 mg) and *A. andreanum* (19.40 mg).

FW/DW x 100 index was highest in the case of Nitsch medium with respect to both the species and was least in the case of MS medium.

It has been found from the present investigations that growth parameters like root length, number of roots per seedling, shoot length, fresh weight, dry weight and the ratio between fresh weight and dry weight were well supported by Nitsch medium as compared to any other media. Therefore it is clear that Nitsch medium is the best for *in vitro* culture of anthuriums. These findings are in conformity with those reported by Swaminathan (1986), Geier (1986a), Nirmala (1989), Randhawa (1990) and Hemanthakumar (1994). Among the two species studied *A. crystallinum* showed

higher values for all the growth parameters as compared to *A. andreanum*. This difference is mainly due to the genotypic effect which is also previously observed by Pierik (1975).

The assessment of requirement in nutrient medium composition the ingredients was in the order of inorganic salt mixtures, organic substances and natural complexes (Murashige, 1974). Although the composition of MS and Nistsch media are almost similar, the variation in the results, may be due to the specific requirement of explant or due to the change in the ionic composition than the mere quantity of the nutrient used in the medium.

5.4 Effects of different adjuvants

A large number of complex adjuvants have been used in different culture media, coconut water, yeast extract, peptone, banana pulp, tomato juice, potato extract and barley malt are most commonly used (Arditti, 1979). The determination of best adjuvants that promote best seed germination and subsequent growth are very important (Fig. 4). The effects of different adjuvants used in the current study are discussed below.

The results indicated that the seed germination was highest in Nitsch medium without the supplementation of adjuvants. However, among the adjuvants applied coconut water was the best in encouraging highest germination, followed by banana pulp, wheat and ragi malt. These studies clearly brought about the fact that adjuvants are not probably required for seed germination. This may be due to the reason that probably the artificially formulated Nitsch medium showed more stimulatory effect for germination. Some of the natural adjuvants were found inhibitory for seed germination, eg., Pierik *et al.*, (1988) indicated that banana pulp affected germination rate in orchids.

In the case of *A. crystallinum* maximum number of seeds germinated on the medium supplemented with coconut water (4.70) as compared to the Nitsch medium alone. But in the case of *A. andreanum* more number seed germinated on Nitsch

medium without adjuvants. It clearly showed that different genotypes responded differently for media and nutrient concentrations.

This means to say that the media requirement was totally different for both the species. This was also reported earlier by Pierik (1975) in anthurium.

First leaf initiation was early in the case of both Nitsch without adjuvants and medium supplemented with wheat malt (32 DAS), followed by banana pulp and ragi malt and was late when coconut water (36 DAS) was supplemented. Maximum number of seedlings initiated first leaf in the case of *A. andreanum* (4.70) on medium containing coconut water whereas *A. crystallinum* (4.30) on Nitsch medium.

Medium supplemented with banana pulp induced early initiation of second leaf (46 DAS) in both the species, but highest number of seedlings initiated second leaf in the case of *A. andreanum* (4.60) on medium supplemented with coconut water, while that of *A. crystallinum* (4.30) on Nitsch medium alone (4.60) and medium supplemented with coconut delayed second leaf initiation (52 DAS).

Both banana pulp and wheat malt induced early third leaf initiation (76 DAS) and was late on Nitsch medium alone (84 DAS) in both the species. Maximum number of seedlings initiated third leaf on medium supplemented with coconut water in the case of *A. crystallinum* (4.10 and 76 DAS) and in the case *A. andreanum* (4.40 and 80 DAS), followed by Nitsch in both the species. But medium supplemented with banana pulp was found to be good for *A. andreanum* (3.50 and 76 DAS) whereas *A. crystallinum* was good on wheat malt (3.40 and 76 DAS). Least number of seedlings initiated third leaf in medium supplemented with ragi malt in both the species.

Early fourth leaf initiated in the case of medium supplemented with banana pulp (100 DAS) in both the species and it was late in coconut water (116 DAS) in both species also. Maximum number of seedlings initiated fourth leaf in both the species on medium supplemented with coconut water. While media supplemented with ragi malt and wheat malt, did not support further growth. Instead the seedlings start drying in both the species. So, medium supplemented with coconut water was found

to be superior as compared to that of Nitsch medium alone or medium supplemented with ragi malt and wheat malt with respect to both the sepcies.

