STANDARDISATION OF MEDIA AND CONTAINERS FOR *EX-VITRO* ESTABLISHMENT OF ANTHURIUM PLANTLETS PRODUCED BY LEAF CULTURE

By

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Dedicated to my
most beloved Father

DECLARATION

I hereby declare that this thesis entitled 'Standardisation of media and containers for *ex vitro* establishment of Anthurium plantlets produced by leaf 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, fellowship or other similar title, of any other University or Society.

College of Agriculture,
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CERTIFICATE

Certified that this thesis entitled 'Standardisation of media and containers for ex vitro establishment of Anthurium plantlets produced by leaf culture' is a record of research work done independently by Shri. Ajithkumar, P.V. under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to him.

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INTRODUCTION

1. INTRODUCTION

Anthurium, Flaming Plant or Painters Palette is one of the most important commercial ornamental crop of the modern world. In a floral arrangement anthurium flowers contribute to the elegance and attractiveness which are prerequisites for a quality design. They are valued for their colourful long lasting flowers and handsome foliage. The showy portion of inflorescence composed of the spadix, a compact cylindrical spike crowded with small bisexual flowers and the spathe, a large conspicuously pigmented bract at the base of the spadix.

The most popular and economically important species of the genus are Anthurium andreanum Lind and A sherzerianum Schott. A andreanum is a native of Columbia and is grown almost exclusively for cutflower production. Major production centres are in the tropical regions of the world because they are suitable for hot tropical weather. Growing of anthuriums on a commercial scale has very recently been started in different countries. Hawaii is the largest producer of anthuriums in the U.S.A. Other leading producers of anthurium are Holland, South American and European countries.

In India the anthurium industry is still in its infancy. Kerala with its unique tropical humid climate is

highly congenial for anthurium cultivation. Recently there has been an increasing awareness among growers about the potentialities of this new commercial flower crop. However availability of high quality planting materials and marketing of the flowers are the limiting factors for developing the cutflower industry in Kerala.

Anthuriums are commonly propagated by seeds and also vegetaively by suckers or cuttings. The length of time taken for conventional method of propagation has been a serious draw back in anthurium cultivation. The recent development of efficient micropropagation techniques opened up entirely new and promising prospects to meet the market demand and to optimise the income for the growers. Methods of in vitro propagation mainly through somatic organogenesis, have been standardised for A andreanum (Pierik et al 1974 b; Pierik, 1976; Pierik et al 1979; Sreelatha, 1992.) Although methods have been standardised there is possibility for improving the propagation efficiency and establishment of in vitro derived plantlets of anthurium under natural environment.

In the present study the influence of the different media and containers on ex vitro establishment of anthurium plantlets were taken into account. This study will bring to light the influence of the various media and containers on

the vegetative growth parameters at the early stages of establishment of *in vitro* produced plantlets. Good vegetative growth being the preliminary factor for further development and flowering.

The specific objective of the study is to develop suitable methods to plantout *in vitro* generated plantlets of *A andreanum* varieties and to standardise suitable containers and media for *ex vitro* establishment.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Plant propagation using tissue culture techniques, more commonly know as micropropagation is being applied to an ever increasing number of plant species. Transplanting and re-establishing aseptically propagated plants under non aseptic condition is still one of the main problems in the micropropagation of many plant spices. Ex vitro establishment of plantlets gained importance, consequent to the commercialisation of micropropagation.

This review encompasses the research works on in vitro propagation of Anthurium andrenum through somatic organogenesis, characteristics of in vitro plantlets that causes problems in ex vitro establishment, measures to overcome these problems; rooting, and acclimatization of cultured plantlets; and the influence of size of microcuttings, potting media, containers, and interaction of media and containers were discussed.

2.1 In vitro Culture

According to Murashige (1974) there are three possible routes for in vitro propagule multiplication.

a. Enhanced release of axillary buds.

b. Production of adventetious shoots through organogenesis and

c. 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 that is callus mediated somatic organogenesis 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 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 route of multiplication is slow and the mortality of plants at planting out to soil is high.

Somatic organogenesis

Somatic organogenesis can be direct or callus mediated (Evans et al., 1981) Levels of growth regulating substances in the culture medium, particularly auxins higher than those necessary to stimulate the direct formation of adventetious shoots generally give rise to the proliferation of caltus from the explant. On the other hand if the concentration of hormones, especially auxins is lowered in

the medium it results in the formation of adventetious shoots. (Shoot morphogenesis / somatic organogenesis) or embryos (Somatic embryogenesis) (Skoog and Miller 1957, Hussey 1986).

Although callus may be obtained from any species, only in some plants can be regenerated. Even when totipotent callus has been obtained, extended proliferation by repeated subculture may result in the reduction and eventual loss of regenerative capacity. The reasons for this have only occassionally been investigated in detail, but reduction in shoot forming ability is often paralleled by an increase in the proportion of polyploid or aneuploid cells (Smith and Street 1974).

As a clonally propagated slow maturing crop (2-3 years from seed to seed) Anthurium andreanum is a prime candidate for improvement using biotechnology. Establishment of callus from a variety of tissue cultured explants like leaf lamina, petiole, inflorescence stalk, spathe, and spadix has previously been described for cultivars of A andreanum available in Holland (Kuehnle and Sugii, 1991; Leffring et al., 1976; Pierik, 1975, 1976, Pierik et al., 1974 b) and in the Republic of South Africa (Finnie and Van staden 1986)

Pioneering studies were conducted by Pierik and collaborators (1974 a,b). They succeeded in the induction of

regeneration, first from embryo and seedling tissue and later from non meristematic parts of mature plants. A modified M S medium supplemented with a cytokinin (PBA) was used for optimum growth of callus tissue at 25°C in darkness.

Callus multiplication was observed best in a liquid medium (Pierik 1975, Pierik et al., 1975). For this leaf pieces with the callus was transferred to a liquid medium which was placed on a shaker rotating at 120 rpm. Based on detailed studies a scheme was proposed for the micro propagation of A andreanum and A scherzerianum (Pierik, 1976, Pierik and Steegmans, 1975, Perik et al., 1979)

Leffring and Soede (1978, 1979 a,b) made use of shoot proliferation as a means of multiplication from leaf callus derived shoots. Addition of 2ip at 3 mg/l to the medium resulted in wide spread shoot formation. The scheme of Kunisaki (1980) avoids an initial callus step by using shoots developed from auxiliary bud explant as starting material. Novak and Nepustil (1980) observed that callus with a high capacity for regeneration were derived from leaf explants of flowering plants.

Finnie and Van Staden (1986) achieved plantlet regeneration using a modified MS medium at 25±2°C with 16 h light and 8 h dark cycle. Keller et al., (1986) obtained callus from leaf explants on MS medium supplemented with 2 mg

kinetin/l. An embryogenic mode of plant regeneration was described only for early stages in spadix derived callus of A scherzerianum Schott (Geier, 1990).

2.2. Characteristics of in vitro plantlets that causes problems in ex vitro establishment

Debergh and Maene (1981) defined 4 Stages in micropropagation

Stage 0 — Preparation of stock plants under hygienic condition.

Stage 1 — Establishment of aseptic cultures,

Stage 2 — Multiplication of micropropagules (Shoot proliferation)

Stage 3a — Preconditioning and preparation of propagules for stage 3b

Stage 3b — Rooting and re establishment of propagules in soil

Here the problems encountered in the final stage of micropropagation and measures to overcome these pitfalls were described.

Success of micropropagation depends on the field establishment of in vitro derived plantlets. With in the in vitro system, plantlets are heterotrophic and get very favourable condition for their growth. During ex vitro establishment plantlets have to switch over to autotrophic

nutrition involving normal photosynthetic activity and water relations.

It has been reported that the leaves of plantlets developed *in vitro* are smaller and thinner than those developed *in vivo* (Lee *et al.*, 1988). Physicologically leaves grown in tissue culture have been shown to be incapable of significant photosynthesis (Langford and Wainwright, 1987), the stomata are unable to close and, as cuticular wax on the leaf surface is minimal, are unable to control water loss (Grout and Aston 1978).

Donnelly and Vidaver (1984) found that rasberry leaves produced *in vitro* were smaller, thinner, had a less compact arrangement of palisade and mesophyll cells and fewer epidermal hairs than those developed *in vivo*. Anatomical studies have demonstrated that leaves of *in vitro* propagated plants have a less ordered palisade layer and relatively more spongy mesophyl than field grown Plans (Brainerd *et al.*, 1981, Donnelly *et al.*, 1985) The large internal air space results in excess water loss from the leaves. Stomatal density is also greater in leaves of *in vitro* plantlets than in leaves of acclimatized or green-house grown plants.

In cauliflower, the palisade mesophyll and palisade cells were found to be limited in the *in vitro* grown leaves (Grout and Aston, 1978). In the case of plum plantlets, the

palisade cell depth and mesophyll air space were significantly less in the in vitro grown leaves than in the field grown leaves (Brainerd et al., 1981)

Grout and Aston (1978) reported that cauliflower plants in in vitro has lower levels of chlorophyll and carbondioxide fixation activity than seedlings of comparable age. Under in vitro conditions, micropropagated plants are not photoautotrphic but mixotrophic or heterotrophic. Leaves existing in culture made only a small or negative photosynthetic contribution following transplanting and the first new leaves formed after transplanting had an intermediate capacity (Donnelly and Vidaver 1984), only new leaves initiated following transfer to soil had full photosynthetic competence and successful acclimatization was dependent on such leaves.

Grout and Aston (1978) observed that the transition zone between shoot and root was abnormal in micropropagated cauliflower shoots. According to Sutter (1981) a continuous vascular connection between the shoot and root was critical for efficient water flow for reducing the mortality during stress conditions. In vitro grown plantlets, when much callus was produced at the shoot base, roots often originated from the callus and were not strongly connected to shoots. The vascular connection between roots and shoots was found to

be proper when the callus production was the minimum at the shoot base (Cheng and Voqui, 1977; Arnold and Eriksson, 1984; Patel et al., 1988).

Roots, that are produced in agar are hairless, easily damaged on transfer and have limited ability to function in composts (Wainwright, 1988). Debergh and Maene (1981) found that in vitro produced roots die soonafter transfer and new in vivo adopted roots are quickly produced to sustain the plant in the non sterile environment. Therefore, rooting directly in vivo is preferable on economic as well as physiological grounds, however greater environmental control can be maintained in tissue culture, and with the more detailed sequence of auxin treatments that some of the more difficult to root species requires, thin roots initiation at least (Zimmerman and Fordham, 1985) in tissue culture.

2.3 Measures to overcome the in vitro problems in ex vitro establishment of plants

The most important factor controlling the success rate during the transition of plants or shoots from in vitro to in vivo condition is the intrinsic quality of the plant material (Debergh, 1991). The environment in the in vitro condition is at very high humidity, low light level and usually constant temperature. Plants leaving these

environment are very poorly adapted to resist the low relative humidity, higher light levels and more variable temperature found outside. In order to adjust with the outside conditions different types of in vitro interventions practiced.

2.3.1. *Control of high humidity in vitro*

Attempts to facilitate hardening-off prior to propagules leaving the culture environment have involved reducing the relative humidity inside the culture vessel by increasing ventilation or increasing the osmotic strength of the media (Short et al., 1987). In almost hermatically closed containers the water retention capacity can be controlled by applying a paraffin layer on top of the medium (Wardle et al., 1983) Short et al., (1987) observed that addition of Polyethylene glycol (PEG) in rooting medium help ex vitro establishment. PEG reduced the humidity with in the culture vessel which in turn caused wax depositions on the leaves.

Dillen and Buysens (1989) developed a modified plastic lid to escape water vapour from the container. They used autoclavable paper under the perforated polycarbonate lid, and could stop evaporation after a while by sealing the holes in the lid.

2.3.2. *Improving photosynthetic behaviour*

Reducing the sucrose levels in the culture medium has increased the photosynthetic ability of rose leaves in culture (Langford and Wainwright 1987), but maintaining sucrose levels or even increasing these levels in the culture medium prior to weaning has in practice been shown to be beneficial in optimizing microplant size after hardening off (Hainwright and Scrace, 1988).

Kozai (1990) proposed photoautotrphic micropropagation based on photoautotrphic tissue culture with all carbon derived from carbondioxide. In this type of culture the growth and development are largely influenced by the physical and environmental factors which include light and CO_2 as major components.

2.4 Rooting

The propagules can be rooted when in tissue culture (roted in vitro) and the rooted plantlet transferred to soil, alternatively the propagule can be treated like a soft-wood cutting and rooted in a non-sterile, high humidity, low-light environment.

2.4.1 *In vitro rooting*

There are different techniques to induce roots in vitro, but differ with plant species. An optimum amount of carbohydrate is required for achieving maximum in vitro rooting in Jack fruit (Rahman and Blake 1988). Meiraziv (1979) reported that in vitro propagated gladiolus plantlets subcultured to a pre-transplanting medium with lower levels of nutrients promoted root development and increased ex vitro establishment percentage.

Hainwright and Scrace (1989) studied the effect of sucrose concentration and the type of of carbohydrate in in vitro plant growth. Two to four per cent sucrose concentration gave the maximum shoot lehgth, fresh weight, and dry weight of plantlets and registered 97.5 per cent in vitro establishment. Among the different carbohydrates tested, sucrose, glucose and maltose were on par, while sorbitol was the least effective.

Desjardins and Tiessen (1985) observed that very low sucrose concentration in the medium reduced the rooting percentage. At higher sucrose concentration the rooting percentage and subsequent shoot growth were better and the time required for rooting was found to be reduced.

Leshem (1983), Marin and Gella (1987) and Short et al., (1987) reported that higher concentration of agar in rooting medium increased the ex vitro establishment of plantlets; but reduced the rooting. Williams and Taji (1989) reported that higher concentrations of gelrite increased the field establishment of plantlets.

The type of auxin used for rooting has been found to influence root morphology and plant survival. Williams and Taji (1989) reported that when NAA and NOA were used, the roots produced were thin, IBA produced thicker roots which reduced the establishment of the plantlets during transplanting.

Renjit and Kester (1988) reported that GA at lower concentrations improved the rooting of tissue cultured cherry root stocks. However, rooting did not occur in the absence or at higher concentration of GA.

2.4.2 *Ex vitro rooting*

The rooting directly in ex vitro is preferable on economic as well as physiological grounds. It will reduce cost of production because it combines the two steps of stage 3 of micropropagation. In vitro produced roots become non-functional after the plantlets were transferred to potting mixture or soil (Debergh and Maene 1981) so physiologically

also it is beneficial. Various techniques are developed for ex vitro rooting.

2.4.2.1 Two-step process

Here first the microcuttings inserted in a root initiation medium then transferred to potting mixture. Micropropagated shoots of apple first inserted in a liquid medium containing sucrose and auxin to induce root initiation, 3-7 days after that shoots transferred to a potting mixture cause elongation of initiated roots and additional roots also developed (Zimmerman and Fordham 1985).

An even simple technique is to add a layer of liquid medium over the agar surface in proliferating cultures. With this pre-treatment, in vitro proliferated shoots of Magnolia soulangeana were successfully rooted in in vivo (Maene and Debergh 1985a, 1985b). Interestingly, adding sterile water alone increased rooting just as much as adding an auxin solution, a sucrose solution, or a combination of auxin and sucrose. The technique worked particularly well with several species of herbaccous plants.

2.4.2.2 One - step process

It is similar to conventional cutting propagation simply treating the cut basal ends of micropropagated shoots

with an auxin carried on talc powder before inserting them into a rooting medium or plug and place them under mist or high humidity conditions worked successfully with blackberry (Broome and Zimmerman 1978), blueberry (Zimmerman and Broome, 1980b) and apple (Zimmerman and Broome 1980c; Simmonds 1983).

Root initiation occurs so readily on microcuttings of some crops that no auxin treatment is required, eg. blackberry (Zimmerman and Broome 1980 a), gardenia (Economu and Spanoudake, 1985) and many clones of azalea and rhododendron (Mc Cown and Lloyd, 1983; Economou and Read 1986 a, 1986 b, Ettinger and Preece, 1985).

2.4.2.3 Plug system

In one-step process and two-step process due to invariable distribution of roots, disturbance and damage of root is more during further transplanting. To minimise this plug-systems are now used. A rigid tube like container made of biodegradable substance like paper folded with appropriate potting mixture, after rooting the whole container can be planted and uniformity of rooting is not so critical to success (Mc Cown 1986, Standardi and Catalano, 1984) but this method depends upon the root system of the plants to develop sufficiently to form a stable plug for transplanting. These plug system provide the growing environment during rooting, acclimatization and the early growth of rooted cuttings.

2.5 Acclimatization

Acclimatization is the terminology used to indicate adaptation of plantlet from in vitro conditions to ex vitro conditions in the green house or in the field (Donnelly and Vidaver 1988; Preece and Sutter, 1990). It is also called 'weaning' or 'hardening-off'.

Acclimatization environment is the major contributing factor to successful establishment of micropropagules. Control of environmental factors like humidity, temperature, light, gaseous exchange etc., are essential, for this modified glass house environment is usually used. (Wardle et al., 1983, Brkowska 1984; Desjardins et al., 1987).

