

## STANDARDIZATION OF MICRO-PROPAGATION TECHNIQUE FOR *ANTHURIUM ANDRAEANUM* IN ANDAMAN ISLAND

V. Baskaran\*, K. Abirami, K. Venkatesan and P. Simhachalam

ICAR-Central Island Agricultural Research Institute (CIARI), Port Blair,  
Andaman and Nicobar Islands

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**Abstract:** Anthuriums are popular cut flowers with attractive spathe colours and highly popular as cut flower and potted plant in International markets. The humid tropical coastal climate of Andaman and Nicobar Islands is very congenial for growing anthurium. Availability of planting material is a major constraint for commercial cultivation of anthurium in the Island. Micro-propagation is the option for rapid multiplication of planting material with production of true to type, disease free quality planting material. The present study was therefore undertaken to standardize the micro-propagation of anthurium using MS media. Callus induction, shoot & root multiplication using leaf and apical shoot buds of *Anthurium andraeanum* explants was achieved with modified MS medium supplemented with 1.0 mg/l 2, 4-D and 1 mg/l BAP. Explants of young leaves and apical shoot buds showed callus formation at 75 and 63 days respectively after inoculation and the callus formation percentage was maximum in apical shoot bud (48.42 %) whereas in leaves it was 35.25%. Maximum number of shoots and percentage of rooted shoots per explant observed in apical shoot buds  $18.64 \pm 1.89$  and 95% respectively).

**Key words:** Anthurium, Andaman and Nicobar Islands, Micro-propagation, Callus, Explant

### INTRODUCTION

The genus *Anthurium*, a native to the tropics of Central and South America (Gantait *et al.*, 2008) belonging to the family Araceae, includes more than 600 species covering vast diversity within the family. The anthurium is a perennial herbaceous plant usually cultivated for its attractive, long-lasting flowers. The present day flowering anthuriums are mostly hybrids of different species involving mainly *Anthurium andraeanum* and *Anthurium scherzerianum*. *Anthurium* is either grown for showy cut flower or for their unusually attractive foliage. They are very popular with flower arrangers because of the bold effect and lasting quality of flowers. The spathe colour of species has been mainly used for the identification of the cultivars (Kobayashi *et al.*, 1987). In order to meet the market demand and preferences in terms of colours, shades, floral sizes and shapes, a huge variability available in *Anthurium* cultivars with respect to an array of spathe colours (ranging from red, orange, pink, coral and white) and belonging to the three spathe categories- namely, standard (single-coloured heart-shaped), obake (bicolours of green with another major anthocyanin colour) and tulip-type are utilized and cultivated across the regions of the world. The production of a single flower from each leaf axil takes place during the sympodial phase and was exploited for commercial flower production, because of the commercial flower is an inflorescence composed of the spadix (a spike of minute flowers closely arranged round a fleshy axis) and the spathe (a large sheathing bract enclosing the flower cluster) (Higakiet *et al.*, 1984). The commercial production of ornamental pot plants has a great potential in international markets. In the global

market, *Anthurium* cultivars with valued flowers are the second beside the Orchids among tropical cut flowers (Dufour and Guerin 2003; Chen *et al.*, 2003). The methods used for propagation of *Anthurium* are through seeds, division of suckers and top cutting (Mahanta and Paswan, 2001). Commercial propagation is by tissue culture. Seed propagation is slow in *Anthurium* which may take 3 years to bloom (Hamidah *et al.*, 1997; Viegas *et al.*, 2007). Vegetative propagation has a disadvantage of scarcity of planting material. Propagation by tissue culture techniques appears as an alternative to increase the production and the micropropagation of *Anthurium* is commercially used (Hamidah *et al.*, 1997; Martin *et al.*, 2003). Micropropagation is a suitable way to produce a large number of progeny plants which are genetically identical to the stock plant in a short time and, has some features to be chosen in commercial production such as multiplicative capacity in a relatively short time, healthy and disease-free production capacity and ability to generate population during a year. Micropropagation of *Anthurium* has been achieved by using variety of explants viz., leaf, petiole, spadix, spathe, seed, lateral bud and shoot tips. Plant regeneration of *A. andraeanum* is achieved through adventitious shoots formation from callus and direct shoot regeneration from lamina explants. The possibility of tissue culture in *Anthurium* was first reported by Pierik *et al.*, (1974), and the use of liquid culture for proliferation of callus (Martin *et al.*, 2003), the liquid or raft culture instead of solid medium for regeneration of adventitious shoots from leaf explants (Teng, 1997) were also reported. Vargas *et al.*, (2004) established an alternative method for regeneration of *Anthurium* plants by using *in-vitro* plants (derived from germinated seed) and plantlets (obtained from

