In Vitro PROPAGATION OF ANTHURIUM

NIRMALA K. S.

DIVISION OF HORTICULTURE
UNIVERSITY OF AGRICULTURAL SCIENCES
BANGALORE

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NIRMALA K. S.

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University of Agricultural Sciences, Bangalore
in partial fulfilment of the requirements
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DIVISION OF HORTICULTURE
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BANGALORE

CERTIFICATE

This is to certify that the thesis entitled "*In vitro* PROPAGATION OF ANTHURIUM" submitted in partial fulfilment of the requirements for the degree of MASTER OF SCIENCE(HORTICULTURE) in FLORICULTURE to the University of Agricultural Sciences, Bangalore, is a record of research work carried out by MISS. NIRMALA, K.S., 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.

Bangalore.
December 1989

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I am extremely thankful to all my friends and staff members of the Division of Horticulture and well wishers for their encouragement, suggestions and kind co-operation.

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(NIRMALA, K.S.)

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My sincere thanks are due to members of my advisory committee,Dr.K.M.Bojappa,Professor and Head of the Department of Horticulture,University of Agricultural Sciences,Bangalore, Dr.N.Vijay Kumar,Associate Professor of Horticulture, University of Agricultural Sciences,Bangalore and Dr.G.P.Satyavati, Associate Professor of Botany,University of Agricultural Sciences,Bangalore for their valuable suggestions during the course of this investigation.

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CONTENTS

LIST OF TABLES

LIST OF PLATES

LIST OF FIGURES

INTRODUCTION

I. INTRODUCTION

Anthurium (Anthurium andreanum Lind.) belongs to the family Araceae and has more than 500 known species (Criley,1986). It is a native of tropical zones of Central and South America. Some of the other better-known members of this family are Philodendron, Monstera, Pothos, Taro, Calla, Aglonema, Dieffenbachia etc.

Anthuriums are grown for their long lasting and attractive flowers. The structure which is commonly called the flower is a combination of colourful modified leaf (Spathe) and hundreds of small flowers on the pencil-like protrusion(Spadix). The flowers are arranged in a series of Spirals. Spadix and Spathe are borne on a leafless stalk or peduncle (Singh,1987).

A. andreanum is an epiphyte with somewhat vining habit of growth,anchoring itself by means of aerial roots to its support. It produces flowers all the year round. Inflorescence emerges from each leaf axil. The sequence of leaf,flower and new leaf is maintained through entire life of the plant and the interval between leaf emergence are shortened or lengthened with changes in the environmental conditions.

A desirable anthurium plant should grow vigorously and be a prolific producer of suckers and flowers. Short internodes are preferred in order to limit the height of the plants. A desirable Spathe is heart shaped with symmetrical overlapping or fused lobes. A gently reclining Spadix will facilitate packing and delivery.

Anthurium andreanum was first brought to Hawaii from London in 1889 by Mr.S.M.Damon(Higaki and Watson,1973). After 90 years of cultivation and hybridization,the Hawaiian anthurium is one of the Island's principle ornamental export to the main land U.S.,Canada,Japan,Italy,Germany and other countries. It is also grown in a large scale in Holland and Mauritius. It is gaining importance in the cut-flower trade in the international market (Singh,1987).

Since last decade area under anthurium cultivation is steadily increasing. In Holland, the area under production increased by 19 per cent and the flower production exceeded 30,000 units (Holdgate,1977).

While the first anthurium introductions were pastel coloured flowers, there has been a constant change in the popularity of colours. To-day,there are hundreds of different Anthurium hybrids but,only a few are commercially acceptable.

Some of the varieties which are grown commercially are of red,orange,white,pink and coral colours. Cultivars of Obake (Bicoloured Spathe), Novelties (tulip type),Brown spathe and Double flowering anthuriums are receiving considerable importance. Although lot of breeding work is going on in different parts of the world, creation of yellow and blue anthuriums are still a challenge for the breeders.

In anthurium, plants derived from seeds show substantial variation in the most important aspects of colour, quality, annual yield and time of first flowering. There is a strong need for vegetatively produced stocks which conventional means cannot supply. The application of tissue culture will enable the breeder to provide the anthurium growers with the elite clones necessary to improve yield,quality and disease resistance.

Anthurium cut flower industry in India is still in its infancy. There is no serious effort to grow them on commercial scale. Added to this, the propagation of Anthurium by conventional means is very slow. The seed viability and germination percentage is also very low (Singh,1987). Seeds are viable only upto 2 or 3 days and germination percentage is as low as 20-30 per cent. Hence, there is a need to standardise a quicker method of propagation which may be achieved through in vitro techniques.

The present experiment was conducted with the following objectives:

a) To standardise the media for *in vitro* germination of *A. andreanum* seeds.

b) To standardise the efficacy of explant for *in vitro* propagation of anthurium.

c) To standardise the media for callus formation and differentiation of tissue.

REVIEW OF LITERATURE

II. REVIEW OF LITERATURE

A perusal of earlier literature reveals that there is very little information on anthurium in general and in vitro propagation in particular. Thus a brief review of work in relevance to all the aspects of the crop is listed below, dealt under different titles.

2.1 BOTANY:

The name anthurium is derived from the greek word 'anthos' = flower and 'oura' = tail , referring to the morphology of the Spadix.

Anthurium Lind. is known to have about 578 known species of which 50 are in cultivation and 10 to 15 are known to the trade (Bailey,1963). Of those that are in cultivation, there are several varieties or hybrids, as the species seems to cross readily. The leaf blade of A. andreanum Lind. is drooping and cordate, Spathe is cordate-ovate,thick in texture, 15-25 cm long,widely open-spreading; Spadix is 7.5 cm to 10 cm long, yellowish with white band marking the zone in which stigmas are receptive and which shows protogyny (Criley, 1986).

Kamemoto and Nakasone (1963) described the flower of Anthurium andreanum as hermaphrodite with 2 carpelled ovary and four anthers. They have also discussed the sequence of leaf and inflorescence production.

Christensen(1971) studied the morphology of growth and flower formation in Anthurium scherzerianum schott. and A. andreanum Lind. He described that the plants have a juvenile phase during which a vegetative bud is produced in the leaf axil, but in the subsequent generative phase, a flower bud is produced. These buds become dormant after initiation. The flower development depends on breaking of dormancy. A. scherzerianum plants which remain in vegetative phase for longer than normal,branch rapidly and are known as bush plants in commerce.

Croat (1980) studied the flowering behaviour of Neotropical Anthurium species and reported that more than 30 species flowered frequently under green house condition.

Sheffer and Croat (1983) evaluated the chromosome number in Anthurium species from North, Central and South America. The chromosome counts ranged from 2n=24 to 2n=66 with 2n=30 being the most common number. Aneuploidy, polyploidy and presence of B chromosomes are the basic features of the genus. Rehena Ali (1979) studied the cytology of different Anthurium species and reported a natural Triploid in A. crystallinum.

Mari and Kamemoto(1983) reported the presence of B chromosomes in A. warocqueanum, their analysis revealed the presence of 1-3 B chromosomes in the complement. However, no phenotypic effect was observed due to the presence of B chromosomes.

The morphological and anatomical aspects of Anthurium andreanum were studied by Higaki and his co-workers (Higaki et al,1984).

Ray (1987) described and classified the leaf types in Araceae including Anthurium, on the basis of their morphology.

2.2 Breeding:

Kamemoto and Nakasone(1963) worked on evaluation and improvement of anthurium clones. Of 113 clones evaluated, 13 were recommended for commercial cut flower production including 'Haga White', the orange variety 'Nitta', and the red varieties 'Kaumana', 'Ozaki', 'Kansako' No.1 and 'Hirsoe'.

Kamemoto et al, (1968) carried out breeding for the improvement of Anthurium. Two high yielding seedling selections, a white and a pink, were named 'Uniwal' and 'Marian Seefurth' respectively, and introduced into the trade. Three bicoloured clones,white green (UH 8), rose opal green (UH 16) and coral green (UH 39) were released later.

Kamemoto and his co-workers (1969) described 3 new cultivars of Anthurium and 2 seedling selections 'Anuenue' and 'Chameleon' as suitable for cut flower production.

Kamemoto and Sheffer (1978) developed a new hybrid A. scherzerianum x A. wendlinzerii. The 2 species were crossed successfully to produce a hybrid with a greyish - orange spathe. Other characterstics such as the length and colour of Spadix and the length and position of the leaf blade were intermediate between the highly contrasting characterstics of the parental species. Fertility in hybrids was very good,indicating close relationship of the 2 species.

Maurer (1979) has discussed the techniques of cross-pollinating Anthurium scherzerianum and also the presence of recessive characters (A = with anthocyanin, a= without anthocyanin, B = whole Spathe coloured, b= Spotted Spathe). When the parents were Aa/Bb the descendents were 9 red (AB), 3 red spots on white (Abb) and 4 white (aaB and aabb). The deficit in white plant was provisionally attributed to their lack of vigour.

Kamemoto et al, (1988) studied the genetics of major spathe colour in anthurium. They identified two major genes M, O for all the major colours for anthurium e.g. Red,Orange,Pink, Coral and White. Red and Pink result when both M and O are present. Orange and Coral result when only O is present,the double recessive mmoo results in white, pink is heterozygous for both M and O. Crosses between two pinks will produce off springs in ratio of 9 red, 3 pink to orange-coral, 4 white.

Gajek and Schwarz (1980) have described the best cultivars for cut flowers; mid sized 'Iga Gold' with a shining red Spathe and a white spadix with a yellow tip and the compact "Elirina" with a vermillion light salmon spathe and a Sulphur yellow Spadix.

Schmidt and Lavterbch(1985) have recognised 2 cultivar groups (a) Miniature cultivars - generally under 20 cm tall with narrow leaves and short petioles,including cultivars 'Oud orange', 'R.nata' and 'Amazone' and (b) larger plants with broad leaves and long petioles including cultivars 'Lachs', 'Flamenco', and $K_2$6.

Bhatt and Desai (1989) have listed different cultivars which are suitable both as cut flowers and pot plants,which are mostly hybrids of different *Anthurium* species, involving mainly *A. andreanum* and *A. scherzerianum.*

2.3 Cultural aspects:

Rapsey and Carr (1969) described growing of *Anthurium* in Trinidad and Tobago including propagation, and cultural practices,pests and disease control etc. Christensen (1973) reported that root trimming of *Anthurium scherzerianum* while picking reduced final plant size, but did not retard flowering. Lefring (1975) concluded on the basis of his experiments that plants grown under shade receiving at least 45 per cent of available light in the green house resulted in increased growth rate and average flower production.

Higaki and Rasmussen (1979) reported that plants of 'Ozaki Red'when sprayed with PBA,BA or Ethephon at a concentration of 100, 500, 1000 or 1500 mgl^{-1} induced adventitious bud formation. Maximum shoot formation was produced with BA at 1000 mgl^{-1} (3.6 shoots/plant) followed by PBA at 1500 mgl^{-1}(2.2 shoots/plants) and Ethephon at 1000 mgl^{-1} (1.8 shoots/ plant). Control plants exhibited no adventitious shoot formation.

Higaki and his Coworkers (1979) recommended a spacing of 45 x 45 cm to accommodate 12,000 plants per acre or a closer planting of 30cm x 30cm, to provide 25,000 plants per acre. When closer spacing is followed, for maximum production, heavy pruning should be done every fourth year, to provide proper air-circulation. Rigid leaf pruning and spray schedule should be followed to control diseases etc. They also suggested the application of fertilizers such as 5-10-10 or 10-20-20 or 16-16-16, at the rate of approximately 135 kg of Nitrogen per year. Slow releasing or pelletised fertilizers are better as they cause less damage.

Voogt (1979) studied the effect of saline water on the development of Anthurium andreanum. The cut flower yield of Anthurium declined progressively as salinity of the water increasec

Water containing Sodium Chloride was particularly detrimental. There was a marked difference in salt sensitivity between cultivars. When desalinized water or rain water is to be used extra calcium application is necessary.

In trials with cv.'Brazil Red' and 'Antiqua', Strauer(1980) reported that culture on rock wool(blocks and mattings) is satisfactory, if a suitable nutrient solution with a high K content is used.

Hetman <u>et al</u>,(1981) studied the effect of substrate on physical properties of <u>Anthurium andreanum</u> assessing for plant height, number of leaves and leaf area coefficient. The best results were obtained on a substrate of peat + Sphagnum, Peat + Perlite in equal proportions.

Schenk and Brundert (1981) studied the effect of temperature on <u>Anthurium andreanum</u> hybrids. 5 cultivars were exposed to air temperature of 13 ^{o}C, 19 ^{o}C or 22 ^{o}C with a constant peat substrate temperature of 22 ^{o}C.Vars. 'Horning Orange' and 'Horning Rubin'' were normally temperature neutral but in 'Horning Rubin' the total number of flowers per plant was reduced at 22 ^{o}C. For the three light red 'vogel' cultivars yield and quality of flowers were best at 19 ^{o}C at which temperature 'Vogel' No.202 produced 7 flowers per plant, the highest yield. At 10 ^{o}C 'Vogel' plants died, and at 13 ^{o}C they showed some leaf necrosis. Plant vigour increased with rising temperature.

Turski and his co-workers (1983) studied the effect of different substrates on growth of Anthurium andreanum. The best substrates were those in which the basic component was high peat.

Tesi and Faro (1985) studied the use of powdered bark as a substrate for growing Anthurium in pots. They concluded that Anthurium could be successfully grown in a bark based medium.

2.4. In vivo Seed Germination and Storage:

Beele (1971) described the method of seed sowing and germination. The best germination resulted from picking the berries at the orange-red stage and fermenting them for four days in water at 22 °C to separate the seeds from the pulp. Storage was sometimes necessary because berries from the same inflorescence ripened at different times. But the maximum time for storage of fermented seed in water was five days. This reduced germination from 99% to 80% and 10 days in water it reduced it to 53%. The best substrate for germination (95%) was peat. Seedling growth,especially root development was better in white peat + perlite than in peat + perlite or conferous litter + Perlite.

Fresh seeds of Anthurium scherzerianum hybrids germinated in light or darkness at 10 - 35 °C. Germination occured after 5-7 days after drying and storing at 20°C for 24 h. Germination under favourable conditions were 70-75% (Bachthaler,1977).

Bachthaler (1978) studied the germination of Anthurium scherzerianum hybrids at different stages of berry ripening. Seeds extracted from (1) green/unripe (2)Reddish/half ripe (3) red ripe and (4)Reddish brown or over ripe berries were placed in petridishes on damp sterile sand and kept at 25 °C for 12 h. of light. In the first three groups all the seeds showed germination but in group four only 42% germinated. Group two and three were the first to germinate and were the most suitable for commercial seed production.

Bachthaler (1979) also worked on storage of seeds of Anthurium scherzerianum. Seeds stored within the berries at 3°C suffered cold injury and fungal infection. The best storage temperature was 10°C, and after 6 weeks 60% of seeds germinated. Seeds from berries treated with thiram just before storage, 95% germinated after 12 weeks at 10°C and 60% after 16 weeks.

Maurer and Brandes (1979a) described the method of seed extraction of Anthurium scherzerianum hybrids. The seeds treated with 13% sodium carbonate at 20°C for 25 hours or in 6% sodium carbonate +1% pectinase solution at 26-36°C for 5 hours were the 2 methods resulting in seeds being clean enough to sow singly.

Maurer and Brandes (1979b) also worked on storage of seeds of Anthurium scherzerianum hybrids. Seeds were stored as spadices, in berries or as cleaned seeds in dry conditions. Seeds left on spadices or berries became too dry or rotted. In cleaned seeds, germination decreased. Some seeds stored at 20°C germinated but seeds stored at 5°C became non-viable. Seed stored in water rotted.

Bachthaler (1980) studied the possibility of storing seeds of Anthurium scherzerianum hybrids. Berries stored at 10°C for upto six weeks germinated in four days whereas those stored at 5°C and 2°C took five and seven to eight days respectively. Germination was 100% in seeds from berries stored at 10°C for three weeks. Seeds from berries treated with thiram germinated well (in four days) after 16 weeks storage. After 12 weeks storage at 10°C seeds from untreated berries failed to germinate, where as germination was above 90% in seeds from treated berries.