2.5.1 *Humidity*

The largest single factor that results in the poor post-transfer growth and survival of in vitro raised plants is the drop in relative humidity from near hundred per cent in the culture vessels to much lower values in the glass house or in the field. (Grout and Aston 1977., Wetizstein and Sommer, 1982). Since plantlet lack effective stomatal closure mechanism, waterloss will be high at lower relative humidity leading to increased field mortality (Donnelly et al., 1987).

Three major methods of controlling relative humidity are the polythene tent, misting and fogging. Poole and Conover (1983) found that in order to provide humidity, intermittent misting of the plantlets was better than growing them under tents. The increased growth observed under misting, might be due to the increased availability of light. However, Sutter and Hutzell (1984) reported that the use of humidity tent was advantageous. Ramesh (1990) found that individual pots covered with polythene cover is the best device to control humidity in establishment of jack plantlets.

The advantages of fogging outlined by Press (1983) include greater flexibility in relative humidity control.

2.5.2 *Temperature*

The effect of temperature on ex vitro establishment of plantlets has been worked out in many crops. The optimum temperature range depend on the crop. Tropical crops requires a temperature of 30 ± 2°C. For the subtropical crops, 27 ± 2°c and for temperate crops, 25°c or above were found lethal (Hughes, 1981, Appelgren and Heide, 1972).

2.5.3 *Light*

In order to minimise shock to the plantlets during acclimatization light intensity should be kept low at first

and then increased gradually (Dunstan and Turner 1984). Supplementary lighting during ex vitro establishment increased the shoot growth and dry weight of tissue cultured strawberry plantlets (Desjardins et al., 1987).

Read and Economou (1982) reported that quality of light influenced the rooting of micro cuttings raised in vitro, in azaleas, the rooting of micro cuttings was promoted when the shoots were cultured under far-red light for two weeks prior to planting them in appropriate rooting medium.

2.5.4 *Gaseous levels in the growth chamber*

Lakso et al. (1986) found that in carbondioxide enriched environment dry weight of micro cuttings increases, root growth and leaf area also improved drastically. Desjardins et al. (1987) observed that carbondioxide enriched environment had no effect during the early period of establishment in strawberry, but net assimilation rate was found significantly increased after 20-30 days. Reuther (1986) found that in vitro plants did not respond to carbondioxide concentration during their initial post transplanting period.

There were certain other physical components which has considerable influence on ex vitro establishment of plantlet produced through tissue culture. They include size

of plantlets, type of potting media, type of container and interaction of media and containers.

2.6 Microcutting size

The size of microcuttings at the time they were planted had a significant effect on survival. Zhang and Sholtz (1989) reported that survival rate increased with microcutting length in the case of Euphorbia fulgens plantlets. Microcuttings of E fulgens $\geq$ 31 mm long survived at a rate 4.7 fold higher than those $<$ 31 mm. However, Poole and Conover (1983) reported that plant size of dieffenbachia propagules does not influence survival rate, but longer plants performed better than smaller ones.

Ramesh (1990) reported that 3.0 cm, long shoots (with three to four leaves) of jack plantlets were ideal for rooting and recorded 100 per cent rooting, (5.5 roots per shoot) and good rooting intensity.

2.7 Potting media

The potting medium used for potting the in vitro produced plantlets has been observed to be an important factor determining ex vitro establishment. Anderson (1980) reported that thorough washing of the plantlets to remove the

traces of nutrient medium and sterilising the potting mixture eliminated serious problems of fungal infection.

Geier (1990) observed that Anthurium andreanum plantlets bearing at least two well developed roots can be established without loss in a peat/sand mixture interspersed with styrofoam granules. Devi (1992) found that the most suitable potting medium for ex vitro establishment of dendrobium plantlets was a mixture of brick and charcoal pieces (1:1 v/v).

Damiano (1979) reported that either pure peat or a mixture of 1:1 sand and peat was suitable as the potting medium for the ex vitro establishment of strawber plantlets. The pH of the medium had to be regulated between 5.6 and 7.0. Mallika et al., (1992) observed that in vitro rooted plantlets of cocoa showed high survival percentage in the ex vitro condition in a potting mixture consisting of sterilised soilrite and soil.

Vermiculite and sand (1:1 v/v) mixture was identified as the best medium for planting out the plantlets of cardomom, supporting 91.7 per cent survival. (Reghunath 1989). Bhasker (1991) found that the most suitable potting medium giving maximum plant survival percentage (90.0) of banana was 1:1 (v/v) mixture of vermiculite and sand.

Kyte and Briggs (1979) observed that a porous potting mixture of sterile peat, perlite and composted barks in 1:1:1 ratio was the best for rooting tissue cultured rhododendrons. In the case of guava plantlets, when a mixture of sterile soil, sand and compost in 3:3:1 ratio was used for planting, only 10 per cent of the plantlets survived (Nair et al., 1983).

Mieraziv (1987) found that the most suitable substrate for ex vitro establishment of carnation plantlet was peat + perlite (1:1 or 1:2) followed by peat + perlite + sand (1:1:1). The rooting rate of rose plantlets in cellulose, sand, expanded clay, perlite, vermiculite, Florafort (peat) and TKS-1 (peat)with sucrose was 100, 100, 20, 85, 100, 15 and 80 per cent respectively (Aldrufeu 1984). Tan (1983) successfully transplanted in vitro rooted plantlets of Bougainvillea glabra to pots containing vermiculite and supplied with quarter strength liquid MS after further development they were transplanted to soil.

Nathan et al. (1992) reported that Heliconia psithacorum plantlets transplanted to pots containing a medium of vermiculite and peat (1:1) and placed under high humidity and low light intensity recorded 90 per cent survival after 4 weeks. Anderson (1984) found vermiculite to be suitable for rooting nephrolepis plantlets. Pena and

Biutrago (1984) reported that sterilised slag medium was ideal for planting coffee plantlets.

The survival rate of asparagus plantlets in sterile vermiculite-sand mixture (2:1 ratio), vermiculite-sand-peat mixture (2:1:2 ratio) and garden loam was 60.0, 57.1 and 78.9 per cent, respectively (Li.1985). The lower survival rate observed in the two vermiculite mixture was due to greater water accumulation and incidence of disease.. But Smith et al., (1989) observed highest survival percentage of euonymus plantlets when transferred to pots containing vermiculite.

In vitro rooted plantlets of African violet were successfully transplanted to pots containing 2:1 mixture of sphagnum peat and soil (Ioannou, 1987).

Reuther (1986) reported that sterile granular rock wool was a better potting medium for asparagus, gerbera, pelargonium and saintpaulia plantlets. In pelargonium, 95 per cent survival of plantlets resulted when perlite medium was used as potting medium (Aldrufeu 1987).

Drew (1988) reported that 90 per cent of papaya plantlets survived when a mixture of sterile peat, perlite and polystrene beads (1:1:1 ratio v/v) was used as the potting medium. Pandey and Rajeevan (1987) found that 80 per cent of papaya plantlets were survived when planted in 1:1 mixture of sand and farm yard manure. Good survival of

rooted papaya plantlets (85 per cent) with small roots or root initials were observed when transferred to containers (4x4 cm) with a medium mix containing peat and polysterine sheredded flakes (1:1 v/v) and kept for 2-3 weeks in humid chamber (Reuveni et al., 1990). Ramesh (1990) observed that sand was the best potting medium among the ten media tried for jack plantlets.

2.8 Container

The influence of containers on ex vitro establishment of tissue cultured plantlets had studied to a limited extent so far. The type and size of container had considerable influence on ex vitro performance of plantlets. Yang and Clore (1974) found that asparagus plantlets survived far better when potted up in soil if they were first transplanted into Jiffy-7 peat pots and grown under intermittent mist for 5-8 days, rather than transferred successively into 10 and 16 cm diameter pots with soil. Lloyd et al., (1988) observed that transplantation of rose plantlets to soil was improved by rooting plantlets in cellulose plugs and transferring plantlets to soil while still in the plugs.

Kyte and Briggs (1979) reported that the depth of soil was important as the survival rate of tissue cultured

rhododendrons were found to be better, in 10 cm pots than in shallow trays. Tubular polythene bags of 15 cm diameter and 20 cm length were identified as the best container for ex vitro survival of banana plantlets (Bhaskar, 1991).

Ramesh (1990) found that plastic pots (5.0 x 5.0 x 7.5 cm size) was the best container for ex vitro survival of jacks plantlets.

2.9 Interaction of potting media and containers

Le and Collet (1981) reported that the stage of rooting (in beakers on an agar medium) and weaning on to a horticultural substrate were successfully combined by transferring microcutting of African violet from testubes directly to plastic boxes containing sterilised white peat or vermiculite and supplemented with distilled water and after two weeks with 80 per cent strength MS medium.

Skirvin and Chu (1979) successfully transferred rooted plantlets of rose directly from the culture tubes to clay pots containing vermiculite. The rooted plantlets of salicornia transplanted into 0.6 liter plastic pots containing a mixture of 1:1:1 peat, vermiculite and perlite were survived better inside a humid polythine tent in the greenhouse. (Chiwon, [illegible], 1992).

Bunn and Dixon (1992) observed that pineapple li
plantlets transferred to 100 ml black plastic pots containi
equal volumes of peat that was pasteurized at 70 c for 1
and unpasteurised perlite recorded 69.8 per cent ± 8.5 p
cent rooting after two months.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

Investigations on suitability of media and containers for better establishment of Anthurium andreanum plantlets produced in the Plant Tissue Culture Laboratory, Department of Horticulture, College of Agriculture, Vellayani were carried out during 1991-1993.

The materials and methods used for in vitro production of anthurium plantlets, in vitro treatments to improve the efficiency of rooting and the ex vitro treatments for standardisation of media and containers to increase the field establishment of plantlets and to minimise the cost of production of tissue culture plants of anthurium have been described.

3.1. In vitro production of anthurium plantlets

Method of in vitro propagation through callus mediated somatic organogenesis of leaf tissues for A andreanum had already standardised (Pierik 1976, Pierik et al 1979). This technique was further modified by Sreelatha (1992). The same method was used for producing anthurium plantlets to supply the required number of plantlets for the experiment.

The chemicals used were of analytical grade from British Drug House (BPH), Sisco Research Laboratories (SRL),

PLATE 1 Callus initiating from the leaf segments

PLATE 2 Callus differentiating to plantlets


1


2

Merck or Sigma. Standard procedures (Biondi and Thorpe, 1981.) were adopted for preparation of the media. The pH of the media was adjusted between 5.6 and 5.8. Borosil brand testubes of O.D and length (mm) of 25x100 and glass bottles (excelbrand) of 250 ml were used. Sterilization of the media and glasswares were done at 15 psi for 20 minutes. All aseptic manipulations were carried out in laminar airflow chamber.

Young leaves, three to four days after unfurling (1/2-2/3 of its final length) were excised from adult plants and used as explant source for in vitro multiplication. The leaves were washed with sterile water 3-4 times, then with wetting agent 'Laboline'. Again the explants were treated with fungicide solution (0.1 per cent Bavestin) for 30 minutes. The leaves were then surface sterilized with 1 per cent sodiumhypochlorite for 20 minutes and rinsed 5-6 times with sterile distilled water. Leaf discs of 1-1.5 cm^2 with at least one main vein were made from the basal portion of the leaves using sterile blade. The explants were then transferred to the callus initiation media.

For callus initiation modified MS medium developed by Pierik 1976 (appendix-II) was used. Composition of media used for various stages of in vitro production of anthurium plantlets are given in Table-1.

Table-1. Composition of media used for various stages of *in vitro* production of *Anthurium andreanum* plantlets.

No.	Stages of *in vitro* production	Composition of medium
1	Callus initiation	Modified MS medium supplemented with 2, 4-D 0.08 mg/l, BA-1.0 mg/l, sucrose 30 g/l and agar 6 g/l.
2	Callus multiplication	MS (macro elements 1/4) supplemented with BA 1.0 mg/l, sucrose 30 g/l and agar 6 g/l.
3	Spront regeneration	MS medium supplimented with IAA 2.0 mg/l, BA 0.5mg/l, sucrose 30 g/l and agar 6 g/l.
4	Shoot proliferation growth and rooting	Medium 3

Cultures were incubated at 26±2°C, relative humidity ranging from 55 to 65 percent with 16h photoperiod (40 μ $Em^{-2}s^{-1}$) except in the cases were complete darkness was required. Cultures for callus initiation and callus multiplication were kept in darkness.

PLATE 3 Shoot and leaf regeneration from callus

PLATE 4 Shoot proliferation


3


4

Sprout regenerated from the callus were sub cultured after 30 days. Intact plantlets were used for raising individual plants. The remaining portion along with the undifferentiated callus was subjected for repeated multiplication process for maintaining a stock of multiple shoots. Shoots regenerated via somatic organogenesis rooted spontaneously. However inorder to improve the rooting efficiency and establishment percentage, various trials on in vitro rooting were carried out with basal MS medium.

3.1.1 *Size of shootlets on in vitro rooting*

The influence of the size of shoots on in vitro rooting was studied. The treatments involved shootlets of 1.0 cm (with single leaf), 2.0 cm (with two leaves) and 3.0 cm (with two to three leaves) length.

3.1.2 *Standardisation of growth regulators for in vitro rooting*

Explants made from shoot proliferating cultures were used as the explant source. The various levels of auxin and cytokinin tried for rooting of anthurium shoots were listed in Table-2.

Table-2. Levels of auxin and cytokinin used for root induction in anthurium plantlets *in vitro*

Basal medium-MS

Treatment No	Treatment	Level
T_1	BA+NAA	0.5 ppm + 1.0 ppm
T_2	BA+NAA	0.5 ppm + 0.5 ppm
T_3	BA+NAA	0.5 ppm + 0.2 ppm
T_4	BA+IAA	0.5 ppm + 1.0 ppm
T_5	BA+IAA	0.5 ppm + 2.0 ppm
T_6	BA+IAA	1.0 ppm + 1.0 ppm

3.1.3 *Standardisation of agar level for in vitro rooting*

The effect of various levels of agar (0.4,0.5,0.6,0.7 and 0.8 per cent) on rooting of anthurium shoots excised from shoot proliferating cultures were studied.

3.1.4 *Effect of various levels of sucrose for in vitro rooting*

The effect of five levels of sucrose (1.5,3.0,4.0,4.5 and 5 per cent) on rooting of anthurium shoots from shoot proliferating cultures were studied.

Observations on the number of days taken for root initiation, number of roots produced, average length of roots and nature of roots produced were recorded 20 days after root initiation.