The results from the current investigations clearly indicated that Nitsch medium was the best to support the production of first and second leaves, followed by the addition of adjuvants such as coconut water, banana pulp, wheat and ragi malt. Whereas, at later stages, namely, third and fourth leaf stages the media supplemented with coconut water followed by Nitsch medium alone and the media supplemented with banana pulp, wheat and ragi malts were found beneficial in this order. This indicates that probably the availability of nutrients makes the difference for the subsequent growth. As the nutrients availability is maximum in Nitsch medium, most of the seedlings attained second leaf, followed by the next best release of nutrients by coconut water, banana pulp, wheat and ragi malts. Probably slow release of nutrients might be also a reason as in the case of wheat and ragi malts in which case the nutrients were in the form of unavailable starch.

The main benefit from using coconut milk is due to its capacity to provide highly active natural cytokinin like growth substances (Kuraishi and Okumura, 1961).

Steward and Caplin (1951) showed that there was a synergistic action between, 2,4-D and coconut milk in stimulating the growth of potato tuber tissue. Synergistic effects of coconut milk was also reported by George (1993). Similarly, coconut milk improved callus growth of seedling explant of peppermint and spearmint (Lin and Staba, 1961). The occurrence of gibberllin like substances in coconut milk was reported by Radley and Dear (1958).

Arditti (1979) reported the early seed germination of orchids in the case of medium supplemented with coconut water, whereas, banana pulp induced the seed germination and all the subsequent stages of growth were enhanced as compared to the control. Similar effects of coconut milk and banana pulp was also reported by Shobhana and Rajeevan (1993) in orchids.

The present study also reveal that early seed germination of both the species of anthurium when medium supplemented with coconut water and banana pulp also induces early growth in both the species.

The report of Swaminathan (1986) indicated that coconut water was inhibitory for growth till second leaf stage but it supported subsequent growth of seedlings, whereas banana pulp was significantly enhanced seed germination and subsequent growth of seedling.

Root length was maximum on the Nitsch medium (7.43 cm), followed by medium supplemented with cocount water (7.02 cm) and banana pulp (6.07 cm). Medium supplemented with ragi malt was inhibiting for root growth followed by wheat malt (0.84 and 3.13 cm, respectively). Both the species responded in different manner with respect to root length. *A. crystallinum* produced highest root length on Nitsch medium (9.28 cm), whereas *A. andreanum* produced highest root length on medium supplemented with coconut water (7.00 cm).

Banana pulp helped in the formation of more number of roots per plant (3.70) followed by coconut water, Nitsch medium alone and wheat malt, but ragi malt inhibited the root formation. Banana pulp induced more number of roots in both *A. crystallinum* (4.10) and *A. andreanum* (3.30). The favourable effect of banana pulp was also previously reported by Shobana and Rajeevan (1993) in orchids.

The benificial effects of coconut water and banana pulp were also marked in the case of other growth parameters like root length, number of roots per plant, shoot length, root to shoot ratio, fresh weight, dry weight and ratio between fresh and dry weights. And the inhibitory influences were also recorded for all these parameters when media supplemented with wheat and ragi malts.

The probable reasons for the favourable effects of coconut water may be that it can both act as a auxin or cytokinin in the culture media apart from supplying aminoacids, organic acids, vitamins, sugars, alcohols and minerals as previously identified by Kuraishi and Okumura (1961), Leetham (1982) and George (1993).

With regard to the beneficial effects banana pulp stands next in the order. It is advantageous to use banana pulp for induction of early growth. Similar response was also reported by Swaminathan (1986) in anthurium. This adjuvant might have released the nutrients slowly as compared to coconut water. Pierik *et al.*(1988) attributed the stimulatory effect on seedling growth in which case banana pulp in the media stabilised the pH as they observed in orchids.

Inhibitory effects of both ragi and wheat malts with respect to seedling growth parameters has not been explained by any of the research workers in *in vitro* culture studies. But some research workers used barley starch and corn starch as alternative gelling agents. Medium supplemented with barley starch induced early adventitious shoots formation from potato discs in three weeks instead of the 5-14 weeks which it took on an agar solidified medium (Sorvari, 1986).