Szendel and his co-workers (1981) evaluated the conditions for germination of Anthurium andreanum and Anthurium scherzerianum seeds. Seeds of A. andreanum harvested at three maturity stages and those of A. scherzerianum at one maturity stage (light orange) were germinated on three substrates at pH ranging from 4 to 8 in light or darkness at 18, 24 or 28°C. In A. andreanum the best germination was obtained on peat substrate, at pH 4 or 5, in light at 28°C using seeds harvested at an early maturity stage (yellow-green to light orange). A. scherzerianum seeds germinated best on peat having pH 4.

2.5 Flower development:

Watson and Shirakawa (1967) studied the morphology of Anthurium and related it to shelf life of the flowers. They described the stages in the development of individual flowers on the spadix of 'Ozaki Red'.They also suggested that picking when the stigmas are receptive should be avoided as water loss is greater at this period.Dipping the spadix in Parafin wax was found to reduce water loss considerably and enhance keeping quality of flowers.

Akamani and Goo (1972) related the physical charcters of Anthurium to its vase life. They concluded that vase life of 'Kaumana' flowers varied from 8-10 to 18-21 days in water at 79-87^{o}F and 54-74% RH. Vase life was inversly related to petiole length and diameter and flower weight.

Iwata and his co-workers (1979) studied the Anthocyanins of Anthurium andreanum cvs. and identified them as pelargonidin - 3 - rhamnosyl glucoside and cyanidin-3-rhamnosyl glucoside. Both pigments were present in red cvs. 'Ozaki', 'Kaumana', 'Kozohara', 'Kansako No.1' and 'Nakazawa' and in the pink cv.'Marian Seefurth'. The orange cv.'Nitta' and coral coloured cv.'Tateishi coral' contained only Pelargonidin 3-rhamnosyl glucoside. Later they also reported that a predominance of cyanidin-3-rhamnosyl glucoside resulted in pink to dark red colours, whereas a predominance of pelargonidin 3-rhamnosyl-glucoside resulted in coral to orange colour (Iwata et al., 1985).

Singh (1987) described harvesting and grading of Anthurium flowers. Flower of commerce is harvested when approximately three-quarter of the stigma along the spadix becomes receptive. Different grades are given based on total spadix length and width.

Grade		Spadix measure
Miniature		< 8 cm
Small	-	8 to 10 cm
Medium	-	10 to 13 cm
Large	-	13 to 15 cm
Extra Large	-	> 15 cm

Akamine and Goo (1981) worked on controlled atmosphere storage of anthurium flowers and found that cold storage at 13 ^{o}C successfully extended the storage life of cut anthurium flower cultivar 'Ozaki', but where refrigeration facilities were not available, storage in 2-10% O_2 could be used advantageously at ambient temperature of 24-25 °C.

Kalkman(1983) concluded that vase life was longest with flowers cut when the spadix was almost completely white. The average vase life for flowers cut at this stage in winter and summer was 21.5 to 25.2 days respectively. 'Avo-Cintha' and 'Avo-Ingrid' had the largest average vase life of 31.3 and 25.4 days respectively in winter and 29.1 and 28.9 days respectively in summer.

Paull (1983) suggested the use of waxes for extending post harvest vase life of anthuriums.Of the eight products used to coat the flower,FMC-819,Carnuba based wax was most effective, increasing the vase life from 18 days in the untreated control to 36 days.

Paull and his co-workers (1985) described the physiological changes associated with senescence of cut anthurium flowers. Silver pulsing (for 40 minutes) of Anthurium andreanum,Cv. 'Ozaki Red', flower stem was done to modify the senescence process. Florets on the spadix continued to open for 5-10 days after harvest in both treated and non-treated flowers. In both, respiration rate was low until senescence began 8 days after harvest. The rate of increase in respiration of silver treated flower was lower than that of controls. In all cases ethylene production remained low throughout the post harvest life of the flowers. 10 days from harvest spathe colour began to change from red to blue with no significant changes in anthoyanin ratios. Tissue pH rose from 5.2 to 5.6. The concentration of tissue phenolics increased during senescence and intensified the colour change by co-pigmentation. Tissue starch level declined by about 25%. The ratio of sugars in the stem,spathe and spadix remained constant with a slight decline in concentration during post-harvest life. Silver pulsing of the stem reduced stem plugging and thus reduced the rate of change of all the senescence process observed.

Paull (1987) also studied the effect of storage duration and temperature on cut Anthurium flowers. The optimum storage temperature for *Anthurium andreanum* flowers of the cultivar 'Kaumana', 'Nitta' and 'Ozaki' was between 14 and 17^{o}C. A Silver Nitrate pulse (4 mM for 40 mins.) given immediately after harvest increased post harvest life of stored flowers. Maximum vase life was achieved with Ag^{+} treated flowers packed for 3 days.

2.6. In vitro Propagation

Fang and Tanabe(1986) gave an effective method of dis-infestation procedure for anthuriums which included air drying of plant stems and roots for four days at room temperature. This resulted in easy removal of leaf coverings of buds. Along with air drying, a soak for 30 mins in liquid detergent reduced contamination upto 60%.

Pierik and his co-workers (1974) described a method for the vegetative propagation of Anthurium andreanum. The callus was induced from excised embryos and young parts of adult plants by growing them on modified Murashige and Skoog's medium, supplemented with PBA 0.1-1mgl^{-1}. Optimum growth of callus tissue was obtained at 25^{o}C when the cultures were grown in darkness. The rate of growth was highly variable amongst callus clones obtained from different genotypes. Callus could be subcultured and induced to form adventitious sprouts, especially in light. These sprouts regenerated roots on the basic culture medium. The most essential factors influencing callus induction, callus subculture and plantlet formation appeared to be the same for both embryos and tissues of adult plants. The identical behaviour of juvenile and adult callus tissues demonstrates that differentiated tissues or adult plants rejuvenate when going through the callus phase.

Pierik and Steegmans(1975) gave a detailed method for vegetative propagation of Anthurium scherzerianum in vitro. The procedure includes callus formation on leaf segments, shoot formation in the callus and the rooting of cuttings of these shoots using a solid agar medium. A liquid medium also gave good results for this species in trials with a shaking machine. Pierik (1975)reported that the growth of callus tissue from adult A. andreanum plants was best in a modified Murashige and Skoog's medium. Genotype A 42-3 reached a fresh weight multiplication rate of 30.7 when grown for five weeks at 25 ^{o}C and in continuous darkness. Clone A 42-3 appeared to have hardly any cytokinin requirement for callus growth.

Pierik(1976) gave the procedure for production of plantlets through tissue culture as:callus induction on excised leaf segments, callus subculture on solid or in liquid media, adventitious sprout formation in callus on solid media and rooting of excised sprouts. The optimum culture media are defined for each stage. The most pronounced and limiting factor for each phase was found to be the presence of a cytokinin. Another basic requirement for effective propagation appeared to be the concentration of macro elements,specially Ammonium Nitrate,which promoted adventitious sprout formation at low concentration.

Fersing and Lutz(1977) reported that Anthurium scherzerianum and A. andreanum explants produced many shoots when cultivated in vitro in the presence of Benzyl Adenine and 2,4-D. Yeast extract stimulated shoot growth of A. scherzerianum but restricted it in A. andreanum. Root growth on leafy shoots was spontaneous phenomenon in A. andreanum but required stimulation in A. scherzerianum.

Lefring and Soede (1978) described propagation of anthurium based on side shoot formation from callus grown on a nutrient medium,which is found to be commercially preferable to that based on continuous callus division. Lefring and Soede (1979) reported that BA added to Murashige and Skoog's medium was found to produce almost no shoots on callus while addition of 3 mg 2-ip resulted in wide spread shoot formation.

Pierik and his co-workers (1979) also studied the effect of various factors on callus formation on excised leaf explants of Anthurium andreanum. The explants were devoid of mid vein, obtained from young,soft and just expanding leaves. They were cultured on a basic nutrient agar medium to which were added growth substances (Adenine,BA, Kinetin, 2-ip,Zeatin or 2,4-D) and Ammonium Nitrate,Ammonium Sulphate or Sodium Nitrate in various concentrations.The effect of using the surfactant Tween 20 during leaf sterilization and of growing explants in light and/or darkness for 20 weeks were also investigated. In all the cases formation of callus at the margins of the leaf explants preceeded the regeneration of sprouts. There was a strong positive correlation between callus formation and shoot regeneration. Regeneration was optimal under the following conditions: addition of 0.25-1.00 ml l^{-1} Tween 20 during leaf sterilization,adding a growth substance (adenine 0.1 mgl^{-1};Zeatin 1mgl^{-1} ,or 2,4-D 0.08 mg l^{-1}),culture in darkness and in light,and including 206 mg l^{-1} Ammonium Nitrate in the medium.The promoting effect of low levels of Ammonium Nitrate on shoot regeneration in callus was caused by the NH_4^+ ion and not by the NO_3^- ion.

Kunisaki (1980) reported that high yields of viable A. andreanum cultures were obtained using small explants of vegetative buds and a long disinfection soak in Sodium hypochlorite. Explants were grown into plantlets on Modified Murashige and Skoog medium supplemented with 15% coconut water. The cultures were maintained at 25-28°C under low illumination. Best results were obtained when stem sections from aseptically grown plantlets were cultured in medium containing 6-benzylamino purine (BA) at 0.2 mg l^{-1}. There was no further increase in number of shoots at higher BA concentrations but callus growth increased and shoots were stunted.

Novak and Nepustil (1980) described large scale propagation of Anthurium andreanum cv.'Iga Gold II' by callus culture. They gave the composition of media for callus induction and cultivation, for plant regeneration and rooting. Callus clones with a high regeneration capacity were derived from leaf explants of flowering plants.

According to Geier(1982) Spadix segments have a much higher capacity for regeneration than segments of leaf petiole, inflorescence stalk or spathe. However, during initial stages of culture they showed considerable stability and rarely formed callus or shoots. The spadix segments of A. scherzerianum were cultured on a Modified Nitsch medium (Ammonium nitrate 100 mg l^{-1} Benzyl adenine 1 mg l^{-1} and 2,4-dichlorophenoxy acetic acid 0.1 mg l^{-1}). The cultures were kept in darkness. Callus and shoot formation occured rarely in the primary cultures but were induced and promoted by repeated dissection and transfer of the tissue onto fresh medium. Highly organogenic cultures producing numerous shoots were obtained after 3 to 6 transfers. Shoots developed either by transformation of flower initials still present in the cultures, or directly from callus or from callus-derived embryoids(which closely resembled zygotic embryos). True-to-type plants were obtained by rooting the shoots on hormone free medium and exposing the plantlets to light (2000 lux for 14 h/day). Kraft et al. (1983) reported that Anthurium andreanum cvs. 'Ellrina' and 'Porzellan' were propagated through tissue culture and the full year required for explants to develop into rooted plants was reduced to 5 months, by taking several crops of shoots from the callus. Rooting occured after four weeks in cytokinin free medium.

Finne and Staden (1986) obtained plantlets on modified Murashige and Skoog medium at $25 \pm 2\,^{o}C$ with 10h light/8h dark cycle in Anthurium andreanum.

The advantages of vegetative propagation of Anthurium scherzerianum were discussed by Geier (1986b). Ease of propagation using cuttings from young leaves(upto 25 cm long) declined in older leaves. At first callus formation was stimulated in darkness on agar medium A containing 0.1 mg l^{-1} 2,4-D and 1 mg l^{-1} BA. After transfer into Agar medium B without 2,4-D and with less BA (0.2 - 0.5 mg l^{-1}) but increased Ammonium Nitrate (from 200 to 720 mg l^{-1}), further callus and shoots developed. Rooting of shoots required four weeks of light (14h at 2500 lux/day) on agar medium C without auxin or cytokinin. Shoot development also occured on medium B where shoots became much branched and formed adventitious shoots. In callus tissue genetic mutations were more frequent than in meristems. Great variability in regeneration ability was shown by five genotypes depending on Ammonium Nitrate supply. Some genotypes were more easily propagated from flower spikes than leaf cuttings.

Geier(1986a) also discussed the factors affecting plant regeneration from leaf segments of A. scherzerianum. Regeneration was found to be highly dependant on genotype and leaf age. Once the leaf hardening started,regeneration decreased. The position on the leaf from where the explant was excised,segments either without or including a part of midrib showed no difference in response,except that segments from the proximal portion including a part of midrib produced significantly less shoots than explants from all other parts of the leaf. The culture media was Nitsch's basal medium with minerals,vitamins,Sucrose and agar. Ammonium Nitrate level had significant effect on callus and shoot formation. A low level of 200 mg l^{-1} was beneficial. Shoots isolated from callus cultured on a medium containing BA + 2,4-D readily formed roots when transferred to basal medium with no growth regulator. Ammonium Nitrate at 720 mg l^{-1} accelerated root formation compared with 200 mg l^{-1} Ammoium Nitrate. Shoots multiplied on a medium containing BA alone were more difficult to root.

Keller et al.(1986) reported that leaf explants formed callus in 1.5 - 2 months on Murashige and Skoog's medium supplemented with 2 mg Kinetin l^{-1}. Plantlets were formed over a fairly long period. The multiplication rate for the cvs. 'Ellrina' and 'Lys Gold' was nine fold and six fold respectively.

A study was undertaken to standardize a proper media and to find out the effect of growth regulators, vitamins and additives on Anthurium andreanum Seed Germination and Subsquent Growth by Swaminathan (1986). Nitsch medium was found to be the best of all the four different media used. NAA (0.1, 1 and 5 mg l^{-1}) did not have any effect. IBA at all concentrations delayed germination but promoted subsequent growth after 1st leaf stage. GA_3 did not affect initial seed germination, but subsequent growth was enhanced. 2,4-D also had similar effect. Thiamine directly affected germination and subsequent growth. Seedlings were much greener and healthier with 5 mg l^{-1} of Thiamine. The germination and growth of seedlings were good when the media was supplemented with Banana pulp. Effect of Colchicine was inhibitory and lethal except at 5% concentration.

Zens and Zimmer (1986) concluded that formation of callus and adventitious shoots by shoot tip explants increased significantly by changing the NH_4-N : NO_3-N ratio from 1:1 to 1:5. The increase in productivity differed between clones. In liquid media fresh weight production within six weeks was three times higher than on solid media with both NH_4- N: NO_3- N ratios.

Zens and Zimmer (1988) developed clones of Anthurium scherzerianum using in vitro culture techniques. After 12 weeks multiple shoot formation was found in 82-93% of seeds of A. scherzerianum germinating under sterile conditions on a Murashige and Skoog agar medium with phytoharmones (NAA & BA). Seeds produced caulogenic callus or callus with new shoots. Productivity depended on genotype and it decreased with increasing NH_4: NO_3 ratio in the medium.

MATERIAL AND METHODS

III. MATERIAL AND METHODS

The experiments were conducted at Indian Institute of Horticultural Research,Hessaraghatta,Bangalore. The plants were maintained in the humidity controlled Glasshouse attached to the laboratory. Standard cultural practices for growing the plants were followed.

3.1 PLANT MATERIAL

Anthurium andreanum Lind. the common anthurium was taken for the studies. The species is of columbian origin and is commonly known as 'Tail flower' or 'Wax flower'. It grows erect with long heart shaped green leaves, and coloured cordate spathe which is wavy and puckered with pendent Spadix (Plate I a,b).

3.1.1 Floral biology and pollination

Flowers in Anthurium are very small. They are bisexual and are closely congested in cylindrical spikes and are arranged in series of spirals on the Spadix. The flowers have four tepals. Stamens are opposite to the tepals. Stigmas are sessile, shiny and slimy when receptive. Flowers are protogynous,hence cross pollination is obligatory. Pollination was done by transferring the pollen from the pollen parent to the receptive stigma by a brush. The time taken from pollination to harvest of the berries is about 120-130 days.

Plate-I *Anthurium andreanum* Lind showing Spathe(a) and Spadix(b)

Plate-I *Anthurium andreanum* Lind showing
Spathe(a) and Spadix(b)


a
b

3.1.2 *Harvesting of berries*

The fruits(berries) were harvested when they were ripe and orange-red in colour.(Plate II). There were 2-3 seeds in each berry.

3.2 EXTRACTION OF SEEDS

The fruits were used immediately after harvest for the experiments. The seeds were extracted by removing the pulp either by hand or by soaking them in 13 per cent sodium carbonate solution or one per cent pectinase for five hours at room temperature(Maurer and Brandis, 1979 a).