3.2 Ex vitro establishment

Inorder to standardise suitable growing media and containers for ex vitro establishment of anthurium plantlets five media and five containers were selected for the experiment

3.2.1 *Media*

a. Sand

(i) Coarse (ii) Fine

Good quality coarse riversand without clay was washed with tapwater 3-4 times. The fine sand particles were then seived through 1 mm seive. The fine and coarse sand were taken separately, dried and sterilised at 15 psi for 20-25 minutes.

b. Charcoal

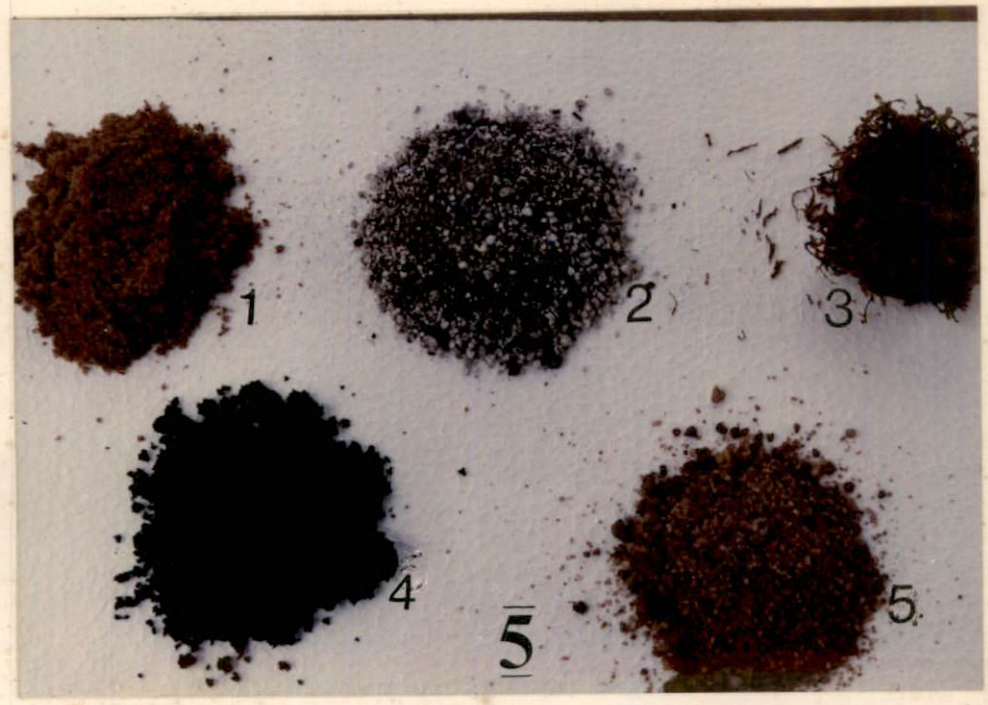
Freshly burnt hard wood charcoal was broken into small gravel size. The charcoal powder were then sterilised at 15 psi for 20-25 minutes.

c. Soil rite

Soilrite is a mixture of irish peatmoss, expanded perlite and exfoliated vermiculite with good aeration and

PLATE 5 Media used in the experiment
1. Fine sand 2. Soilrite 3. Sphagnum moss
4. Charcoal powder 5. Coarse sand

PLATE 6 Containers used in the experiment
1. Pot tray with netted pot inside 2. Plastic pot
3. Polythene cover 4. Paper pot 5. Mud pot



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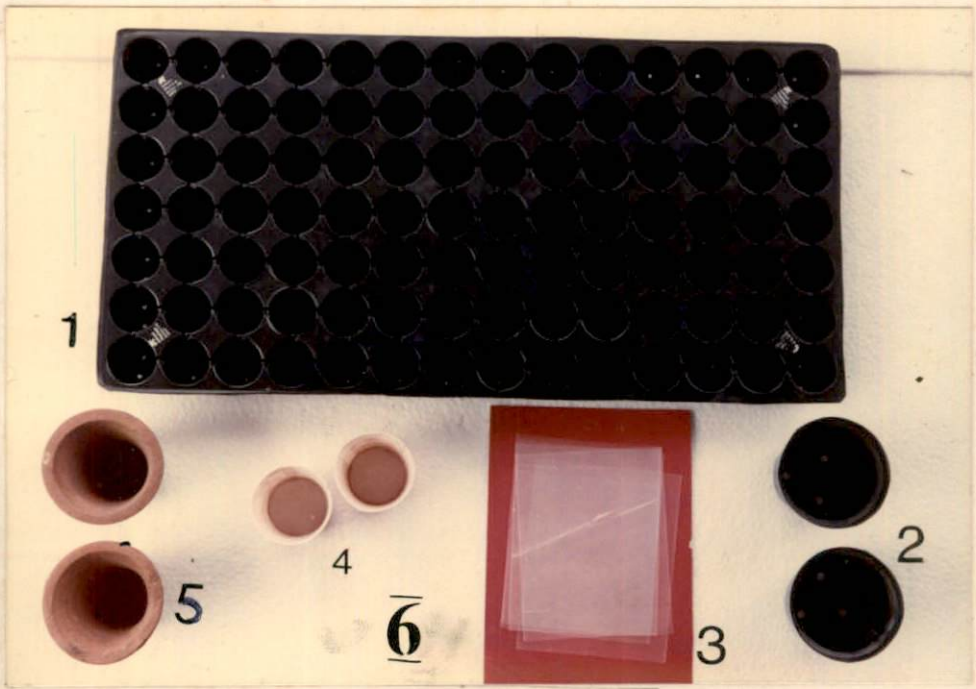

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water holding capacity. Commercial grade soilrite was purchased and sterilised at 15 psi for 20-25 minutes.

d. Sphagnum moss

Comercial sphagnum moss is the dehydrated young residue of living portion of acid-bog plants in the genus Sphagnum such as S. papillosum, S. capillaceum and S. palustre. Good quality dry sphagnum moss was collected and cleaned by removing debris and treebark and used as such.

3.2.2 *Containers*

a. Mudpot

Round clay pot of 3" diameter with good drainage holes at bottom.

b. Plastic pot

Round plastic pot of 3" diameter with drainage holes at bottom

c. Polythene cover

Polythene cover (200 gauge) of 3" diameter with holes at bottom.

d. Paperpot

Round paperpot of 2" diameter with holes at bottom.

e. Plastic trays with netted pot 1" diameter

The five media and the five containers constituting 25 treatment combinations were tried for the present investigations.

PLATE 7 Plants arranged inside the hardening chamber

PLATE 8 Plants in containers arranged treatment wise


7


8

3.2.3 *Treatments*

Following were the 25 treatments tried.

1. C_1M_1 Mud pot- coarse sand
2. C_2M_1 Plastic pot-coarse sand
3. C_3M_1 Paper pot-coarse sand
4. C_4M_1 Polythene cover-coarsesand
5. C_5M_1 Netted pot-coarsesand
6. C_1M_2 Mud pot-finesand
7. C_2M_2 Plastic pot-finesand
8. C_3M_2 Paper pot-finesand
9. C_4M_2 Polythene cover-finesand
10. C_5M_2 Netted pot-finesand
11. C_1M_3 Mud pot-charcoal
12. C_2M_3 Plastic pot-charcoal
13. C_3M_3 Paper pot-charcoal
14. C_4M_3 Polythene cover- charcoal
15. C_5M_3 Netted pot- charcoal
16. C_1M_4 Mud pot-soilrite
17. C_2M_4 Plastic pot-soilrite
18. C_3M_4 Paper pot-soilrite
19. C_4M_4 Polythene cover-soilrite
20. C_5M_4 Netted pot-soilrite
21. C_1M_5 Mud pot-sphagnum moss
22. C_2M_5 Plastic pot- sphagnum moss
23. C_3M_5 Paper pot- sphagnum moss
24. C_4M_5 Polythene cover-sphagnum moss
25. C_5M_5 Netted pot-sphagnum moss

3.2.4 *The experimental design*

The design used for the experiment was completely randomised design in5x5 factorial experiment. For each treatment ten plantlets were used.

3.2.5 *Preparation of plantlets for transplanting*

In vitro rooted lumps of plantlets were taken out of the bottle and carefully separated each other using forceps. The roots were carefully washed with sterile tap water until they were free of agar. The plantlets were then treated with 0.1 per cent Bavestin for 15 minutes and singled out from the shoot cluster with roots. The uniform sized plants were selected from the lot and used for the study.

3.2.6 *Potting of plants*

The pots were filled with the respective potting media according to the treatments. The plantlets were planted at the centre of the pots. The plants were irrigated with distilled water till water oozes from the drainage hole.

3.2.7 *Humidity control*

A tunnel like structure made by bricks on the side and a frame made by angle iron was used. The top of the

frame was covered with polythene sheets (350 gauge) to provide high humidity inside the chamber. Diffused sunlight was given by using synthetic high density polythene shade net (75 per cent shade). The container with the plants were arranged treatment wise inside the tunnel. The frame could be partially lifted to regulate humidity inside the chamber. The light intensity within the chamber during mid day was 25 μ $Em^{-2}S^{-1}$, the temperature range was 27 ± 2°c. High humidity was provided in the chamber during first week of transplanting by frequent spraying of water and completely closing the chamber. The humidity could be gradually reduced to ambient levels by reducing the water spray and slightly opening the frame.

3.2.8 *Cultural management*

The plantlets were irrigated daily with distilled water for the first one week, then with tap water. For the first two days after transplanting, complete shade was provided by covering the chamber with newspaper over the polythene sheet. A nutrient solution containing half concentration of MS mineral salts having a pH 5.7 was given as additional nourishment once in a fortnight.

3.3 Observations recorded

The following observations were recorded during the growth phase in humidity chamber upto two months after planting.

3.3.1 *Survival percentage*

Influence of the size of shoots on ex vitro establishment under different media and containers were observed. The treatments involved shoots of 1.5-2 cm (with one fully opened leaf and one root), 2-2.5 cm (with two fully opened leaf and one root) and 2.5-3cm (with 3-4 fully opened leaves and two or more roots) length. Observations on the number of plants survived and percentage of survival at weekly intervals were recorded upto one month.

Based on the result of survival percentage, the optimum plantlet size was fixed, and following observations were recorded on uniform size microcuttings transplanted. Five plants from each treatment were randomly selected for this purpose.

3.3.2 *Number of leaves*

The total number of fully opened leaves borne at fortnightly intervals was counted and recorded.

3.3.3 *Height of plants*

Height of plants were recorded at monthly intervals.

3.3.4 *Area of the new leaves*

Leaf area of the fully opened new leaves were computed at fortnightly intervals. The length and maximum width of leaves were measured separately and leaf area was computed based on length - breadth method.

The relationship between leaf area (Y) and the length (L) and breadth (B) of leaf was estimated from a sample of fifty leaves.

The relationship was found to be

$$Y = 0.9409\ L^{0.7241}\ B^{0.6897}$$

or

$$\log Y = -0.0609 + 0.7241 \log L + 0.6897 \log B$$

This relationship was utilised for estimating the leaf area.

3.3.5 *Petiole length*

Petiole length of mature fully opened leaves were recorded at fortnightly intervals.

3.3.6 *Number of root and root length*

Number of roots and average total root length of plants were recorded two months after planting at the time of transplanting into bigger pots.

3.4 Statistical analysis

The data generated from the study were subjected to analysis of variance (Panse and Sukhatme 1978).

RESULTS

4. RESULTS

Investigations were carried out to develop suitable methods to plant out in vitro generated plantlets of Anthurium andreanum and to standardise suitable containers and media for ex vitro establishment. The results of the studies are presented.

4.1 In vitro rooting studies

4.1.1 *Size of shootlets on in vitro rooting*

In vitro rooting in relation to size of shootlets were investigated. The minimum days for root initiation (10.24) and maximum number of roots/shoots (3.88) were observed in 3cm long shoots with three leaves (Table 3). The corresponding values were 10.66 and 3.68 for 2cm long shoots with two leaves and 12.62 and 2.18 for 1cm long shoots with one leaf.

4.1.2 *Strength of auxin and cytokinin on in vitro rooting*

The influence of cytokinin and auxin on in vitro rooting were studied. Combination of BA and IAA recorded minimum time for root initiation and have more number of roots per shoots than combination of BA and NAA (Table 4) BA 0.5ppm + IAA 2ppm recorded minimum time (11.4 days) for root initiation and maximum roots per shoots (3.88). The

Table 3. Effects of size of shootlets on *in vitro* rooting

Treatment No.	Treatment	Days taken for root initiation	*No. of roots/ shoots
T_1	1 cm shoot with one leaf	12.62	2.18
T_2	2 cm shoot with two leaves	10.66	3.68
T_3	3 cm shoot wth three leaves	10.24	3.88

* Average of six observations

Table 4. Effects of plant growth substances on *in vitro* rooting

Basal medium : MS

Treatment No.	Treatments	Days for root initiation	Root/ Shoot*	Remarks
T_1	BA 0.5 ppm + NAA 1.0 ppm	18.00	2.88	Small thick roots of 1.5 - 2 cm
T_2	'' + NAA 0.5 ppm	14.40	2.72	Small thick roots of 1.5 - 2 cm
T_3	'' + NAA 0.2 ppm	12.62	2.82	Small thick roots of 2 - 2.5 cm
T_4	'' + IAA 1.0 ppm	11.80	3.68	Basal thick roots of 3 - 3.5 cm
T_5	'' + IAA 2.0 ppm	11.40	3.88	Fleshy long roots of 4 - 4.5 cm
T_6	BA1.0 ppm + IAA 1.0 ppm	14.26	3.52	Thin long roots of 4 - 4.5 cm

* Average of 6 observations

PLATE 9 Nature of root induction by IAA and BA treatment combinations

PLATE 10 Nature of root induction by NAA and BA treatment combinations


9


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combination of BA 0.5ppm and NAA 1ppm took maximum time (18 days) for root initiation, and number of roots per shoots was minimum in a combination of BA 0.5ppm + NAA 0.2ppm. Combination of BA and IAA produced long roots (3 - 4.5 cm) while BA and NAA combination produced short and thick roots of 1.5 to 2.5cm long (plate 9;10).

4.1.3 *Strength of agar on in vitro rooting*

The optimum concentration of agar on in vitro rooting of anthurium plantlets were carried out. Number of days for root initiation was minimum (10.54) when 0.7 per cent agar was used (Table 5). However the number of roots per shoot was maximum (4.2) when 0.4 per cent agar was used, which was found to reduce when concentration of agar was increased. More than 3cm long roots were produced when agar concentration of 0.7 to 0.8 per cent was used. However the root length was only less than 2cm when 0.4 per cent agar was used.

4.1.4 *Concentration of sucrose on in vitro rooting*

The role of sucrose concentration on in vitro rooting of plantlets was studied. The number of days for root initiation was 10.18 for 1.5 per cent, 12.32 for 3 per cent, 14.88 for 4 per cent,16.26 for 4.5 per cent and 17.88 for 5 per cent. Sucrose at 5 per cent level recorded maximum number of roots per shoots (4.52). While sucrose at 1.5 per

Table 5. Effects of concentration of agar on *in vitro* rooting

Basal medium : MS

Treatment No.	Treatments (per cent)	Days for root initiation	Root/Shoot*	Remarks
T_1	0.4	25.42	4.2	Slender roots of < 2 cm
T_2	0.5	20.36	3.6	Slender roots of 2 - 2.5 cm
T_3	0.6	14.58	2.96	Slender roots of 2 - 2.5 cm
T_4	0.7	10.54	2.98	Slender roots of more than 3 cm
T_5	0.8	12.58	2.96	Slender roots of more than 3 cm

* Average of 6 observations

Table 6. Effects of concentration of sucrose on *in vitro* rooting

Treatment No.	Treatments (per cent)	Days for root initiation	Root/ Shoot*	Remarks
T_1	1.5	10.18	2.28	Slender roots of 2.5 - 3 cm
T_2	3.0	12.32	4.22	Slender roots of 2.5 - 3 cm
T_3	4.0	14.88	4.28	Slender roots of 2.5 - 3 cm
T_4	4.5	16.26	4.50	Slender roots of 2 - 2.5 cm
T_5	5.0	17.88	4.52	Medium thick roots of < 2 cm

* Average of 6 observations

cent level recorded least number of roots/shoots (2.28). Length of roots were found to be reduced on increase of sucrose concentration in the medium. Concentration of 1.5 per cent,3.0 per cent and 4.0 per cent produced slender roots of 2.5-3cm length. While at 5 per cent level, thick roots of less than 2cm length were produced (Table 6).

4.2 Ex Vitro Establishment

Standardisation of media and containers

4.2.1 *Micro cutting size*

The influence of micro cuttings on survival of plantlets under different media and containers were studied. Microcuttings (2.5 - 3 cm long) with three to four leaves and two or more roots recorded highest survival percentage in all the twentyfive treatment combinations of media and containers, when observed one month after transplanting (Table 9). Out of the 25 treatments, twenty recorded 90-100 per cent survival. The lowest survival was recorded in the treatments C_3M_5 (Paperpot-sphagnum moss), C_2M_5 (plastic pot-sphagnum moss), C_4M_5 (polythene cover-sphagnum moss) and C_5M_5 (Nettedpot-spagnum moss) with 60,65,70 and 75 per cent establishment respectively.

The plantlets of 1.5 to 2cm long with one leaf and one root recorded lowest survival percentage (upto 40%) in

PLATE 11 Plants in different media inside a pot tray with netted pot after two weeks of planting out

PLATE 12 Plants in different media inside a pot tray with netted pot after four weeks of planting out

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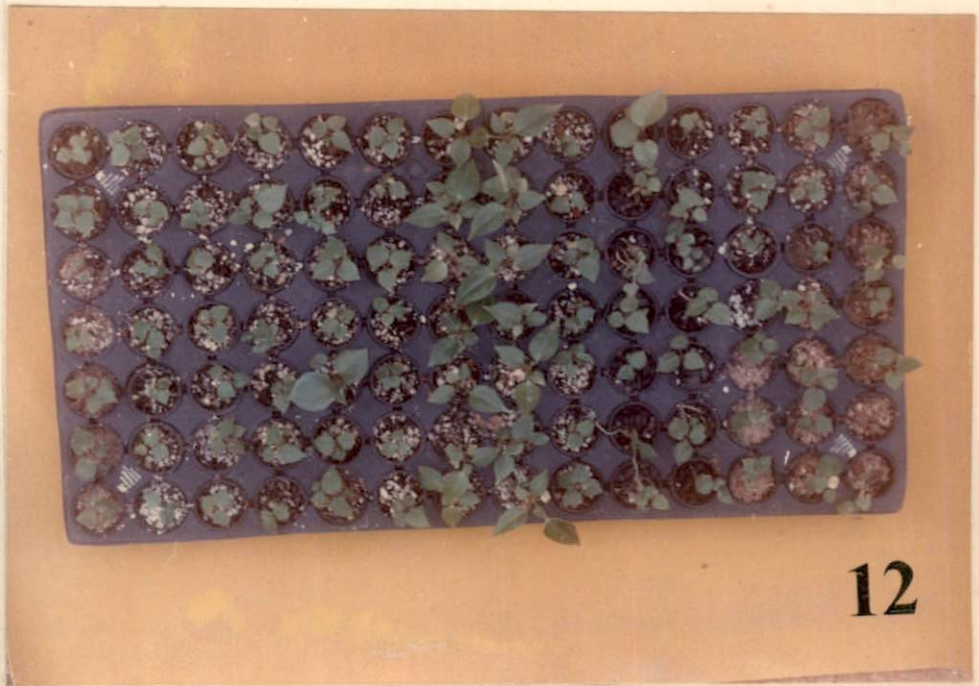

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all the twentyfive treatment combinations of media and containers when recorded at the fourth week of transplanting (Table 7).

