COST - EFFECTIVE METHODS AND DEVICES FOR HOME SCALE ADOPTION OF PLANT TISSUE CULTURE

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DECLARATION

I hereby declare that this thesis entitled Cost-effective methods and devices for home scale adoption of plant tissue culture is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship or other similar title, of any other University or Society.

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LIST OF ABBREVIATIONS

Auxins:

IAA	Indole acetic acid
2,4-D	2,4 - dichlorophenoxyacetic acid

Cytokinins:

BA	Benzyladenine
PBA	Benzyltetrahydropyranyl adenine

Media:

MS	Murashige and Skoog (1962)
MMS	Murashige and Skoog (1962) medium modified by Pierik (1976)

Others:

AR	Analytical Reagent
LR	Laboratory Reagent
BDH	British Drug House
SRL	Sisco Research Laboratories
CD	Critical difference
NS	Not significant
ca.	approximately

INTRODUCTION

1. INTRODUCTION

Tissue culture has become a very useful and potential method for rapid propagation of plants, especially for the high value ornamentals and other herbaceous plants. Currently tissue culture is one of the fastest developing areas in biological sciences. This technique has now been commercialised and several private sector firms have started producing orchids, anthurium, banana, cardamom and several other high value ornamental plants in large scale for internal market as well as for export. These firms utilise sophisticated infrastructure, expensive equipment and skilled technicians, and their capital input is very high. Hence the technology is still confined mainly to research institutions and big commercial firms. There is no effective mechanism to transfer the technology to the needy individuals for utilisation.

In Kerala, where unemployment among educated youth is a major problem, home scale production of plants through tissue culture is a good proposition for self employment. However the proposition will be viable only if the technology becomes simple and the high capital requirement is reduced substantially. A simpler but efficient and feasible protocol if developed, utilising cheaper substitutes for the expensive items of plant tissue culture, will defenitely help in bringing down the cost of plant production enabling home scale practice of *in vitro* culture.

The primary step in the development of protocol for tissue culturing a plant species or variety is the standardisation of culture medium. Here the chemical composition of nutrient medium necessary to induce tissue development, differentiation, induction of plantlets and rooting are precisely defined. This part of plant tissue culture demands the expertise of experienced research personnel working in well equipped laboratories. Once the protocol is made available it can be adapted suitably using cheaper substitutes for cost-effective micropropagation.

In the present study Anthurium andreanum Lind. (Pink) was taken as the test plant. The protocol of in vitro propagation mainly through somatic organogenesis, already standardised by Sreelatha (1992) was followed.

The objectives of the study were

i. to develop or select cost-effective devices and culture conditions for home scale adoption of plant tissue culture.

ii. to investigate the influence of various cost-effective equipment, commercial grade chemicals and culture conditions on the in vitro establishment and growth of anthurium plants.

iii. to work out the economics of cost-effective method of tissue culture compared to the expensive conventional method.

REVIEW OF LITERATURE

2.REVIEW OF LITERATURE

The plant tissue culture technology, though proved to be a very viable proposition for rapid propagation and improvement of a number of useful plant species, has not yet become a handy tool for even an educated and progressive farmer or nursery man for multiplying his elite collection of plant species. The major reasons for the situation are the high capital investment required for establishing a tissue culture facility, the sophistication of the methods, necessity of employing skilled technicians and the resultant high cost of the output.Hence the greatest requirement for plant micro-propagation to be a viable and profitable business venture is reduction of production costs. The high cost of establishment is accounted by expensive equipment like the electronic balance, laminar airflow chamber, autoclave, water distillation unit, pH meter, air conditioner and culture trolleys. Other major expenditure are on account of culture containers, high labour costs and analytical grade chemicals.

Oki (1981) reported that costs of micropropagation can be reduced by modifications which would save time, labour and materials. If production costs are reduced educated youth and housewives can adopt this technology for self employment with minimum expenditure. The following is a review of literature on

cost-effective methods and low cost devices in micropropagation, followed by works in the micropropagation of Anthurium spp.

2.1 Equipment and glassware

According to Raju (1993) the expensive laminar airflow unit utilised as a sterile work-bench for inoculation of cultures can be substituted by a small, less expensive hood which can be made at home. Razdan (1993) reported that the simplest type of transfer area is an enclosed plastic box (glove box) fitted with UV light. The floor surface can be sterilised by swabbing with 95 per cent ethyl alcohol, when in operation. Ordinarily a small wooden hood may also be used for tissue culture work. The hood must have a glass or plastic door, either sliding or hinged and should be dust proof. The transparent glass or plastic door enables the worker to see inside and make the necessary manipulations. The glove box has limited applications, as relatively few transfers can be effected in it. The use of a wooden or plastic box fitted with ultraviolet light or fumehood has also been reported by Kumar and Seeni (1994).

Tissue culture media,glassware and tools are sterilised by autoclaving. Instead of an autoclave the domestic pressure cooker can be used for this. The limitation is the volume of the

pressure cooker. Also, the cooking time has to be increased by about 15 per cent for complete sterilisation (Raju, 1993 ; Kumar and Seeni, 1994).

The electronic balance used to weigh out minute quantities of chemicals can be replaced by a simple common balance which costs much less. By way of dilution of stock solutions any desired level of chemical can be added to the culture medium without difficulty (Raju,1993).

The pH meter can be substituted by pH indicating paper. Instead of the refrigerator, ice packed thermocol boxes will be sufficient (Kumar and Seeni,1994).

The type of culture vessel or container used in tissue culture work is a major item of expenditure. The container used must be economical, suited for plant growth and could be easily handled and transported (Rajeevan and Pandey, 1986). Expensive borosilicate glassware which cost over Rs.2.50 per cm^2 of culture surface can be replaced by any clear, colourless bottles,which cost much less than one paise per cm^2 (Raju,1993).

According to Raju and Kavitha (1996) almost all the items of sophisticated equipment could be replaced by simple and cheaper alternatives which will in no way affect the quality of the plants produced. Chemical balance replaced by a small two pan balance,

autoclave by pressure cooker, laminar flow by inoculation hood, air-conditioned culture rooms and culture trolleys fitted with flourescent tube lights, replaced by open, but shaded areas in the house premises, analar grade chemicals with commercial quality chemicals and borosilicate glassware with used soda glass bottles, all contribute to the low capital and recurring expenses.

2.2 Labour

Anderson et al. (1977) reported that the cost of labour was the most expensive item in the micropropagation of broccoli plants. The specific advantage of a high multiplication rate for cost reduction was stressed by a number of workers including Anderson et al. (1977) and De Fossard (1981). High multiplication rate and successful in vivo rooting are equally important for reducing the cost of tissue culture plants. Encouraging result with non-sterile rooting conditions has been achieved, though they are not yet good enough for commercial success (Rajeevan, 1983). Tissue culture operations if limited only to establishing the cultures and multiplying shoots that can be handled as cuttings in large number by non-skilled workers,then the technology can become still cheaper.

The major cost in plant propagation by tissue culture is manual labour. This is especially pronounced in the latter stages (elongation and rooting), when individual shoots are manipulated. Some of the very expensive transfer work can be avoided by supplementing cultures with liquid medium. Liquid media can be added to established, exhausted cultures, instead of transplanting the tissues to a fresh medium (Maene and Debergh, 1985). This has been proved in cultures of Cordyline, Philodendron, Magnolia and Spathiphyllum.

Rajeevan and Pandey (1986) reported that in papaya, in vitro rooting and hardening are the most expensive stages. Also a number of problems may arise such as insufficient rooting, delayed growth due to poor root functioning and root damage during planting (Maene and Debergh, 1983). A higher multiplication rate, if achieved, can reduce the number of subcultures and thus reduce the labour charges during multiplication to only 13 per cent of the laboratory costs (Rajeevan and Pandey,1986).

Chu and Kurtz (1990) reported that labour costs account for 40 - 60 per cent of production costs. The greatest requirement for plant micropropagation to be a viable and truly profitable business venture is reduction of production costs. To enter markets currently propagated by seed, such as vegetable crops, the

micropropagated material must be able to compete favourably in cost with seed-derived transplants. Seeds of many crops cost less than 0.01 cent each and transplants are currently produced for less than one cent each and sold for approximately three cents each. These costs are extremely low when compared to rooted liners produced by tissue culture which sell for 40 - 60 cents each. The future success of the micropropagation industry depends on the reduction of production costs as much as possible without any decrease in product quality.

2.3 Culture medium

Wang and Hu (1980) tried to replace the costliest, but not indispensable component, agar in culture media. A medium solidified with gelling agents similar to agar as well as liquid media with various support matrices such as filter paper and glasswool have been used in place of agar. Kelco (1982) reported that gelrite a self gelling hydrocolloid which forms rigid, brittle, transparent gel in the presence of soluble salts can substitute agar. Gelrite is favoured over agar because it costs less per litre of medium and its clarity makes it easy to observe plant growth and bacterial contamination. But some cultivars showed `vitrification' (a physiological disorder of glassiness of plant parts) which could be reversed or halted by using an appropriate mixture of gelrite and agar (Kevers et al.,1984). Agar

is the costliest ingredient and often contains impurities that may affect the growth of the cultured plant cells and organs (Heller, 1953; Debergh, 1983). But among gelling agents, agar has been used widely because of its convenient gelling properties, stability and resistance to metabolism during use (Henderson and Kinnersley, 1988). Moreover local accumulation of heat, hindrance to the access of dissolved oxygen to the cultured plant parts in media, contamination of media through agar sticking to the neck of the culture vessels etc are reported to be other disadvantages of agar (Tulecke and Nickell, 1960 ;UNIDO, 1988 ; Comline, 1989). (Matsumoto and Yamaguchi (1989) reported the use of polymeric substances as support matrices in place of agar. Sagawa and Kunisaki (1990) reported that to improve shoot growth and rooting especially among woody dicots, the concentration of agar can be reduced to as low as 0.3 per cent or it can be completely avoided.)

Bera and Roy (1991) reported an effective low cost micropropagation method in Tylophora indica. In the culture medium, commercial grade sugar and single distilled water were successfully used along with different combinations of auxins and cytokinins. The plant was regenerated directly from stem segments with pre-existing shoot buds and indirectly through callus. The overall cost when calculated showed that the direct

way of micropropagation from leaf segments was the most useful low cost method in this plant material.

Yet another area where the cost can be reduced is by using chemicals of low purity levels. For standardisation of the protocol, analytical or equivalent grade of chemicals, may become necessary for preparing culture media of high purity, but after developing the protocol efforts can be made to replace them with the commercial grade chemicals if appreciable difference in the response of the plant material does not occur. When commercial grade chemicals are used their assay as well as the level of impurities are to be considered to make necessary adjustments in the basic composition. In many such cases use of micronutrients can be avoided without change in tissue response (Raju, 1993 ; Kumar and Seeni 1994).

Bhattacharya et al. (1994) tested efficacy of sago (from Metroxylon sagu) and isabgol (from Plantago ovata) as gelling agents and that of filter paper, nylon cloth, polystyrene foam and glasswool cloth as support matrices in the micro propagation of chrysanthemum plantlets. The performance of those low cost gelling agents and matrices were found satisfactory and could compare well with that of agar. Glasswool cloth was however found to be the best matrix. For a given quantity of medium sago

and isabgol costed about 1/8th and 1\10th of the cost of agar respectively. The corresponding costs of the matrices were also less than that of agar.