Henderson and Kinnersley (1988) used starch instead of agar and reported the growth of wild carrot and embryonic carrot callus cultures was found to be better on media gelled with corn starch than with agar. Probable reason that could be attributed for the decrease in seed germination and subsequent seedling growth is that the nutrients would not have been provided in the available form or they would have been released slowly as compared to coconut water and banana pulp.

5.6 Callus induction

Seeds were cultured initially on Nitsch medium and medium supplemented with BAP (1.0 ppm) and combination of BAP with 2,4-D at various concentrations. Of the different treatments tried (0, 1, 2, 4 and 6 ppm) in the present study. Maximum number of seeds initiated callus on medium supplemented with one ppm each BAP and 2,4-D (3.15) at 7 WAI and as the concentration of 2,4-D increased number of seeds initiating callus was decreased and the quantity of callus also decreased. Among the two species, highest number of seeds initiated callus in the case of *A. andreanum* (3.70), followed by *A. crystallinum* (2.70) on medium supplemented with BAP + 2,4-D (1 ppm each) and BAP + 2,4-D (1+2 ppm) respectively. But in all the treatments amount of callus (+ +) produced was less. At

seven WAI, all the seeds were transferred to the MS medium containing same concentrations of BAP and 2,4-D.

By the end of nine WAI, highest number of seeds initiated callus on MS medium containing one ppm each of BAP and 2,4-D (3.60) and was least or 1 and 6 ppm of BAP and 2,4-D (1.75). Both the species initiated good amount of callus (+ + + +) as medium containing BAP + 2,4-D (1 ppm each) and also highest number of seeds initiated callus at this level of growth regualtors. *A. andreanum* (3.70), followed by *A. crystallinum* (3.50). The results showed cytokinin is a must for callus induction but in order to get good amount of callus a balanced ratio of cytokinin and auxin was essential.

Pierik *et al.* (1974) reported the formation of small amount of callus from embryos even in the absence of hormones. Addition of 3.2 μm BA to culture medium resulted in consistent callus formation. Cytokinin was found to be essential for callus induction. Supplementing the medium with auxins in addition to cytokinins generally improve both callus induction and growth in subculture. Just like in the current study, a combination of auxin (2,4-D) and cytokinin (BAP) for production of callus has been well established by several research workers in anthuriums from different explants (Weaver, 1972; Geier, 1982; Eapen and Rao, 1985; Zens and Zimmer, 1986; Kuehnle and Sugii, 1991; Cen, *et al.*, 1993; Nirmala and Singh, 1993; Hemanthkumar, 1994 and Sreelatha *et al.*, 1994).

5.7 Callus multiplication and morphogenesis

In the present study BAP and 2,4-D (1 ppm each) helped in the callus multiplication (+ + + +) but rarely shoot formation was observed. Higher concentrations (2,4 and 6 ppm) inhibited callus growth and organogenesis. At 6 ppm callus turned brownish to grey and started drying.

Anthurium seed explants require the combination of BAP and 2,4-D for the induction and multiplication of callus. 2,4-D requirement is low (1 ppm) and higher concentrations could result in death of callus. This may be due to the endogenous

level of 2,4-D.

In the present studies, the presence of 1 ppm BAP induced callus and/shoot, root formation, however, higher concentrations of BAP (2.5 and 5.0 ppm) reduced the number of shoots as well as roots.

Pierik *et al.* (1979) showed that callus and sprout formation occurred when Zeatin was added at 1 mg/l followed by BA at 1 mg/l, kinetin at 1 mg/l and 2iP at 10 mg/l. Sprout formation was promoted when 0.08 mg/l 2,4-D in combination with BAP was added to culture medium in anthurium. Therefore current findings also indicated that combination both BAP and 2,4-D is essential at lower concentrations (1 ppm each) for the induction of callus and regeneration of shoots and roots.

Geier (1986a) reported that varied response of *A. scherzerianum* genotypes for callusing and differentiation.