The plantlets of 2 to 2.5 cm long with two leaves and one or two roots recorded varying responses to different treatment combinations of media and containers (Table 8). The treatments C_5M_2 (Nettedpot-fine sand) and C_5M_4 (Nettedpot-soilrite) recorded 90 per cent survival and C_5M_1 (Nettedpot-coarse sand) recorded 80 per cent survival. Among the various containers used, plants in the netted pots recorded highest survival percentage (90.0%), when observed four weeks after transplanting (plate 11;12). While plants in the medium M_5 (sphagnum moss) recorded lowest survival percentage (upto 40 per cent).

Microcuttings (1.5 - 2 cm long) with one fully opened leaf and one root showed significant differences under various media and containers. During the first week after transplanting (Table 7.1) the plants grown in the container C_1 (mud pot) recorded highest survival rate (33.65) which on par with C_5 (netted pot). The plants in C_4 (polythene cover) recorded least survival rate (15.46). Among the media plants grown in M_1 (coarse sand) recorded highest survival rate (46.12) at the first week of transplanting which was

significantly superior to other containers. The least survival rate (5.21) was on M_3 (charcoal) grown plantlets.

In the second week of the transplanting (Table 7.2) plants grown in the container C_5 (netted pot) recorded highest survival value (24.68) which was superior to all other containers. The lowest survival value (15.46) was recorded on plants in the containers C_4 (polythene cover). In the case of media plants in M_2 (fine sand) recorded highest survival value (32,30) which was superior to all other media. Plants in the media M_3 (charcoal) recorded least survival rate (00.00).

In the third week after transplanting (Table 7.3) also plants grown in the containers C_5 (netted pot) recorded highest survival rate (24.68) which was superior to all other containers. The lowest survival rate (7.84) was on the container C_4 (polythene cover). Among the media used plants in M_2 (fine sand) recorded highest survival rate which was superior to all other media. The least survival value (00.00) was on the media M_3 (charcoal).

The plantlets of 2 to 2.5 cm long with two fully opened leaves and one or two roots had significant influence on survival of plantlets in different media and containers. At one week after transplanting (Table 8.1) survival rate was

maximum (67.93) on C_5 (netted pot) grown plants which was superior to all other containers and least survival rate (48.49) was on C_2 (plastic pot) grown plants. In the case of media plants in M_2 (fine sand) recorded highest survival rate (63.54) which was on par with M_1 (coarse sand) and M_4 (soil rite).

In the second week after transplanting (Table 8.2) C_5 (netted pot) grown plants recorded highest survival rate (58.92) which was significantly superior to other containers. The lowest value (37.06) was on containers C_3 (paper pot). Among the media soil rite grown plants showed highest survival rate (53.8) which was on par with M_2 (fine sand). The least survival value (29.77) was on M_5 (sphagnum moss) grown plants.

At the third week after transplanting (Table 8.3) the plants in the containers C_5 (netted pot) recorded highest survival rate (57.77) which was superior to all other containers. The least response (29.45) was on C_3 (paper pot) grown plants. In the case of media the plants in M_4 (soil rite) recorded highest survival rate which was on par with M_2 (fine sand) and the least survival rate (23.70) was on the media M_5 (sphagnum moss) at the third week of transplanting.

At the fourth week of transplanting (Table 8.4) plants in the container C_5 (netted pot) recorded highest survival rate (57.70) which was superior to all other containers. Plants in the container C_3 (Paper pot) recorded lest survival rate. In the case of media M_4 (soil rite) grown plants recorded highest survival rate (53.8) which was on par with M_2 (fine sand) and the lowest response (23.70) was on the media M_5 (sphagnum moss).

Microcuttings (2.5 - 3 cm long) with 3 to 4 leaves and two or more roots also recorded varying response with different media and containers at weekly intervals in the case of survival of plantlets.

At the first week of transplanting (Table 9.1) plants in the containers C_1 (mud pot) recorded highest survival rate (87.90) which was superior to all other containers. The lowest survival rate (78.13) was on C_5 (netted pot) grown plants. Among the media M_1 (coarse sand) grown plants showed highest survival rate (90.00) which was superior to all other media and the plants grown on M_5 (sphagnum moss) recorded least survival value (65.65).

Second week after transplanting (Table 9.2) C_1 (mod pot) grown plants recorded highest survival rate (84.71) which was superior to all other containers. The lowest

survival rate (72.09) was on C_5 (sphagnum moss) grown plants. In the case of media M_1 (course sand) grown plants recorded highest survival (90.00) which was significantly superior to all other media. C_5 (sphagnum moss) grown -plants recorded least survival rate (58.45).

Third week after transplanting (Table 9.3) C_1 (mud pot) grown plants recorded highest survival rate (84.70) which was superior to other containers. Plants grown on C_5 (netted pot) recorded least survival rate. In the case of media M_1 (coarse sand) grown plants recorded highest survival rate (87.41) which was superior to all other media. The plants grown on C_5 (sphagnum moss) showed least survival value (56.97).

If the fourth week after transplanting (Table 9.4) Plants in the containers C_1 (mud pot) recorded highest survival rate (84.71) which was superior to all other containers. The least survival rate (68.0) was on the containers C_5 (netted pot). As in the case of third week the media showed same response in the fourth week also with highest survival rate (87.41) was on M_1 (course sand) grown plants which was superior to all other media and the least survival rate was on M_5 (sphagnum moss) grown plants.

Table 7. Effects of microcutting size on survival of plantlets under different media and containers (1.5 - 2 cm height with one fully opened leaf and one root)

Treatments	I Week No. of plants survived	per cent*	II Week No. of plants survived	per cent	III Week No. of plants survived	per cent	IV Week No. of plants survived	per cent
C_1M_1	8	80.0	4	40.0	2	20.0	0	00.0
C_2M_1	6	60.0	5	50.0	3	30.0	3	30.0
C_3M_1	4	40.0	2	20.0	0	00.0	0	00.0
C_4M_1	6	60.0	6	60.0	4	40.0	4	40.0
C_5M_1	2	20.0	0	00.0	0	00.0	0	00.0
C_1M_2	4	40.0	4	40.0	3	30.0	2	20.0
C_2M_2	4	40.0	2	20.0	2	20.0	2	20.0
C_3M_2	5	50.0	4	40.0	4	40.0	4	40.0
C_4M_2	2	20.0	2	20.0	0	00.0	0	00.0
C_5M_2	6	60.0	4	40.0	4	40.0	4	40.0
C_1M_3	2	20.0	0	00.0	0	00.0	0	00.0
C_2M_3	0	00.0	0	00.0	0	00.0	0	00.0
C_3M_3	0	00.0	0	00.0	0	00.0	0	00.0
C_4M_3	0	00.0	0	00.0	0	00.0	0	00.0
C_5M_3	0	00.0	0	00.0	0	00.0	0	00.0
C_1M_4	4	40.0	0	00.0	0	00.0	0	00.0
C_2M_4	5	50.0	2	20.0	0	00.0	0	00.0
C_3M_4	2	20.0	2	20.0	2	20.0	2	20.0
C_4M_4	0	00.0	0	00.0	0	00.0	0	00.0
C_5M_4	5	50.0	5	50.0	5	50.0	4	40.0
C_1M_5	0	00.0	0	00.0	0	00.0	0	00.0
C_2M_5	0	00.0	0	00.0	0	00.0	0	00.0
C_3M_5	0	00.0	0	00.0	0	00.0	0	00.0
C_4M_5	0	00.0	0	00.0	0	00.0	0	00.0
C_5M_5	5	50.0	4	40.0	4	40.0	4	40.0

* Average of 10 observations

Table 7. Effects of microcutting size on survival of plants under different media and containers (1.5 - 2 cm height with one fully opened leaf and one root)

7.1. At the first week of transplanting

	C_1	C_2	C_3	C_4	C_5	Mean M
M_1	80.69 (63.90)	60.00 (50.74)	39.83 (39.13)	60.00 (50.74)	19.13 (26.06)	(46.12)
M_2	39.86 (39.13)	40.00 (39.21)	50.00 (44.98)	20.00 (26.55)	60.14 (50.28)	(40.14)
M_3	19.31 (26.06)	00.00 (00.00)	00.00 (00.00)	00.00 (00.00)	00.00 (00.00)	(5.212)
M_4	39.86 (39.13)	50.00 (44.98)	20.00 (26.55)	00.00 (00.00)	50.00 (44.98)	(31.13)
M_5	00.00 (00.00)	00.00 (00.00)	00.00 (00.00)	00.00 (00.00)	50.00 (44.98)	(8.99)
Mean C	(33.65)	(26.99)	(22.13)	(15.46)	(33.37)	

Media	F (4,50) -	290.64^{**}	CD 3.07
Container	F (4,50) -	51.44^{**}	CD 3.07
Media x Container	F (16,50) -	38.03^{**}	CD 6.85

7.2. At the second week of transplanting

	C_1	C_2	C_3	C_4	C_5	Mean M
M_1	40.00 (39.21)	50.00 (44.98)	20.00 (26.55)	60.00 (50.74)	00.00 (00.00)	(32.30)
M_2	39.85 (39.13)	20.00 (26.55)	40.00 (39.22)	20.00 (26.55)	40.00 (39.21)	(34.13)
M_3	00.00 (00.00)	00.00 (00.00)	00.00 (00.00)	00.00 (00.00)	00.00 (00.00)	(00.00)
M_4	00.00 (00.00)	20.00 (26.55)	20.00 (26.55)	00.00 (00.00)	50.00 (44.98)	(19.62)
M_5	00.00 (00.00)	00.00 (00.00)	00.00 (00.00)	00.00 (00.00)	40.00 (39.22)	(7.84)
Mean C	(15.67)	(19.62)	(18.47)	(15.46)	(24.68)	

Media	F (4,50)	- 1229.69**	CD 1.21
Container	F (4,50)	- 77.70**	CD 1.21
Media x Container	F (16,50)	- 294.80**	CD 2.70

7.3. At the third week of transplanting

	C_1	C_2	C_3	C_4	C_5	Mean M
M_1	20.00 (26.55)	30.00 (33.19)	00.00 (00.00)	40.00 (39.21)	00.00 (00.00)	(19.79)
M_2	30.00 (33.19)	20.00 (26.55)	40.00 (39.21)	00.00 (00.00)	40.00 (39.21)	(27.63)
M_3	00.00 (00.00)	00.00 (00.00)	00.00 (00.00)	00.00 (00.00)	00.00 (00.00)	(00.00)
M_4	00.00 (00.00)	00.00 (00.00)	20.00 (26.55)	00.00 (00.00)	50.00 (44.98)	(14.31)
M_5	00.00 (00.00)	00.00 (00.00)	00.00 (00.00)	00.00 (00.00)	40.00 (39.22)	(7.84)
Mean C	(11.95)	(11.95)	(13.15)	(7.84)	(24.68)	

Media	F (4,50)	- 1278.70**	CD 0.85
Container	F (4,50)	- 454.31**	CD 0.85
Media x Container	F (16,50)	- 645.75**	CD 1.89

Table 8. Effects of microcutting size on survival of plantlets under different media and containers (2 - 2.5 cm height with 2 fully opened leaf and one or two root)

Treatments	I Week No. of plants survived	per cent*	II Week No. of plants survived	per cent	III Week No. of plants survived	per cent	IV Week No. of plants survived	per cent
C_1M_1	7	70.0	5	50.0	5	50.0	5	50.0
C_2M_1	6	60.0	5	50.0	5	50.0	5	50.0
C_3M_1	8	80.0	6	60.0	4	40.0	4	40.0
C_4M_1	6	60.0	4	40.0	3	30.0	3	30.0
C_5M_1	9	90.0	8	80.0	8	80.0	8	80.0
C_1M_2	6	60.0	6	60.0	4	40.0	4	40.0
C_2M_2	7	70.0	5	50.0	5	50.0	5	50.0
C_3M_2	8	80.0	6	60.0	6	60.0	6	60.0
C_4M_2	7	70.0	5	50.0	5	50.0	5	50.0
C_5M_2	10	100.0	9	90.0	9	90.0	9	90.0
C_1M_3	5	50.0	5	50.0	4	40.0	4	40.0
C_2M_3	6	60.0	3	30.0	3	30.0	3	30.0
C_3M_3	4	40.0	2	20.0	0	00.0	0	00.0
C_4M_3	6	60.0	5	50.0	5	50.0	5	50.0
C_5M_3	6	60.0	4	40.0	4	40.0	4	40.0
C_1M_4	6	60.0	5	50.0	5	50.0	5	50.0
C_2M_4	5	50.0	5	50.0	5	50.0	5	50.0
C_3M_4	8	80.0	7	70.0	7	70.0	7	70.0
C_4M_4	7	70.0	6	60.0	6	60.0	6	60.0
C_5M_4	9	90.0	9	90.0	9	90.0	9	90.0
C_1M_5	5	50.0	4	40.0	2	20.0	2	20.0
C_2M_5	4	40.0	4	40.0	2	20.0	2	20.0
C_3M_5	2	20.0	0	00.0	0	00.0	0	00.0
C_4M_5	2	20.0	2	20.0	2	20.0	2	20.0
C_5M_5	6	60.0	5	50.0	4	40.0	4	40.0

* Average of 10 observations

Table 8. Effects of microcutting size on survival of plants under different media and containers (2 - 2.5 cm height with two fully opened leaves and one or two roots).

8.1. At the first week of transplanting

	C_1	C_2	C_3	C_4	C_5	Mean M
M_1	77.99 (61.99)	60.00 (50.75)	80.00 (63.41)	60.00 (50.75)	90.00 (71.54)	(59.69)
M_2	60.00 (50.75)	70.00 (56.77)	80.00 (63.41)	70.00 (56.77)	100.00 (90.00)	(63.54)
M_3	50.00 (44.98)	60.14 (50.83)	40.00 (39.21)	60.14 (50.83)	60.14 (50.83)	(47.34)
M_4	60.64 (51.12)	50.00 (44.98)	80.69 (63.90)	70.00 (56.77)	90.00 (71.54)	(57.66)
M_5	50.00 (44.98)	39.86 (39.13)	20.00 (26.55)	20.00 (26.55)	68.39 (55.77)	(38.60)
Mean C	(50.77)	(48.49)	(51.30)	(48.33)	(67.93)	

Media	F (4,50) -	18.91^{**}	CD 6.67
Container	F (4,50) -	12.37^{**}	CD 6.67
Media x Container	F (16,50) -	2.46	CD 14.41

8.2. At the second week of transplanting

	C_1	C_2	C_3	C_4	C_5	Mean M
M_1	50.00 (44.98)	50.00 (44.98)	60.64 (51.12)	39.86 (39.13)	80.69 (63.90)	(48.825)
M_2	60.00 (50.75)	50.00 (44.98)	60.14 (50.83)	50.00 (44.98)	93.31 (74.98)	(53.30)
M_3	50.00 (44.98)	29.67 (32.99)	20.00 (26.55)	50.00 (44.98)	40.00 (39.21)	(37.75)
M_4	50.00 (44.98)	50.00 (44.98)	70.00 (56.77)	60.14 (50.83)	90.00 (71.54)	(53.82)
M_5	40.00 (39.21)	39.86 (39.13)	00.00 (00.00)	20.00 (26.55)	50.00 (44.98)	(29.77)
Mean C	(44.98)	(41.41)	(37.06)	(41.30)	(58.92)	

Media	F (4,50)	-	27.99^{**}	CD 5.65
Container	F (4,50)	-	17.89^{**}	CD 5.65
Media x Container	F (16,50)	-	5.08^{**}	CD 12.64

8.3. At the third week of transplanting

	C_1	C_2	C_3	C_4	C_5	Mean M
M_1	50.00 (44.98)	50.00 (44.98)	39.86 (39.13)	30.00 (33.19)	80.69 (63.90)	(45.24)
M_2	39.86 (39.13)	50.00 (44.98)	60.14 (50.83)	50.00 (44.98)	93.13 (74.98)	(50.98)
M_3	40.00 (39.21)	29.67 (32.99)	00.00 (00.00)	50.00 (44.98)	40.00 (39.21)	(31.28)
M_4	50.00 (44.98)	50.00 (44.98)	70.00 (56.77)	60.14 (50.83)	90.00 (71.54)	(53.82)
M_5	20.00 (26.55)	20.00 (26.55)	00.00 (00.00)	20.00 (26.55)	39.36 (38.84)	(23.70)
Mean C	(38.97)	(38.90)	(29.45)	(40.11	(57.70)	

Media	F (4,50)	-	55.37^{**}	CD 4.97
Container	F (4,50)	-	34.73^{**}	CD 4.97
Media x Container	F (16,50)	-	7.37^{**}	CD 11.10