Kumar and Seeni (1994) reported that instead of double glass distilled water, rain water or filtered water can be used in culture media and thereby the expense on water distillation can be avoided.

2.4 Culture conditions

According to Raju (1993) cultures can be incubated in any available space where the cultures will get diffused light. Usually verandah is a good place but direct exposure to sun is to be avoided. In extreme cases where such space is not available, cultures can be hung from the branches of trees. This gives a very good environment for the plants to grow in terms of light, temperature and humidity. Except for security reasons this will be an excellent method for incubation. Cultures kept in this manner were found to grow much better than in culture rooms litted with flourescent tubelight. Raju and Kavitha (1995) reported that the recurring expenditure can be kept low by utilising the resources like natural light instead of light from flourescent tubelights, by keeping cultures in the partially shaded open area with plenty of air movement avoiding the need for

air-conditioners and by the use of commercial grade chemicals instead of analytical grade. Instead of the air-conditioned and expensive laboratory space, a small cabin measuring not less than 2 x 2.5 m^2 is sufficient as the exclusive space for the sterile work. For other activities, space available in kitchen or elsewhere can be utilised (Raju and Kavitha, 1996).

2.5 Micropropagation of Anthurium spp.

Anthurium exhibits easy production of shoots and mass multiplication ability in vitro. With tissue culture technology anthuriums are multiplied very rapidly and production has been reported to be higher than those from seedlings (Jayanthi and Gowda,1994 ; Sreelatha,1992 ; Nirmala and Singh,1993). According to Kumar and Seeni (1994), a large number of plants can be produced through tissue culture within a limited period.

As mentioned earlier, for micropropagation of Anthurium spp. first the necessary protocol for multiplication has to be developed. This is the research part of micropropagation carried out by research personnel in well equipped laboratories.

According to Murashige (1974) there are three possible routes for in vitro propagule multiplication viz., enhanced release of axillary buds,production of adventitious shoots through organogenesis and somatic embryogenesis.

In the first route, meristems like shoot tips are cultured which assures genetic uniformity of progeny to a great extent (Chand and Roy, 1980; Rao and Lee, 1986). This method is being used for rapid clonal multiplication. The second route is callus-mediated somatic organogenesis which is not recommended for clonal propagation, but may be ideal for recovery of useful variant lines. Somatic embryogenesis, the third route is limited to a few species, but it results in the most rapid mode of plant regeneration (Evans et al., 1981). All species in which organogenesis and plant formation can be achieved in vitro may not be suited for large scale clonal propagation (Vasil and Vasil, 1980). For some species the process is too expensive. The rate of multiplication is slow and the mortality of plants at planting out to soil is high.

2.5.1 Explant

Micropropagation using leaf and spadix explants is reported for Anthurium andreanum and Anthurium scherzerianum (Pierik et al., 1979; Geier, 1982; Geier, 1986).Several workers obtained plantlet regeneration from callus differentiated from cultured spathe (Pierik, 1975; Finnie and Staden, 1986), leaf (Fersing and Lutz, 1977; Kuehnle et al., 1992) and petiole explants (Finnie and Staden, 1986) in Anthurium andreanum. Similar results were

reported in A. scherzerianum from spadix (Geier and Reuther, 1981) and leaf segments (Geier, 1986). Axillary buds and two noded stem sections from aseptically grown plantlets of Anthurium andreanum were used for production of multiple shoots by Kunisaki (1980). Zens and Zimmer (1988) obtained multiple shoots from seeds of Anthurium scherzerianum by germinating them under sterile conditions. Seeds produced caulogenic callus or callus with new shoots. Soczek and Hemoel (1989) reported that 0.5 cm long shoot tips of Anthurium x Cultorum clones were successfully used as primary explants. The regenerated shoots and callus bottom were used in multiplication. Also, single node fragments from the middle part of shoots (between the nearest node of the bottom and the node from which the youngest leaf was developed) grown in vitro were successfully used. Use of young leaf as explant was reported by Lightbourn and Prasad (1992) and Keller and Brehmer (1986). Rapid regeneration of selected commercially important cultivars of Anthurium andreanum was obtained through in vitro cultured young leaf lamina and petiole explants (Sreekumar et al., 1992). According to Nirmala and Singh (1993) leaf sections with midrib responded well when compared to that without midrib. Spadix segments when used as explants showed very high stability in the initial stages. However callusing was induced by repeated subculturing. Vegetative buds also were used

as explant, however leaf sections were found to be the best explants. Somatic embryogenesis for producing adventitious shoots was reported by Rajashekharan et al. (1994). Formation of nodular type callus from leaf explants of Anthurium andreanum was reported by Kumar et al. (1994). Similar type of callusing from Anthurium watermaliense was reported by Singh and Prakash (1994).

2.5.2 Surface sterilization

According to Pierik et al. (1979) surface sterilization of mature plant parts of A. andreanum was accomplished by dipping in 96 per cent alcohol for a few seconds, followed by treating with 1.0 per cent sodiumhypochlorite (containing a few drops of Tween 20) for 30 minutes. The nodal sections were sterilised by soaking them for 20 minutes in 0.52 per cent sodiumhypochlorite containing two drops of Tween 20. Leaf sheaths were removed under a dissecting microscope and buds excised and soaked in 0.26 per cent sodiumhypochlorite containing two drops of Tween 20 for 15 minutes (Kunisaki, 1980). Surface sterilization of unfolded leaves was obtained by submerging them in 1.0 per cent natriphene and then washing two times in sterile water and then sterilising in 5 per cent chlorox solution for 45 minutes followed by washing three times using distilled water (Hirunratana, 1988). Geier (1990) reported that prior to culturing,plant material was disinfected in

70 per cent ethanol for a few seconds followed by immersion in a solution of sodiumhypochlorite made up to contain 15 g/l active chlorine and supplemented with 0.5 ml/l Tween 20. Young leaves were kept for 10-15 minutes in this solution whereas older leaves were treated for 15-25 minutes. In order to obtain optimum growth in primary cultures,the sterilant had to be removed completely by washing the explant thoroughly. Three consecutive rinses with sterile water for 10,30 and 60 minutes duration are recommended. Sreelatha (1992) reported that young leaf explants should be immersed in 0.1 per cent solution of Bavistin (Carbendazim based fungicide) for 30 minutes. Then they are sterilized in 1.0 per cent sodiumhypochlorite solution for 20 minutes.

2.5.3 Culture media

Pierik et al. (1974) found that in Anthurium andreanum embryos could be cultured on the MS medium (Murashige and Skoog, 1962) modified as MS macroelements at half strength, MS microlements, sucrose 3.0 per cent, MS organic constituents (except Edamin, IAA and Kinetin) and Difcobacter agar at 0.7 per cent. The pH of the media was adjusted to 5.8 before autoclaving. Shoot tips and leaves, aseptically dissected and subcultured on a fresh basic culture medium with PBA at 0.5 - 1.0 mg/l produced abundant callus tissue when kept in the dark. Instead of

sucrose, glucose 4.0 per cent was used. On solid media the addition of NAA (0.1 mg/l) strongly induced root formation. The formation and development of adventitious sprouts quite often occur spontaneously, but was particularly enhanced by transferring to light. Pierik and Steegmans (1976) modified the basic culture medium for callus induction as follows: the glucose concentration was decreased to 3.0 per cent, PBA was added at 1.0 mg/l and 2,4-D at 0.1 mg/l. In the medium for callus subculture glucose concentration was reduced to 2.0 per cent, PBA to 0.1 mg/l and 2,4-D was omitted. For clonal increase, nodal explants of aseptically grown plantlets were placed on agar medium of Murashige and Skoog (MS) basic nutrients supplemented with 0.2 mg/l of BA (Kunisaki, 1977). Pierik et al. (1979) proposed that adventitious sprout formation from leaf explants of Anthurium andreanum was optional under the following conditions: adenine 0.1 mg/l, zeatin 1.0 mg/l, 2,4-D 0.08 mg/l along with MS basic nutrients. When vegetative buds are cultured, MS inorganic salts, 0.4 mg/l thiamine-HCl, 0.5 mg/l each of nicotinic acid and pyridoxine-HCl, 6.0 g/l sucrose and 15.0 per cent (by volume) coconut water was found best (Kunisaki, 1980). Geier (1982) cultured the leaf explants of Anuthurium andreanum in Nitsch and Nitsch (1969) medium with ammonium nitrate reduced to 100 mg/l, BA 1.0 mg/l and 2,4-D 0.1 mg/l. Geier (1986) found that for shoot multiplication, along with Nitsch and Nitsch medium, BA 0.5 mg/l

alone may be used as growth regulator supplement. For rooting, the shoot multiplication medium alone, without growth regulators need be used. Semisolid Nitsch medium containing 0.45 per cent agar and 1.5 mg/l BA was reported best for inducing callus by Sreekumar et al. (1992). For organogenesis the proliferated calli were transferred to a hormone free medium. Callusing responses were negligible in liquid as well as hardened (0.6 per cent agar) media. Sreelatha (1992) reported that MS medium modified by Pierik (1976) supplemented with 2,4-D 0.08 mg/l, BA 1.0 mg/l, sucrose 30g/l and agar 6 g/l could be used for callus induction. For callus multiplication MS (macro elements 1/4) supplemented with BA 1.0 mg/l, sucrose 30 g/l and agar 6 g/l and for sprout regeneration, growth and rooting MS medium supplemented with IAA 2.0 mg/l, BA 0.5 mg/l, sucrose 30 g/l and agar 6 g/l was best. Somatic embryogenesis was reported by Rajashekharan and Kumar (1994) in a medium comprising of modified inorganic salts of Nitsch and Nitsch (1969), Whites (1934) vitamins and hormones such as BAP, Kinetin and 2,4-D. Embryos were germinated in a modified MS medium supplemented with BAP.

2.5.4 Culture Conditions

Finnie and Staden (1986) reported that in Anthurium andreanum plantlet regeneration was achieved best at 25 ± 2°C with a 16 h

light and 8 h dark cycle at an intensity of 27 μE m^{-2} s^{-1}. According to Geier (1990), callus formation in Anthurium andreanum occurred in continuous darkness at 25°C. Shoot development was induced under low illumination of about 20 μE m^{-2} s^{-1}. Sreelatha (1992) found that cultures of Anthurium andreanum were to be incubated in continuous darkness at 26 ± 2°C, relative humidity ranging from 55 - 63 per cent with a 16 h photoperiod except in cases where complete darkness was required. Cultures for callus initiation and callus multiplication must be kept in darkness. According to Singh (1994) callus induction and sub culturing was achieved in total darkness at 25°C while regeneration and shoot formation was best done under continuous fluorescent light. Rajashekharan et al. (1994) reported that cultures were to be incubated in a growth room at 25 ± 2°C day and 18 ± 2°C night temperature and a 16 h photoperiod with light intensity of 1600-2000 lux.

2.5.5 Time required for producing plantlets.

According to Sreelatha (1992) the full cycle from leaf explants into planting out of plantlets takes six to seven months, passing through the various phases of callus induction, callus multiplication, shoot regeneration, proliferation and rooting. Singh (1994) reported that the total duration of the cycle from leaf explant to complete plantlet was 11 months, two months for

callus induction, three months for callus multiplication, four months for sprout induction and leaf development and two months for root formation. Rajashekharan and Kumar (1994) reported that the whole regeneration cycle of somatic embryos into plantlets beginning with the induction of embryos, passing through the phase of induction, maturation and germination, took about three months.