5.8 Multiple shoot induction

In the present study solid MS medium supplemented with various concentrations of BAP (1, 2.5 and 5.0 ppm) induced maximum number of shoots per flask. MS medium supplemented with 1.0 ppm BAP alone (30) and higher concentrations of BAP (2.5 and 5.0 ppm) decreased the number of shoots per flask (25 and 20, respectively). Among the two species, maximum number of shoots per plant was obtained in the case of *A. andreanum* (36), while in the case of *A. crystallinum* only 24 shoots per flask was observed. Kunisaki (1980) recommended a low BAP concentration (0.2 mg/l) for induction of multiple shoots. Soczek and Hampel (1989) also observed that number of shoots decreased with the increased cytokinin levels of BA, Zeatin and 2ip.

As the concentration of BAP increased (1, 2.5 and 5.0 ppm) the shoot length also increased (6.30, 6.87 and 7.62 cm, respectively). But number of roots per shooted callus clump and root length were decreased with increase in concentrations. Similar observations made by Chandrashekar and Singh (1994) that lower

concentration of BAP (0.1 mg/l) recorded least shoot length (1.65 cm) support our findings that higher concentrations of BAP until 2 mg/l was found beneficial for increasing shoot length in anthurium.

Highest number of roots per shooted callus clump was recorded in the case of medium containing 1 ppm BAP (8.90) and was least in 5 ppm BAP (6.75) and as the concentration of BAP was increased the number of roots produced and their length decreased. Among the two species, highest number of roots was recorded by *A. crystallinum* (9.80) and was least in *A. andreanum* (8.0). And highest root length was recorded in the case of *A. crystallinum* (9.98 cm) and was least in the case of *A. andreanum* (6.78 cm).

Similarly, leaf length and leaf breadth were also decreased as the concentration of BAP increased. Highest leaf length was recorded in the case of *A. crystallinum* (1.84 cm) followed by *A. andreanum* (1.40) and they differed significantly. Similarly, leaf breadth was highest in the case of *A. crystallinum* (1.22 cm) and was least in *A. andreanum* (0.85), in the medium supplemented with 1 ppm BAP. In the present study 1 ppm BA alone was found to be good for shoot differentiation. Same results obtained by Lightbourn and Prasad (1990) in *A. andreanum* at 0.2-0.8 mg/l BA alone was sufficient. Eapen and Rao (1985) reported that shoot differentiation was good, when the medium supplemented with BA (1 mg/l) and 2,4-D (0.1 mg/l) in the case of *A. patulum*. Zens and Zimmer (1988) obtained maximum shoot multiplication on MS medium supplemented with NAA and BA from seeds of *A. scherzerianum*. Kuehnle and Sugii (1991) obtained successful plant regeneration of seven cultivars of Hawaiian anthuriums on a modified Pierik medium containing 0.36 M. 2,4-D and 4.4 M BA.

Hemanthakumar (1994) induced differentiation of *in vitro* petiole expalnts on MS medium supplemented with BA and 2, 4-D at 1 and 0.5-1.0 mg/l, respectively. The choice of BAP was reported by Geier (1986a) that among the three cytokinins (BAP, kinetin and 2iP) studied as BAP produced more number of shoots in *A. scherzerianum*.

5.9 Rhizogenesis

Pierik (1976) reported that presence of NAA in the solid medium induced root formation. But best rooting of shootlets was observed on hormone free Nitsch medium and light conditions (Geier, 1982, 1986a). Rooting occured after 4 weeks in cytokinin free medium in *A. andreanum* (Kraft *et al.*, 1983).

Anthurium probably do not require the stimulus of NAA for induction of roots, however, the addition of 1 ppm would help in the production of more number of better quality roots. Similar opinion was expressed by Chandrashekar and Singh (1994) that 1 mg/l NAA was optimal for production of longest roots.

In the present study rooting was observed on hormone free MS medium but the number of roots per shooted callus clump, and length of roots was least as compared to that of medium supplemented with various levels of NAA. Highest number of roots per shooted callus clump and highest root length were observed in the case of medium supplemented with 1 ppm NAA (12.55 and 13.45 cm, respectively). Highest root length was recorded in the case of *A. crystallinum* (14.74 cm) followed by *A. andreanum* (12.15 cms). Similarly, highest number of roots per shooted callus clump was recorded in the case of *A. crystallinum* (13.50) followed by *A. andreanum* (11.60).