8.4. At the fourth week of transplanting

	C_1	C_2	C_3	C_4	C_5	Mean M
M_1	50.00 (44.98)	50.00 (44.98)	39.85 (39.13)	30.00 (33.19)	80.69 (63.90)	(45.24)
M_2	39.85 (39.13)	50.00 (44.98)	60.14 (50.83)	50.00 (44.98)	93.31 (74.98)	(50.98)
M_3	40.00 (39.21)	29.67 (32.99)	00.00 (00.00)	50.00 (44.98)	40.00 (39.21)	(31.28)
M_4	50.00 (44.98)	50.00 (44.98)	70.00 (56.77)	60.14 (50.83)	90.00 (71.54)	(53.83)
M_5	20.00 (26.55)	20.00 (26.55)	00.00 (00.00)	20.00 (26.55)	39.36 (38.84)	(23.70)
Mean C	(38.97)	(38.90)	(29.35)	(40.11)	(57.70)	

Media	F (4,50)	55.37^{**}	CD 4.97
Container	F (4,50)	34.73^{**}	CD 4.97
Media x Container	F (16,50)	7.36^{**}	CD 11.10

Table 9. Effects of microcutting size on survival of plantlets under different media and containers (2.5-3 cm height with 3 or 4 fully opened leaves and two or more roots)

Treatments	I Week No. of plants survived	per cent	II Week No. of plants survived	per cent	III Week No. of plants survived	per cent	IV Week No. of plants survived	per cent
C_1M_1	20	100.0	20	100.0	20	100.0	20	100.0
C_2M_1	20	100.0	20	100.0	20	100.0	20	100.0
C_3M_1	20	100.0	20	100.0	20	100.0	20	100.0
C_4M_1	20	100.0	20	100.0	20	100.0	20	100.0
C_5M_1	20	100.0	20	100.0	19	95.0	19	95.0
C_1M_2	20	100.0	20	100.0	20	100.0	20	100.0
C_2M_2	20	100.0	20	100.0	20	100.0	19	95.0
C_3M_2	20	100.0	12	90.0	12	90.0	18	90.0
C_4M_2	20	100.0	20	100.0	20	100.0	20	100.0
C_5M_2	18	90.0	16	80.0	16	80.0	16	80.0
C_1M_3	20	100.0	20	100.0	20	100.0	20	100.0
C_2M_3	20	100.0	20	100.0	20	100.0	19	95.0
C_3M_3	20	100.0	19	95.0	18	90.0	17	85.0
C_4M_3	20	100.0	19	95.0	19	95.0	19	95.0
C_5M_3	17	85.0	15	75.0	15	75.0	15	75.0
C_1M_4	20	100.0	20	100.0	20	100.0	20	100.0
C_2M_4	20	100.0	19	95.0	18	90.0	18	90.0
C_3M_4	18	90.0	18	90.0	18	90.0	18	90.0
C_4M_4	20	100.0	19	95.0	19	95.0	19	95.0
C_5M_4	20	100.0	19	95.0	19	95.0	19	95.0
C_1M_5	19	95.0	16	80.0	16	80.0	16	80.0
C_2M_5	15	75.0	13	65.0	13	60.0	13	65.0
C_3M_5	15	75.0	12	60.0	12	60.0	12	60.0
C_4M_5	14	70.0	14	70.0	14	70.0	14	70.0
C_5M_5	18	90.0	17	85.0	15	75.0	15	75.0

* Average of 20 observations

Table 9. Effects of microcutting size on survival of plants under different media and containers (2.5 - 3 cm height with 3-4 fully opened leaves two or more roots)

9.1. At the first week of transplanting

	C_1	C_2	C_3	C_4	C_5	Mean M
M_1	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	(90.00)
M_2	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	90.00 (71.54)	(86.31)
M_3	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	85.00 (67.19)	(85.43)
M_4	100.00 (90.00)	100.00 (90.00)	90.40 (71.92)	100.00 (90.00)	100.00 (90.00)	(86.38)
M_5	96.72 (79.53)	75.11 (60.05)	75.00 (59.98)	70.08 (56.82)	90.40 (71.92)	(65.65)
Mean C	(87.90)	(84.01)	(80.39)	(83.36)	(78.13)	

Media	F (4,50)	- 221.97^{**}	CD 1.85
Container	F (4,50)	- 32.62^{**}	CD 1.85
Media x Container	F (16,50)	- 30.75^{**}	CD 4.15

9.2. At the second week of transplanting

	C_1	C_2	C_3	C_4	C_5	Mean M
M_1	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	(90.00)
M_2	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	80.16 (63.52)	(81.01)
M_3	100.00 (90.00)	100.00 (90.00)	95.00 (77.05)	95.00 (77.05)	75.11 (60.05)	(78.83)
M_4	100.00 (90.00)	95.00 (77.05)	90.40 (71.92)	95.00 (77.05)	96.72 (79.52)	(79.11)
M_5	80.16 (63.52)	65.06 (53.74)	60.04 (50.77)	70.08 (56.82)	85.24 (67.38)	(58.45)
Mean C	(84.71)	(80.16)	(72.26)	(78.18)	(72.09)	

Media	F (4,50) -	257.20^{**}	CD 2.05
Container	F (4,50) -	55.73^{**}	CD 2.05
Media x Container	F (16,50) -	24.81^{**}	CD 4.59

9.3. At the third week of transplanting

	C_1	C_2	C_3	C_4	C_5	Mean M
M_1	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	95.77 (77.05)	(87.41)
M_2	100.00 (90.00)	100.00 (90.00)	90.00 (71.54)	100.00 (90.00)	80.15 (63.52)	(81.01)
M_3	100.00 (90.00)	100.00 (90.00)	90.00 (71.54)	95.00 (77.05)	75.11 (60.05)	(77.72)
M_4	100.00 (90.00)	90.40 (71.92)	90.40 (71.92)	95.00 (77.05)	96.71 (79.52)	(78.09)
M_5	80.16 (63.52)	65.05 (53.74)	60.04 (50.77)	70.08 (70.08)	75.00 (59.98)	(56.97)
Mean C	(84.70)	(79.13)	(71.15)	(78.18)	(68.02)	

Media	F (4,50)	- 242.04^{**}	CD 2.09
Container	F (4,50)	- 81.72^{**}	CD 2.09
Media x Container	F (16,50)	- 19.86^{**}	CD 4.68

9.4. At the fourth week of transplanting

	C_1	C_2	C_3	C_4	C_5	Mean M
M_1	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	95.00 (77.05)	(87.41)
M_2	100.00 (90.00)	95.00 (77.05)	90.00 (71.54)	100.00 (90.00)	80.16 (63.53)	(78.42)
M_3	100.00 (90.00)	95.00 (77.05)	85.23 (67.38)	95.00 (77.05)	75.11 (60.05)	(74.31)
M_4	100.00 (90.00)	90.40 (71.92)	90.40 (71.92)	95.00 (77.05)	96.71 (79.53)	(78.09)
M_5	80.16 (63.52)	65.06 (53.74)	60.03 (50.77)	70.08 (56.82)	75.00 (59.96)	(56.97)
Mean C	(84.71)	(73.95)	(70.32)	(78.18)	(68.02)	

Media	F (4,50)	- 213.97^{**}	CD 2.18
Container	F (4,50)	- 75.05^{**}	CD 2.18
Media x Container	F (16,50)	- 13.40^{**}	CD 4.87

4.2.2 *Leaf production*

Different media and containers showed significant influence on the production of new leaves in the ex vitro condition in all the four fortnights.

4.2.2.1 First fortnight

During the first fortnight after transplanting, the plants grown in the container C_2 (plastic pot) recorded largest number of leaves (3.68) which was on par with C_1 (mud pot) and C_4 (polythene cover). The plants in C_3 (paper pot) produced least number of leaves (3.24) (Table 10.1).

Among the different media used, plants in M_4 (soilrite) produced largest number of leaves (3.84) in the first fortnight after transplanting which was on par with M_1 (coarse sand) and plants in the medium M_3 (charcoal) produced least number of leaves (3.24).

There was significant interaction between media and containers in the case of leaf production at first fortnight. The treatment combinations C_4M_1 (polythene cover-coarse sand) C_1M_4 (mud pot-soilrite) and C_5M_4 (nettedpot-soilrite) produced largest number of leaves (4.0). It was

also observed that with media M_1 (coarse sand) and M_2 (fine sand) plants grown in C_4 (polythene cover) gave the maximum number of leaves followed by C_1 (mud pot). In media M_3 (charcoal) and M_5 (sphagnum moss) plants in the container C_2 (plastic pot) were found to be the best, while with M_4 (soilrite), all the containers have more or less same response. The treatment combinations C_5M_3 (nettedpot-charcoal), C_3M_3 (paper pot-charcoal), C_3M_2 (paper pot-fine sand), C_4M_3 (polythene cover-charcoal) and C_5M_2 (nettedpot-fine sand) produced least number of leaves (3.0) (plate 13).

4.2.2.2 Second fortnight

During the second fortnight after transplanting, the treatment C_2 (plastic pot) produced more number of leaves (4.52) which was on par with C_1 (mud pot) and C_4 (polythene cover) (Table 10.2). Plants in the container C_3 (paper pot) produced least number of leaves (3.84).

In the case of medium, M_4 (soilrite) was the best treatment (4.80) which was superior to all other treatments. The plants in the media M_5 (sphagnum moss) produced least number of leaves (3.80) in the second fortnight.

PLATE 13 Number of leaves on anthurium plantlet as influenced by media and containers at first fortnight after transplanting
a. Polythene cover - Coarse sand
b. Netted pot - Fine sand

PLATE 14 Number of leaves on anthurium plantlet as influenced by media and containers at second fortnight after transplanting
a. Mud pot - Soilrite
b. Paper pot - Sphagnum moss


a
b
13


a
b
14

There were significant interaction between media and containers for production of leaves (Table 10.2). The treatment combination C_1M_4 (mud pot-soilrite) produced highest number of leaves (5.20). It could also be noted that under medium M_1 (coarse sand), no significant difference in the case of leaf production with various containers. But under M_2 (fine sand), C_4 (polythene cover) was found to be the best container which was on par with C_1 (mud pot) and C_2 (plastic pot). Under M_3 (charcoal) the container C_2 (plastic pot) was the best which was on par with C_1 (mud pot) and under M_5 (sphagnum moss) the container C_5 (netted pot) was the best which was on par with C_2 (plastic pot) and C_1 (mud pot). However under M_4 (soilrite) all the containers showed more or less same response with respect to leaf production at second fortnight after transplanting. The treatment combination with least number of leaves (2.8) was registeed by C_3M_5 (paper pot-sphagnum moss) (Plate 14).

4.2.2.3 Third fortnight

Among the different containers (Table 10.3) C_2 (plastic pot) produced highest number of leaves (5.16) which

was on par with C_1 (mud pot) and C_4 (polythene cover). The plants in C_3 (paper pot) showed least response (4.12).

Plants grown in the medium M_4 (soilrite) produced largest number of leaves (5.52) during the third fortnight which was superior to all other media. The least response (4.20) was observed for plants in M_3 (charcoal).

Significant interaction between media and containers were also observed (Table 10.3). The treatment combination C_1M_4 (mud pot-soilrite) showed highest influence on leaf production (6.0). It was found that with medium M_1 (coarse sand), plants grown on container C_4 (polythene cover) was the best which was on par with C_1 (mud pot). With the medium M_2 (fine sand), the best leaf production rate was on C_4 (polythene cover) which was on par with C_2 (plastic pot) and C_1 (mud pot). With the medium M_3 (charcoal) the best container was C_2 (plastic pot) followed by C_1 (mud pot) and with M_5 (sphagnum moss) the container C_5 (netted pot) was the best which was on par with C_2 (plastic pot) and C_1 (mud pot). However, the plants on M_4 (soilrite) produced more or less same response in all the containers in respect of leaf production. The treatment

Table 10.1. Effects of media and containers on production of leaves in the first fortnight.

	C_1	C_2	C_3	C_4	C_5	Mean M
M_1	3.8	2.8	3.4	4.0	3.2	3.64
M_2	3.6	3.4	3.0	3.8	3.0	3.36
M_3	3.4	3.8	3.0	3.0	3.0	3.24
M_4	4.0	3.8	3.6	3.8	4.0	3.84
M_5	3.2	3.6	3.2	3.2	3.8	3.40
Mean C	3.6	3.68	3.24	3.56	3.40	

Media	F (4,100) -	7.260^{**}	CD 0.269
Container	F (4,100) -	3.860^{**}	CD 0.269
Media x Container	F (16,100) -	1.985^{*}	CD 0.557

Table 10.2. Effect of media and containers on Production of leaves in the second fortnight.

	C_1	C_2	C_3	C_4	C_5	Mean M
M_1	4.6	4.2	4.2	4.8	4.2	4.4
M_2	4.4	4.4	3.8	4.8	3.4	4.16
M_3	4.2	4.8	3.8	3.6	3.2	3.92
M_4	5.2	4.8	4.6	4.8	4.6	4.80
M_5	3.8	4.4	2.8	3.4	4.6	3.80
Mean C	4.44	4.52	3.84	4.38	4.00	

Media	F (4,100) -	9.072^{**}	CD 0.370
Container	F (4,100) -	4.755^{**}	CD 0.370
Media x Container	F (16,100) -	2.675^{**}	CD 0.826

Table 10.3. Effect of media and containers on Production of leaves in the third fortnight.

	C_1	C_2	C_3	C_4	C_5	Mean M
M_1	5.0	4.6	4.6	5.8	4.4	4.88
M_2	5.0	5.2	3.8	5.8	4.0	4.76
M_3	4.8	5.4	4.2	4.0	2.6	4.20
M_4	6.0	5.6	5.0	5.6	5.4	5.52
M_5	4.8	5.0	3.0	3.8	5.2	4.36
Mean C	5.12	5.16	4.12	5.00	4.32	

Media	F (4,100) -	10.265^{**}	CD 0.499
Container	F (4,100) -	9.154^{**}	CD 0.499
Media x Container	F (16,100) -	3.730^{**}	CD 1.003

Table 10.4. Effect of media and containers on Production of leaves in the fourth fortnight.

	C_1	C_2	C_3	C_4	C_5	Mean M
M_1	5.6	5.2	5.0	6.6	5.2	5.52
M_2	5.6	6.0	4.4	6.6	4.4	5.40
M_3	5.8	6.2	4.2	4.6	3.0	4.84
M_4	7.0	6.6	5.6	6.6	6.2	6.40
M_5	5.2	5.4	3.8	4.4	6.0	4.96
Mean C	5.84	5.88	4.68	5.76	4.96	

Media	F (4,100) -	11.409^{**}	CD 0.508
Container	F (4,100) -	9.486^{**}	CD 0.508
Media x Container	F (16,100) -	3.590^{**}	CD 1.136

combination which produced least number of leaves (2.6) in the third fortnight was C_5M_3 (netted pot-charcoal).

4.2.2.4 Fourth fortnight

Transplanting in the container C_2 (plastic pot) at the fourth fortnight (Table 10.4) produced largest number of leaves (5.88) which was on par with C_2 (plastic pot) and C_4 (polythene cover). The lowest number of leaves (4.68) were produced by plants in the container C_3 (paper pot).

Among the different media used, M_4 (soilrite) was found to be the best treatment (6.40) which was superior to all other media. Plants in the media M_3 (charcoal) produced least number of leaves (4.84) in the fourth fortnight.

The treatment combination of media and containers showed significant influence on leaf production. The highest number of leaves (7.0) were produced by plants in the treatment combination C_1M_4 (mud pot soilrite). It was also observed that along with medium M_1 (coarse sand), plants in the container C_4 (polythene cover) produced largest number of leaves which was on par with C_1 (mud pot). Along with medium M_2 (fine sand) the best container was C_4 (polythene cover) which was on par with C_2 (plastic pot) and C_1 (mud pot). In medium M_3 (charcoal) and M_5 (sphagnum moss) the container C_2 (plastic pot) showed highest influence which was on par with

C_1 (mud pot). With medium M_4 (soilrite) all the containers showed more or less same rate of leaf production except with C_3 (paper pot). The plants in the treatment combination C_5M_3 (netted pot - charcoal) produced least number of leaves (3.0).

4.2.3 *Plant height*

One month after transplanting of anthurium plantlets to exvitro condition, the media and containers showed significant influence on plant height (Table 11.1). Among the different containers C_1 (mud pot) found to be the best treatment (3.28 cm) which was on par with C_4 (polythene cover) and C_2 (plastic pot). The lowest height (2.84 cm) was recorded in the case of C_5 (netted pot) grown plants.

The plants grown on the medium M_4 (soilrite) showed the maximum response (3.41 cm) and all other media were inferior to it. The least height (2.78 cm) was showed by plants in the treatment M_5 (sphagnum moss) in the first month after transplanting.

Significant interaction was noticed between media and containers in the case of plant height after one month (Table 11.1). The plants in the treatment combination C_1M_1 (mud pot-coarse sand) and C_1M_4 (mud pot-soilrite) recorded maximum heights (3.54 cm). It was also observed that under M_1 (mud pot), plants with maximum height was in the container

C_1 (mud pot) which was on par with C_4 (polythene cover) and C_3 (paper pot). Under M_2 (fine sand), all the containers showed more or less same response except in the case of C_5 (netted pot) grown plants; and in medium M_3 (charcoal) the plants grown on container C_1 (mud pot) recorded maximum height which was on par with C_2 (plastic pot) and C_4 (polythene cover). Plants in the medium M_4 (soilrite) and M_3 (sphagnum moss) showed no significant difference with respect to plant height on various containers after one month. The lowest responses (2.6 cm) were showed in combinations C_3M_5 (paper pot-sphagnum moss), C_5M_3 (netted pot-charcoal) and C_5M_2 (netted pot-fine sand).