3.2.1 Sterilization of seeds

The seeds were thoroughly washed and sterilized by soaking in 4% sodium hypochlorite (BDH) solution for one hour.

3.2.2 Media

The following different media were used in the present study.

1. Nitsch - 1969	(Table 1)
2. Vacin and Went - 1949	(Table 2)
3. Murashige and Skoog-1962	(Table 3)
4. Knudson C -1951	(Table 4)

Plate-II Close up of <u>Anthurium</u> <u>andreanum</u> spadix showing mature berries.

Plate-II Close up of *Anthurium andreanum* spadix showing mature berries.

3.2.3 Preparation of media

Stock solution of micronutrients, vitamins and growth regulators required were prepared by dissolving known quantity of each chemical separately in known volume of water. They were stored in dark coloured bottles in refrigerator at $4^{o}C$.

For preparing the nutrient media, required quantity of solutions of micronutrients, growth regulators and vitamins were taken into 500 ml boiling flask. Required quantity of macro-nutrients were also added and dissolved. The pH was adjusted between 5.5 - 5.8 by using lN NaOH or lN HCl. For preparing the solid media, required quantity of agar was added to the hot solution. Care was taken not to over heat the agar as it gets charred. 80 ml of the prepared media was poured into 150 ml Erlenmeyer's flasks and plugged with cotton plug and kept for autoclaving.

During the preparation of stock solutions, growth regulators like IAA, NAA and 2,4-D were first dissolved in a minimum quantity of ethanol and the required volume was made up with distilled water. Kinetin and tricalcium phosphate were first dissolved in lN HCl and the volume made up with distilled water.

Na_2. Fe.EDTA was prepared from Na_2 EDTA dissolved and heated in water with $FeSO_4 . 7H_2O$.

3.2.4 Autoclaving

Horizontal type of autoclave was used. The flasks were autoclaved at a pressure of 1.06 kg/cm^2 (121°C) for 15 minutes. The flasks were then taken out and were allowed to cool. They were kept staked in the culture room at 25 $\pm$ 2°C. Inoculation of seeds and explants was done after 4-5 days ensuring that the flaks were free from contamination.

3.2.5 Inoculation of seeds

The sterilized seeds were sown in flasks containing different media under aseptic conditions in the inoculation room provided with UV tubes.

3.2.6 Care of culture

The cultured flasks were kept in the culture room at 25 $\pm$ 2°C with light intensity of 2000 lux for 16 hours.

3.2.7 Recording of data

Data was recorded on:

1. Germination percentage of seeds
2. Number of days taken for germination
3. Number of days taken for first ,second, third and fourth leaf stage.
4. Fresh weight-dry weight ratio of seedlings.

Dry weight was taken by drying the plants at their fourth leaf stage for 48 hours at $60^{o}C$ in the oven.

3.3. TISSUE CULTURE

Standard tissue culture methods were adopted (Pierik, 1987) with the following adoptation.

3.3.1 Explants

The different explants used for the present studies were:

a) Leaf sections

b) Spadix segments

c) Vegetative buds

3.3.2 Sterilization of explants

3.3.2.1 Leaf sections: For leaf culture, young unfolding leaves were selected. They were dipped in alcohol for few seconds, immersed in water with few drops of Tween 20 for 1 hour washed thoroughly with sterile water to remove the traces of Tween 20. The leaves were later immersed in a solution of 0.1% mercuric chloride for 30 minutes and were cut into pieces of 1 cm^2 with or without midrib.

3.3.2.2. Spadix segments: Young spadices which were still covered with Spathe were selected. For sterilization they were dipped in alcohol for few seconds. Immersed in water with few drops of Tween 20 for 1 hour after which they were rinsed in sterile water thoroughly to remove traces of Tween 20; immersed in a solution of 1 per cent sodium hypochlorite for 30 minutes. At this stage, the spathe was removed and spadix was once again immersed in a solution of 1 per cent sodium hypochlorite for 30 minutes. They were then cut into pieces of 0.8 - 1.0 cm length and kept ready for inoculation.

3.3.2.3. Vegetative buds: Vegetative buds from young plants were collected by cutting out nodal sections of about 0.5 - 1 cm^3. They were first dipped in alcohol for few seconds, soaked in 0.5% sodium hypochlorite solution with 2 drops of Tween 20 for 20 minutes. Leaf coverings were later removed under a dissection microscope. The excised buds were soaked in 0.25 per cent sodium hypochlorite solution with 2 drops of Tween 20 for 45 minutes. Each bud was disinfected separately.

3.3.3. Media

The following different media were used in the present studies.

1. Nitsch - 1969 (Table 1)
2. Vacin and Went - 1949 (Table 2)
3. Murashige and Skoog - 1962 (Table 3)
4. Morel - 1948 (Table 5)
5. Modified Nitsch-Geier - 1986a (Table 6)
6. Modified Murashige and Skoog - - Kunisaki - 1980 (Table 7)
7. Modified Murashige and Skoog - Pierik - 1976 (Table 8)

Media were used either in liquid or solid form.

3.3.4 Preparation of media

As given under 3.2.4

3.3.5 Autoclaving

As given under 3.2.5

3.3.6 Inoculation

The inoculation of explants were done in inoculat chamber under perfect aseptic conditions.

Table 1. Composition of Nitsch medium (Nitsch, 1969)

Elements	Qty./lt.
Inorganic	
Ammonium nitrate	720 mg
Potassium nitrate	950 mg
Calcium chloride	166 mg
Magnesium sulphate	185 mg
Potassium phosphate	68 mg
Boric acid	10 mg
Manganese sulphate	25 mg
Zinc sulphate	10 mg
Sodium molybdate	0.25 mg
Copper sulphate	0.025 mg
Ferrous sulphate	27.8 mg
Sodium EDTA	37.3 mg
Organic	
Inositol	100 mg
Nicotinic acid	5 mg
Pyridoxin HCl	0.5 mg
Thiamine HCl	0.5 mg
Glycine	2 mg
Folic acid	0.5 mg
Biotin	0.05 mg
Sucrose	20 g
Distilled water to make upto	1000 ml
Agar	9 g

Table 2. Composition of Vacin and Went medium (Vacin and Went, 1949)

Elements	Qty./lt.
Calcium phosphate	200 mg
Potassium nitrate	525 mg
Magnesium sulphate	250 mg
Potassium phosphate	250 mg
Ammonium sulphate	500 mg
Manganese sulphate	250 mg
Ferric tartarate	28 g
Sucrose	20 g
Distilled water to make upto	1000 ml
Agar	9 g

Table 3. Composition of Murashige and Skoog medium (Murashige and Skoog,1962)

Elements	Qty./lt.
Major	
Ammonium nitrate	1650 mg
Potassium nitrate	1900 mg
Calcium chloride	440 mg
Magnesium sulphate	370 mg
Potassium phosphate	170 mg
Sodium EDTA	37.3 mg
Ferrous sulphate	27.8 mg
Minor	
Boric acid	6.2 mg
Manganese sulphate	22.3 mg
Zinc sulphate	8.6 mg
Potassium iodide	0.83 mg
Sodium molybdate	0.25 mg
Copper sulphate	0.025 mg
Cobalt chloride	0.025 mg
Organic	
Glycine	2.0 mg
Inositol	100 mg
Nicotinic acid	0.5 mg
Pyridoxin HCl	0.5 mg
Thiamine HCl	0.1 mg
Sucrose	30 g
Distilled water to make upto	1000 ml
Agar	10 g

Table 4. Composition of Kundson C medium (Kundson,1951)

Elements	Qty/lt
Calcium nitrate	1 g
Potassium phosphate	0.25 g
Magnesium sulphate	0.25 g
Ammonium sulphate	0.50 g
Ferrous sulphate	0.0025 g
Manganese sulphate	0.0075 g
Sucrose	20.00 g
Distilled water to make upto	1000 ml
Agar	8.5 g

Table 5. Composition of Morel's medium(Morel, 1948)

Elements	Qty/lt
Calcium nitrate	500 mg
Magnesium sulphate	125 mg
Potassium nitrate	125 mg
Ferrous sulphate	2.5 mg
Potassium phosphate	125 mg
Kinetin	8 mg
Indole-3- acetic acid	1 mg
Naphthalene acetic acid	1 mg
Glucose	40 g
Distilled water to make upto	1000 ml
Agar	9 g

Table 6. Composition of modified Nitsch medium (Geier,1986 a)

Elements	Qty/lt
Inorganic	
Ammonium nitrate	200 mg
Potassium nitrate	950 mg
Calcium chloride	166 mg
Magnesium sulphate	185 mg
Potassium phosphate	68 mg
Boric acid	10 mg
Manganese sulphate	25 mg
Zinc sulphate	10 mg
Sodium molybdate	0.25 mg
Copper sulphate	0.025 mg
Ferrous sulphate	27.8 mg
Sodium EDTA	37.3 mg
Organic	
Inositol	100 mg
Nicotinic acid	5 mg
Pyridoxin HCl	0.5 mg
Thiamine HCl	0.5 mg
Glycine	2 mg
Folic acid	0.5 mg
Biotin	0.5 mg
Sucrose	20 g
Benzyl aminopurine	1 mg
2,4-Dichlorophenoxy acetic acid	0.1 mg
Distilled water to make upto	1000 ml
Agar	9 g

Table 7. Composition of modified Murashige and Skoog medium (Kunisaki, 1980)

Elements	Qty/lt
Ammonium nitrate	1650 mg
Potassium nitrate	1900 mg
Calcium chloride	440 mg
Magnesium sulphate	370 mg
Potassium phosphate	170 mg
Sodium EDTA	37.3 mg
Ferrous sulphate	27.8 mg
Boric acid	6.2 mg
Manganese sulphate	22.3 mg
Zinc sulphate	8.6 mg
Potassium iodide	0.83 mg
Sodium molybdate	0.25 mg
Copper sulphate	0.025 mg
Cobalt chloride	0.025 mg
Thiamine HCl	0.4 mg
Pyridoxin HCl	0.5 mg
Sucrose	20 g
Coconut water	15% (150 ml/l)

Table 8. Composition of modified Murashige and Skoog medium (Pierik,1976).

Elements	Qty/lt
Ammonium nitrate	825 mg
Potassium nitrate	950 mg
Calcium chloride	440 mg
Magnesium sulphate	370 mg
Potassium phosphate	85 mg
Sodium EDTA	25 mg
Boric acid	6.2 mg
Manganese sulphate	22.3 mg
Zinc sulphate	8.6 mg
Potassium iodide	0.83 mg
Sodium molybdate	0.25 mg
Copper sulphate	0.025 mg
Glycine	2.0 mg
Inositol	100 mg
Nicotinic acid	0.5 mg
Pyridoxin HCl	0.5 mg
Thiamine HCl	0.1 mg
Glucose	30 g
Benzyl aminopurine	1 mg
2,4-Dichlorophenoxy acetic acid	0.08 mg
Distilled water to make upto	1000 ml
Agar	7 g

3.3.6.1. Leaf sections: The leaf sections were finally rinsed in sterile water and inoculated on the medium under aseptic conditions.

3.3.6.2. Spadix segments: Spadix segments were again rinsed in sterile water and inoculated on the nutrient medium under aseptic conditions.

3.3.6.3. Vegetative buds: The disinfected vegetative buds were taken out and were directly placed on the nutrient medium under aseptic conditions.

3.3.7 Culture conditions

The cultures were kept under light or dark conditions as required by the experiment.The intensity of light was 2000 lux for 16 hours. Temperature of 25 $\pm$ 2 ^{o}C was maintained. Cultures which required darkness were kept in BOD incubator callon 152-6 c.F. with light control. Liquid cultures which required shaking were put on Emenvee rotary shaker 80 rpm.

3.3.8 Recording of data

Observations were recorded on:

1. Callus initiation

2. Extent and frequency of callus development

3. Nature and colour of callus

4. Regeneration and plantlet formation

5. Survival in the field

3.4 TRANSPLANTING IN THE FIELD

3.4.1 Seedlings

Young seedlings were taken out from the flasks, thorough washed with water to remove all traces of media and were transplanted to community pots having 1:1 mixture of tree fern fibre and leaf mould (Plate III). The young seedlings were kept in shade and were watered 2-3 times a day to maintain the humidity The seedlings were given 1-2 sprays of Benlate after 15-20 days as prophylactic measure.

3.4.2 Mericlones

Young mericlones were transplanted to the Jiffy pots which were kept moist and under shade.

3.5. STATISTICAL ANALYSIS

The data was analysed to rank the means from the highest to the lowest and identify the appropriate treatments as advocated by Mize and Chun (1988) for tissue culture experiments.

Plate -III A seedling transplanted to the pot.

Plate -III A seedling transplanted to the pot.

EXPERIMENTAL RESULTS

IV. EXPERIMENTAL RESULTS

The results of the experiment conducted on Anthurium andreanum are reported in this chapter.

A preliminary study was conducted on floral biology of Anthurium andreanum. The cross pollinated protogynous flowers took about 120-130 days from pollination to maturity of the berries in different cultivars (Table 9).

4.1 Seed culture studies.

The effect of different methods of extraction and effect of different nutrient media on seed germination and subsequent growth are reported below.

4.1.1. Effect of Method of extraction of seeds.

Methods like hand pulping and extraction,treating the berries with Sodium carbonate or pectinase were used in the present study. Data on percent seed germination and number of days taken for seed germination were recorded(Table 10). Subsequent growth such as days taken for formation of first leaf,second leaf,third leaf and fourth leaf were also recorded (Table 11).

In all respects Hand pulping of the berries was found to be the best method of extraction as compared to treating the berries with one percent pectinase or 13 per cent Sodium carbonate(Plate IV). Percent germination was highest (92.18) when seeds were

Table 9. Floral biology of *Anthurium andreanum*

Plant Number	Total number of days taken for			Number of days taken for pollination to harvest
	Bud opening	Stigmatic receptivity	Pollen anthesis	
175	15	20	27	126
92	17	24	29	113
153	10	12	17	126
91	12	15	19	128
103	11	25	29	112
148	10	16	22	143
41	17	19	14	183
149	8	12	17	146
40	9	12	17	118
127	11	17	21	110
Average	12	17.2	21.2	130.5

Table 10. Effect of method of extraction of seeds on percent germination and days taken for germination

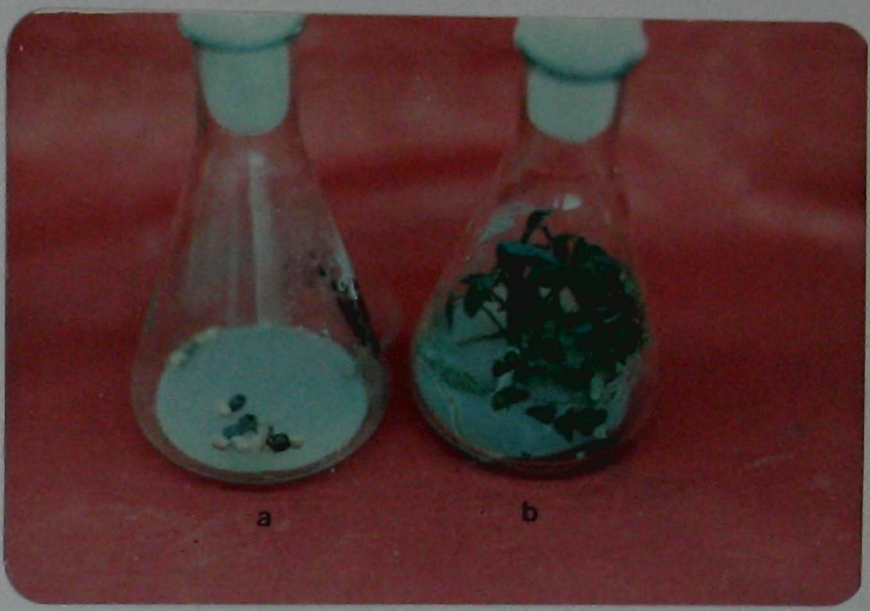
Extraction Method	Percent Germination	Average number of days taken for germination
Hand Pulping	92.18	8.8
Treatment with Pectinase(1%)	65.88	12.8
Treatment with Sodium Carbonate(13%)	49.4	12.8

Table 11. Effect of method of extraction of seeds on number of days taken for first, second, third and fourth leaf stage.