5.10 Transplanting and hardening of plantlets

In vitro plantlets when they produced four leaves and 2-3 roots were weaned into mist chamber, greenhouse and room conditions. It has been found that in both the species establishment rate under three conditions was cent per cent. This indicates the suitability of the stages of weaning for successful establishment. However, further research showed that among different conditions tested (mist chamber, greenhouse and room conditions), mist chamber provided the most congenial conditions. This effect was clear from the highest shoot length, root length, leaf length and leaf breadth recorded by both the species as compared to greenhouse or room conditions.

There are no reports available in the literature regarding the weaning conditions in anthurium. However, it is probable that the highest growth rate (shoot length, root length, leaf length and leaf breadth) achieved in the mist chamber was due to the fact that the plants transplanted did not suffer from the transplanting stress, as the relative humidity level of mist chamber was high. However, it is also possible to wean anthurium plants even at room conditions.

SUMMARY

VI. SUMMARY

Anthuriums are becoming more popular because of their attractive foliage and flowers and gaining importance in the new market horizons. Advantages of anthurium are that they can be grown in environments similar to the requirements of most of the other foliage and flowering plants.

The propagation of anthurium is by seeds, divisions and cuttings. They are very slow growing and also seeds lose their viability quickly and germination process *in vitro* is also very slow. The present investigations on both *A. andreanum* and *A. crystallinum* were conducted at Indian Institute of Horticultural Research, Hessaraghatta, Bangalore, for standardising the best nutrient medium and adjuvants for *in vitro* seed germination and subsequent growth of the seedling, callusing, differentiation and acclimatization of plantlets.

Seed germination in the present investigations was initiated as early as three DAS in both the species and continued upto 14 DAS. The earliest *in vitro* seed germination was supported by Morel's medium (7 DAS), followed by 1/2 strength Nitsch, Nitsch, MS and VW (9, 10, 13 and 13 DAS, respectively) media and was late on KC medium (14 DAS) in both the species studied.

The study clearly brought out the effects of different media on subsequent seedling growth. Nitsch medium was found to be the best to support subsequent seedling growth of anthurium plantlets after *in vitro* seed germination. The plants attained second leaf stage by 48 DAS and the fourth leaf stage by 116 DAS.

Nitsch medium was also found to be effective in enhancing the other growth parameters like root length (9.70 cm), number of roots per plant (3.30), shoot length (3.24 cm), fresh weight (498.70 mg/plant), dry weight (50.60 mg/plant) and FW/DW X 100 index (985.20).

The investigations showed that early (10 DAS) seed germination was supported by Nitsch medium supplemented with coconut water. But the subsequent seedling growth (fourth leaf stage) was early on Nitsch medium supplemented with banana pulp (100 DAS).

Among the different adjuvants (coconut water, banana pulp, wheat and ragi malt) supplemented to Nitsch media, coconut water and banana pulp showed effective in increasing the growth parameters of anthurium plantlets. The Nitsch medium supplemented with ragi malt and wheat malt was inhibitory to seed germination and subsequent seedling growth.

MS medium supplemented with 1 ppm each of BAP and 2,4-D was found to be good for callus induction (3.15) and further growth. As the concentrations of 2,4-D increased proportionately amount of callus produced also decreased and higher concentrations (6 ppm) resulted in browning and drying of callus in both the species investigated.

The medium supplemented with 1 ppm BAP increased the number of shoots per flask (36), root length (9.98 cm), number of roots per shooted callus clump (8.90), leaf length (1.84 cm) and leaf breadth (1.22 cm). Higher concenrations of BAP (2.5 and 5.0 ppm) reduced the number of shoots and also inhibited the rooting in both the species studied.

MS medium supplemented with 1 ppm NAA enhanced root length (13.45 cm) and number of roots per shooted callus clump (12.55). However, rooting was also observed in NAA free medium in both the species studied.

Survival percentage of seedlings under all the three environmental conditions was 100 per cent. However, seedlings grown under mist chamber were superior for most of the growth parameters such as shoot length (6.49 cm), root length (10.29 cm), leaf length (3.66 cm) and leaf breadth (2.33 cm) in both the species studied.

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* Original not seen.

APPENDICES

APPENDICES

Appendix-1: Composition of Murashige and Skoog medium (Murashige and Skoog, 1962).