The different treatments had significant influence on plant height when observed two months after transplanting (Table 11.2). Among the different treatments with containers, C_1 (mud pot) was found to be the best (3.792 cm) which was on par with C_4 (polythene cover) and C_2 (plastic pot). Plants in C_5 (netted pot) showed least plant height (3.124 cm).

The largest plants (4.188 cm) were observed in the media M_4 (soilrite) which was found to be superior to other media and least height (2.928 cm) was recorded in M_5 (sphagnum moss) after two months.

Table 11.1. Effect of media and containers on height of plants - one month after transplanting.

	C_1	C_2	C_3	C_4	C_5	Mean M
M_1	3.54	2.70	3.12	3.18	2.72	3.052
M_2	3.26	3.08	2.88	3.36	2.60	3.036
M_3	3.34	3.14	2.66	2.86	2.60	2.920
M_4	3.54	3.52	3.18	3.50	3.34	3.416
M_5	2.74	2.84	2.60	2.78	2.96	2.784
Mean C	3.284	3.056	2.888	3.136	2.844	

Media	F (4,100) -	11.924^{**}	CD 0.190
Container	F (4,100) -	7.027^{**}	CD 0.190
Media x Container	F (16,100) -	2.087^{*}	CD 0.425

Table 11.2. Effect of media and containers on height of plants - two months after transplanting

	C_1	C_2	C_3	C_4	C_5	Mean M
M_1	4.26	3.30	3.78	3.94	3.06	3.668
M_2	3.74	3.68	3.28	4.26	2.84	3.560
M_3	3.80	3.44	2.82	3.14	2.20	3.080
M_4	4.12	4.44	3.90	4.34	4.14	4.188
M_5	3.04	3.06	2.20	2.96	3.38	2.928
Mean C	3.792	3.584	3.196	3.728	3.124	

Media	F (4,100) -	27.504^{**}	CD 0.267
Container	F (4,100) -	10.289^{**}	CD 0.267
Media x Container	F (16,100) -	3.585^{**}	CD 0.596

Significant interaction between media and containers were observed for plant height at two months after transplanting (Table 11.2). The plant height was maximum (4.44 cm) on C_2M_4 (plastic pot-soilrite). On medium M_1 (coarse sand), maximum height was recorded for plants grown on container C_1 (mud pot) which was on par with C_4 (polythene cover) and C_3 (paper pot). On medium M_2 (fine sand) largest plant height was on C_4 (polythene cover) grown plants which was on par with C_1 (mud pot) and C_2 (plastic pot) and on medium M_3 (charcoal) the highest response was on container C_1 (mud pot) grown plants which was on par with C_2 (plastic pot). However, along with medium M_4 (soilrite) all the containers showed equal response in the case of plant height. The medium M_5 (sphagnum moss) also showed more or less same response under various containers except on C_3 (paper pot) grown plants which was inferior to others. The lowest heights (2.0 cm) were recorded in the combination C_3M_3 (paper pots-sphagnum moss) and C_5M_3 (netted pot-charcoal) (Plate 15).

4.2.4 *Leaf Area*

The media and containers had significant influence on increasing the area of new leaves emerged at fortnightly intervals in the ex vitro establishment of anthurium plantlets.

4.2.4.1 First fortnight

During the first fortnight after transplanting (Table 12.1) maximum leaf area (1.114 cm^2) was observed in C_1(mud pot) which was found to be on par with C_2 (plastic pot) and C_4 (polythene cover). The minimum leaf area (0.973 cm^2) was recorded in C_3 (paper pot).

Plants grown in M_2 (fine sand) were found to be the best treatment (1.219 cm^2) among the media. The least leaf area (0.970 cm^2) was observed in plants grown on M_1 (coarse sand) in the first fortnight.

There were significant interaction between media and containers with reference to area of new leaves formed at the first fortnight (Table 12.1). Plants grown in the treatment combination C_1M_2 (mud pot - fine sand) were found to be the best (1.321 cm2). It was also found that with media M_1 (coarse sand) all other containers except C_3 (paper pot) showed more or less same response. Under M_2 (fine sand) no significant difference was observed with various containers except in the case of C_5 (netted spot) which was inferior to other containers. Under M_3 (paper pot) the plants grown on C_2 (plastic pot)_recorded maximum leaf area which was on par with C_1 (mud pot) and C_5 (netted pot). Under M_5 (Sphagnum moss), all the containers showed response on par except C_2 (plastic pot) grown plants which was

inferior to others. With media M_4 (soilrite) no significant difference were observed in the case of area of new leaves by using various containers. The least influence on leaf area was in the combination C_3M_1 (paper pot-coarse sand).

4.2.42 Second fortnight

During the second fortnight after transplanting (Table 12.2), the plants grown in C_2 (plastic pot) showed maximum leaf area (1.498 cm^2) which was on par with C_1 (mud pot) and C_4 (polythene cover). The minimum leaf area (1.226 cm^2) was recorded for plants grown on C_5 (netted pot).

In the case of media, the highest leaf area (1.619 cm^2) was recorded in M_2 (fine sand) which was on par with M_4 (soilrite). The lowest leaf area (1.187 cm^2) of newly formed leaves was recorded in media M_5 (sphagnum moss).

Significant interactions were observed between the media and containers with reference to the area of new leaves formed at second fortnight after transplanting (Table 12.2). The plants in the treatment combination C_2M_4 (plastic pot-soilrite) recorded maximum leaf area (2.083 cm^2). It was also found that in media M_1 (coarse sand), all the containers showed more or less same leaf area. In media M_2 (fine sand) all the containers showed almost equal response except in the case of C_5 (netted pot) grown plants which was inferior to others. In media M_3 (paper pots) the plants grown on C_2

(plastic pot) recorded highest leaf area which was on par with C_1 (mud pot). In media M_5 (sphagnum moss) plants in the container C_5 (netted pot) recorded maximum leaf area which was on par with C_4 (polythene cover) and C_1 (mud pot). But in media M_4 (soilrite) the plants grown on C_2 (plastic pot) alone recorded highest area of new leaves which was superior to all other containers. The least response (0.980 cm^2) was observed in the combination C_3M_1 (paper pot-coarse sand).

4.2.4.3 Third fortnight

During the third fortnight after transplanting a significant difference in the area of newly formed leaves with different media and containers used were observed (Table 12.3).

Among the containers used maximum area (1.982 cm^2) was recorded in C_4 (polythene cover) which was on par with C_2 (plastic pot). Lowest leaf area (1.538 cm^2) was recorded in C_3 (paper pot).

Plants grown in M_4 (soilrite) recorded maximum leaf area (2.147 cm^2) which was on par with the plants grown in media M_2 (fine sand) at third fortnight. The least value of leaf area (1.438 cm^2) was observed in M_5 (sphagnum moss).

There were significant interactions between media and containers for the area of new leaves formed at third

PLATE 15 Size of anthurium plantlets as influenced by media and containers at two months after transplanting
a. Plastic pot - Soilrite
b. Paper pot - Sphagnum moss
c. Netted pot - Char coal

PLATE 16 Leaf area of anthurium plantlets as influenced by media and containers after three fortnights
a. Plastic - Soilrite
b. Paper pot - Sphagnum moss


a
b
c
15


a
16
b

fortnight (Table 12.3). The treatment combination C_2M_4 (plastic pot-soilrite) recorded maximum leaf area (2.865 cm^2). It was also observed that along with the medium M_1 (coarse sand), plants grown on C_4 (polythene cover) were found to be superior to all the other containers in respect of leaf area. Along with medium M_2 (fine sand) no significant difference with various containers were noticed except with C_5 (netted pot) which was inferior to other containers. In medium M_3 (charcoal), plants grown on C_2 (plastic pot) recorded largest leaf area which was on par with C_1 (mud pot). In the medium M_4 (soilrite) plants in the container C_2 (plastic pot) showed maximum response which was on par with C_4 (polythene cover) and in medium M_5 (sphagnum moss) plants grown on C_5 (netted pot) recorded largest leaf area which was on par with C_4 (polythene cover).

4.2.4.4 Fourth fortnight

Significant influences were exhibited between media and containers with respect to leaf area of newly formed leaves at fourth fortnight - after transplanting (Table 12.4). In this stage, plants grown on container C_2 (plastic pot) recorded the highest leaf area (2.841 cm^2) which was on par with C_4 (polythene cover). The leaf area was lowest (2.045 cm^2) in plants grown on C_3 (paper pot).

Table 12.1. Effect of media and containers on area of new leaves produced - in the first fortnight

	C_1	C_2	C_3	C_4	C_5	Mean M
M_1	1.034	0.997	0.721	1.144	0.953	0.970
M_2	1.321	1.308	1.218	1.186	1.063	1.219
M_3	1.120	1.147	0.864	0.877	1.052	1.012
M_4	1.101	1.191	1.143	1.231	0.946	1.122
M_5	0.993	0.883	0.917	1.092	0.969	0.972
Mean C	1.114	1.106	0.973	1.106	0.997	

Media	F (4,100) -	11.034^{**}	CD 0.091
Container	F (4,100) -	4.381^{**}	CD 0.091
Media x Container	F (16,100) -	2.238^{**}	CD 0.204

Table 12.2. Effect of media and containers on area of new leaves produced - in the second fortnight

	C_1	C_2	C_3	C_4	C_5	Mean M
M_1	1.390	1.159	0.980	1.423	1.035	1.197
M_2	1.752	1.700	1.735	1.543	1.365	1.619
M_3	1.484	1.515	1.203	1.150	1.118	1.294
M_4	1.410	2.083	1.498	1.606	1.262	1.572
M_5	1.191	1.033	1.064	1.296	1.350	1.187
Mean C	1.445	1.498	1.296	1.403	1.226	

Media	F (4,100) -	30.070^{**}	CD 0.105
Container	F (4,100) -	8.669^{**}	CD 0.105
Media x Container	F (16,100) -	5.572^{**}	CD 0.235

Table 12.3. Effect of media and containers on area of new leaves produced - in the third fortnight

	C_1	C_2	C_3	C_4	C_5	Mean M
M_1	1.687	1.271	1.314	2.109	1.260	1.528
M_2	2.017	2.200	2.062	2.153	1.609	2.008
M_3	1.945	2.232	1.347	1.686	1.352	1.712
M_4	1.834	2.685	2.078	2.327	1.813	2.147
M_5	1.360	1.408	0.891	1.635	1.897	1.438
Mean C	1.768	1.960	1.538	1.982	1.586	

Media	F (4,100) -	24.927^{**}	CD 0.170
Container	F (4,100) -	11.314^{**}	CD 0.170
Media x Container	F (16,100) -	5.398^{**}	CD 0.380

Table 12.4 Effect of media and containers on area of new leaves produced - in the fourth fortnight

	C_1	C_2	C_3	C_4	C_5	Mean M
M_1	2.266	1.810	1.870	2.537	1.613	2.019
M_2	3.203	3.331	2.581	3.284	2.025	2.885
M_3	2.435	3.061	1.526	2.489	1.352	2.172
M_4	2.762	4.202	3.356	3.606	2.821	3.349
M_5	1.779	1.798	0.891	2.083	2.464	1.803
Mean C	2.489	2.841	2.045	2.800	2.055	

Media	F (4,100) -	41.247^{**}	CD 0.281
Container	F (4,100) -	14.661^{**}	CD 0.281
Media x Container	F (16,100) -	4.526^{**}	CD 0.628

Among the media, the plants grown on M_4 (soilrite) recorded highest leaf area (3.349 cm^2) which was significantly superior to other media. The the lowest response (1.803 cm^2) was observed in medium M_5 (sphagnum moss).

Significant differences were observed with interaction of media and containers on leaf area (Table 12.4) of newly formed leaves at fourth fortnight. Plants grown on C_2M_4 (plastic pot-soilrite) gave the highest leaf area (4.202 cm^2). It was found that with medium M_1 (coarse sand) the highest leaf area was on C_4 (polythene cover) grown plants which was on par with C_1 (mud pot). With medium M_2 (fine sand) and M_3 (charcoal) the best container was C_2 (plastic pot) which was on par with C_4 (polythene cover) and C_1 (mud pot). With the medium M_4(soilrite), C_2 (plastic pot) grown plants showed maximum leaf area which was on par with C_4 (polythene cover)and with medium M_5 (sphagnum moss) the container C_5 (netted pot) recorded maximum response which was on par with C_4 (polythene cover). Plants grown in the treatment combination C_3M_5 (paper pot-sphagnum moss) recorded the lowest leaf area (0.891 cm^2) (Plate 16).

4.2.5 *Petiole length*

The media and the containers had varying influence on petiole length of newly formed leaves of anthurium plants at fortnightly intervals.

4.2.5.1 First fortnight

Media and containers showed significant influence on petiole length of newly formed leaves at first fortnight after transplanting (Table 13.1). Plants grown in the container C_4 (polythene cover) recorded highest petiole length (1.796 cm) which was significantly superior to all other treatments. The C_3 (paper pots) grown plants recorded lowest peteole length (1.392 cm).

Among the different media M_2 (fine sand) grown plants had highest petiole length (1.688 cm) which was on par with M_4 (soilrite) while M_1 (coarse sand) grown plants gave least response (1.404 cm).

Significant differences were observed between interaction of media and containers with respect to petiole length of new leaves at first fortnight after transplanting (Table 13.1). The plants grown in the treatment combination C_4M_4 (polythene cover-soilrite) recorded highest petiole length (2.14 cm). It was also found that for medium M_1 (coarse sand) and in medium M_2 (fine sand), the best container with respect to peteole length was C_4 (polythene cover) which was on par with C_1 (mud pot). For medium M_3 (charcoal) all the containers showed almost equal response except in C_3 (paper pot) grown plants which was inferior to other containers and under the medium M_4 (soilrite) the best

container was C_4 (polythene cover) which was significantly superior to other containers. While in medium M_5 (sphagnum moss) no significant difference was observed in the case of petiole length with various containers. The lowest petiole length (1.08 cm) was recorded in the treatment combination C_2M_1 (plastic pot - coarse sand).

4.2.5.2 Second fortnight

During the second fortnight after transplanting, significant differences with respect to petiole length of newly formed leaves were observed in the case of media only (Table 13.2). No significant differences were observed between containers, and interaction between media and containers.

Plants grown in media M_4 (soilrite) recorded highest petiole length (1.848 cm) which was on par with M_2 (fine sand). Lowest petiole length (1.484 cm) was recorded for plants grown in M_5 (sphagnum moss) (Table 13.2).

4.2.5.3 Third fortnight

Significant differences were observed between media and containers with respect to petiole length of newly formed leaves (Table 13.3). Plants grown in container C_4 (polythene cover) recorded highest petiole length (2.020 cm) which was on par with C_1 (mud pot) and was superior to all other

Table 13.1. Effect of media and containers on petiole length of new leaf produced - in the first fortnight

	C_1	C_2	C_3	C_4	C_5	Mean M
M_1	1.62	1.08	1.30	1.74	1.28	1.404
M_2	1.74	1.66	1.62	2.10	1.32	1.688
M_3	1.68	1.76	1.22	1.34	1.48	1.496
M_4	1.40	1.52	1.30	2.14	1.56	1.584
M_5	1.24	1.32	1.52	1.66	1.66	1.480
Mean C	1.536	1.468	1.392	1.796	1.460	

Media	F (4,100)	-	2.575^{*}	CD 0.189
Container	F (4,100)	-	5.354^{**}	CD 0.189
Media x Container	F (16,100)	-	2.426^{**}	CD 0.423

Table 13.2. Effect of media and containers on petiole length of new leaf produced - in the seconf fortnight

	C_1	C_2	C_3	C_4	C_5	Mean M
M_1	1.76	1.28	1.36	1.84	1.44	1.536
M_2	2.06	1.42	1.82	1.96	1.70	1.792
M_3	1.62	1.78	1.40	1.54	1.40	1.548
M_4	1.80	1.78	1.78	1.96	1.92	1.848
M_5	1.44	1.36	1.42	1.54	1.66	1.484
Mean C	1.736	1.524	1.556	1.798	1.624	

Media	F (4,100)	-	4.755^{**}	CD 0.212
Container	F (4,100)	-	2.004	CD -
Media x Container	F (16,100)	-	0.977	CD -

Table 13.3. Effect of media and containers on petiole length of new leaf produced - in the third fortnight

	C_1	C_2	C_3	C_4	C_5	Mean M
M_1	2.38	1.64	1.90	1.82	1.86	1.920
M_2	1.62	1.92	1.78	2.48	1.76	1.912
M_3	1.86	1.88	1.86	1.92	1.14	1.732
M_4	2.16	1.76	2.04	2.06	1.92	1.988
M_5	1.66	1.58	1.16	1.82	2.00	1.644
Mean C	1.936	1.756	1.748	2.020	1.736	

Media	F (4,100) -	3.953^{**}	CD 0.203
Container	F (4,100) -	3.214^{*}	CD 0.203
Media x Container	F (16,100) -	3.178^{**}	CD 0.453

Table 13.4. Effect of media and containers on petiole length of new leaf produced - in the fourth fortnight

	C_1	C_2	C_3	C_4	C_5	Mean
M_1	2.06	1.62	1.62	2.06	1.78	1.828
M_2	2.12	1.70	2.06	2.30	1.82	2.000
M_3	2.10	2.10	1.80	1.92	1.14	1.812
M_4	2.38	2.56	2.42	2.08	2.32	2.352
M_5	1.66	1.82	1.20	1.96	1.98	1.724
Mean C	2.064	1.960	1.820	2.064	1.808	

Media	F (4,100) -	9.416^{**}	CD 0.226
Container	F (4,100) -	2.381	CD -
Media x Container	F (16,100) -	2.410^{**}	CD 0.506

containers have more or less same response on petiole length. Plants grown in C_5M_3 (netted pot-charcoal) recorded least petiole length (1.14 cm).