2.5.6 Hardening and planting out .

Physical, chemical and biological properties of the potting media and atmospheric conditions are important in the establishment of in vitro regenerated plantlets, which have been planted out. Damino (1979) reported that either pure peat or mixture of 1:1 sand and peat was suitable as the potting medium for strawberry plantlets. Kyte and Briggs (1979) observed that porous potting mixture of peat : perlite : composted bark (1:1:1) was the best for rooting rhododendrons. Geier (1990) observed that plantlets of anthurium could be established without losses in a peat or sand medium. Sand as the potting media was found to be the best for jack plantlets by Ramesh (1990). Sreelatha (1992) had also reported sand as the best potting medium for anthurium plantlets.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

The investigations on `Cost-effective methods and devices for home scale adoption of plant tissue culture' were carried out at the Plant Tissue Culture Laboratory of the Department of Horticulture, College of Agriculture, Vellayani during 1993-95. The test plant selected for the study was Anthurium andreanum Lind. (Pink).

Anthurium constitutes the largest genus of the family Araceae, with approximately 600 species distributed in the American tropics. Several species are grown as ornamentals in botanical gardens, private collections as on a larger commercial scale. By far, the most popular and economically important species are Anthurium andreanum Lind. and Anthurium scherzerianum Schott. Anthurium andreanum is a native of Columbia. It possesses dark green, heart-shaped foliage with long petioles. The attractive inflorescence is composed of the spadix, a compact cylindrical spike crowded with small bisexual flowers and the spathe which is a large conspicuously pigmented bract at the base of the spadix.

In this chapter, the materials and methods used for in vitro production of anthurium plantlets using different equipment, containers and culture conditions intended to minimise the cost of production of tissue culture plants of anthurium are described. The conventional in vitro culture techniques and cost- effective in vitro culture techniques are compared. The method of in vitro

propagation of Anthurium andreanum through callus mediated somatic organogenesis of leaf tissues has already been standardised (Pierik, 1976; Pierik et al., 1979). This technique was further modified by Sreelatha (1992). The same protocol was adopted for the in vitro production of anthurium callus to supply the required material for the experiment.

3.1 In vitro culture techniques.

The description of mother plants used as explant source are given in Table 1.

3.1.1 Explant and preparation of explant.

Young leaves, just unfurled and reddish brown in colour were selected as the explants. Care was taken to see that the leaf surface is free from any damage. Prior to culturing, the leaves (after removing petiole) were washed thoroughly in running tap water. They were then washed in tap water containing one or two drops of `Labolene' (a wetting agent) followed by washing with distilled water. The wetting agent was washed off completely from the leaf surface.

3.1.2 Surface sterilisation and inoculation.

Surface sterilisation of plant material was carried out inside a laminar airflow chamber. The leaf surface was first wiped with 70 per cent ethanol. Each leaf was then cut into 2-3 pieces and surface sterilised with freshly prepared sodium hypochlorite solution (1.0 per cent) for 15-20 minutes or 0.1 per cent mercuric chloride solution for seven minutes. The explants, after surface sterilisation, were subjected to repeated washing (at least five times) with sterile distilled water. They were then cut into pieces of 1.0 cm^2 size, placed with their abaxial surfaces on the callus - initiating medium. Contact between the cut edges and the medium was assured.

3.1.3 Culture medium.

For callus initiation, modified MS medium developed by Pierik (1976) was used. For callus multiplication and shoot regeneration, Murashige and Skoog (1962) medium (Table 2) was used. Variations in hormone combinations in MS media used for various stages of *in vitro* production of anthurium plantlets are given in Table 3.

Table 1. Description of Anthurium andreanum plants used as explant source*

Plant No.	Number of leaves	Number of suckers	Mean no.of flowers **	Height (cm)	Spread (cm)
1	14	5	2	75	45
2	6	2	1	90	45
3	8	3	2	60	30
4	5	2	2	30	45
5	7	3	2	45	30
6	8	3	2	75	60
7	8	2	3	60	50
8	6	2	2	60	45
9	5	2	2	45	30
10	9	3	2	75	45

* Pink variety grown in large mud pots (30 cm diameter at the top with three large holes at the bottom) filled with brick pieces, coconut husk and charcoal maintained under shade (15 per cent) and with moderate watering (twice daily) during non-rainy days. Fed by a solution of complex fertilizer mixture (20:20:20 NPK) at the rate of 15 g per plant at monthly intervals. Flowers are of medium size, with pink coloured spathe and candle-like spadix.

** Produced at tri-monthly interval.

Table 2.Composition of media utilised for in vitro production of Anthurium andreanum plantlets.

Ingredients	Quantity (mg/l)		Grade of chemicals			
	MS*	MMS**	AR	Purity (%)	LR	Purity (%)
Macronutrients						
KNO_3	1900.0	950.0	Merck	99.0	Ranbaxy	98.0
NH_4NO_3	1650.0	825.0	BDH	99.5	Ranbaxy	98.0
$MgSO_4 .7H_2O$	370.0	370.0	BDH	99.5	Ranbaxy	99.0
KH_2PO_4	170.0	85.0	SD Fine	99.5	Glaxo	98.0
$Ca Cl_2. 2H_2O$	440.0	440.0	Qualigens	99.5	Qualigens	98.0
Micronutrients						
H_3BO_3	6.200		SRL	99.5	Qualigens	99.5
$MnSO_4.H_2O$	22.300		Merck	99.0	SD Fine	98.0
$ZnSO_4.7H_2O$	8.600		BDH	99.5	CDH	99.0
KI	0.830		Qualigens	99.8	Qualigens	99.5
$Na_2 MoO_4.2H_2O$	250.000		SRL	99.0	-	-
$FeSO_4. 7H_2O$	27.800		BDH	99.0	Ranbaxy	98.0
Na EDTA	37.300		Qualigens	99.5	Qualigens	98.0
$CoCl_2. 6H_2O$	0.025		SRL	99.0	-	-
$CuSO_4. 5H_2O$	0.025		BDH	99.0	Ranbaxy	98.5
Vitamins						
Thiamine.HCl	0.1		SRL	98.5		
Pyrodoxine.HCl	0.5		SRL	99.5		
Nicotinic acid	0.5		SRL	99.0		
Others						
Glycine	2.0		SRL	99.5		
Myo-inositol	100.0		SRL			
Sucrose	30.0 g		SRL			
Agar	6.0 g		SRL			

*MS- Murashige and Skoog (1962)medium

**MMS-Murashige and Skoog (1962) medium as modified by Pierik (1976).

AR - Analytical Reagent

LR - Laboratory Reagent

Table 3. Variations in hormones and their combinations in MS media utilised for *in vitro* production of *Anthurium andreanum* plantlets.

Stages of *in vitro* propagation	Composition of medium
Callus initiation	Modified MS +2,4-D 0.08 mg/l+BA-1.0 mg/l + sucrose 30 g/l + agar 6.0 g/l.
Callus multiplication	MS (macroelements1/4) + BA 1.0 mg/l+ sucrose 30 g/l + agar 6.0 g/l
Shoot regeneration	MS + IAA 2.0 mg/l + BA 0.5 mg/l + sucrose 30 g/l + agar 6.0 g/l
Shoot proliferation, growth and rooting	do

In the conventional method, the chemicals used were of analytical grade (AR) (source: British Drug House (BDH)/ Sisco Research Laboratories(SRL)/ Merck/ Sigma) of high purity (Table 2). In the cost-effective method, the chemicals used were of labortary grade (LR) (source: Ranbaxy/ Glaxo/ SD Fine/ Qualigens) of comparatively lesser purity (Table 2). Standard procedures (Biondi and Thorpe,1981) were adopted for the preparation of the media. The pH of the media was adjusted to 5.8. In semisolid media Difcobacter agar (BDH) was used at the rate of 0.6 per cent. Borosil brand test tubes of length 25x100 mm and Erlenmeyer flasks (100,150 and 250 ml) were used. Sterilisation of media and glassware was done at 121^{o}C and 1.01 kg/cm^{2} for 20 minutes and 45 minutes,2 respectively. All aseptic manipulations were carried out in a laminar airflow chamber.

3.1.4 Culture conditions

Cultures for callus initiation and callus multiplication were incubated in continuous darkness at 26 $\pm$ 2^{o}C and at a relative humidity ranging from 55 - 65 per cent in an air-conditioned culture room. The callus so obtained was used for shoot regeneration studies using

conventional as well as cost-effective methods. For shoot regeneration and multiplication in the conventional method the cultures were incubated at 26 ± 2° C at 55 - 65 per cent relative humidity with a 16 h photoperiod of light intensity 3000 lux.

3.2 Cost-effective methods and devices.

In the new method, the aim was to develop alternatives for reducing the high cost involved in the four major items of expenditure in mass tissue culture viz. equipment, chemicals, containers/glassware and culture conditions.

3.2.1Physical factors.

3.2.1.1 Equipment.

The following low cost equipments were tested and compared for their efficiency against expensive equipments conventionally used in plant tissue culture.

3.2.1.1.1 Comparison of transfer hood and laminar airflow cabinet

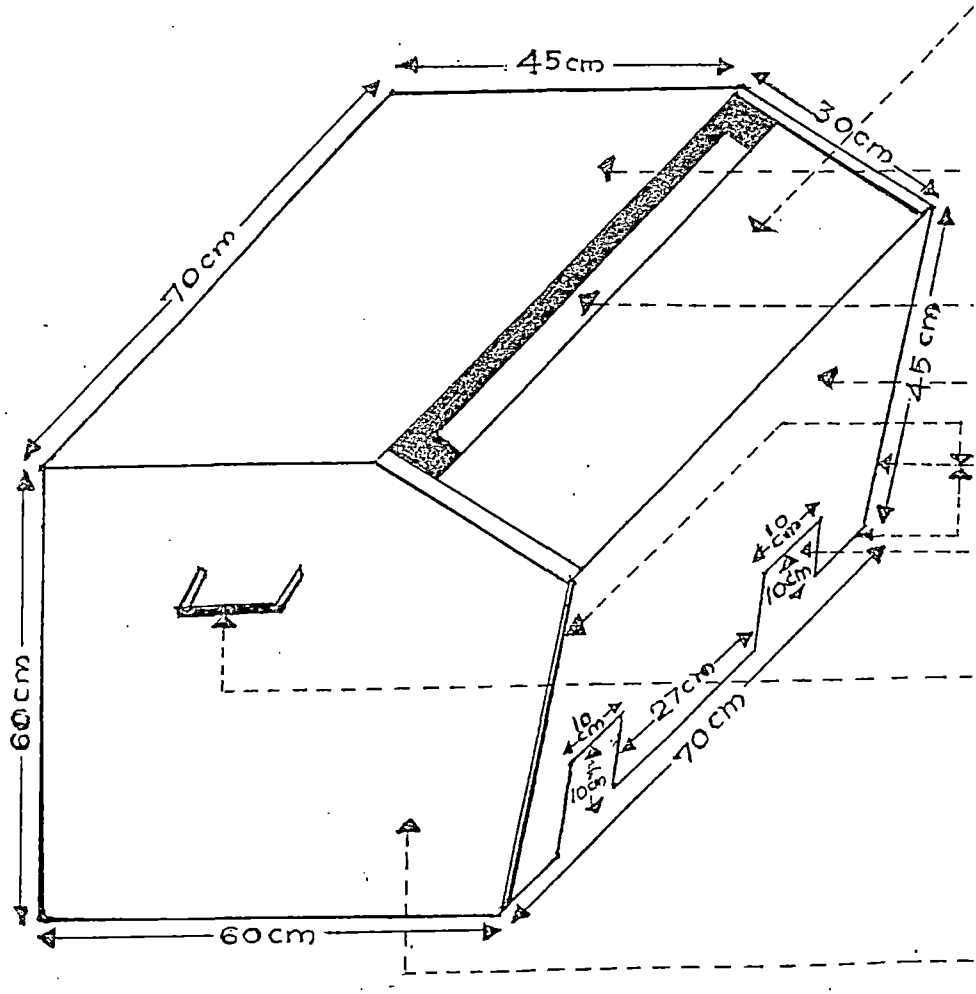
Fabricated transfer hood made of cheap wood having laminated inner surfaces was tested against the laminar airflow cabinet. Dimensions of the transfer hood are given in Fig.1. The laminated inner surface was wiped well or