Method	Average number of days taken for			
	First leaf	Second leaf	Third leaf	Fourth leaf
Hand Pulping	20.6	39.4	60.74	90.2
Treatment with Pectinase(1%)	24.6	40.8	69.8	-
Treatment with Sodium Carbonate(13%)	23.6	45.4	-	-

Plate-IV Effect of Sodium Carbonate(a) and hand pulping(b) on seed germination and subsequent growth.

Plate-IV Effect of Sodium Carbonate(a) and hand pulping(b) on seed germination and subsequent growth.


a
b

hand pulped and extracted than by treating with pectinase (65.88) or Sodium carbonate (49.4).

Average number of days taken for germination of seeds was less when the seeds were Hand pulped(8.8 days) than on treating with Pectinase (12.8 days) or Sodium carbonate (12.8 days).

The average number of days taken for first leaf formation was 20.6 days on hand pulping, 24.6 days on treating with pectinase and 23.6 days on treating with Sodium carbonate.

The second leaf formed after 39.4 days on hand pulping,40.8 days on treating with pectinase, and 45.5 days on treating with Sodium carbonate.

The third leaf formation was after 60.74 days on hand pulping and 69.8 days on treating with pectinase. However, seeds treated with Sodium carbonate did not show any growth after 2nd leaf.

The average number of days taken for fourth leaf formation on hand pulping of seeds was 90.2 days while seeds treated with Sodium carbonate and pectinase did not show any subsequent growth and slewly died out.

Fresh and Dry weight of seedlings were recorded after 120 days and Fresh Weight : Dry weight indices were calculated (Table 12), by using the formula -

$$\text{Weight Index} = \frac{\text{Fresh weight}}{\text{Dry weight}} \times 100$$

Table 12. Effect of method of extraction of seeds on Fresh Weight, Dry weight of Seedlings and their index $\left(\text{Index} = \frac{\text{Fresh weight}}{\text{Dry weight}} \times 100\right)$ after 120 days

Method	Fresh weight (mg)	Dry weight (mg)	Index
Hand Pulping	0.2126	0.0241	882.16
Treatment with Pectinase(1%)	0.217	0.02422	1114.78
Treatment with Sodium Carbonate(13%)	0.1332	0.01782	747.475

Fresh weight of seedlings were comparatively equal, when treated with pectinase (0.217 mg) or when hand pulped (0.2126mg), while it was low in the seedlings treated with Sodium Carbonate (0.133 mg).

Dry weight of the seedlings also showed a similar trend. It was almost the same for the seedlings obtained after pectinase treatment (0.0242 mg) and hand pulping (0.0241 mg) while it was less in the seedlings with Sodium carbonate treatment (0.0178 mg).

The fresh weight and dry weight Index was more for the seedlings obtained by treating with pectinase (1114.78) followed by those obtained by hand pulping (882.16) and by treating with Sodium Carbonate (747.475).

4.1.2. Effeft of different media:

The experiments were conducted to find out the effect of different media on seed germination and subsequent growth in the cultivars of Anthurium andreanum.

Four different media were used for the present study. (a) Nitsch medium (1969); (b) Murashige and Skoog medium (1962); (c)Knudson C medium (1951) and (d)Vacin and Went medium (1949)(Plate V).

Data recorded on percent seed germination and number of days taken for seed germination are given in the Table 13.

Plate-V. Effect of different nutrient media on anthurium seed germination.

1. Nitsch
2. MS
3. Vacin and Went
4. Knudson C.

Plate-V. Effect of different nutrient media on anthurium seed germination.

1. Nitsch
2. MS
3. Vacin and Went
4. Knudson C.


1
2
3
4

Table 13. Effect of different nutrient media on percent germination and number of days taken for germination in *Anthurium andreanum*

Media	Percent germination	Average number of days taken for germination
Nitsch	93.33	6.5
Murashige & Skoog	86.3	13.75
Vacin and Went	76.8	8.75
Knudson C.	65.18	13.25

Nitsch medium was found better over the other three media used (Plate VI). Percent seed germination was highest on Nitsch medium (93.33) followed by Murashige and Skoog's(86.3). Vacin and Went (76.8) and Knudson C medium (65.18). Similarly, early seed germination was noticed on Nitsch medium (6.5 days) followed by Vacin and Went (8.75 days), Knudson C (13.25 days) and Murashige and Skoog's medium (13.75 days).

Subsequent growth was recorded in terms of number of days taken for the development of first leaf, second leaf,third leaf and fourth leaf stage (Table 14) (Plate VII).

First leaf formed after 23.25 days on Nitsch medium, 27.75 days on Knudson C medium, 33.25 days on Vacin and Went medium and 38.25 days on Murashige and Skoog's medium on an average.

The development of second leaf took about 40 days on Nitsch's medium, 41 days on Knudson C medium, 51.5 days on Murashige and Skoog's medium and 54.75 days on Vacin and Went medium.

A totally different trend for the 3rd and 4th leaf stage was seen in the media used.

Third leaf development was faster on Murashige and Skoog's medium which took 65.75 days,while on Knudson C it was 68.5 days,on Vacin and Went, 69.5 days and on Nitsch medium 72.5 days.

Plate-VI. Healthy anthurium seedlings on
Nitsch medium.

Table 14. Effect of different nutrient media on number of days taken for first, second, third and fourth leaf stage.

Media	Average number of days taken for			
	First leaf	Second leaf	Third leaf	Fourth leaf
Nitsch	23.25	40	72.5	87.75
Murashige & Skoog	38.25	51.5	65.75	86.75
Kundson C	27.75	41	68.5	89
Vacin & Went	33.28	54.75	69.5	86.5

Plate-VII. Anthurium seedlings raised on Nitsch medium showing different stages of growth.

Plate-VII. Anthurium seedlings raised on Nitsch medium showing different stages of growth.

The average number of days taken for fourth leaf formation was 86.5 days on Vacin and Went medium, 86.75 days on Murashige and Skoog's medium, 87.75 days on Nitsch medium and 89 days on Knudson C medium.

Fresh weight and Dry weight of the seedlings were recorded after 120 days and Fresh weight by dry weight Index was calculated (Table 15).

The highest weight of fresh seedlings was noticed on Nitsch medium (0.227 mg) followed by Murashige and Skoog medium (0.225 mg), Knudson C medium (0.223 mg) and Vacin and Went medium (0.215 mg).

The Dry weight of the seedlings was more on Murashige and Skoog's medium (0.0285 mg) followed by Vacin and Went (0.0266 mg), Nitsch (0.026 mg) and Knudson C medium (0.025 mg).

Fresh weight by dry weight Index was highest on Knudson C medium (880.90) followed by Nitsch (878.823), Vacin and Went (807.360) and Murashige and Skoog's medium (788.091).

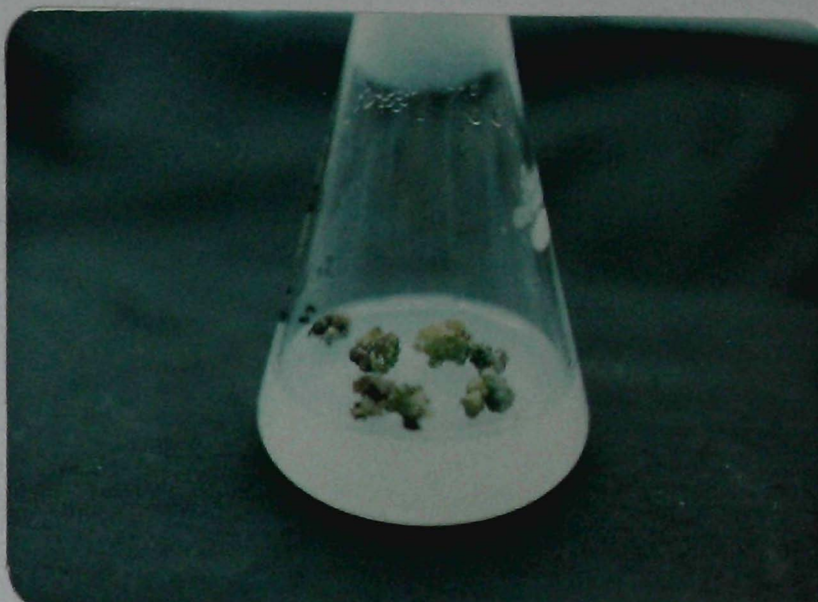
Callusing in seeds: Small percentage of seeds (1.5%) inoculated on to Nitsch medium started callusing in about 4-6 weeks (Plate VIII,IX) and continued to grow till 1-2 sprouts formed. The callus was green to cream or yellow in colour. It took about 7-8 months for the formation of seedlings from callus.

Table 15. Effect of different nutrient media on Fresh weight, Dry weight of seedlings and their Index (Index $= \frac{\text{Fresh weight}}{\text{Dry weight}} \times 100$) after 120 days

Media	Fresh weight (mg)	Dry weight (mg)	Index
Nitsch	0.227	0.02583	878.823
Murashige & Skoog	0.225	0.02855	788.091
Knudson C	0.223	0.025315	880.901
Vacin & Went	0.215	0.02663	807.360

Plate-VIII. Anthurium seeds showing initiation of callus on Nitsch medium.

Plate-IX. Anthurium seeds showing profuse callusing on Nitsch medium.



4.2 Tissue culture studies:

Different explants like leaf section,spadix segments and vegetative buds were used for the present study. Effect of different nutrient media on these explants are reported below:

4.2.1 Leaf sections.

Leaf sections of $1 cm^2$ were inoculated on 5 different nutrient media (Plate X) like - Nitsch, Murashige and Skoog, Vacin and Went, Morels medium and modified Nitsch medium(Geier,1986a). There was no change in the explant when inoculated on 1st four media even after 3 months of incubation in dark.

Cream or green coloured globular calli formed on Nitsch medium (Geier,1986a)supplemented with $1 mg\ l^{-1}$ BA and $0.1 mg\ l^{-1}$ 2,4-D (Table 16) with less of Ammonium Nitrate. Cultures were kept in dark (Plate XI and XII).

Of the 37 different genotypes tested, only two genotypes IIHR No.77 and 83 could callus with very good frequency showing the effective genotypic response of different cultures for callusing (Table 17).

4.2.2. Spadix segments.

The spadix segments were inoculated on 5 different nutrient media like Nitsch,Murashige and Skoog,Vacin and Went, Morel's medium and Modified Murashige and Skoog's medium(Kunisaki,1980).

Plate-X. Leaf section inoculated on Modified Nitsch medium.

Plate- X. Leaf section inoculated on
Modified Nitsch medium.

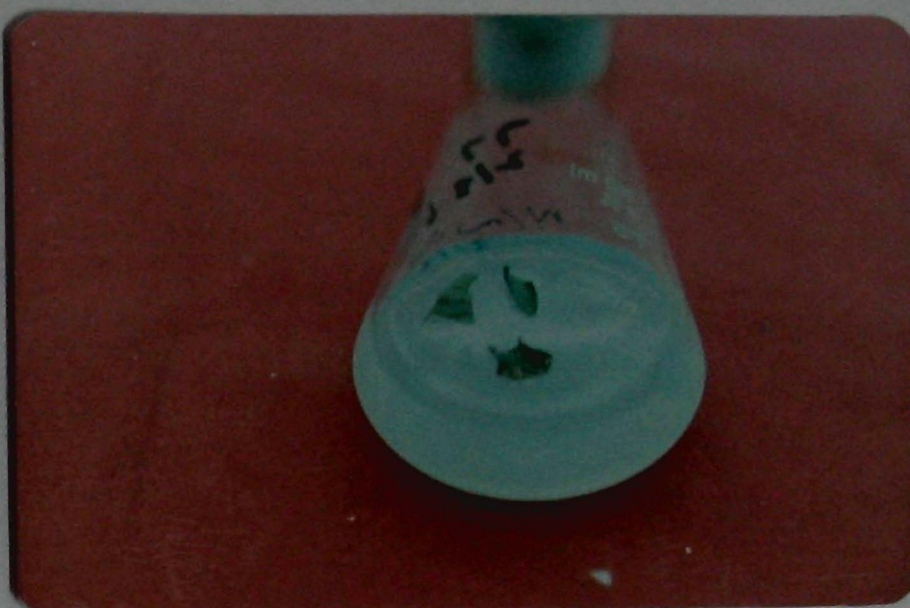


Table 16. Effect of different nutrient media on leaf sections

Medium	Extent of callusing	Frequency	Nature	Colour	Callus differentiation	Plantlet formation	Survival in the field
Nitsch	-	Nil	-	-	-	-	-
Murashige and Skoog	Enlargement of sections			No callusing		-	-
Morel	Enlargement of sections			No callusing		-	-
Vacin and Went	-	-	-	-	-	-	-
Modified Nitsch	+++	69%	Globular	Cream or Green	>10	<15	>8

Degree of callusing

+ = Moderate

++ = Good

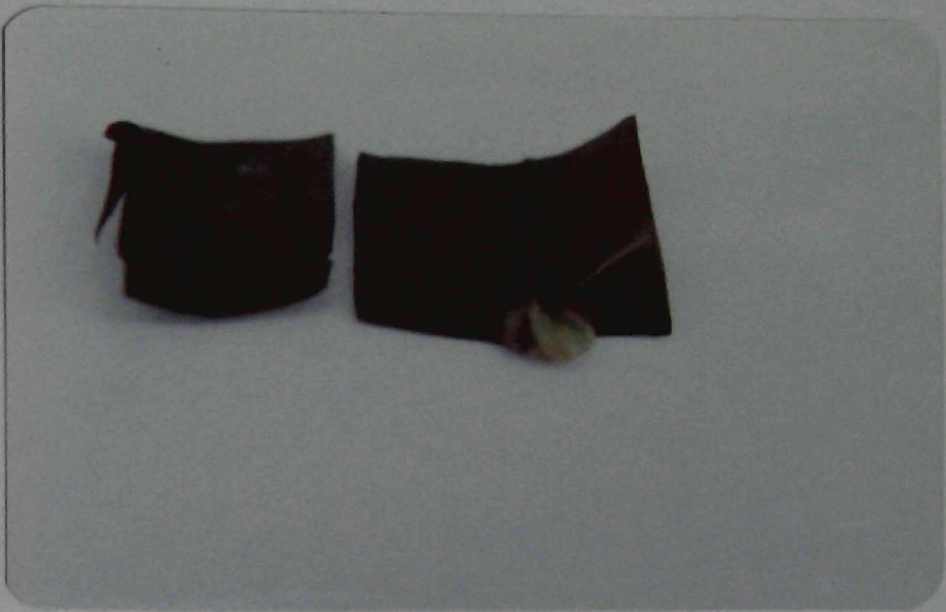
+++ = Excellent

Plate-XI. Leaf sections showing initiation of callus on Modified Nitsch medium.

Plate-XII. Initial stages of differentiation in leaf callus on Modified Nitsch medium.

Plate-XI. Leaf sections showing initiation of callus on Modified Nitsch medium.

Plate-XII. Initial stages of differentiation in leaf callus on Modified Nitsch medium.