4.2.5.4 Fourth fortnight

During the fourth fortnight after transplanting, significant differences between media, and interaction between media and containers with respect to petiole length of newly formed leaves were observed. No significant difference was observed among various containers used (Table 13.4).

Among the media, plants grown on M_4 (soilrite) recorded highest petiole length (2.352 cm) which was significantly superior to other media. The lowest petiole length (1.724 cm) was observed in plants grown on M_5 (sphagnum moss).

Among the treatment combinations of media and containers (Table 13.4), plants grown on C_2M_4 (plastic pot-soilrite) recorded highest petiole length (2.56 cm). At this stage it was found that except in the case of media M_3 (charcoal) all the other media had no influence on petiole length of new leaves with various containers. But with M_3 (charcoal) except the plants grown on container C_5(netted pot) all other containers show more or less same response. The C_5 (netted pot) grown plants recorded least response.

containers. The least peteole length (1.736 cm) was recorded for plants grown in C_5 (netted pot).

Among the media, highest petiole length (1.988 cm) was recorded by plants grown in M_4 (soilrite) which was on par with M_1 (coarse sand) and M_2 (fine sand). Least response (1.644 cm) was observed in M_5 (sphagnum moss) grown plants.

Significant interaction between media and containers were observed in the case of petiole length of newly formed leaves at third fortnight (Table 13.3). The treatment combination with highest peteole length (2.48 cm) was recorded for plants grown on C_4M_2 (polythene cover-fine sand). At this stage along with medium M_1 (coarse sand), the plants grown on C_1 (mud pot) was found to be the best and significantly superior to all other containers. Along with medium M_2 (fine sand) the plants grown on the container C_4 (polythene cover) recorded maximum petiole length and was superior to other containers. Along with media M_3 (charcoal) the plants under various containers recorded more or less same petiole length except on C_5 (netted pot) grown plants which was inferior to other containers. Along with medium M_5 (sphagnum moss) the container C_5 (netted pot) recorded maximum petiole length which was on par with C_4 (polythene cover). However, with medium M_4 (soilrite) all the

Plants grown on the treatment combination C_5M_3 (netted pot-charcoal) recorded least petiole length (1.14 cm).

4.2.6. *Root production*

Two months after transplanting, anthurium plantlets showed significant influence on media and containers with respect to rate of root production (Table 14). During this stage, among the containers C_4 (polythene cover) was found to be the best (5.44) which was on par with C_1 (mud pot), C_2 (plastic pot) and C_5 (netted pot). The plants in the container C_3 (paper pot) produced lowest number of roots (3.68).

Among the different media used, M_4 (soilrite) was found to be the best treatment (5.720) which was on par with M_2 (fine sand) and was significantly superior to all other media. Minimun number of roots (4.240) were produced by plants in the medium M_1 (coarse sand) (Fig. I).

There was no interaction between media and containers for the production of roots at two months after transplanting.

4.2.7 *Root length*

Media and containers showed significant influence on root length of anthurium plants at two months after

Table 14. Effect of media and containers on root production

	C_1	C_2	C_3	C_4	C_5	Mean M
M_1	5.0	4.8	3.2	4.0	4.2	4.24
M_2	5.8	5.4	4.0	6.4	4.6	5.24
M_3	5.4	4.4	3.6	5.0	4.6	4.60
M_4	6.4	5.6	4.8	6.8	5.0	5.72
M_5	4.4	4.8	2.8	5.0	5.2	4.44
Mean C	5.40	5.0	3.68	5.44	4.72	

Media	F (4,100)	-	6.691^{**}	CD 0.662
Container	F (4,100)	-	9.113^{**}	CD 0.662
Media x Container	F (16,100)	-	0.870	CD -

Table 15. Effect of media and containers on root length

	C_1	C_2	C_3	C_4	C_5	Mean M
M_1	12.960	11.860	5.600	7.640	8.600	9.332
M_2	16.420	13.280	10.360	17.620	11.000	13.736
M_3	12.740	12.700	8.660	10.920	11.820	11.368
M_4	17.340	15.200	12.200	21.880	13.920	16.108
M_5	10.800	13.040	7.900	12.540	15.020	11.860
Mean C	14.052	13.216	8.944	14.120	12.072	

Media	F (4,100)	-	21.740^{**}	CD 1.531
Container	F (4,100)	-	15.203^{**}	CD 1.531
Media x Container	F (16,100)	-	3.399^{**}	CD 3.424

transplanting (Table 15). Among the different containers, C_4 (polythene cover) was found to be the best treatment (14.12cm) which was on par with C_1 (mud pot), C_2 (plastic pot) and C_5 (netted pot). Plants grown in C_3 (paper pot) produced plants with shortest root length (8.944cm).

In the case of different media, M_4 (soilrite) produced roots with highest length (16.108cm) which was on par with M_2 (fine sand) and was significantly superior to all other media. The treatment M_1 (coarse sand) produced plants with least root length (Fig. II).

There were significant interactions between media and containers in the case of root length at two months after transplanting (Table 15). The treatment combination C_4M_4 (polythene cover soilrite) produced longest roots (21.88cm). From the data analysed it was also noted that along with media M_1 (coarse sand) the container C_1 (mud pot) grown plants produced roots with maximum length which was on par with C_2 (plastic pot). Along with medium M_2 (fine sand) plants in the container C_4 (polythene cover) had maximum root length which was on par with C_1 (mud pot). Along with medium M_3 (charcoal) and M_5 (sphagnum moss) all the containers except paper pot had more or less same response on root length. However along with medium M_4 (soilrite) the plants grown in container C_4 (polythene cover) alone was superior compared to other container, with respect to root length.

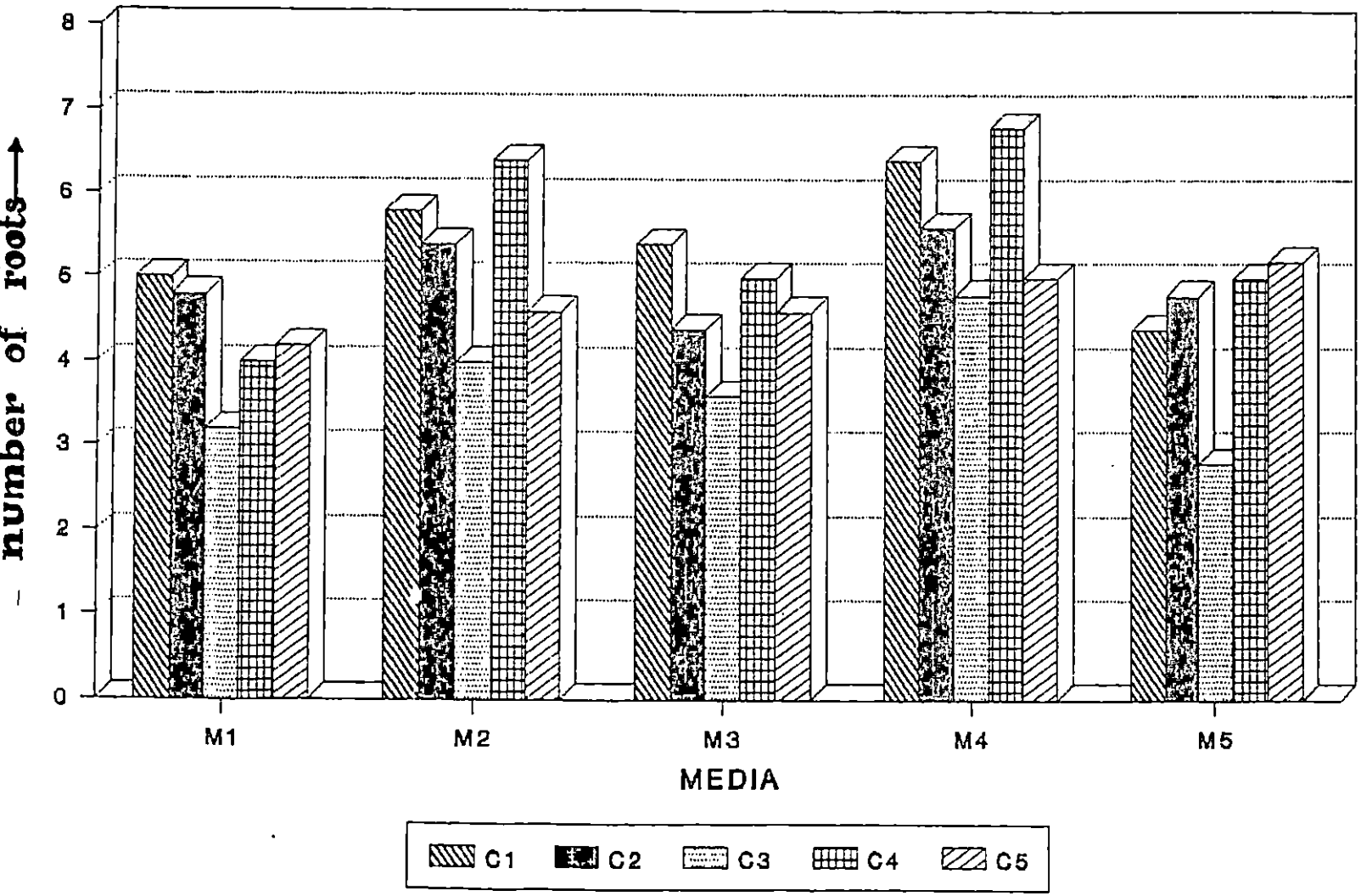

Fig. 1. Effect of media and containers on root number

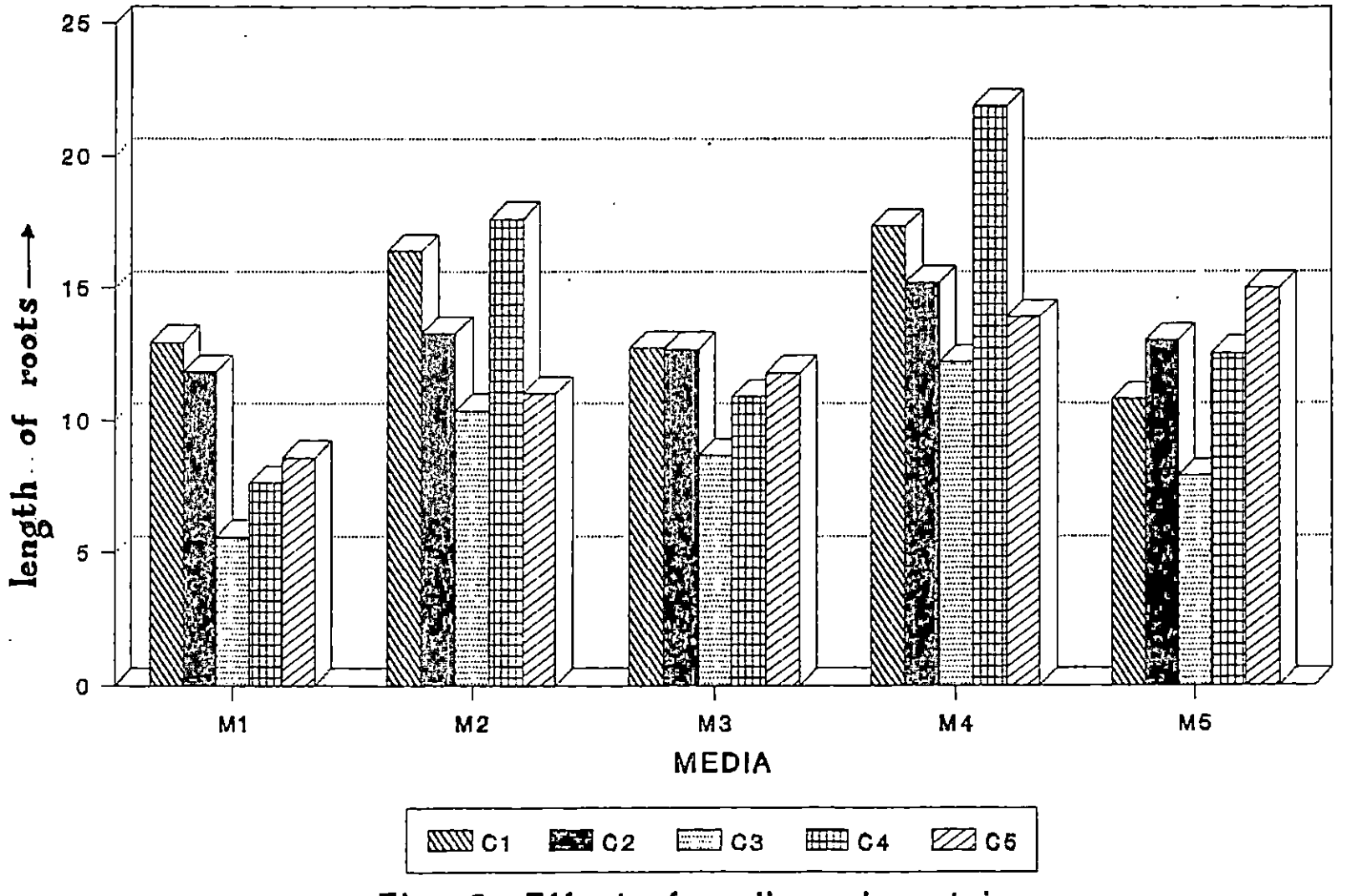

Fig. 2. Effect of media and containers on root length

DISCUSSION

5. DISCUSSION

The potentialities of plant tissue culture in rapid multiplication and crop improvement have provided a substantial impetus for biotechnological research. The plants produced by tissue culture are generally more expensive than conventionally produced plants. The cost of transfer of laboratary regenerants to soil has been estimated to be 40-80 per cent of total production cost. This transfer step is time consuming, labour intensive and may vary with the species or even varieties. The survival rate often determines whether or not the technology is economically feasible. Serious field mortality is often encountered while planting out. The present investigations carried out at College of Agriculture, Vellayani were mainly aimed for selection of suitable media and containers for ex vitro establishment of Anthurium andreanum plantlets. Attempts were also made for improving in vitro rooting efficiency of anthurium plantlets. The outcome of the investigations are discussed in the following pages.

Debergh and Maene (1981) pointed out that rooting in vitro was the most labour intensive part of micropropagation. In the present studies various factors influencing in vitro rooting such as microcutting size, plant growth substances, and other medium supplements like agar, and sucrose levels were standardised.

In vitro rooting of anthurium plantlets was favoured when comparatively larger sized shoots were used. Plantlets of 3 cm length with at least three leaves recorded shortest time (10.24 days) for root initiation and produced maximum number of root per shoot compared to smaller shoots. Ramesh (1990) has also reported that, compared to smaller sized shoots of jack plantlets, large sized shoots produced 100 per cent rooting with maximum number of roots per shoot. Higher food reserves and increased auxin production in the plantlet might have caused such a response.

In the case of plant growth substances, combination of BA 0.5 ppm and IAA 2 ppm was the best with respect to initiation of roots within the shortest period of time (11.4 days) with maximum number of root per shoot (3.88). While combination of BA and NAA took more time for root initiation, and number of roots per shoot was less compared to BA and IAA combination. IAA treated plants produced thin long roots, which help the plants to survive better when planted out, while plants in the medium containing NAA produces short thick roots. Lane (1979) also reported that NAA usually give rise to short thick roots. On contrary to the present observation, Williams and Taji (1989) reported that when NAA and NOA were used the roots produced were thin.

Increased agar concentration in the rooting medium helps the *ex vitro* establishment of plantlets but reduced the

rooting (Leshem, 1983, Marin and Gella, 1987, Short *et al.*, 1987). In anthurium, plantlets, shortest time (10.54 days) for root initiation was 0.7 per cent concentration of agar, and the number of roots per shoot decreases by increasing the agar concentration in the medium, while the length of root increases along with increase in agar concentration. Agar is not a totally inert material and contains impurities that can influence the *in vitro* rooting (Debergh, 1983; Hu and Wang, 1983).