sprayed with 95 per cent ethyl alcohol. Also, any material taken inside the hood was wiped with alcohol. Hands were inserted inside the hood through two holes made on the sliding glass door in front of the hood (Plate I). In the conventional laminar airflow cabinet, a small motor blows air into the unit first through a coarse filter, where large dust particles are seperated and subsequently passes through a 0.34 μm HEPA filter. The air coming out of the fine filter is ultraclean, free from fungal or bacterial contaminants and its velocity (27 ± 3 m/min) adequately prevents the microbial contamination of the working area. The laminar airflow cabinet used for the study was of the make `Thermadyne' make worth Rs. 40,000. The rate of contamination, when the two types of transfer hoods were used, was recorded.

3.2.1.1.2 **Comparison of pressure cooker and electric autoclave**

Ordinary pressure cooker (make: `Prestige') of capacity 22 l worth Rs. 2000 was tested against the electric autoclave. The rate of contamination in the two cases was noted. In the electric autoclave sterilisation was carried out at 121^{o}C and 1.01 kg/cm^{2} for 15 - 20 minutes. The

Fig. 1. DESIGN OF TRANSFER HOOD
(Scale 1:10)



---------Fixed glass window

-------Wood + White mica

---------Flourescent tube light

---------Perspex sliding glass cover

---------Aluminium single channel

---------Hole

-------Handle

--------- Wood + White mica

Plate I. Fabricated inoculation hood

autoclave used for the present study was a make of M/s `Fork Scientific Industries, Delhi', worth Rs. 20,000.

3.2.1.1.3 **Comparison of water distillation unit and water filter**

Ordinary candle type water filter of 16 l capacity costing Rs. 1000 was tested against the electric water distillation unit. Every fortnight the candle inside the water filter was sterilised by immersing in boiling water for 15 minutes and then replaced in the filter. This was found necessary to check the growth and multiplication of microrganisms in the water. The electric water distillation unit used for the study had an output capacity of 16 l/day. The unit was a make of `Bhann Scientific Instruments'; Bangalore, worth Rs. 30,000. Single filtered, boiled and single filtered, double filtered, boiled and double filtered water and rain water were tested against double glass distilled water.

3.2.1.1.4 **Comparison of the effect of natural light and artificial light**

For incubation , culture vessels were either stacked on ordinary wooden racks placed in partially open verandahs or they were hung in such places from overhead supports like

stretched steel or nylon wires and were subjected to ordinary diffused sunlight of intensity ranging from 2500 - 3000 lux. This was tested against flourescent tube illuminated culture racks of light intensity 3000 lux.

3.2.1.1.5 Comparison of ordinary balance and electronic balance

Ordinary common balance (goldsmith type) with weights worth Rs. 110, was tested against electric/electronic balance. The comparison of precision of balances is given in Table 12. The electronic balance used was of make `Sartorius,' model No. A 1205 worth Rs. 35,000. The mean of the difference of ten different weights taken in ordinary balance and the reading in electronic balance for the same weights was calculated and recorded. For weighing sucrose and agar the ordinary digital balance or common balance could be used. The low cost weighing equipment are shown in Plate II.

3.2.1.1.6 Comparison of pH indicator paper and pH meter

pH indicator paper was tested against a pH meter in measuring the pH of culture medium. The pH indicator paper used was of brand `Qualigens' with a pH range of 5.5 to

Plate II. Low cost weighing equipment

a Digital balance

b Ordinary balance (gold-smith type)

c Weight box with weights

a

b

c

7.5. The pH meter was of make `Philips' worth Rs. 6000. Here also the mean of the difference between pH measured using pH meter and pH indicator paper was recorded. The comparison of precision of pH measured using pH indicator paper and pH meter is given in Table 13.

3.2.1.1.7 **Comparison of ice box and refrigerator**

Insulated type ice box of make:'Hi-Cool'(Brite) having 16 l capacity worth Rs. 1200 was compared against a refrigerator (make: `Godrej' of 165 l capacity). Ice cubes were filled in polythene bags, sealed and loaded inside the ice box along with bottles of stock solutions and the lid was closed airtight. The quantity of ice required to attain a particular temperature and number of days for which the temperature could be maintained were noted.

3.2.1.1.8 **Comparison of borosilicate glassware and ordinary glass ware**

Ordinary colourless glass bottles and jars were tested against expensive borosilicate glassware. The glassware were first subjected to a `breaking-in' procedure to remove the toxic materials like silicon-dioxide contained in them. For this purpose the items of glassware were

first washed thoroughly in tap water. After filling with tap water they were autoclaved for 20 minutes. They were then immersed in a hot detergent solution and washed thoroughly,using tap water. One more autoclaving (after filling with distilled water) for 20 minutes was done. They were dried in an oven and before using, rinsed with distilled water. The low cost devices for media preparation, storage and sterilisation are shown in Plate III.

3.2.2 Chemical factors

3.2.2.1 Chemicals.

Laboratory reagent grade (LR) chemicals were tested against expensive analytical reagent grade (AR). The suppliers of laboratory reagent grade chemicals were M/s Ranbaxy and M/s Qualigens. The response of the regenerating calli in media containing the above two grades of chemicals were compared and results recorded.

3.2.2.2 Carbon source

Commercial grade crystal sugar and confectionary grade sugar cubes of `Glaxo' make were tested against costly analytical grade sucrose.

Plate III. Low cost devices for media preparation, storage and sterilisation

a Domestic pressure cooker

b Water filter (candle - type)

c Ordinary glass bottles and jars

d Ice box (Hi-Cool, 16 l)

a

b

c

d

3.2.2.3 Support matrix

Commonly available playing marbles (diameter ca.12 mm) were used as support matrix for plantlets instead of agar. After filling the basal 1/4th portion of the containers with marbles, liquid medium (MS) was poured and plantlets inserted in between the marbles for support. Growth rate was observed and compared with that on agar.

3.2.3 Environmental factors

Cool, partially open verandah was tested against an air-conditioned culture room (Plate IV). In the partially open verandah, the cultures were incubated at 28 ± 2°C temperature and 65 - 85 per cent relative humidity. The mean day time light intensity was 2500 lux. The temperature, relative humidity and light intensity varied slightly depending on weather conditions. Growth observations under the above conditions were compared with those under flourescent light in the air-conditioned culture room.

3.2.4 Hardening and planting out

Rooted plantlets were separated and washed in tap water to remove the traces of agar sticking to the roots. They were then planted out in sterilised sand and

Plate IV. Incubation on wooden shelf in partially open verandah

sprinkled with cold water twice daily. Rooting and establishment of plantlets were recorded.

3.3 Observations recorded

3.3.1 In vitro phase

The following observations were recorded during the in vitro growth phase upto eight weeks after culture.

3.3.1.1 Contamination percentage

The rate of contamination, if any, while using cost-effective equipment and media components of reduced purity level was observed.

3.3.1.2 Number of shoots

The number of shoots newly formed, after subculturing to the media constituted by different cost-effective components, was counted and recorded at fortnightly intervals.

3.3.1.3 Length of shoots

The length of the longest shoot produced in each treatment was recorded at fortnightly intervals.

3.3.1.4 Number of leaves

The number of leaves newly produced was recorded at fortnightly intervals.

3.3.1.5 Width of the biggest leaf

The width at the middle of the biggest leaf, produced by each callus mass after eight weeks of culture was recorded.

3.3.1.6 Number of roots

The number of roots produced after eight weeks of culture was recorded.

3.3.2 Ex vitro phase

3.3.2.1 Per cent rooting and establishment of plants under ex vitro conditions.

The per cent loss during various stages of hardening of plantlets after planting out was noted and the per cent rooting and establishment was recorded.

3.3.3 Economics of plant production and cost of producing a plantlet.

The economics of plant production and cost of producing a plantlet through conventional and cost-effective methods of tissue culture was worked out. The recurring and non-recurring items of expenditure were worked out separately. The total annual expenditure for the conventional as well as the cost-effective method was calculated.

$$\text{Per plant cost} = \frac{\text{Total annual expenditure}}{\text{Number of plants produced annually}}$$

3.4 Statistical analysis

Statistical analysis was done in completely randomised design wherever necessary. The critical difference was calculated and the different treatments were compared at five per cent level of significance.

RESULTS

4.RESULTS

Attempts were made to develop cost-effective methods and devices for home scale adoption of plant tissue culture using Anthurium andreanum Lind. (Pink) as the test plant. Certain low cost equipment were either fabricated or procured and tested for their efficiency in comparison to the conventional, expensive method. The results of the study are presented in this chapter.

4.1 Explant choice and season of collection

Explant choice was made as reported by Sreelatha (1992). The callusing potential of different types of explants and the effect of different months of explant collection on microbial contamination and callusing are presented in Tables 4 and 5 respectively. Among different types of explants tried, only the young leaf segments produced callus. Callusing was observed on young leaf explants, 45 - 75 days after culture on modified MS medium supplemented with 2,4-D 0.08 mg/l and BA 1.0 mg/l. Significant variation was observed on the response of explants with respect to the month of explant collection and inoculation (Table 5). Callusing percentage of leaf explants was maximum during the month of May (60.00) followed by April (52.63). No callusing was observed during August.

Table 4. Callusing potential of different explants of *Anthurium andreanum*

Basal medium : Modified MS + 2,4-D 0.08 mg/l + BA 1.0 mg/l

Explant	Callusing* (%)	Remarks
Leaf	58.33	Masses of light yellow coloured callus were formed
Petiole	0.600	Only slight swelling of cut ends was observed
Candle segment	0.00	do

Culture period : 60 days

* Mean of 12 replications

Table 5. Effect of month of explant collection on microbial contamination and callusing of explants

Basal medium : Modified MS + 2,4-D 0.08 mg/l + BA 1.0 mg/l

Month	Contamination* (%)	Survival* (%)	Callusing* (%)
January 1995	20.00	80.00	12.50
February 1995	13.30	86.67	19.23
March 1994	28.57	57.14	16.67
April 1994	16.67	63.33	52.63
May 1994	10.00	83.33	60.00
June 1994	50.00	50.00	13.33
July 1994	10.00	70.00	20.00
August 1994	32.25	28.12	0.00
September 1994	71.40	25.71	50.00
October 1994	8.33	89.50	9.30
November 1994	26.67	70.00	23.81
December 1994	16.67	66.60	20.00

Culture period : 1994 March - 1995 February.