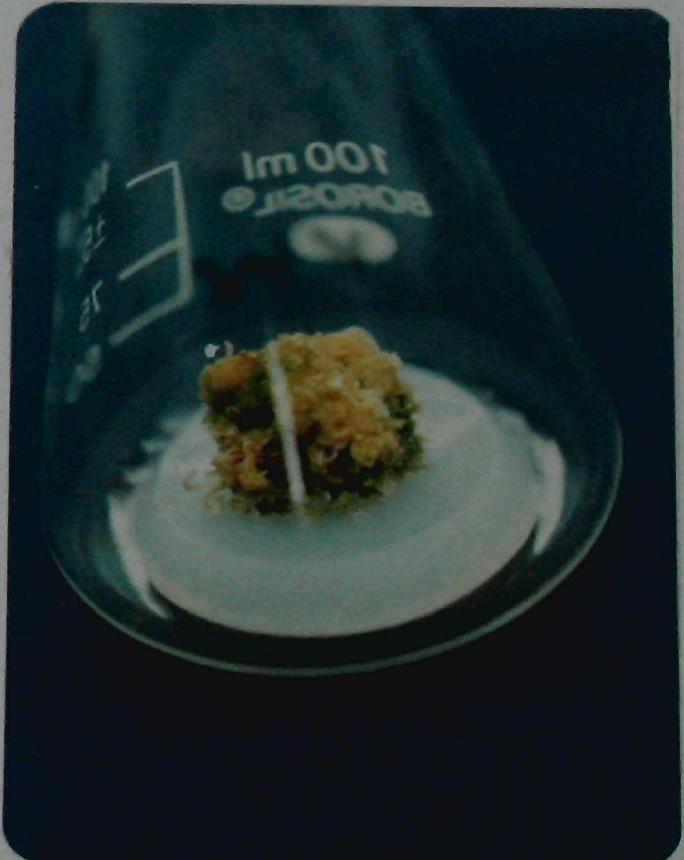

100 ml

Table 17. Response of genotype to callusing/sprouting using leaf section as explant, in Anthurium andreanum

Sl.No.	Cultivar Number	Number of explant	Media	Formation of	
				Callus	Sprout
1	28	4	Nitsch	-	-
2	41	2	"	-	-
3	56	2	"	-	-
4	119	7	"	-	-
5	41	2	"	-	-
6	22	8	"	-	-
7	27	7	"	-	-
8	52	7	"	-	-
9	28	4	Murashige and Skoog	-	-
10	41	2	"	-	-
11	56	2	"	-	-
12	119	7	"	-	-
13	50	7	"	-	-
14	63	3	"	-	-
15	52	3	"	-	-
16	56	5	"	-	-
17	28	4	Morel	-	-
18	41	4	"	-	-
19	119	7	"	-	-
20	22	5	"	-	-
21	50	3	"	-	-
22	27	3	"	-	-

1	2	3	4	5	6
23	52	7	Morel	-	-
24	63	3	"	-	-
25	28	4	Vacin and Went	-	-
26	41	4	"	-	-
27	56	2	"	-	-
28	119	7	"	-	-
29	32	5	"	-	-
30	27	7	"	-	-
31	63	7	"	-	-
32	27	7	"	-	-
33	77	8	Modified Nitsch	+++	+
34	55	5	"	-	-
35	83	8	"	+++	+
36	9	4	"	+	-
37	13	5	"	++	+

\+ = Callus/Sprouts formed

\- = Callus/ Sprouts not formed

Degree of callusing

\+ = Moderate

++ = Good

+++ = Excellent

They showed very high stability on all media. On Nitsch medium, only little expansion at the base of the segments was noticed. However, there was no further growth. On Morel and Vacin and Went medium, the enlargement of the unfertilized ovaries were noticed after 55 days. They formed pseudo berries in 150 days (Table 18). The berries did not have any seeds but contained good amount of pulp.

On Modified Murashige and Skoog's medium (Kunisaki,1980), further supplemented with 2,4-D - 0.1 mgl^{-1} , BA-1 mgl^{-1} and Agar - 8 gl^{-1}, the spadix segments produced callus which was cream in colour (Table 18). It took 20 days for callusing. Similar result was also obtained when the spadix was inoculated on Murashige and Skoog's medium (Murashige and Skoog,1962). Callusing occured under dark conditions (Plate XIII and XIV). Of the 33 different genotypes tested, only 3 genotypes, IIHR No.55, 162, 41 callused with good frequency (Table 19).

4.2.3 Vegetative buds.

Vegetative buds were inoculated on Nitsch, Murashige and Skoog, Morel and Vacin and Went medium and modified Murashige and Skoog's medium (Kunisaki,1980). Both liquid and solid medium were tried. Vegetative buds produced callus on liquid Vacin and Went medium under diffused light conditions.

Table 18. Effect of different nutrient media on Spadix segments

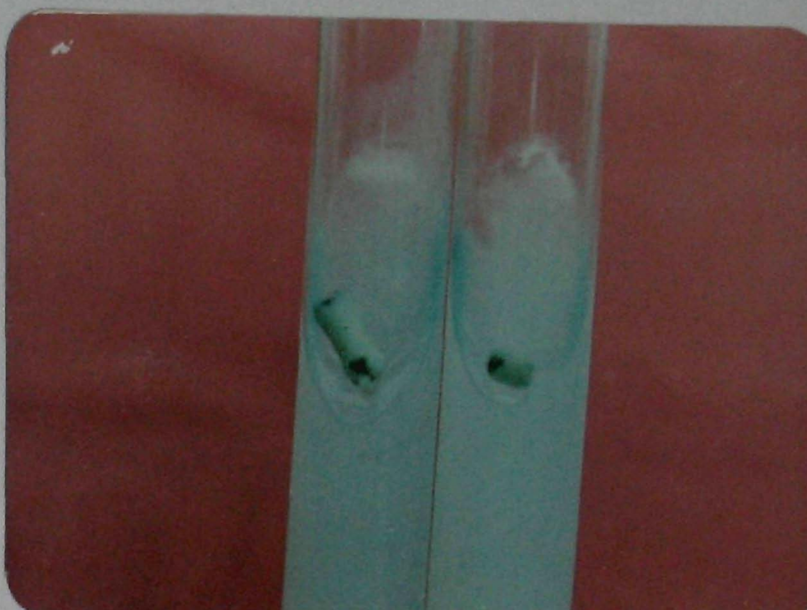
Medium	Extent of callusing	Frequency	Nature	Colour	Callus differen- tiation	Plantlet formation	Survival in the field
Nitsch	Nil	-	--	-	-	-	-
Murashige and Skoog	++	40%	Globular	Cream- is white	>10	>8	>5
Vacin and Went	Formation of pseudo berries				No callus	-	-
Morel	Formation of pseudo berries				No callus	-	-
Modified Murashige and Skoog	++	60%	Globular	Cream white	>15	>10	>8

Degree of callusing

+ = Moderate
++ = Good
+++ = Excellent

Plate-XIII. Spadix segments on Modified MS medium.

Plate-XIV. Callus formation on Spadix segments on Modified MS medium



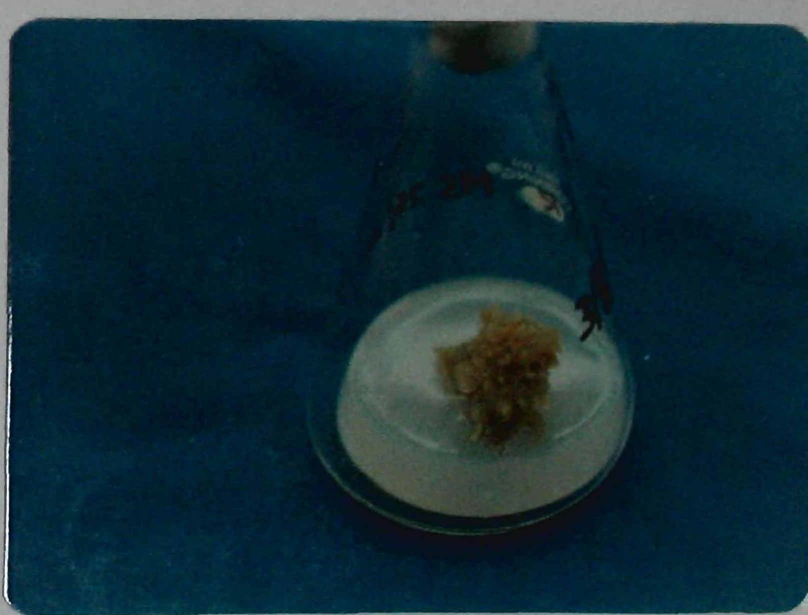


Table 19 . Response of genotype to callusing /sprouting using Spadix segments as explants in *Anthurium andreanum*

Sl.No.	Cultivar Number	Number of explant	Media	Explant forming Callus	Explant forming Sprouts
1	3	2	Nitsch	-	-
2	36	1	"	-	-
3	20	3	"	-	-
4	107	4	"	-	-
5	171	6	"	-	-
6	170	4	"	-	-
7	3	2	Murashige and Skoog	-	-
8	36	7	"	+	+
9	20	3	"	+	-
10	107	4	"	+	+
11	91	6	"	-	-
12	56	2	Murashige and Skoog	-	-
13	3	2	Morel	-	-
14	36	2	"	-	-
15	25	2		-	-
16	52	5		-	-
17	49	4	"	-	-
18	107	5	"	-	-
19	20	3	Vacin and Went	-	-
20	52	5	"	-	-

1	2	3	4	5	6
21	41	6	Vacin and Went	-	-
22	171	7	"	-	-
23	1	4	"	-	-
24	107	4	Modified Murashige and Skoog (Kunisaki, 1980)	+	-
25	23	3	"	-	-
26	220	4	"	-	-
27	100	4	"	-	-
28	55	4	"	++	+
29	123	5	"	-	-
30	40	2	"	-	-
31	57	2	"	-	-
32	162	4	"	++	+
33	41	4	"	++	+

+ = Callus/Sprout formed

- = Callus/Sprout not formed

Degree of callusing

+ = Moderate

++ = Good

+++ = Excellent

On Modified Murashige and Skoog medium(Kunisaki,1980), the buds turned green in about 5 weeks. For another one week there was increase in its size, but there was no further growth (Table 20).

Of the 36 different genotypes tested,IIHR No.52 and 104 showed very good frequency for callusing (Table 21).

4.3. **Efficacy of the explant.**

For the present study, explants like Leaf sections,Spadix segments,Vegetative buds were used. Leaf sections were better than Spadix segments or vegetative buds.The frequency and extent of callusing was high when leaf sections were used as explants.

4.4 **Survival of plantlets in the field.**

Among the plantlets formed from leaf as explant,more than 53 per cent survived in the field,while plantlets formed from Spadix segments more than 80 per cent, and from Vegetative buds more than fifty per cent survived in the field (Plate XV).

Table 20. Effect of different nutrient media on vegetative buds

Medium	Extent of callusing	Frequency	Nature	Colour	Callus differen-tiation	Plantlet formation	Survival in the field
Nitsch	Nil	-	-	-	-	-	-
Murashige and Skoog	Nil	-	-	-	-	-	-
Vacin and Went	+++	40%	Cottony	Brownish cream	<15	<12	>6
Morel	Nil	-	-	-	-	-	-
Modified Murashige and Skoog		Size of the buds increased					

Degree of callusing

+ = Moderate

++ = Good

+++ = Excellent

Table 21. Response of genotype to callusing/sprouting using vegetative buds as explants in *Anthurium andreanum*

Sl.No.	Cultivar number	Number of explant	Media	Formation of Callus	Formation of Sprouts
1	15	2	Nitsch	-	-
2	98	2	"	-	-
3	40	1	"	-	-
4	5	1	"	-	-
5	15	3	"	-	-
6	107	2	"	-	-
7	104	3		-	-
8	52	2	"	-	-
9	51	1	Murashige and Skoog	-	-
10	65	2	"	-	-
11	22	2	"	-	-
12		1	'	-	-
13	105	4	"	-	-
14	95	2	"	-	-
15	73	2	"	-	-
16	110	1	"	-	-
17	40	2	"	-	-
18	52	5	Vacin and Went	+++	+
19	34	3	"	-	-
20	104	4	"	+++	+

1	2	3	4	5	6
21	73	4	Vacin and Went	-	-
22	103	2	"	-	-
23	112	2	"	-	-
24	15	2	"	-	-
25	105	3		-	-
26	178	3	Modified Murashige and Skoog (Kunisaki, 1980)	+	-
27	105	2	"	+	-
28	34	2	"	-	-
29	65	1	"	-	-
30	2	4	"	-	-
31	51	3	"	-	-
32	47	3	"	-	-
33	55	4	"	+	-

+ = Callus/Sprout formed

- = Callus/Sprout not formed

Degree of callusing

+ = Moderate

++ = Good

+++ = Excellent

Plate-XV. Tissue raised plants transferred to Jiffy pellets showing good shoot and root growth.

DISCUSSION

V. DISCUSSION

The present studies were conducted to standardise nutrient media for the *in vitro* germination of anthurium seeds and also to study the efficacy of different explants and nutrient media for the tissue culture propagation of different cultivars, the results obtained therein are discussed in the present chapter.

5.1 Anthurium seeds:

Anthurium is a cross pollinated crop, the berries which take 4-5 months to mature are usually picked up before they are fully mature for *in vitro* sowing. Each berry contains 2-3 seeds which are surrounded by pulp. The anthurium seeds lose their viability very fast.

As compared to majority of the angiosperms seeds which contain a bipolar embryo at maturity, the embryo in anthurium remains small, reduced and lacks proper differentiation at the time of dispersal(Natesh and Rao,1984). Intraseminal growth and after ripening are reported in different species (Raghavan,1980). The improper development of embryo makes them heterotropic and make them dependent on the metabolites present in surrounding cells or endosperm. This indicates that the nutrition requirement of anthurium seeds is very complex and highly specific in nature. This may be the main reason for the low germination of anthurium seeds *in vivo*.

In vitro germination of anhturium seeds on different nutrient media gives an opportunity to study the nutrition requirement of developing embryo at different stages of development especially the heterotropic phase and autotrophic phase which are essential features of anthurium seed development(Raghavan,1980). This will also help to select the right medium to overcome intraseminal growth,dormancy and enhance seed germination as compared to *in vitro* germination.

5.1.1 **Method of extraction of seeds:**

A perusal of earlier literature reveals(Maurer and Brandes 1979 a) that different methods have been used by earlier workers for the extraction of *Anthurium scherzerianum* seeds like hand pulping,treating the berries with sodium carbonate or Pectinase. Experiments were conducted to study the effect of these methods on germination,percentage of germination and subsequent development of *Anthurium andreanum* seeds. It was observed that hand pulping and thorough washing of seeds in running water was the best method.It gave high and early germination and subsequent growth(Fig.1).This may be due to washing off of some enzyme or inhibitors present in the seeds. On the other hand treatment with Sodium Carbonate and Pectinase delayed the germination and showed inhibitory effect not only in seed germination but also in subsequent growth(Fig.2a, 2b and 3).