Elements	Quantity (mg/l)
Inorganic: Major	
1) Ammonium nitrate	1650
2) Potassium nitrate	1900
3) Calcium chloride	440
4) Magnesium sulphate	370
5) Potassium phosphate	170
6) Sodium EDTA	37.3
7) Ferrous sulphate	27.8
Minor	
8) Boric acid	6.2
9) Manganese sulphate	22.3
10) Zinc sulphate	8.6
11) Potassium iodide	0.83
12) Sodium molybdate	0.25
13) Copper sulphate	0.025
14) Cobalt chloride	0.025
Organic	
1) Myo-inositol	100
2) Nicotinic acid	0.5
3) Pyridoxin-Hcl	0.5
4) Thiamine-Hcl	0.1
5) Glycine	2
6) Sucrose	30,000
7) Distilled water to make up to volume (ml)	1000
8) Agar	9000

Appendix-2: Composition of Vacin and Went medium (Vacin and Went, 1949).

Element	Quantity (mg/l)
Inorganic	
1) Potassium nitrate	525
2) Ammonium sulphate	500
3) Potassium phosphate	250
4) Magnesium sulphate	122.1
5) Calcium phosphate	200
6) Manganese sulphate	5.08
7) Ferric tartarate	28
Organic	
1) Sucrose	30,000
2) Distilled water to make up to volume (ml)	1000
3) Agar	9000

Appendix-3: Composition of Nitsch medium (Nitsch, 1969)

Elements	Quantity (mg/l)
Inorganic	
1) Ammonium nitrate	720
2) Potassium nitrate	950
3) Magnesium sulphate	185
4) Calcium chloride	166
5) Potassium phosphate	68
6) Boric acid	10
7) Manganese sulphate	25
8) Zinc sulphate	10
9) Sodium molybdate	0.25
10) Cupric chloride	0.025
11) Ferrous sulphate	27.8
12) Sodium EDTA	37.3
Organic	
1) Myo-inositol	100
2) Nicotinic acid	5
3) Pyridoxin Hcl	0.5
4) Thiamine Hcl	0.5
5) Glycine	0.5
6) Folic acid	0.05
7) Biotin	0.05
8) Sucrose	30,000
9) Distilled water to make up to volume (ml)	1000
10) Agar	9000

Appendix-4: Composition of Knudson C medium (Knudson, 1951)

Elements	Quantity (mg/l)
Organic	
1) Calcium nitrate	1000
2) Potassium phosphate	250
3) Magnesium sulphate	250
4) Ammonium sulphate	500
5) Ferrous sulphate	2.5
6) Manganese sulphate	7.5
Organic	
1) Sucrose	30,000
2) Distilled water to make up to volume (ml)	1000
3) Agar	9000

Appendix-5: Composition of modified Morel's medium (Morel, 1964).

Elements	Quantity (mg/l)
Organic	
1) Calcium nitrate	500
2) Potassium nitrate	125
3) Magnesium sulphate	125
4) Ferrous sulphate	25
Inorganic	
1) Adenine sulphate	8
2) Glucose	40,000
3) Distilled water to make up to volume (ml)	1000
4) Agar	9000

Abbreviations of the terms used in the text:

BAP/BA	:	6-Benzylamino purine or 6-Benzylamine.
cm	:	Centimeter.
2,4-D	:	2,4-Dichlorophenoxy acetic acid.
DAS	:	Days after sowing
EDTA	:	Ethyl Diamine Tetra Acetic acid.
g	:	Grams.
IAA	:	Indole-3-acetic acid
IBA	:	Indole-3-butyric acid.
2ip	:	2-Isopentenyl adenine or 6-(γ,γ-dimethylallylamino) purine
KC	:	Knudson-C medium.
l	:	Liter.
mg	:	Milligram.
ml	:	Milliliter.
mg/l	:	Milligram per liter.
mm	:	Millimeter.
μM	:	Micromolar.
MS	:	Murashige and Skoog medium.
NAA	:	α-Naphthalene acetic acid.
%	:	Per cent.
PBA	:	6-(benzyl amino)-9-(2-tetra hydronyl) -9 H-purine.
pH	:	Hydrogen ion concentration.
psi	:	Pounds per square inch.
VW	:	Vacin and Went medium.
V/V	:	Volume by volume.
WAI	:	Weeks after inoculation