* Mean of 12 replications

4.2 Surface sterilisation of leaf explants

The results of surface sterilisation of leaf explants using 1.0 per cent sodiumhypochlorite and 0.1 per cent mercuric-chloride are presented in Tables 6 and 7. Treatment with mercuric chloride for eight minutes resulted in lowest contamination rate (11.1 per cent), followed by sodium-hypochlorite for 17 minutes (10.7 per cent). The rate of callusing was also high in the above treatments (62.0 and 70.0 per cent respectively) (Table 7).

4.3 Culture medium

The basal medium utilised for the present study was MS (1962) (Table 2), as standardised by Sreelatha (1992). The modifications tried in the basal medium and their influence on callus initiation, callus multiplication, shoot regeneration, shoot proliferation, growth and rooting are presented in Table 3. Callusing was observed on young leaf explants, 45 - 75 days after culture, on modified MS medium supplemented with 2,4-D 0.08 mg/l and BA 1.0 mg/l along with sucrose 30 g/l and agar 6 g/l. For callus multiplication MS (macro elements x 1/4) medium supplemented with BA 1.0 mg/l, sucrose 30 g/l and agar 6 g/l was used. For shoot regeneration, shoot proliferation, shoot growth and rooting MS medium supplemented with IAA 2.0 mg/l,

Table 6. Surface sterilisation of Anthurium andreanum leaf explants

Basal medium : Modified MS + 2,4-D 0.08 mg/l + BA 1.0 mg/l

Sterilant	Duration (minutes)	Contamination* (%)	Remarks
Sodium-hypochlorite (1.0 %)	15	58.30	Leaf margin showed browning
	16	33.30	Small brown patches on leaves
	17	16.70	do
	20	0.00	Marked browning on leaves
Mercuric-chloride (0.1 %)	5	25.00	Leaves remained healthy green.
	6	18.18[a]	do
	7	16.67	do
	8	11.10[b]	do
	9	0.00	No response
	10	0.00	do

Culture period : 60 days

* Mean of 12 replications

a Mean of 11 replications

b Mean of 9 replications

Table 7. Effect of sodiumhypochlorite and mercuricchloride on callusing of leaf explants.

Basal medium: Modified MS + 2,4-D 0.08 mg/l + BA 1.0 mg/l

Sterilant	Duration (minutes)	Callusing* (%)
Sodium-hypochlorite (1.0 %)	15	60.00
	16	62.50
	17	70.00
	20	41.67
Mercuric-chloride (0.1 %)	5	22.20
	6	44.40
	7	60.00
	8	62.00[a]
	9	0.00
	10	0.00

Culture period : eight weeks

* Mean of 12 replications

a Mean of 9 replications

BA 0.5 mg/l, sucrose 30 g/l and agar 6 g/l was used. The different stages of plant regeneration from leaf tissues of Anthurium andreanum are shown in Plate V. The composition of culture medium in both conventional and cost-effective methods was the same. However in the cost-effective method, attempts were made to substitute the expensive analytical reagent grade chemicals, carbon source, double glass distilled water and support matrix (agar) with suitable less expensive items. The results of the trials are given in the following pages.

4.3.1 Grade of chemicals

The influence of AR and LR grades of chemicals used to supplement macro and micro elements to the culture medium (as per Table 2) in shoot regeneration from leaf callus is presented in Table 8.

Number of shoots.

The rate of shoot regeneration in the medium containing LR grade chemicals was significantly lower than that in the medium containing AR grade chemicals. The number of shoots produced in the medium containing AR grade chemicals was 11.5, while that in the medium containing LR grade chemicals was 9.5. The shoots produced were green and healthy in the former whereas they were stunted in the latter.

Plate V. Stages of plantlet regeneration from leaf tissues of Anthurium andreanum cultured in vitro

a Callus formation

b Shoot regeneration

c Elongated shoots with roots

a

b

c

Number of leaves.

The rate of leaf production was also significantly lesser in the medium containing LR grade chemicals compared to that in the medium containing AR grade chemicals. The number of leaves produced in the medium containing chemicals of LR grade was 9.3 whereas that of AR grade was 15.8. The leaves produced were small and greenish-yellow in the former medium whereas in the latter they were green and healthy (Plate VI).

Length of shoot and width of leaf

Similar results were observed in the case of length of shoot and width of leaf. Both the length of shoot and width of leaf were significantly lesser in the medium containing LR grade chemicals compared to the medium containing AR grade chemicals.

Number of roots

No roots were produced in the medium containing LR grade chemicals whereas rooting was observed in the medium containing AR grade chemicals.

It is evident from the above results that the AR grade chemicals in the culture medium cannot be effectively replaced by LR grade chemicals for successful *in vitro* culture of *Anthurium andreanum*.

Table 8. Effect of Laboratory Reagent (LR) grade and Analytical Reagent (AR) grade chemicals on shoot regeneration and growth from leaf callus

Basal medium : MS + IAA 2.0 mg/l + BA 0.5 mg/l

Grade of chemicals	Number of shoots regenerated*	Number of leaves*	Length of shoot(cm)*	Width of leaf(cm)*	Number of roots*
AR	11.80	15.80	2.68	1.22	3.00
LR	9.50	9.30	1.77	0.68	0.00
CD(5 %)	1.83	3.40	0.37	0.34	-
SEM ±	0.82	1.52	0.16	0.15	-

Culture period : eight weeks

* Mean of six replications

Plate VI. Shoot regeneration of Anthurium andreanum in media containing AR grade and LR grade chemicals.

a Shoot regeneration in AR grade chemical

b Shoot regeneration in LR grade chemical

a

b

4.3.2 Carbon source

The effect of the quality of carbon source (sucrose) on regeneration and growth from leaf callus is presented in Table 9. All the cultures exhibited cent per cent survival as well as growth, in all the treatments tried. Significant variation was observed in the number of shoots and roots produced. The length of shoot and width of leaf also differed, significantly (Plate VII).

Number of shoots

Maximum number of shoots was produced in the medium containing AR grade sucrose compared to commercial grade crystal sugar and confectionary grade sugar cubes. Significant difference was exhibited in the rate of shoot production by commercial grade crystal sugar and confectionary grade sugar cubes, compared to AR grade sucrose. Commercial grade crystal sugar and confectionary grade sugar cubes produced 5.3 and 6.1 shoots respectively whereas AR grade sucrose producee 9.1 shoots.

Number of leaves

With respect to the rate of leaf production there was no significant difference between treatments. Commercial grade crystal sugar and confectionary grade sugar cubes produced

10.3 and 12.8 leaves respectively compared to AR grade sucrose which produced 13.5 leaves.

Length of shoot

In the case of the length of shoot produced, no significant difference was exhibited between confectionary grade sugar cubes (25 mm) and AR grade sucrose (24 mm). But there was significant difference between commercial grade crystal sugar (8 mm) and AR grade sucrose and also between commercial grade crystal sugar and confectionary grade sugar cubes.

Width of leaf and number of roots

The influence of sugar cubes (confectionary grade) and crystal sugar (commercial grade) on the width of leaf and rate of root production was significantly inferior to that of AR grade sucrose. The width of leaf was maximum in AR grade sucrose (9.4 mm) followed by confectionary grade sugar cubes (4.5 mm) and commercial grade crystal sugar (2.3 mm). Similarly the number of roots was maximum in AR grade sucrose (3.60) followed by sugar cubes (2.30) and crystal sugar (0.66). Therefore it could be concluded that sugar cubes could be used to replace sucrose, while crystal sugar could not

Table 9. Effect of quality of carbon source (sucrose) on shoot regeneration and growth from leaf callus

Basal medium : MS + IAA 2.0 mg/l + BA 0.5 mg/l

Carbon source	Number of shoots regenerated*	Number of leaves*	Length of shoot* (cm)	Width of leaf*(cm)	Number of roots*
Sucrose (AR grade)	9.10	13.50	2.40	0.94	3.60
Sugar cubes (confectionary grade)	6.10	12.80	2.50	0.45	2.30
Crystal sugar (commercial grade)	5.30	10.30	0.80	0.23	0.66
CD (5 %)	2.80	NS	0.94	0.27	1.18
SEM ±	1.35	2.19	0.44	0.13	0.56

Culture period: eight weeks

* Mean of six replications

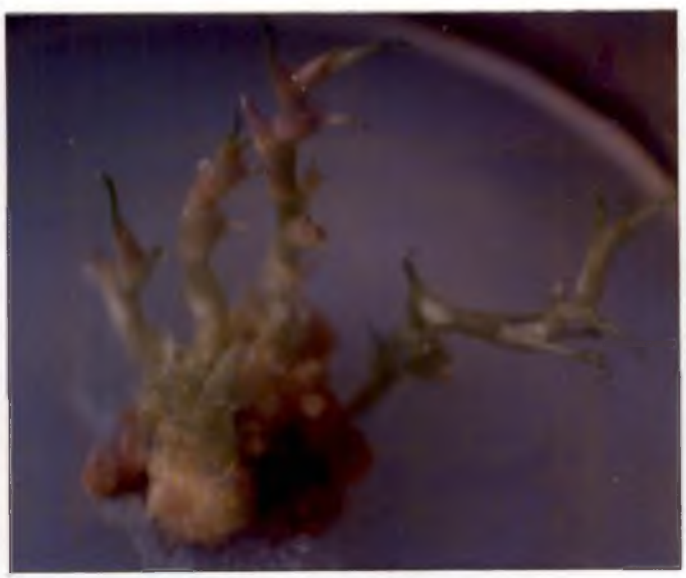
Plate VII. Shoot regeneration of Anthurium andreanum in media containing various carbon sources

a Shoot regeneration in AR grade sucrose

b Shoot regeneration in confectionary grade sugar cubes

c Shoot regeneration in commercial grade sugar

a



b

c

4.3.3 Sources of water

The effect of different sources of water used for preparing culture medium (as substratum) on shoot regeneration and growth from the leaf callus of Anthurium andreanum was studied. All the treatments resulted in cent per cent survival of the cultures. The results are presented in Table 10 (Plate VIII).

Number of shoots.

Among all the treatments tried, maximum regeneration of shoots (10.3) was obtained when double glass distilled water was used as culture medium substratum followed by rain water (9.5). There was significant difference in the rate of shoot production between double glass distilled water and all other sources of water, except rain water.

Number of leaves

The rate of leaf production was maximum (13.5) when double glass distilled water was used. Significant difference was exhibited between double glass distilled water and all other sources of water, except rain water. Double glass distilled water produced 13.5 leaves, double-filtered water produced 6.5, boiled and double-filtered water produced 6.6, boiled and single-filtered water produced 9.0 and rain water produced 12.1 leaves.

Length of shoot and width of leaf

Double glass distilled water produced 16.7 mm long shoots while rain water produced 12.2 mm long shoots. Also the width of leaf was maximum in double glass distilled water (7.3 mm) followed by rain water (6 mm). No significant difference was exhibited between double glass distilled water and rain water, with respect to length of shoot and width of leaf. Significant difference in these growth parameters could be observed in all other treatments when compared to double glass distilled water.

Number of roots

Double glass distilled water produced maximum number of roots (3.0) followed by rain water (2.33). No significant difference was observed in the rate of root production between double glass distilled water and rain water. All the other sources of water failed to produce any roots.