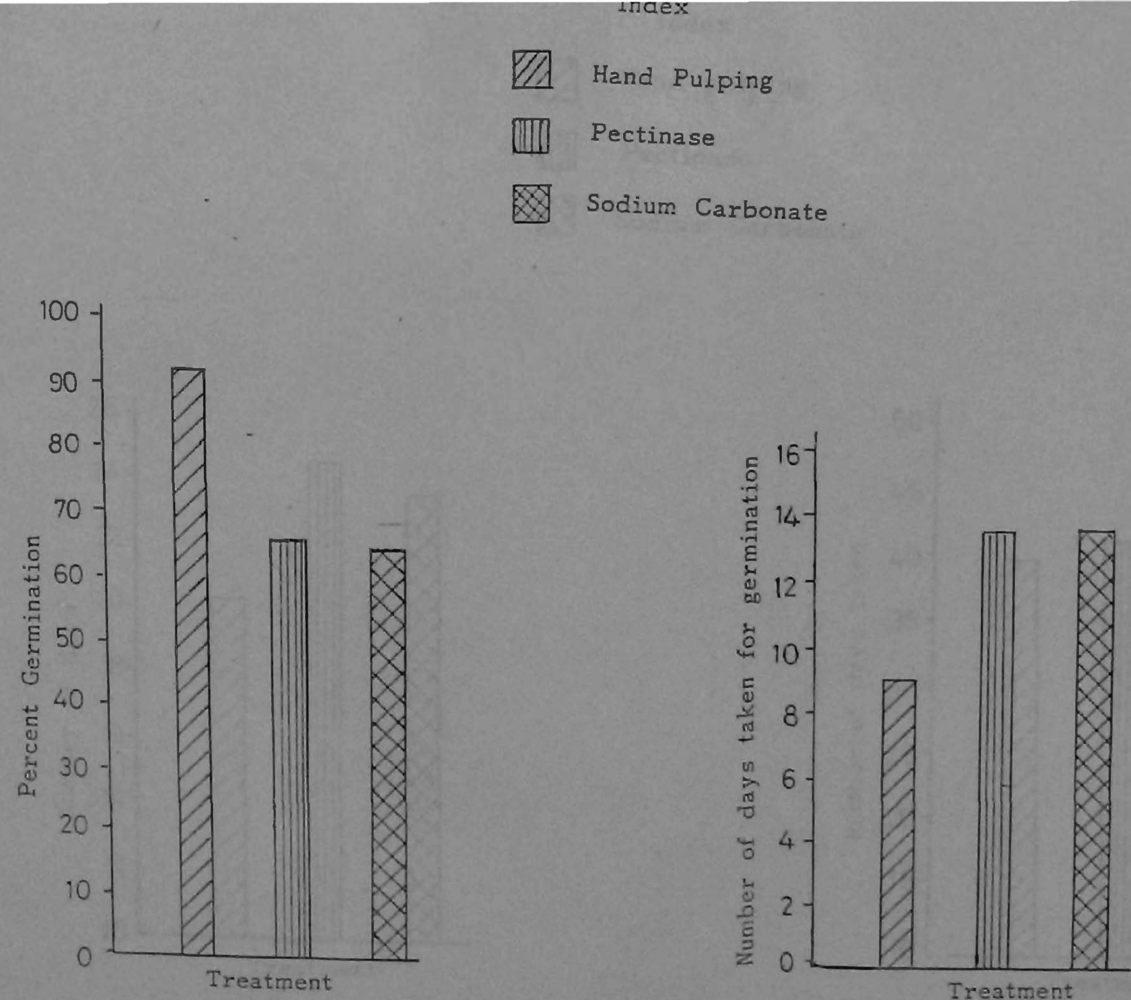
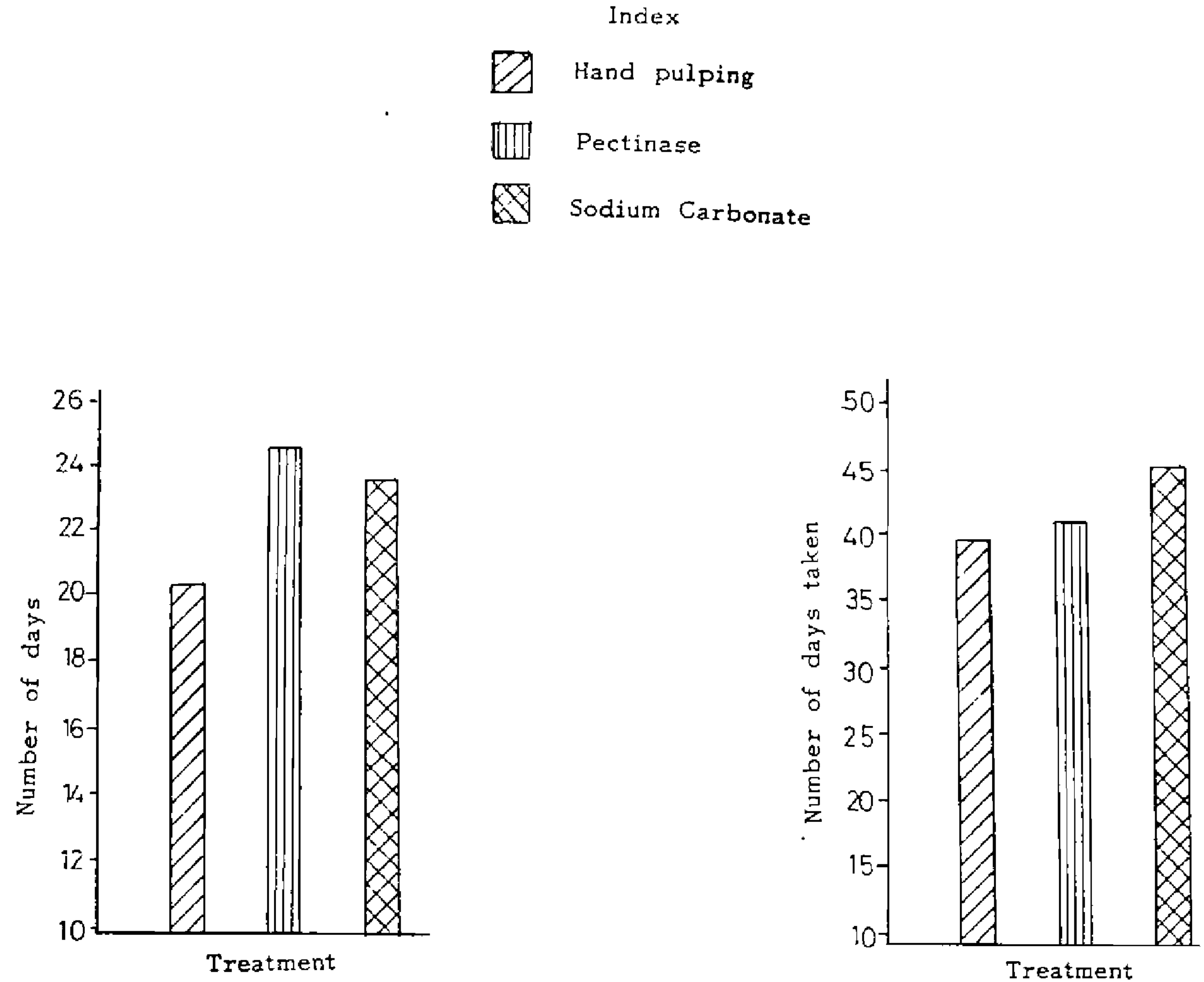


Fig.1. EFFECT OF METHOD OF EXTRACTION ON <u>Anthurium</u> SEED GERMINATION.



First Leaf Stage

Second Leaf Stage

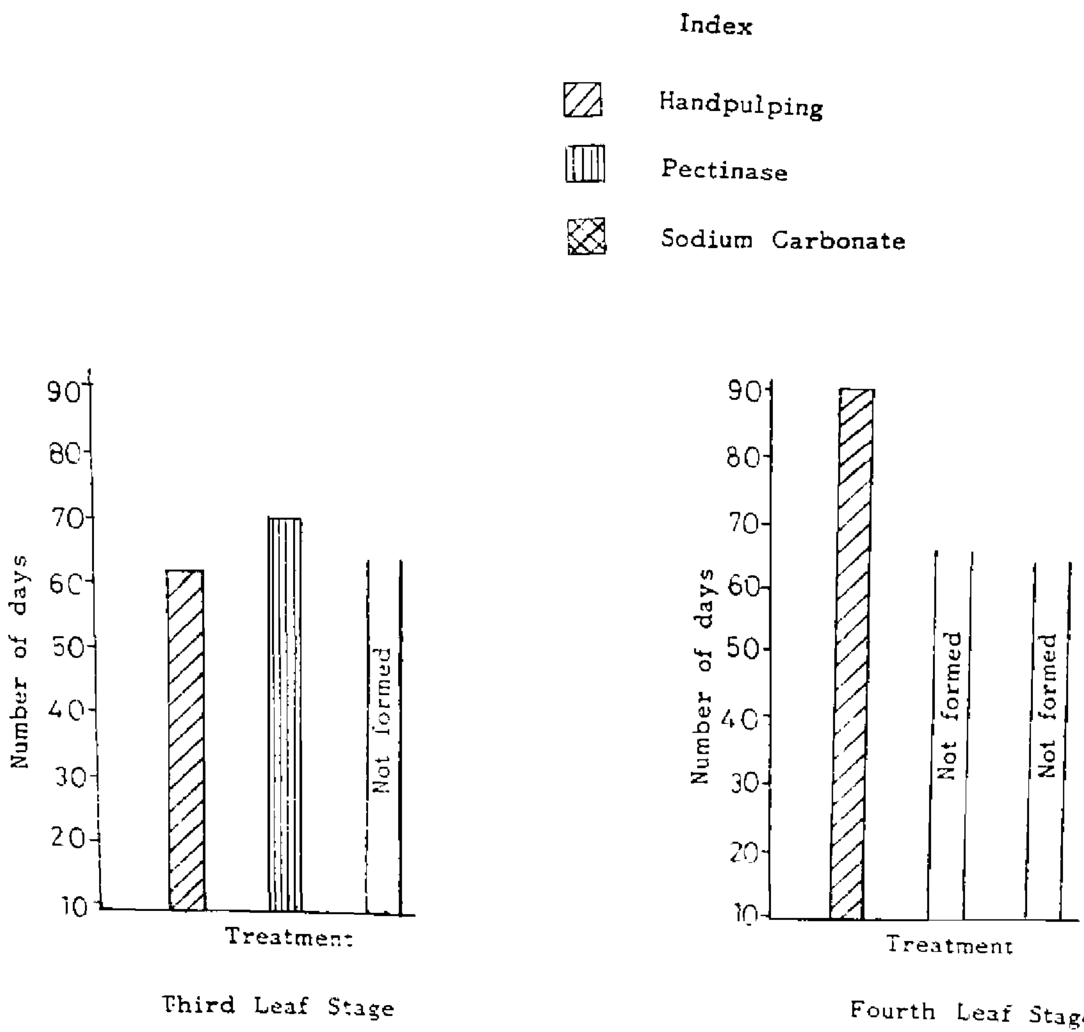
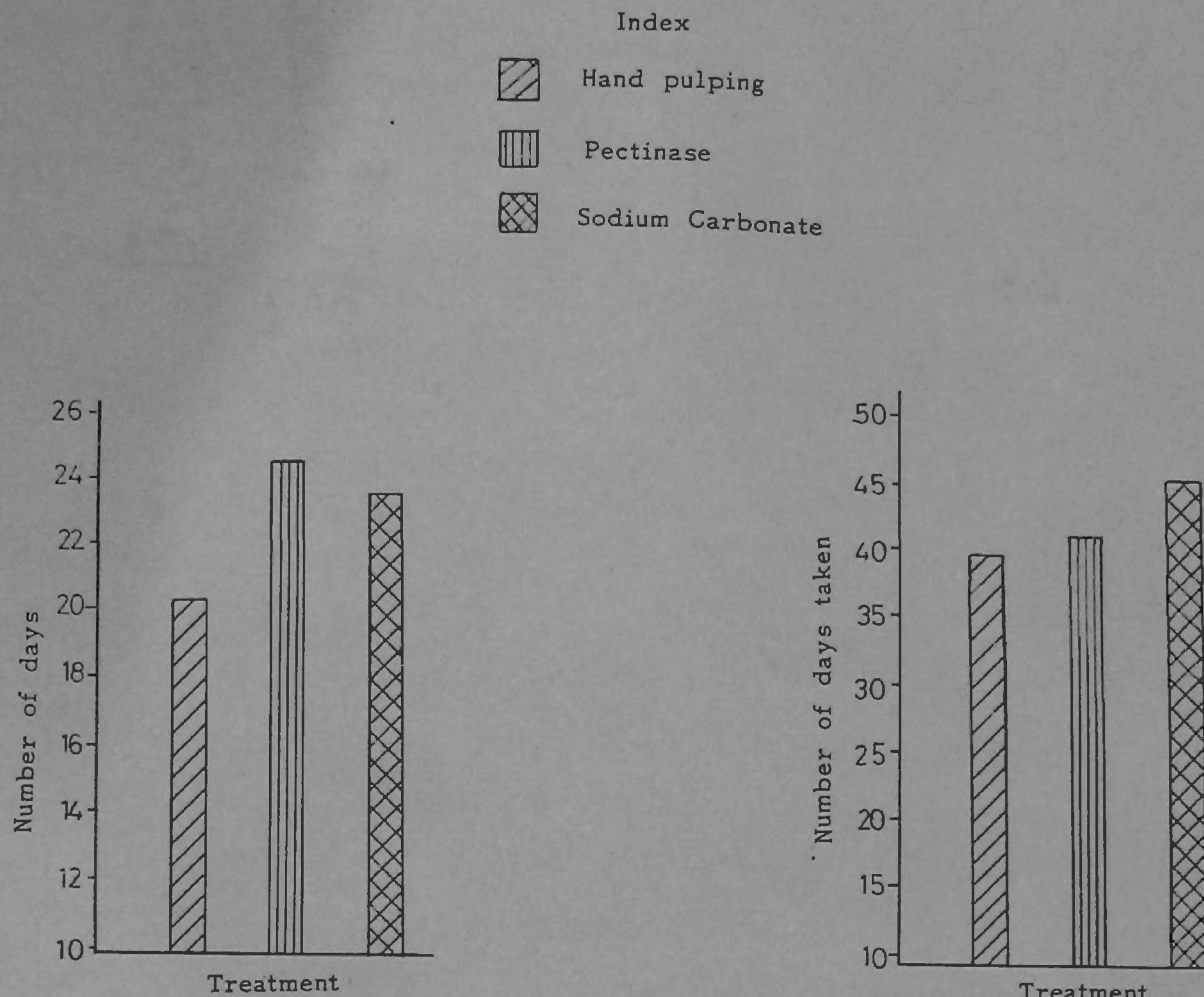


Fig.2b. EFFECT OF METHOD OF EXTRACTION ON *Anthurium* SEEDLING DEVELOPMENT.



First Leaf Stage

Second Leaf Stage

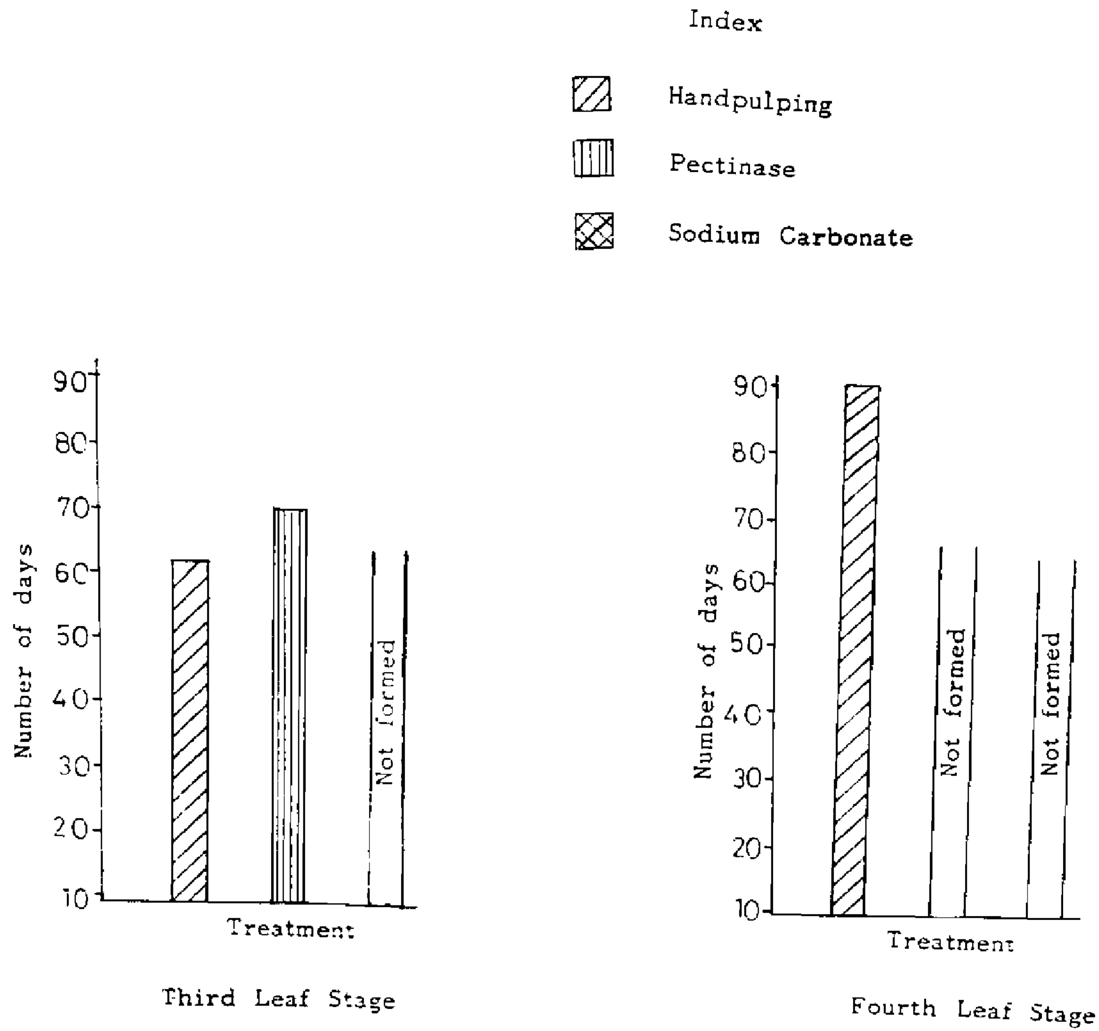
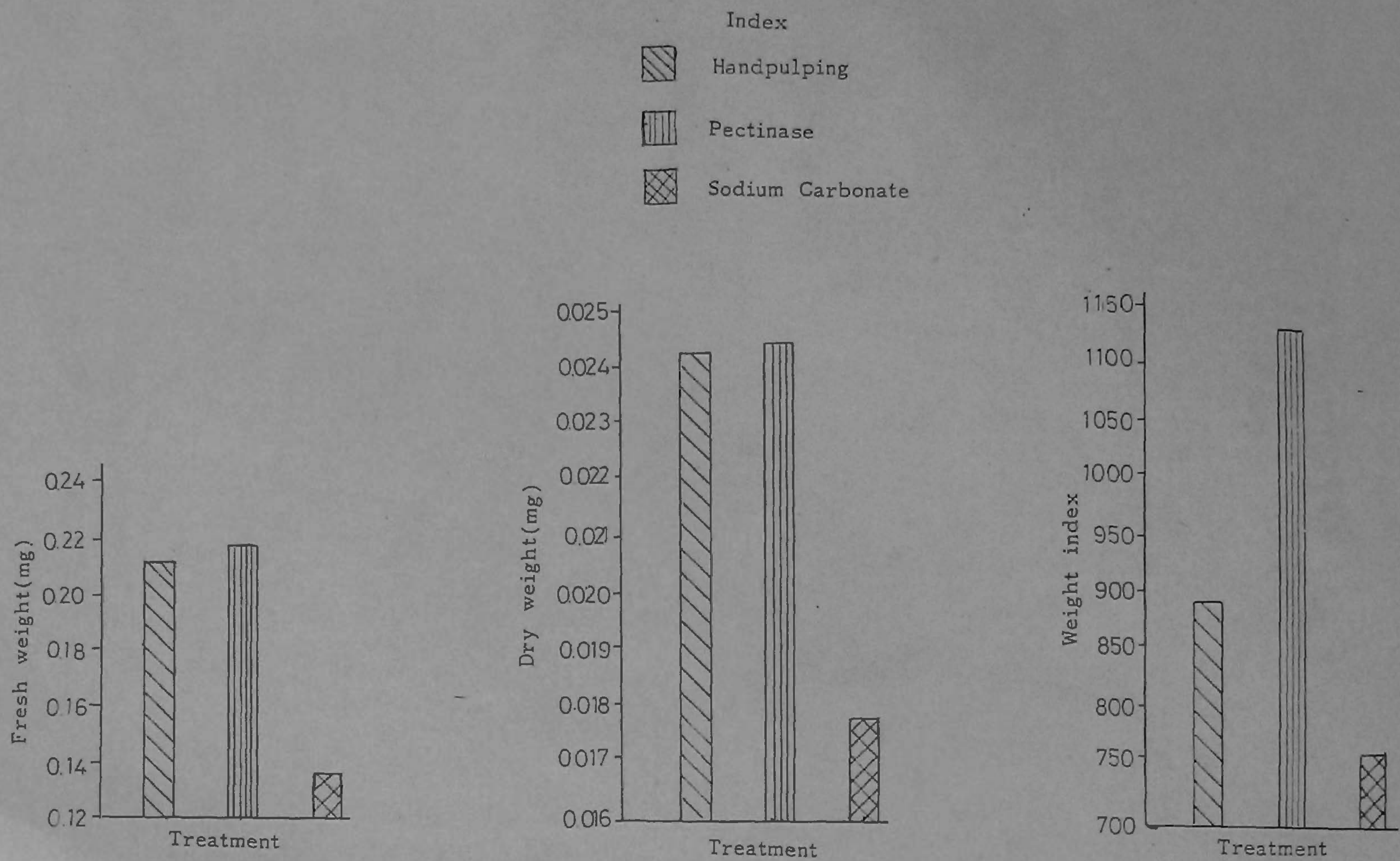