Lowering the sucrose level in the culture medium is advantageous for *ex vitro* establishment as it helps the plantlets to switch over from heterotrophic to autotrophic growth (Conner and Thomas, 1982). The present study revealed that lowering sucrose level reduces the time required for root initiation. Desjardins and Tiessen (1985) found that very low sucrose concentration in the medium reduced the rooting percentage. It has also been found from the present studies that lowering the sucrose concentration in the medium reduced number of roots per shoot. Sucrose level maintained at normal level of MS medium (3%) took less time for root initiation (12.32 days) and more number of roots per shoot (4.22) compared to all other levels above and below.

In order to standardise the media and containers for *ex vitro* establishment of anthurium plantlets five media

paper pot, polythene cover, and netted pot were used. In an attempt to unravel the possible influence of media and containers and its interaction on 25 combinations of media and containers, parameters like survival percentage, number of leaves, hight of plant, area of new leaves, petiole length of new leaves, root number and root length were studied.

The survival percentage of plantlets with respect to plant size were specifically studied at weekly intervals. The results pertaining to the effect of plantlet size on survival percentage under different media and containers indicated that plantlets with a minimum of 2.5-3 cm size with 3-4 leves and two or more roots recorded 90.0 to 100.0 per cent survival irrespective of media and containers, except one with sphagnum moss as medium. The reason for the comparatively low survival percentage (up to 75 %) in sphagnum moss might be the less compactness of sphagnum moss with plants in the early stages. Sphagnum moss is light in weight and has a high water holding capacity (Hartman and Kester, 1986).

The number of leaves is basically a genetic factor which could be modified by physical conditions. In the present study it was found that different media and containers significantly influenced the production of new

leaves for the ex vitro establishment of anthurium plantlets. In the case of containers, plants raised in plastic pots recorded highest number of leaves in all the four fortnights. This might be due to the ability of plastic pot to maintain optimum moisture level with in the potting medium by preventing water loss through walls. Similar results was observed by Ramesh (1990). He found that plastic pot was the best suited container for planting out of jack plantlets. Soilrite was identified as the best potting media out of the five media tried in the case of leaf production, plants grown on soilrite recorded highest number of leaves in all the four fortnights. Significant interaction was recorded between media and containers with respect to leaf production. It has been found that with medium soilrite the containers had no significant influence in leaf production except at fourth fortnight after transplanting. At this stage, paper pot grown plantlets were found to be inferior to other containers. Soilrite was an ideal potting medium for maintaining an optimum moisture level and sufficient aeration to the root zone of plantlets. It was also recorded that with medium coarse sand or with medium fine sand the plants in the container polythene cover recorded highest number of leaves in all the four fortnights. This might be due to the interaction of sand with polythene cover by maintaining most suited condition for production of leaves.

With charcoal medium, plastic pot grown plants recorded maximum leaf production and with medium sphagnum moss, netted pot and plastic pot grown plants have greatest influence on leaf production. The reason for this is due to the positive interaction of charcoal and sphagnum moss with plastic pot.

As in the case of number of leaves, plant height also showed different response to the media and containers. Both these treatments at one and two months after transplanting C_1 (mud pot) outdid other containers and M_4 (soilrite) outdid other media by producing plants with largest hight. Significant interaction were also recorded between media and containers. Along with medium coarse sand or charcoal the best container was found to be mud pot at one month and two months after transplanting with respect to plant height. The superiority of this combination could be explained by a good support and supply system provided by the media in conjunction with the container mud pot. The less moisture holding capacity of coarse sand and or charcoal along with mud pot also has to be taken into account. In the case of medium fine sand at first month after transplanting no significant influence was shown by containers except netted pot which had least response on plant hight. This might be due to the high compactness of fine sand in small sized (one inch) netted pot because of the

partialy epiphytic nature of anthuriums and its aeration requiremnt for normal growth. However along with medium soilrite or with medium sphagnum moss containers showed no influence on plant hight at monthly intervals. This could be explained by the superiority of soilrite and sphagnum moss with the capacity to provide optimum aeration for plant growth without any interaction with containers.

The treatments which produced shortest plants were those raised on sphagnum moss and when netted pots were used as the containers. In this treatment excess moisture content and attack of termites on moist sphagnum moss might be the reason for the failure.

Leaves are the photosynthetic apparatus of the plants which synthesize carbohydrates and store for developmental aspects of plants. Hence the more the leaf area the more could be the photointerception and stored energy. So the media and container which could help the plants in producing larger leaves could be treated as better for their establishment.

In the present study, differential response with media and containers were recorded at each fortnight in the case of leaf area of anthurium plants. At the first fortnight after transplanting C_1 (mudpot) recorded maximum leaf area. At the second and fourth fortnights, C_2 (plastic

pot) was the best container and at the third fortnight C_4 (polythene cover) grown plants gave the the highest leaf area. In the case of media, first and second fortnights after transplanting, M_2 (fine sand) grown plants gave maximum leaf area but at third and forth fortnights after transplanting M_4 (soilrite) grown plants were superior to other media. Thus the medium soilrite once again proved its superiority as a potting medium for ex vitro establishment. Media and containers also showed interaction in the case of area of new leaves at fortnightly intervals. Along with the media coarse sand, the plants grown in polythene cover recorded highest leaf area at the third and fourth fortnights. This could be explained by the fact that the low moisture holding capacity of coarse sand is compensated by polythene cover by maintaining the moisture at the optimum level. Along with the medium fine sand upto third fortnight after transplanting, containers showed no influence on leaf area of new leaves, but at the fourth fortnight plastic pot grown plants were found to be the best with maximum leaf area. With medium charcoal or with medium soilrite the plastic pot grown plants recorded maximum leaf area in all the four fortnights. This is attributed to the better water holding capacity and aeration of charcoal and soilrite in plastic pot. Charcoal can retain enough moisture and air, preventing unwanted acid build up (Battacharjee, 1985). But with medium sphagnum moss the largest leaf area was recorded

on netted pot grown plants. This is supposedly due to the less compactness and water holding capacity of sphagnum moss on small sized (one inch) netted pot so that the plants obtained optimum moisture and aeration. Bose and Battacharjee (1980) reported that layers of sphagnum moss in the compost of orchids retain more moisture than osmunda and was found to be a good material for those orchids that require constant moisture supply.

The plants grown on media M_5 (sphagnum moss) and container C_3 (paper pot) showed poor result in the case of leaf area of new leaves at fortnightly intervals. This might be due to constant high moisture content of sphagnum moss and deterioration of paper pot owing to continuous moisture supply.

Length of petiole is another important morphological character of anthurium plants which has considerable influence on growth of plants. The petiole is cylindrical, smooth and its base forms a sheath around the stem (Higaki et al., 1984). The media and containers which could help plants in producing leaves with more petiole length is considered better because of its higher photointerception capacity. The present study also showed varying response with different media and containers at fortnightly intervals. The containers showed no significant influence on length of petiole at the second and forth

fortnights after transplanting. Among the media, M_4 (soilrite) grown plants gave the highest petiole length from second fortnight onwards. This again proved superiority of soilrite as a media for ex vitro establishment of anthurium plantlets. Significant interactions were recorded between media and containers in the case of petiole length at first, third and forth fortnight after transplanting. With the medium coarse sand, fine sand or soilrite, plants grown in polythene covers recorded highest petiole length. This could be explained by the fact that polythene cover can retain moisture and there by providing enough humidity for the ex vitro establishment. At the forth fortnight after transplanting except in case of charcoal all other media have no influence in petiole length with various containers. This could be explained that in the case of petiole length rather than containers, media have better influence at fortnightly intervals. The plants in the medium sphagnum moss recorded least influence in petiole length. This might be due to the high water content above optimum level in sphagnum moss.

The roots of anthurium are cylindrical, fleshy, epiphytic and adventitious, and the epidermis is developed as velamen (Higaki et al., 1984). Dycus and Kundson (1957) indicated that the principal role of velamen is mechanical protection and water conservation. Hence the roots have vital role in the growth of anthurium plants. The media and

containers which help to produce more roots and large roots are considered better. In the present investigation a differential response was observed in the case of media and containers with respect to root production and length of root at two months after transplanting. The plants in the containers C_4 (polythene cover) and media M_4 (soilrite) recorded maximum number of roots and length of roots. Significant interaction between media and containers were observed only in the case of root length. Along with the media, fine sand or soil rite, the plants grown on the container polythene cover recorded maximum root length. This might be due to the optimum depth of media in polythene cover and favourable interaction with polythene cover and media. Kyte and Briggs (1979) observed that depth of soil was important, as the survival rate of tissue cultured rhododendrons was found to be better in 10 cm pots rather than in shallow trays. In the case of coarse sand, mud pot grown plants have highest influence on root length. This is because of positive interaction of coarse sand with mud pot by maintaining optimum conditions for root development. With charcoal or sphagnum moss, the containers showed no significant influence on root development except in the case of paper pot which was inferior to other containers. This might be due to the depth of potting media in paper pot below the optimum level and also unfavourable interaction with charcoal and sphagnum moss.

SUMMARY

6. SUMMARY

The *in vitro* rooting factors and the suitability of various containers and potting media on *ex vitro* establishment of anthurium plantlets were investigated. The study was conducted during 1991-93 at the Plant Tissue Culture Laboratory of the Department of Horticulture, College of Agriculture, Vellayani.

The protocol developed by Sreelatha (1992) was adopted for *in vitro* production of Anthurium plantlets.

The salient findings of the study are summarised below.

Shoots of 3.0 cm long (with three leaves) were ideal for *in vitro* rooting and recorded minimum days for root initiation (10.24) and maximum number of roots per shoot.

Combination of BA 0.5 ppm + IAA 2.0 ppm recorded minimum time (11.4 days) for root initiation and maximum roots per shoot. IAA treated shoots produced thin long roots while NAA treated shoots produced short thick roots.

Agar at 0.7 per cent level recorded minimum days (10.54) for root initiation, while number of roots per shoot

decreased by increasing the agar concentration. Sucrose level maintained at normal level in MS medium (3 per cent) took less time for root initiation and produced more number of roots per shoot.

2.5 - 3.0 cm long plantlets with 3-4 leaves and two/ more root was identified as the optimum size for transplanting supporting highest survival percentage (90.0 - 100.0) in all the twenty five treatment combinations of media and containers.

Among the containers used plasticpot grown plants and among the media, soitrite grown plants recorded maximum number of leaves in all the four fourtnights while treatment combinations of mud pot and soilrite recorded highest rate of leaf production at second, third and fourth fortnigh after transplanting.

At one month and two months after transplanting tallest plants were produced in the contianers mud pot and in the media soilrite.

At first fortnight after transplanting mudpot grown plants recorded maximum leaf area. At second and fourth fortnight, plastic pot was the best container and at third

fortnight polythene cover grown plants gave highest leaf area. In the case of media, first and second fortnight after transplanting fine sand, and third and fourth fourtnight after transplanting seilrite grown plants gave maximum leaf area.

The containers showed no significant influence on length of petiole at second and fourth fortnight after transplanting, while among the media soitrite grown plants gave the highest petiole length from second fortnight ownwards.

The plants grown in polythine cover with media soilrite recorded maximum number of roots and length of roots at two months after transplanting.

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* - Orginals not seen

APPENDICES

APPENDIX - I

Composition of Murasige and Skoog (1962) medium

Particulars	Quantity per litre	Weight taken	Volume made up	Volume pipetted
Solution A				
NH_4NO_3	1650 mg	16.5 g	250 ml	25 ml
KNO_3	1900 mg	19.0 g		
$MgSO_4.7H_2O$	370 mg	3.7 g		
KH_2PO_4	170 mg	1.7 g		
Solution B				
$CaCl_2 2H_2O$	440 mg	8.8 g	100 ml	5 ml
Solution C				
H_3BO_3	6.2 mg	620 mg	100 ml	1 ml
$MnSO_4H_2O$	22.3 mg	2.23 g		
$ZnSO_4 7H_2O$	8.6 mg	860 mg		
KI	0.83 mg	83 mg		
$Na_2MoO_4 2H_2O$	250 mg	25 mg		
Solution D				
$FeSO_4 7H_2O$	27.8 mg	2.78 g	500 ml	5 ml
NaEDTA	37.3 mg	3.73 g		
Solution E				
$CoCl_2 6H_2O$	0.025 mg	12.5 mg	250 ml	0.5 ml
$CuSO_4 5H_2O$	0.025 mg	12.5 mg		
Solution F				
Glycene HCl	2.0 mg	200 mg	100 ml	1 ml
Nicotinicacid	0.5 mg	50 mg		
Phyridoxine HCl	0.5 mg	50 mg		
Thiamine HCl	0.1 mg	10 mg		
Inositol	100 mg			
Sucrose	30 g			
Agar	6 g			
pH	5.6 - 5.8			

APPENDIX - II

Composition of Murasige and Skoog (1962) medium modified by Pierik (1976)

Particulars	Quantity per litre	Weight taken	Volume made up	Volume pipetted
Solution A				
NH_4NO_3	825 mg	8.25 g	250 ml	25 ml
KNO_3	950 mg	9.50 g		
$MgSO_4.7H_2O$	370 mg	3.7 g		
KH_2PO_4	85 mg	850 mg		
Solution B				
$CaCl_2 2H_2O$	440 mg	8.8 g	100 ml	5 ml
Solution C				
H_3BO_3	6.2 mg	620 mg	100 ml	1 ml
$MnSO_4H_2O$	22.3 mg	2.23 g		
$ZnSO_4 7H_2O$	8.6 mg	860 mg		
KI	0.83 mg	83 mg		
$Na_2MoO_4 2H_2O$	250 mg	25 mg		
Solution D				
$FeSO_4 7H_2O$	27.8 mg	2.78 g	500 ml	5 ml
NaEDTA	37.3 mg	3.73 g		
Solution E				
$CoCl_2 6H_2O$	0.025 mg	12.5 mg	250 ml	0.5 ml
$CuSO_4 5H_2O$	0.025 mg	12.5 mg		
Solution F				
Glycene HCl	2.0 mg	200 mg	100 ml	1 ml
Nicotinicacid	0.5 mg	50 mg		
Phyridoxine HCl	0.5 mg	50 mg		
Thiamine HCl	0.1 mg	10 mg		
Inositol	100 mg			
Sucrose	30 g			
Agar	6 g			
pH	5.6 - 5.8			

ABBREVATIONS

ABA	-	Abscisic acid
BA	-	Benzyl adenine
GA	-	Gibberellic acid
2ip	-	2 isopentenyl adenine
IAA	-	Indole acetic acid
IBA	-	Indole butyric acid
2, 4-D	-	2, 4 - dichloro phenoxy acetic acid
NAA	-	Naphthalene acetic acid
NOA	-	Naphthoxy acetic acid
CD	-	Critical difference
OD	-	Optical density
MS	-	Murashige and Skoog

STANDARDISATION OF MEDIA AND CONTAINERS FOR *EX-VITRO* ESTABLISHMENT OF ANTHURIUM PLANTLETS PRODUCED BY LEAF CULTURE

By

AJITHKUMAR. P. V.

ABSTRACT OF THE THESIS
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ABSTRACT

Investigations were carried out at the Plant Tissue Culture Laboratory of the College of Agriculture, Vellayani during 1991-1993 to develop suitable methods to plant out Anthurium andreanum plantlets and to standardise media and containers to maximise the ex vitro establishment and growth of in vitro derived plantlets.

Segments of leaf were used as explant for producing required number of plantlets for the study. Various factors influencing in vitro rooting were standardised. Plantlets of 3 cm length with at least three leaves recorded shortest time (10.24 days) for root initiation and produced maximum number of roots per shoot compared to smaller shoots. Combination of BA 0.5 ppm and IAA 2.0 ppm was found to be the best for in vitro rooting. Agar at 0.7 % recorded shortest time (10.54 days) for root initiation and the number of roots per shoots decreased by increasing its concentration in the medium, while the length of root increased along with increase in agar concentration. Sucrose level maintained at normal level in MS medium (3.0 per cent) was found to be the best for in vitro rooting.

In order to standardise the media and containers for ex vitro establishment, media such as coarse sand, finesand, charcoal, soilrite and sphagnum moss and containers

such as mud pot, plastic pot, paper pot, polythene cover and netted pot were used.

Plantlets with at least 2.5-3 cm size (with 3-4 leaves and two or more roots) recorded 90.0 to 100.0 per cent survival irrespective of media and containers. Of the various madia and containers tried plastic pot as the container and soilrite as the media recorded highest number of leaves in the transplanted plants at fortnightly intervals. Both one and two months after transplanting, mud pot outdid other containers and soilrite outdid other media with respect to plant hight. In the case of leaf area at second and fourth fortnight, plastic pot and at third fortnight polythene cover was found to be the best container, and soilrite was the best medium at third fortnight onwards. The containers showed no significant influence on petiole length at second and fourth fortnight but at second fortnight onwards medium soilrite recorded maximum petiole length. The plants grown in polythene cover with media soilrite recorded maximum number of roots and length of roots at two months after transplanting. So it is evident that among the media, soilrite was the best for ex vitro establishment of anthurium plantlets but containers showed no uniform response with various growth factors.