These results indicated that double glass distilled water could be effectively replaced with rain water for use as the substratum of culture medium. Single-filtered water could not be used, since very few cultures, in this treatment regenerated. The others produced green calli with no regeneration of shoots or produced one or two minute shoots without leaves.

Table 10. Effect of different sources of water used as culture medium substratum on shoot regeneration and growth from leaf callus

Basal medium : MS + IAA 2.0 mg/l + BA 0.5 mg/l

Source of water	Number of shoots regenerated*	Number of leaves*	Length of shoot* (cm)	Width of leaf*(cm)	Number of roots*
Double glass distilled water	10.30	13.50	1.67	0.73	3.00
Double filtered water	4.30	6.50	0.28	very small scaly leaves	0.00
Boiled and double filtered water	5.00	6.66	0.28	"	0.00
Boiled and single filtered water	5.80	9.00	0.41	"	0.00
Rain water	9.50	12.10	1.32	0.60	2.33
CD (5 %)	1.81	3.20	0.37	-	-
SEM ±	0.88	1.55	0.18	_	_

Culture period: eight weeks

* Mean of six replications

Plate VIII. Shoot regeneration of Anthurium andreanum in media containing different sources of water

a Shoot regeneration in double distilled water

b Shoot regeneration in rain water

c Shoot regeneration in single-filtered water

d Shoot regeneration in single-filtered and boiled water

e Shoot regeneration in double-filtered water

f Shoot regeneration in double-filtered and boiled water

a

b

c

d

e

f

4.3.4 Support matrix.

The results of using playing marbles (diameter ca.12 mm) in replacing agar (0.6 per cent) as support matrix are presented in Table 11. Growth parameters like number of shoots regenerated and leaves and roots produced were almost similar in both treatments (Plate IX). This indicated that agar, the expensive support matrix in tissue culture, could be substituted with less expensive playing marbles.

4.3.2. Preparation of culture medium

4.3.2.1 Weighing of chemicals

The results of weight measurement of chemicals taken using ordinary balance and electronic balance, to compare their precision are presented in Table 12. The mean of the difference between ten different measurements were computed. It was found that the mean of the difference between measurements of the two balances was not significant. The electronic balance could be effectively replaced by the ordinary gold-smith type balance without sacrificing the precision of weight measurements.

The growth response in culture medium prepared out of stock solutions in which the chemicals were weighed using ordinary balance was not significantly different from that in the medium prepared using chemicals weighed in the electronic balance (Table 14).

It could be concluded that the expensive electronic balance could be effectively replaced by the ordinary (gold-smith type) balance in weighing chemicals for preparation of culture medium.

4.3.2.2 pH adjustment

Both pH indicator paper and pH meter were tested for their efficiency in testing the pH of the medium before sterilisation (Table 13). The mean of the difference between measurements was computed and it was found that the difference between pH measurements made using pH indicator paper and pH meter was not significant. The growth response in culture medium in which the pH was adjusted using pH indicator paper was not significantly different from that of the control in which a pH meter was utilised. The results are presented in Table 15. Results show that the pH meter could be effectively replaced with the pH indicator paper.

4.3.3 Culture vessels

Ordinary colourless glass bottles and jam jars were used in place of expensive borosilicate glassware. The results are presented in Table 16. No significant difference in the growth of cultures was observed in both types of culture vessels, after eight weeks of culture.

Table 11. Comparative efficiency of playing marbles and agaragar as support matrix in culture medium

Type of support matrix	Number of shoots *	Number of leaves*	Number of roots*
Marbles	3.15	3.60	0.80
Agar-agar (0.6 %)	2.86	3.50	0.50
CD (5 %)	NS	NS	NS

Culture period : eight weeks

* Mean of six replications.

Plate IX. Shoot regeneration of Anthurium andreanum in different culture medium support matrices

a Agar-agar as support matrix

b Playing marbles as support matrix

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Table 12. Comparative efficiency of ordinary balance (gold-smith type) and electronic balance* in weighing chemicals

No. of weighing	Reading in ordinary balance (g)	Reading in electronic balance (g)	Mean of difference
1	0.010	0.009	
2	0.020	0.015	
3	0.100	0.097	
4	0.200	0.199	
5	0.500	0.495	
6	1.000	0.998	0.0078(+)
7	2.000	1.999	
8	5.000	4.970	
9	10.000	9.970	
10	20.000	19.980	

* Make : Sartorius Model No. A 1205

Table 13. Comparative effect of pH indicator paper and pH meter in measuring the pH of the culture medium before autoclaving

No. of pH measurement	pH measured using paper*	pH measured using pH meter*	Mean of difference
1	7.00	7.12	
2	6.50	6.60	
3	5.50	5.60	
4	6.00	6.09	0.095(-)
5	5.00	5.11	
6	4.00	4.05	

* Mean of three replications

Table 14. Comparative efficiency of ordinary balance and electronic balance in weighing chemicals for culture media preparation as expressed in the growth of cultures

Type of balance	Number of shoots regenerated*	Number of leaves*	Length of shoot* (cm)	Width of leaf*(cm)
Electronic balance	5.60	8.60	1.85	1.63
Ordinary balance	6.30	6.50	2.00	1.16
CD (5 %)	NS	NS	NS	NS

Culture period : eight weeks

* Mean of six replications

Table 15. Comparative efficiency of pH indicator paper and pH meter in testing pH of the medium as expressed in the growth of cultures

Type of pH measurement	Number of shoots regenerated*	Number of leaves*	Length of shoot* (cm)	Width of leaf* (cm)
pH meter	5.60	8.60	1.85	1.63
pH paper	5.60	6.60	2.00	1.45
CD (5 %)	NS	NS	NS	NS

Culture period : eight weeks

* Mean of six replications

Table 16. Comparative effect of borosilicate glassware and ordinary jam jars in the *in vitro* response of explants.

Type of glassware	No. of shoots regenerated*	No. of leaves*	Length of shoot* (cm)	Width of leaf* (cm)	No. roots*
Borosilicate	10.30	13.50	1.67	0.73	3.00
Ordinary jam jars	9.50	12.10	1.22	0.60	2.33
CD(5 %)	NS	NS	NS	NS	NS

* Mean of six replications

Culture period : eight weeks

4.3.4 Sterilisation of culture medium and containers

In order to reduce the cost of sterilisation of culture medium and glassware a trial was made to substitute the expensive autoclave with the domestic pressure cooker. The comparative efficiency of both the equipments as sterilising devices is presented in Table 17. The domestic pressure cooker was found to be equally efficient as the electric autoclave. The culture medium sterilised using both the devices was totally free from microbial contamination, even after twelve weeks of culture.

4.3.5 Storage of culture medium and stock solutions.

The results of testing the efficiency of ice box (make: Hi-Cool, Brite, 16 l) in comparison to a refrigerator (make: Godrej 165 l) for maintaining low temperature in the storage of stock solutions and culture medium are given in Table 18. The results showed that a temperature of 4°C could be maintained for 24 h in the ice box using 2.5 kg of ice, fully packed with glass bottles containing stock solutions and/or culture medium. After 24 h, the temperature gradually declined and reached 10° C at 48 h. The results indicated that the ice packing in the ice box had to be changed every 48 h to ensure a low temperature (10°C) in the ice box. The ice box could accommodate eight numbers of 500 ml glass bottles with stock solutions and/or culture medium.

Table 17. Comparative efficiency of domestic pressure cooker* and autoclave as a sterilising device**

Basal medium : Modified MS + 2,4-D 0.08 mg/l + BA 1.0 mg/l

Sterilising device	Period of incubation (weeks)	Contamination (%)#
Pressure cooker	2	Nil
	4	Nil
	6	Nil
	8	Nil
	10	Nil
	12	Nil
Autoclave	2	Nil
	4	Nil
	6	Nil
	8	Nil
	10	Nil
	12	Nil

* Make : Hawkins (22 l)

** Made by M/s Fork Scientific Industries, Delhi

\# Mean of 60 cultures in 100 ml Erlenmeyer flask

Table 18. Efficiency of ice box* (16 l)** in maintaining the temperature of stock solutions

Quantity of ice	Temperature (°C)				
	0h	12h	18h	24h	48h
1.0 kg	15	15	15	15	21
1.5 kg	10	10	10	10	15
2.5 kg	4	4	4	4	10

* Make : Hi-Cool (Brite)

** Packed with 8 x 500 ml glass bottles filled with stock solutions

4.4.1 Culture inoculation.

The influence of fabricated transfer hood and laminar air flow cabinet on the rate of culture contamination was studied. The results are presented in Table 19. Both the devices were equally effective in providing contamination free cultures, during the non-rainy season. During the rainy season, the fabricated transfer hood registered a slightly higher rate of contamination (16.6 per cent) compared to that of the laminar airflow cabinet (10 per cent). The results showed that the fabricated transfer hood is suitable for home scale tissue culture. The cost of the device is considerably lower when compared to the laminar airflow chamber (5.0 per cent of the cost of laminar airflow chamber) (Table 23).

4.5 Culture incubation

4.5.1 Light

The effect of artificial flourescent lighting and natural diffused light on the regeneration of calli was compared. Cultures in both treatments showed cent per cent survival and growth. The results are presented in Table 20 (Plate X).

Number of shoots

The rate of shoot production was found to be more when the calli were incubated under artifical light, than under natural diffused light. Significant difference was exihibited between the two treatments, with respect to shoot production. Under natural light 4.8 shoots per culture were produced, while under artifical light 6.16 shoots per culture vessel were produced.

Number of leaves.

The shoots produced less number of leaves when subjected to natural light. The number of leaves produced, when cultures were incubated under natural light differed significantly when compared to cultures incubated under artificial light. Under artificial light, the number of leaves produced per culture vessel was 11.3, while under natural light 7.8 leaves were produced.

Length of shoot and width of leaf

Incubation under natural light was found to be equally favourable with that of artificial light in the length of shoot and width of leaves produced. The length of shoot produced under natural light was 23.5 mm and that under artifical light was 27.7 mm. The corresponding values for width of the leaf were 12 mm and 10 mm for artificial light and natural light respectively.

Table 19. Comparative efficiency of fabricated trasfer hood and laminar airflow cabinet* on the rate of culture contamination during different seasons of inoculation

Basal medium : Modified MS + 2,4-D 0.08 mg/l + BA 1.0 mg/l

Type of transfer hood	Season	Contamination (%)
Fabricated transfer hood	December	0.00[a]
Laminar air-flow cabinet		0.00[a]
Fabricated transfer hood	June	16.60[b]
Laminar air-flow cabinet		10.00[b]

* Make : Thermadyne

Culture period : eight weeks

a Mean of 15 replications

b Mean of 30 replications

Table 20. Comparative effect of diffused light and artificial light on growth and regeneration of leaf callus

Type of light	Number of shoots regenerated	Number of leaves	Length of shoot (cm)	Width of leaf (cm)
Artificial light	6.16	11.30	2.77	1.20
Natural light	4.80	7.80	2.35	1.00
CD (5 %)	1.26	2.30	NS	NS
SEM ±	0.56	1.03	0.32	0.20

Culture period : eight weeks

* Mean of six replication

Plate X. Shoot regeneration of Anthurium andreanum on exposure to diffused sunlight and flourescent tube light.

a Shoot regeneration on exposure to diffused sunlight

b Shoot regeneration on exposure to flourescent tube light

a

b

4.6 Frequency of subculture

The rate of culture establishment and proliferation of callus regenerated shoots of Anthurium andreanum, as influenced by frequency of subculture is presented in Table 21. In the conventional method, one culture vessel initially containing 3.5 shoots proliferated on each subculture (done at four weeks interval) to 13.5, 25.0 and 40.0 shoots. Thus the rate of increase in shoot proliferation, at each subculture declined from 285 per cent in the first subculture to 60 per cent in the last subculture done at the twelth week. Similarly in the cost-effective method, a culture vessel containing 3.4 shoots further multiplied to 10,16 and 24 shoots at four weeks interval between each subculture. The rate of increase in shoot proliferation in this case declined from 185 per cent to 50 per cent.