Fig.2b. EFFECT OF METHOD OF EXTRACTION ON *Anthurium* SEEDLING DEVELOPMENT.



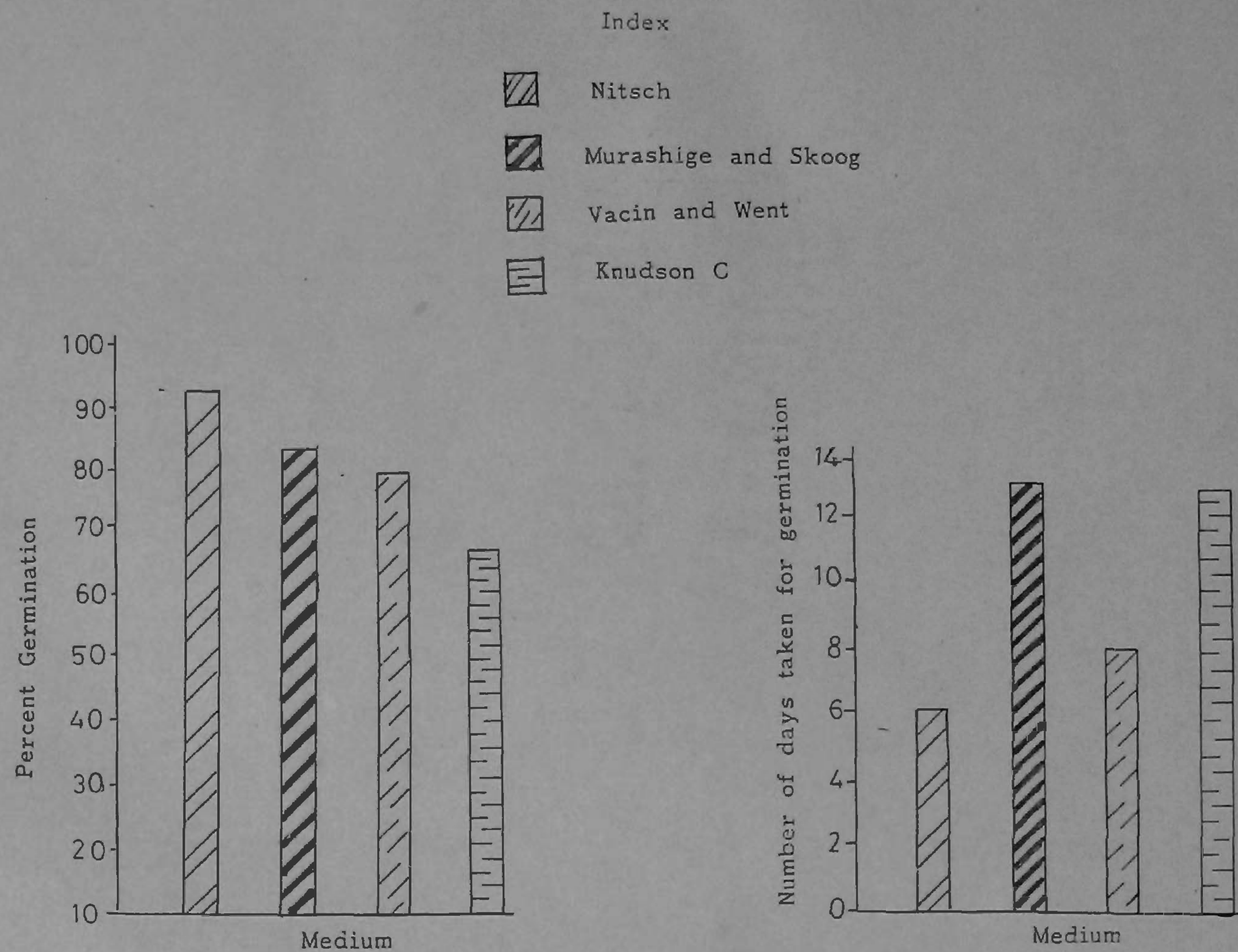
Index
Handpulping
Pectinase
Sodium Carbonate
Fresh weight(mg)
0.24
0.22
0.20
0.18
0.16
0.14
0.12
Treatment
Dry weight(mg)
0.025
0.024
0.023
0.022
0.021
0.020
0.019
0.018
0.017
0.016
Treatment
Weight index
1150
1100
1050
1000
950
900
850
800
750
700
Treatment

5.1.2 *Effect of different media.*

Four different nutrient media were used in the present study. These are Nitsch medium (1969), Vacin and Went (1949), Murashige and Skoog(1962) and Knudson C medium (1951). It was observed that the best seed germination was on Nitsch medium followed by MS medium.

Nitsch medium supported early germination and growth (Fig.4). The seeds germinated within 6.5 days with a high percentage of germination (93.33). However, the subsequent growth of the seedlings was not very good.After 2nd leaf stage the best growth was seen on MS medium(Fig.5a and 5b).

A perusal of earlier literature reveals that not much work has been done on in vitro germination of Anthurium seeds. Pierik et al. (1974) cultured some excised embryos on Murashige and Skoog's medium to induce callus. Though they reported that small percentage of embryo produced callus, they did not report anything on seedling production. Swaminathan(1986) who worked on in vitro seed germination of Anthurium reported that Nitsch medium is the best medium for seed germination and subsequent growth. The present studies also reveal that Nitsch medium is most ideal medium for seed germination. This may be due to the presence of lower concentration of macro and micro elements and organic constituents as compared to MS medium which contain higher



Index
Nitsch
Murashige and Skoog
Vacin and Went
Knudson C
Percent Germination
100
90
80
70
60
50
40
30
20
10
Medium
Number of days taken for germination
14
12
10
8
6
4
2
0
Medium

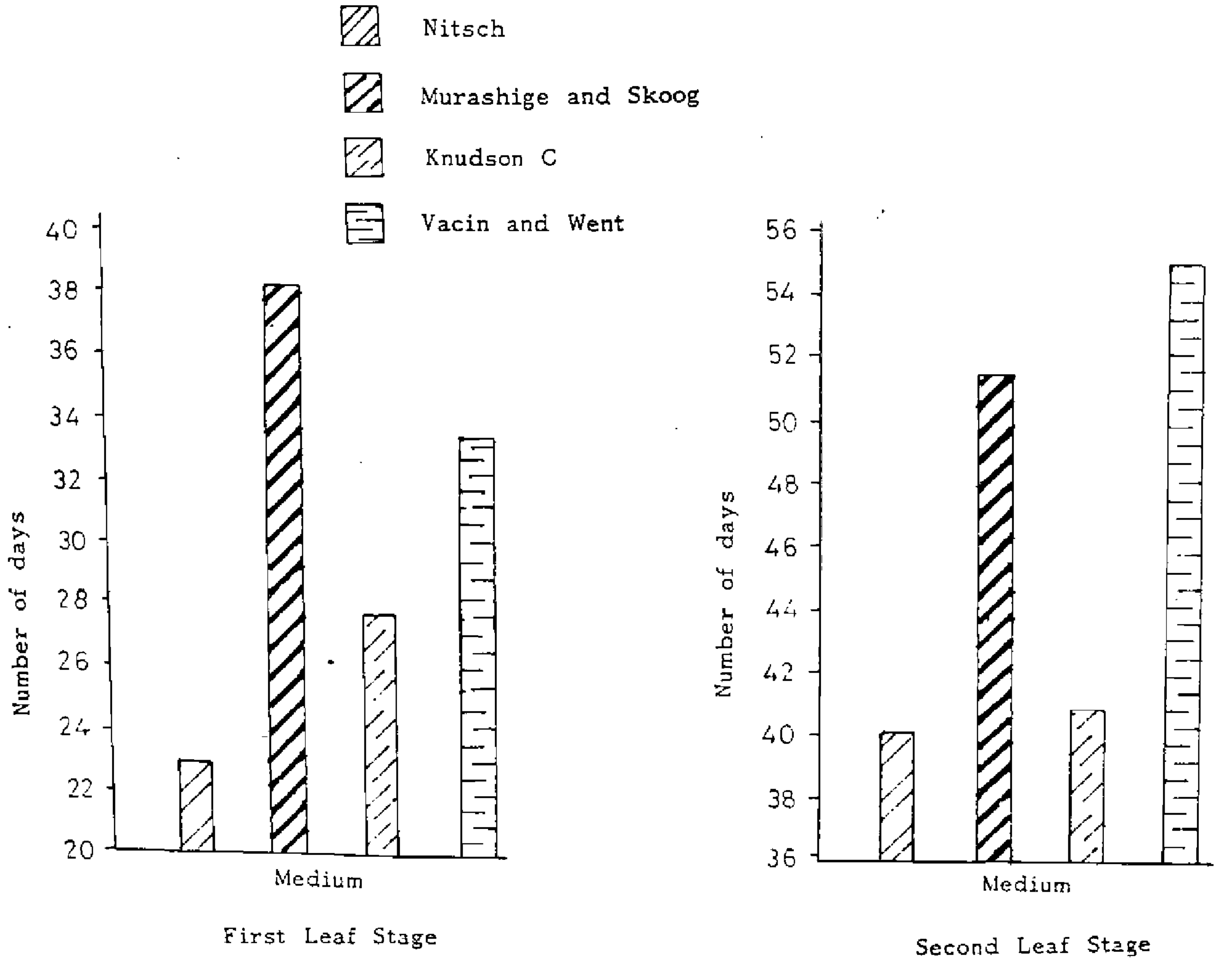
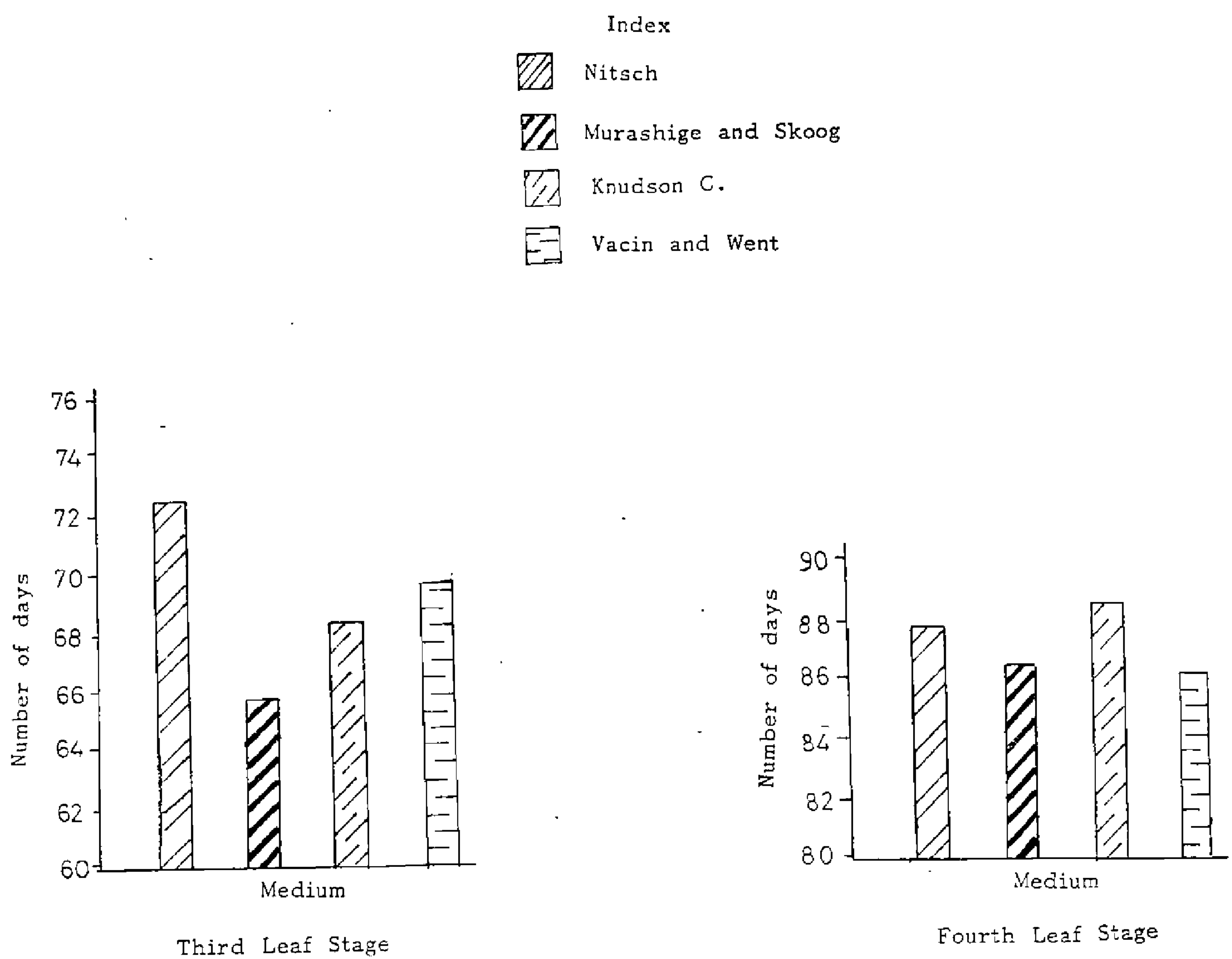


Fig.5a. EFFECT OF NUTRIENT MEDIUM ON <u>Anthurium</u> SEEDLING DEVELOPMENT.


Index
Nitsch
Murashige and Skoog
Knudson C.
Vacin and Went
Number of days
76
74
72
70
68
66
64
62
60
Medium
Third Leaf Stage
Number of days
90
88
86
84
82
80
Medium
Fourth Leaf Stage

concentrations of salts like Ammonium nitrate, Potassium nitrate, Calcium Chloride, Potassium dihydrogen orthophosphate etc. These salts inhibit the seed germination and development upto 2nd leaf stage, however, they are helpful in subsequent growth and development. It also indicates that higher concentration of Calcium and Potassium was inhibitory for anthurium seed germination. The present result also indicated that ammonium nitrate was the best source of nitrogen for the germination and subsequent growth of anthurium seedlings. The fresh weight of the seedlings after 120 days was highest on Nitsch medium, while the dry weight of the seedlings was highest on Murashige and Skoog's medium (Fig.6). These results, thus, indicate that Nitsch and Murashige and Skoog's medium support the plant growth at different stages.

5.2 Tissue culture studies.

Tissue culture propagation is based on the concept of totipotency, a characteristic of all plant cells, however its expression is confined to particular group of cells e.g. meristematic cells. The explant need not contain meristematic cell always, provided its other cells can generate meristematic cells when suitably stimulated. Some of the important factors responsible for tissue culture propagation are:

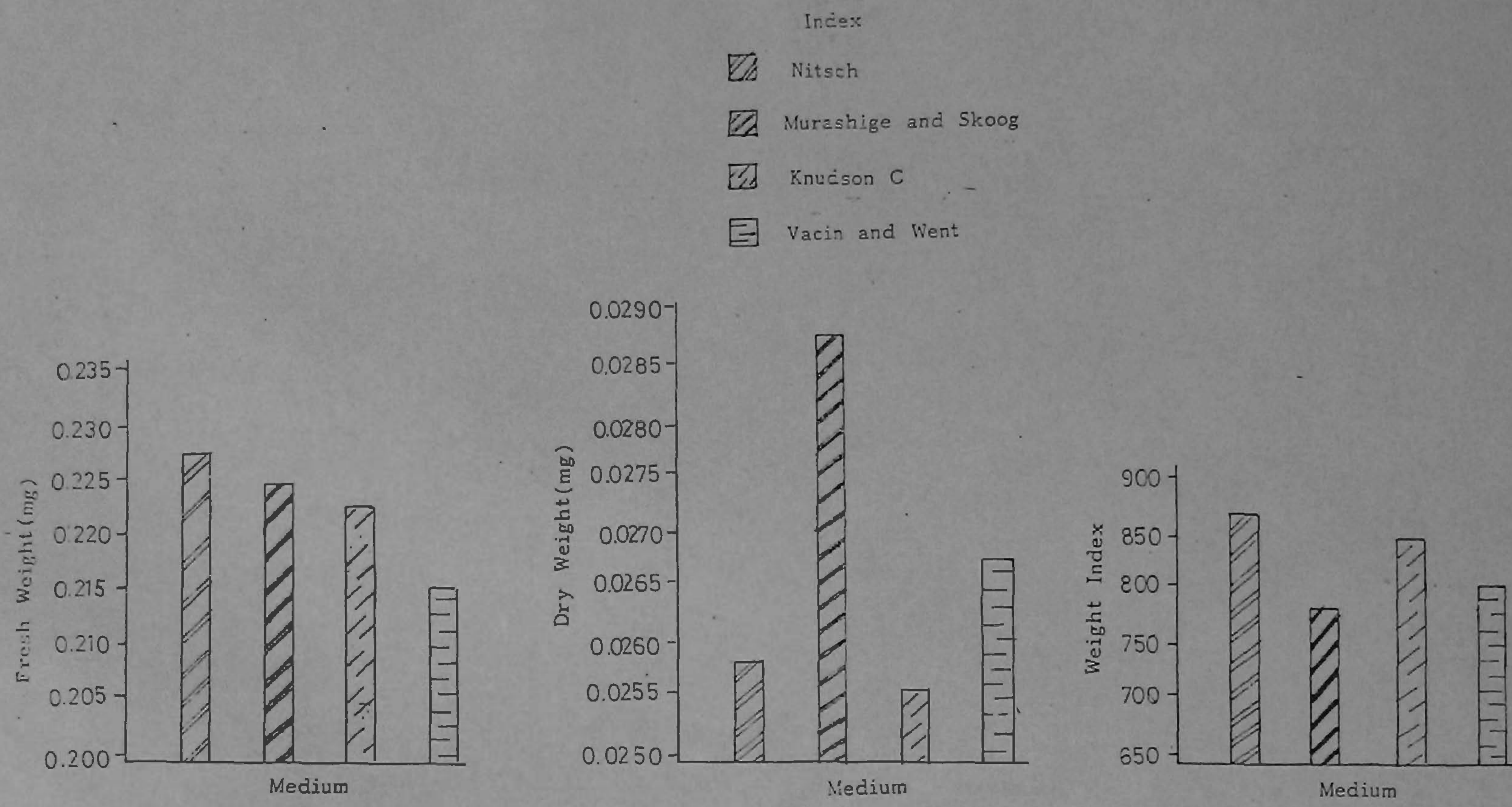


Fig.6. EFFECT OF NUTRIENT MEDIUM ON FRESH WEIGHT, DRY WEIGHT AND WEIGHT INDEX OF SEEDLINGS.

1. Explant: its source,age,preculture treatment,
2. Nutrient media: Chemical composition including growth regulators etc.
3. Culture environment: Light,temperature etc. (Murashige,1977).

Anthurium is known to respond to tissue culture techniques. The flower production in the mericlones has been reported to be higher than those from seedlings(Leeuwen,1980). The technique has been used extensively in mericloning of *A. andreanum* (Pierik *et al.* 1974) and *A. scherzerianum* (Pierik and Steegmans,1975, Geier,1986 b).

5.2.1. Explant:

A perusal of earlier literature reveals that different explants have been used by earlier workers to get callus. The explants are leaf sections,spadix segments,vegetative buds,nodal sections, inflorescence stalk etc.(Eapen and Rao,1985). Geier (1982) found spadix segments to have much higher capacity for regeneration as compared to segments of leaf petiole,spathe etc.

5.2.2. Leaf sections as Explant:

As in other monocotyledonous plants the potential for dedifferentiation is restricted to immature tissues,substantial number of uncontaminated explants consisting of immature tissues are most easily obtained from young leaves,etc. Pierik et al.(1975) suggested a method for the commercial propagation of anthurium. After callus is formed on the leaf sections in liquid MS medium, they are transferred to solid medium. Geier (1986a) successfully used leaf explant for the propagation of A. scherzerianum. Eapen and Rao (1985) reported the efficacy of leaf explant in in vitro propagation of Anthurium patulum. They observed that highest callus formation was seen on the leaf sections cultured with mid vein. Geier (1986a) however did not see any difference in reponse with leaf sections with or without mid rib, except that segments from proximal position produced significantly less shoots, the reason being the improper contact of the cut edge with the medium.

The present studies have shown that frequency of callusing was much higher in leaf sections with mid rib(Table 22).It was also observed that callusing on leaf section was much better when placed with their abaxial surface onto the culture medium. This is in accordance with the results obtained earlier by Geier (1986a).

Table 22. Response of explants for callusing in _Anthurium andreanum_

Explant source	Extent of callusing	Frequency of callus	Plantlet formation
a. Leaf with Mid vein	+++	69%	+
b. Leaf without Mid vein	++	30%	+
c. Spathe	-	-	-
d. Petiole	-	-	-
e. Spadix	++	60%	+
f. Vegetative buds	+++	40%	+

Degree of callusing

+ = Moderate

++ = Good

+++ = Excellent

Older and decoloured leaves were not suitable as explants, only fresh, just unfurled leaves gave the best callus.

One of the major factors influencing leaf as explant seen in the present experiments was the genotype which strongly determines callusing and regeneration ability. Out of 37 genotypes tried, only 2 give good response.

The other factor which influenced the callus formation on leaf section was the extent of illumination. For callus induction and multiplication, total darkness was very essential (Table 23). Illumination was found to enhance shoot and root development at the expense of callus growth.

Concentration of the nurtrients in the medium also affected callusing. Callus formation was observed in the leaf sections when the concentration of Ammonium Nitrate in the Nitsch medium was reduced to 200 mg l^{-1} (Table 24) along with 2,4-D and BA (0.1 mg l^{-1} and 1mg l^{-1} respectively). Geier (1986a) also reported that callusing can be induced from leaf sections on modified Nitsch medium with a low level of Ammonium Nitrate (200 mg l^{-1}) and BA (1mg l^{-1}) and 2,4-D (0.1 mg l^{-1}), while shoots can be multiplied on a medium containing higher level of Ammonium Nitrate (720 mg/lt) and BA (0.5 mg/lt). Root formation occured on the medium with no growth regulators. Callus and shoot generation is effected by NH_4^+ ions and not by NO_3^- ions (Pierik et al., 1979).

Table 23. Effect of nature of light on callus formation and sprout regeneration in *Anthurium andreanum*

Explant	Dark		Light(2000 lux for 16h)	
	Callus	Sprout	Callus	Sprout
Leaf Sections	+	-	-	+
Spadix Segments	+	-	-	+
Vegetative buds	Callused under diffused light			

\+ = Callus/sprout formed

\- = Callus/sprout not formed

5.2.3. Spadix segments as explant.

Spadix segments showed very high stability and did not show any response even after 3 months on Nitsch and Murashige and Skoog's medium. However, on Morel and Vacin and Went medium the ovaries grew to form pseudoberries after 5 months. Geier (1986b) reported that on Modified Nitsch medium Spadix segments of A. scherzerianum showed very high in vitro stability and there was only enlargement of floral organs. Callus and shoot formation rarely occured in the primary cultures. Repeated dissection and transfer of spadice tissues onto fresh medium induced and promoted callusing. However, in the present study, even after repeated dissections, the tissues showed very high stability on Modified Nitsch medium.

Callusing was observed in Spadix segments cultured on Modified Murashige and Skoog medium(Kunisaki,1980) further supplemented with 1mg l^{-1} of BA and 0.1 mg l^{-1} of 2,4-D. Callusing was promoted when the cultures were kept in dark at 25°C (Table 23) on solid medium. The concentration of Ammonium Nitrate played an important role in the callusing of spadix segments. Best results were obtained when concentration of $(NH_4)NO_3$ was 1650 mg l^{-1} while at low levels (200 mg l^{-1}) there was no callus formation (Table 24).

Table 24. Effect of Ammonium Nitrate concentration on callus formation and sprout regeneration in *Anthurium andreanum*

Medium	Concentration of NH_4NO_3	Explant	Formation of callus	Formation of sprout
Modified Nitsch (Geier, 1986 a)	720 mg l^{-1}	Leaf	-	+
Modified Nitsch (Geier, 1986 a)	200 mg l^{-1}	Leaf	+	-
Nitsch (1969)	720 mg l^{-1}	Spadix	-	-
Modified Murashige andSkoog (Kunisaki, 1980)	1650 mg l^{-1}	Spadix	+	+

+ = Callus/Sprout formed

- = Callus/Sprout not formed

5.2.4. Vegetative buds as explant.

A perusal of earlier literature reveals that vegetative buds and shoot apices are not commonly used for tissue culture propagation(Pierik and Steegmans 1975) due to higher level of contamination in cultures. Kunisaki (1980) got high yield of viable A. andreanum cultures using small explants of vegetative buds disinfected with 0.2% sodium hypochlorite. Formation of callus and adventitious shoots by shoot tip explants,was achieved by reducing the concentration of Ammonium nitrate on MS medium.

Present studies have shown that vegetative buds and shoot apices are not very suitable for callus culture and regeneration (Table 22).

Vacin and Went was found to be a more suitable medium for callus induction in vegetative buds than Nitsch, Murashige and Skoog or Morel medium. On Modified Murashige and Skoog medium(Kunisaki,1980),there was only an increase in the size of the vegetative buds.

Diffused light conditions promoted callusing than when cultures were kept in light.Liquid medium was better,as on solid medium the vegetative buds did not show any response(Table 23).

SUMMARY

VI. SUMMARY

The experiments on Anthurium andreanum Lind.were conducted at Indian Institute of Horticultural Research,Hessaraghatta,Bangalore. The plants were maintained in the humidity controlled glass house attached to the Laboratory.

At present,anthurium seeds are grown in open and are chief source of planting material. The germination of seeds in vivo is very low as the seeds lose their viability very fast. Similarly standard methods are not available for the tissue culture propagation of different cultivars.

The present studies were undertaken to standardize the nutrient media for aseptic germination of seeds so as to get higher germination and good subsequent growth.

Experiments were also conducted to standardise micropropagation of selected anthurium cultivars. Different explants like leaf sections,spadix segments and vegetative buds were used on different nutrient media to get callus and plantlets.

A preliminary study showed that the time taken from pollination to harvest of the berries is about 120-130 days in different cultivars. The seeds from the berries were extracted by hand pulping or by treating with pectinase (1%) or with Sodium Carbonate(13) Observations indicated that hand pulping was the best method of seed extraction as it gave good germination and subsequent growth of the seedlings.

The seeds were sown on four different media eg. Nitsch, Murashige and Skoog, Vacin and Went and Knudson C medium. Observations indicated that Anthurium andreanum is very specific in its nutritional requirements at different stages of its growth. Best and early seed germination was noticed on Nitsch medium. Further growth upto 2nd leaf stage was also good on the same medium. However, it was observed that for subsequent stages of development(3rd and 4th leaf), Murashige and Skoog's medium was more suitable. Thus for practical purposes, the seeds can be germinated and grown upto 2nd leaf stage on Nitsch medium and on MS medium for subsequent growth.

Tissue culture studies were conducted using three different explants such as leaf sections, spadix segments and vegetative buds.

Leaf Sections:

$1Cm^2$ sections along with mid rib,taken from young leaves which had just unfolded gave the best result in the form of callusing.The best nutrient medium which supported the callusing was found to be modified Nitsch medium.It was also observed that callusing was induced only when the explant was kept in dark at 25 ± 2^oC.

It was observed that concentration of Ammonium Nitrate played a very vital role in the callusing of leaf sections, lower concentration(200 mg l^{-1}) being the most ideal.

Spadix segments:

0.8 - 1.0 cm long Spadix segments gave best result in the form of callusing on Murashige and Skoog's medium and also Modified Murashige and Skoog's medium. In the initial stages,they showed a very high stability,however,callusing was induced by subculturing them 2-3 times. Further the callusing was induced only when the explants were kept in dark at 25 ± 2^oC.

Vegetative buds:

Vegetative buds callused well on Vacin and Went medium. Callusing was induced when they were cultured in diffused light conditions at 25 ± 2^oC.

It was also observed that anthurium genotypes show varied response in the form of callusing and plantlet formation in different explants. Out of 37 genotypes tried only 2 gave good response.

The present studies have shown that seeds of Anthurium andreanum Lind.can be successfully germinated in vitro on Nitsch medium with a very high percentage of germination and good subsequent growth.

Similarly, it was found that leaf sections are the best explant for micropropagation of anthurium cultivars.

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APPENDIX

APPENDIX

BA	: Benzylaminopurine
2,4-D	: 2,4-Dichlorophenoxyacetic acid
GA_3	: Gibberellic acid
IAA	: Indole-3-acetic acid
IBA	: Indole-3-butyric acid
NAA	: α-Naphthalene acetic acid
2-ip	: 6-(3-Methyl-2 butenylamino)purine
PBA	: (6-(Benzylamino)-9-(2-tetrahydro-pyranyl) 9H-purine)
Na FeEDTA	: Sodium Salt of Ferric Ethylenediamine tetra-acetic acid
MS medium	: Murashige and Skoog's medium
VW medium	: Vacin and Went medium