4.7 Hardening and planting out.

Rooted plantlets were planted out in sterile sand medium and were sprinkled with cold water. There was 30 per cent loss during various stages of hardening (Table 22) in both the conventional and cost-effective methods. The plantlets were saleable three months after hardening. The plants were found to exhibit no difference in the ex vitro establishment irrespective of the method of culture (Plate XI).

Plate XI. Plantlets in plastic pots

Table 21. Influence of continuous subculturing of callus regenerated shoots at four week interval on the shoot multiplication rate.

Culture no.	No. of shoots per culture* Conventional method	Cost - effective method
1	3.5 (0.0)	3.5 (0.0)
2	13.5 (285.0)	10.0 (185.0)
3	25.0 (85.0)	16.0 (60.0)
4	40.0 (60.0)	24.0 (50.0)

* Mean of 12 replications

Data in parantheses indicate percentage increase in number of shoots over the initial culture

4.8 Economics of plant production

The estimated cost of producing an anthurium plantlet through conventional and cost-effective methods of tissue culture is given in Tables 23 and 24. It is estimated that by the conventional method 20,000 plants can be produced annually whereas, adopting the cost-effective method, 12000 plants can be produced per year from 1000 culture vessels.

4.8.1 Non-recurring items

Among the non-recurring items, various expensive items of laboratory equipment (namely the laminar airflow chamber, electric water distillation unit, electronic and digital balances, air-conditioner, electric autoclave, pH meter, refrigerator and heating mantle) accounted for 14.02 per cent of the total cost, whereas in the cost-effective method, they accounted for only 7.63 per cent. The share of glassware comes next, to 11.6 percent of the total cost in the conventional method. In the cost-effective method, the glassware accounted for 9.13 per cent of the total cost. The laboratory and culture room facility accounted for the highest capital input of around one lakh rupees in the conventional method. Considering a 50 year life span for the building, an annual expenditure of Rs.2000/- was estimated in this regard. This accounts for only 1.93 per cent of the total cost. In the cost-effective home scale tissue culture method, a

portion of the house can be converted as a laboratory and culture room. In the green-house phase, humidity maintanance devices contributed to 1.83 per cent of the total cost in the cost-effective method and 3.9 per cent of the total cost in the conventional method.

4.8.2 Recurring items

Among the recurring items, labour (skilled and non-skilled) costs accounted for the highest share (29 per cent) of the total cost in the conventional method. In the cost-effective method labour charges accounted to only 11.42 per cent. The miscellaneous items as aluminium foil, cotton wool, cling film, ethyl or methyl alcohol and fuel and electricity charges accounted to 9.66 per cent of the total cost. In the cost-effective method, this accounted to 11.88 per cent of the total cost. The components of the culture medium accounted to 5.7 per cent of the total cost in the conventional method, while in the cost-effective method it accounted to 7.86 per cent. Small PVC pots and potting mixture contributed to 24.17 per cent of the total cost per year in the conventional method. In the cost-effective method, mud pots and potting mixture contributed to 50.24 per cent of the total cost. The pie diagrams showing the different items of expenditure in the conventional and cost-effective methods of tissue culture are given in Fig. 2 and Fig. 3, respectively.

The total annual expenditure for producing 20000 plants in the conventional method was Rs. 103399. The estimated perplant cost was Rs. 5.16. In the cost-effective method the annual expenditure for producing 12000 plants was Rs. 21895. The estimated per plant cost was only Rs.1.82. Thus the cost per plant in the cost-effective method was found less than half of the cost per plant produced in the conventional method. Hence the cost-effective method of tissue culture can effectively be used to replace the conventional method, in producing anthurium plantlets.

DISCUSSION

5. DISCUSSION

The techniques for culturing plants *in vitro* have always been kept within the confines of the research institutions and commercial firms. The apprehension that tissue culture technology is much beyond the comprehension and that it is too expensive to start with, has kept the nurserymen and farmers (even educated progressive class) away from venturing into plant tissue culture. Due to lack of efforts in developing protocols for micropropagation of plants using limited infrastructure and low recurring expenditure, the technology has not yet come to the grass root level for exploitation (Oki, 1981; Raju and Kavitha, 1996). Had it been possible to simplify the procedure and bring down the expenses of *in vitro* plant culture, it would have been a right choice for the educated, unemployed youth and houswives of our State for generating self employment. The present investigations carried out at the College of Agriculture, Vellayani, was mainly aimed at developing cost-effective methods and devices for home scale adoption of plant tissue culture using *Anthurium andreanum* Lind. (Pink) as the test plant. The results of various invetigations carried out are discussed in this chapter.

Among the different types of explants tried, only the young leaf segments produced callus. The segments of spadix and petioles failed to produce callus. Similiar results were obtained

by Keller and Brehmer (1986), Lightbourn and Prasad (1992) and Sreelatha (1992). Nirmala and Singh (1993) could also obtain good results with leaf sections. For any given species or variety, a particular explant may be ideal for successful plant regeneration. Explants consisting of shoot tips or isolated meristems, which contain mitotically active cells, have been generally successful for callus initiation and subsequent plant regeneration in several species (Murashige, 1974). In the present study, only leaf explants produced callus. Morphological and physiological status of the explants can account for the difference in the response. The less lignified tissues of leaf may facilitate easy de-differentiation process than the tissues of other plant parts.

The season of the year can influence callus initiation from explants, especially when the donor plant is field grown. In the present study also seasonal variation was observed with respect to callus initiation from leaf explants. The variation may be due to changes in the concentrations of endogenous auxins. Seasonal variation in the concentrations of endogenous auxins has been reported by Wodzicki (1978).

Surface sterilisation of explants was best achieved when treated with 0.1 per cent mercuricchloride for eight minutes or 1.0 per cent sodiumhypochlorite for 17 minutes. The rate of callusing was also high in the above treatments (62 and 70 per

cent, respectively). Sreelatha (1992) could successfully sterilise young leaf explants using 1.0 per cent sodium hypochlorite for 20 minutes. However, in the present study, it was found that treatment beyond 17 minutes resulted in severe bleaching of the green epidermal layer of the leaf explants.

Kunisaki (1980) used MS salts at full strength for the induction of multiple shoots in anthurium. In the present study one-fourth strength of the major nutrients of MS medium along with full strength of micro nutrients was found good for the induction of multiple shoots. Reports also show that low salt MS medium is better suited than full strength MS for anthurium tissue culture (Pierik, 1976; Geier, 1982). Low salt requirement of anthurium is reported to be species-specific (Geier, 1982).

The quality or grade of the chemicals used for the preparation of the culture medium, particularly those which contribute the major and minor mineral nutrients, was found to be crucial in achieving successful culture establishment, plant regeneration and their growth. All the growth parameters like number of shoots, number of leaves, length of shoot, width of leaf and number of roots were found adversely affected by the use of LR grade chemicals, when compared to AR grade chemicals. The ill effects of LR grade chemicals may be due to the presence of chemical

impurities and micronutrients unfavourable for plant growth. Raju (1993) and Kumar and Seeni (1994) are also of the opinion that by replacing AR grade chemcials with commercial grade chemicals, it is difficult to achieve good results in anthurium tissue culture, though it reduces slightly the cost of production of plants.

Sugar is indispensable in the culture medium. It is the source of carbon and has an important role in osmoregulation. Bera and Roy (1991) reported that commercial grade crystal sugar can be successfully used in presence of auxins and cytokinins in tissue culture. In the present study confectionary grade sugar was found to be equally good to AR grade sucrose, while commercial grade crystal sugar was not. Confectionary grade sugar costs only about one-fourth the cost of AR grade sucrose. Confectionary grade sugar available on cubes is more refined than commercial grade crystal sugar.

Among the different sources of water namely double glass distilled water, single-filtered, single- filtered and boiled, double-filtered, double-filtered and boiled and rain water, only rain water gave similar results as that of double glass distilled water in shoot regeneration and growth from callus. Single-filtered water was not found good because only a few cultures in this treatment regenerated. This indicates that rain water can be used as a substitute to double glass distilled water. The

favourable attributes of rain water may be due to its purity similar to that of double glass distilled water. Kumar and Seeni (1994) suggested that instead of double glass distilled water, rain water or filtered water can be made use of for preparing culture medium and thereby the use of water distillation unit can be avoided. By avoiding the water distillation unit, 2.9 per cent of the total expenses can be reduced.

Support matrices are used in the culture medium for anchoring plants. The trial to substitute the most expensive component of culture medium ie. agar-agar (Difco grade) with playing marbles (diameter ca. 12 mm) indicated that playing marbles can be effectively substituted for agar-agar. Playing marbles are less expensive, reusable and not prone to contamination even on prolonged storage. However, when compared to semi-solid medium, cultures containing playing marbles are to be handled carefully, as there is a chance for submergence of the plantlets in liquid culture medium due to displacement of marbles on movement or shaking. Bhattacharya et al. (1994) found that the performances of low-cost gelling agents and matrices are satisfactory and compare well with that of agar. Heller (1953) reported satisfactory growth of explants in a liquid medium containing glass beads which can anchor explants. Wang and Hu (1980) also reported various support matrices such as filter paper and glass wool, in liquid media, instead of agar.

In most of the tissue culture laboratories, the chemicals for media preparation are weighed using the expensive electronic balance. In the present study, it was observed that there was no significant difference in the growth response when chemicals were weighed using ordinary (gold-smith type) balance for media preparation. It can be concluded that the expensive electronic balance could be effectively replaced by the ordinary balance. Similar results have been obtained by Raju and Kavitha (1996) who reported that the electronic balance used for weighing chemicals can be replaced by a small double pan balance. By way of diluting stock solutions while preparing culture medium any desired quantity of chemical can be introduced into the medium without difficulty. The cost of electronic and digital balances account to 2.35 per cent of the total cost in the conventional method while in the cost-effective method the cost of ordinary balance accounts to 0.94 per cent of the total cost.

In the trial to test the efficiency of pH indicator paper for measurement of pH of the culture medium, it was found that the pH meter could be effectively replaced with the pH indicator paper without sacrificing the precision of measurement. The mean of the difference between measurements using pH meter and pH indicator paper was only - 0.095. Kumar and Seeni (1994) also opined that the pH meter could be effectively replaced with the pH indicator

paper. The pH meter accounts to 0.58 per cent of the total cost while pH indicator paper accounts to 1.60 per cent of the total cost.

The type of container or culture vessel used in tissue culture work is a major item of expenditure. The container used must be suitable for plant growth, easy to handle and transport and also they should be economical (Rajeevan and Pandey, 1986). The present study revealed that ordinary colourless glass bottles or jam jars can be economically used, instead of expensive borosilicate glassware. Raju (1993) also opined that borosilicate glassware which costs more than Rs 2.50 per cm^2 of culture surface can be replaced by any clear, colourless bottles, which costs less than one paise per cm^2. In the conventional method glassware account for 11.61 per cent of the total cost while in the cost-effective method they account for 9.13 per cent of the total cost.

In the attempt to reduce the expenses of sterilising culture medium and glassware by substituting the expensive autoclave with the domestic pressure cooker, it was found that the domestic pressure cooker was equally efficient as the electric autoclave. Similar results were reported by Raju (1993) and Kumar and Seeni (1994). The only limitation experienced was the volume of the pressure cooker. The culture medium sterilised using both the devices were totally free from microbial contamination, even after

twelve weeks of culture. The electric autoclave accounts for 1.3 per cent of the total cost while the pressure cooker accounts for 0.90 per cent of total cost.

Another expensive equipment used in tissue culture laboratories is the refrigerator which in the present study could be replaced with an ordinary insulated and ice-packed ice box. The device could maintain the low temperature (<10^{o}C) required for storing stock solutions and culture media for about 48 h. However frequent opening of the ice box leads to increase in temperature inside the box, which should be avoided. Kumar and Seeni (1994) also are of the opinion that instead of refrigerators, ice-packed thermocol boxes would serve the purpose. The refrigerator accounts for 0.52 per cent of the total cost while the ice box accounts for 0.55 per cent of the total cost.

Laminar airflow cabinets are expensive devices which provide sterile work benches or transfer hoods for culture inoculation. In the present study, a much efficient and highly economical wooden transfer hood could be fabricated which was equally effective in providing contamination-free cultures compared to that of conventional laminar airflow cabinets. Razdan (1993) had also suggested that a small wooden hood is sufficient for tissue culture work. According to him, the hood can be conveniently

placed on a bench, in a quiet corner of the laboratory. However, such transfer hoods have a limitation that relatively few transfers can be effected in it, that too with less comfort for the operator, when operated for a long time. In a home scale tissue culture unit, however these limitations may be overlooked. Kumar and Seeni (1994) suggested the use of plastic boxes, fitted with ultraviolet light or fumehood. During the rainy season wooden transfer hood exhibited a slightly higher percentage of microbial contamination, due to the high percentage of micro organisms in the atmosphere. The laminar airflow chamber accounts for 3.87 per cent of the total cost while the transfer hood accounts for 0.90 per cent of total cost.

In the trial to assess the effect of artificial fluorescent light and natural diffused light on the regeneration of plantlets from callus, both the treatments gave cent per cent survival and growth. It was evident from the results that artificial fluorescent lighting could be effectively replaced with natural diffused light. Raju and Kavitha (1996) reported that air-conditioned culture rooms and culture trolleys with fluorescent tube light can be replaced by partially shaded areas in the domestic premises. In extreme cases where space is not available, cultures can even be hung from the branches of trees provided their physical protection is ensured (Raju, 1993). In the

conventional method, air-conditioner and culture racks with tube light account for 2.31 per cent of total cost. In the cost-effective method ordinary culture racks account for 0.68 per cent of total cost. Here air-conditioners are not required.

Rooted plantlets when planted out, exhibited 30 per cent loss during various stages of hardening. Anderson (1980) reported that the loss during hardening of tissue culture plant- lets is due to excessive water loss, reduced uptake of water and nutrients, lack of initiation of new shoots and roots and attack of pathogens. The ex vitro establishment of the in vitro generated plantlets is critical for successful clonal multiplication (Collins, 1978). Twenty per cent of Anthurium andreanum plantlets were reported to succumb in a trial of ex vitro hardening by Sreelatha (1992).

The analysis of the economics of plant production revealed that the cost of production of a single anthurium plantlet come to Rs 5.16 in the conventional method whereas in the cost-effective method it could be brought down to Rs 1.82, by resorting to low cost protocol and devices described in the foregoing chapter. Labour costs accounted for the highest share of the total cost (29 per cent) in the conventional method. Chu and Kurtz (1990) reported that labour costs account for 40-60 per cent of production costs, a very high component in the cost of tissue culture. Anderson et al. (1977) also reported that the cost of

labour was the most expensive item in the micropropagation of broccoli plants. Maene and Debergh (1985) reported that manual labour in plant tissue culture can be saved to some extent by supplementing liquid media to exhausted, semi-solid cultures, instead of transplanting the tissues to a fresh medium. In the cost-effective method, home labour is utilised and labour charges for miscellaneous outdoor works is very meagre (11.42 per cent).

In the cost-effective method, pots and potting mixture accounted to 50.21 per cent of the total cost. As the plants are sold in a potted stage, this item of expenditure is recurring as well. It is warranted that further cost reduction has to be effectively applied in this aspect so as to supply tissue cultured plants at competitive rates. Natural low cost, self disintegrating materials like straw, coconut husk, palm leaves etc. can be used for making pots. Such pots can be filled with litter, charcoal, dead wood pieces etc. This saves considerable amount of potting mixture and cost of pots and containers. The cost per plantlet can be further reduced, if a higher multiplication rate is achieved. Rajeevan and Pandey (1986) reported that a higher multiplication rate if achieved can reduce the number of subcultures which saves labour charges during multiplication.

It is worth while to suggest some future prospects from the results of the present investigations. In the present study AR grade chemicals could not be effectively replaced with LR grade chemicals. Further studies in detail has to be taken up in this aspect. In the present study both macro and micro nutrients of AR grade were replaced with LR grade. Further studies have to be taken up by replacing AR with LR for each nutrient, one by one. This is a very important aspect and requires critical study.

In the present study, only Anthurium andreanum Lind. (Pink) has been selected as test plant. Separate low cost protocols must be developed for other commercial crops also.

In the case of support matrix, a more convenient one has to be found out, to overcome the inconvenience while using marbles.

The plants produced through tissue culture are usually more expensive than those produced in conventional propagation methods. Laid up capital, recurring expenses, cost of labour, cost of electric power (back up with generator) etc. add up the production cost to over Rs. 5.0 per tissue cultured plantlet. Conventional methods are required for standardising the protocols. Once this is done, cost reduction can be attempted. The cost-effective methods with reduced capital and recurring expenses, extremely low power consumption and use of domestic labour altogether bring down

the cost of production to less than half of the conventional procedures, thus rendering it highly suitable as an income generating procedure within the domestic confines. Almost all the returns from the sale of plants will be the profit, as the expenses are quiet negligible compared to the returns.

SUMMARY

SUMMARY

Attempts were made to develop cost-effective methods and devices for home scale adoption of plant tissue culture in the plant tissue culture laboratory of the Department of Horticulture, College of Agriculture, Vellayani, using Anthurium andreanum Lind. (Pink) as the test plant.

Segments of leaf, petiole and candle were used as explants for somatic organogenesis. Callus initiation in the different explants was compared. Seasonal influence on callus initiation was studied by collecting explants (leaf) at monthly intervals. The callus obtained was used for further regeneration studies in media prepared from different cost-effective components. Various low-cost equipment were fabricated and tested for their efficiency in comparison to the conventional expensive method.

The salient findings of the above study are:

Among the different types of explants tried, only the young leaf segments produced callus.

Callusing percentage of leaf explants was maximum during the month of May followed by April.

Surface sterilisation of leaf explants with mercuric chloride for eight minutes resulted in lowest contamination rate.

AR grade chemicals in the culture medium could not be effectively replaced by LR grade chemicals for successful in vitro culture of Anthurium andreanum.

Confectionary grade sugar cubes could be used to replace sucrose, while crystal sugar could not.

Double glass distilled water could be effectively replaced with rain water for use as the substratum of culture medium. Singe-filtered water could not be used, since very few cultures in this treatment regenerated.

Agar-agar, the expensive support matrix in tissue culture could be substituted effectively with the less expensive playing marbles of diameter ca.12 mm.

The expensive electronic balance could be effectively replaced by the ordinary (gold-smith type) balance in weighing chemicals for preparation of culture medium.

The pH indicator paper could be used to replace the pH meter, for adjusting pH of the medium before autoclaving.

Ordinary colourless glass bottles and jam jars could be used effectively instead of expensive borosilicate glassware.

The domestic pressure cooker was found to be equally efficient as the electric autoclave for sterilisation of media and containers.

Instead of the refrigerator, the ice box could be used for storage of stock solutions and culture medium. A temperature of $4^{o}C$ could be maintained for 24 h in the ice box using 2.5 kg of

ice, fully packed with glass bottles containing stock solutions and/or culture medium.

The fabricated transfer hood was found equally effective as the laminar airflow cabinet in providing contamination-free cultures during the non-rainy season. During the rainy season, fabricated transfer hood registered a slightly higher rate of contamination compared to that of the laminar airflow cabinet.

Artificial flourescent lighting could be effectively replaced with natural diffused light, for regeneration of calli. Cultures in both treatments showed cent per cent survival and growth.

In the conventional method, the rate of increase in shoot proliferation, at each subculture declined from 285 per cent in the first subculture to 60 per cent in the last subculture done at the twelth week. In the cost-effective method, the rate of increase in shoot proliferation declined from 185 per cent to 50 per cent.

There was 30 per cent loss during various stages of hardening of plantlets.

In the conventional method, the estimated per plant cost was Rs. 5.16 and in the cost-effective method the per plant cost was estimated to be Rs. 1.82.

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COST- EFFECTIVE METHODS AND DEVICES FOR HOME SCALE ADOPTION OF PLANT TISSUE CULTURE

By

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ABSTRACT OF A THESIS

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ABSTRACT

Attempts were made to develop cost-effective methods and devices for home scale adoption of plant tissue culture in the plant tissue culture laboratory of the Department of Horticulture, College of Agriculture, Vellayani, during 1993-95. The test plant selected for the study was Anthurium andreanum Lind. (Pink).

Segments of leaf were used as explant for cullus initiation. The callus so obtained was used for further regeneration studies. Various low cost equipment were fabricated and tested for their efficiency in comparison to the conventional expensive method. One-fourth strength of the major nutrients of MS medium along with full strength of micro nutrients was found good for the induction of multiple shoots.

All the growth parameters were found adversely affected by the use of LR grade chemicals, when compared to AR grade chemicals. Confectionary grade sugar was found to be equally good to AR grade sucrose, while commercial grade crystal sugar was not. Rain water could be used as a substitute to double glass distilled water in the culture medium. Attempts to substitute agar-agar with less expensive playing marbles, as support matrix of the culture medium was also successful.

Ordinary (gold-smith type) balance could be used to replace the expensive electronic balance in weighing chemicals for media preparation. The pH indicator paper could be effectively used

instead of the pH meter, in adjusting pH of the medium. Ordinary colourless glass bottles and jam jars could be economically used, instead of expensive borosilicate glassware.

The domestic pressure cooker was equally efficient as the electric autoclave in sterilising culture medium and containers. The expensive refrigerator could be effectively replaced with ice-packed thermocol boxes. Instead of the laminar airflow cabinet, the fabricated transfer hood could be effectively used.

Attempts to substitute artificial flourescent light with natural light were successful. Rooted plantlets when planted out exhibited 30 per cent loss during various stages of hardening.

The cost of producing a single anthurium plantlet was Rs. 5.16 in the conventional method, whereas in the cost-effective method it could be brought down to Rs. 1.82.