\*Corresponding Author

micro-cuttings culture), which showed callus at the stem base. Propagation from axillary or terminal buds is the most ensurable method to have the highest genetic stability during *in-vitro* propagation of plants. MS media with modifications have been frequently applied in tissue culture of anthurium. Andaman and Nicobar Islands have wide scope for cultivation of anthurium due to the prevailing congenial climatic conditions. The major drawback for cultivation of anthurium in the Island is the non-availability of planting material. Mass multiplication through tissue culture is the only solution for large scale availability of quality planting material in the Island. With this background the present study was conducted to standardize the mass propagation of anthurium using growth regulators in the Andaman Islands.

## MATERIALS AND METHODS

**Plant material:** The variety Tropical Red which is maintained in the anthurium house of Garacharma experimental farm, ICAR-Central Island Agricultural Research Institute (CIARI), Port Blair, Andaman and Nicobar Islands was selected for collection of explants for the present study. The experiment was conducted at the Plant Tissues Culture Laboratory.

**Surface sterilization:** Sterilization methods used in *Anthurium* tissue culture is presented in Table 1. To minimize the contamination caused by fungus, endogen and exogen bacteria. Leaf and apical buds were the explants used for the present study and they were subjected to sterilization immediately after collection. Surface sterilization was done for 1 minute in 70% (v/v) ethanol and soaked in gentamicin solution for 30 minutes then soaked in 20% (v/v) commercial bleach (commercial bleach contains about 5% (v/v) sodium hypochloride) for 12 minutes. Leaves were rinsed three times in sterile distilled water. Sterile leaf explants were sectioned to about 1 cm<sup>2</sup> pieces.

**Media:** After surface sterilization, the leaf and apical shoot bud explants were transplanted into culture media supplemented with 30 g/L sucrose, 8.0 g/l agar-agar and different treatment of 2,4-D and BAP. The pH was adjusted to 5.65 by using NaOH or HCl 1 N solutions before autoclaving. The culture media were sterilized at 121°C and 15 psi (103, 42 kPa) for 20 min. The media differed in concentrations of plant growth regulators and light conditions. Seedlings with 2-3 leaves were used for shoot induction, which were obtained *in-vitro* from the MS culture medium (Murashige and Skoog, 1962) at a half salts concentration (MS). For shoot induction, the same basal medium was used with the addition of the growth regulator 6-benzylaminopurine (BAP), alone or in combination with kinetin (KIN) and indole-3-acetic acid (IAA); the basal medium without growth regulators was used as the control treatment.

The cultures were kept at 23°C ± 2°C under light conditions.

**Shoot micropropagation of Anthurium:** The regenerated shoots longer than 3 cm with a pair of leaves were transferred to the MS medium with 1 mg/L IBA and 2 mg/L BAP plant growth regulators. The shoots developed in the different treatments were transferred after 60 days to growth regulator-free culture medium. The material was maintained at 23°C ± 2°C under constant light, and the number of developed and rooted plants in the treatment was counted. For the adaptation process, 250 plantlets were selected (1.5-3.0 cm). The *in-vitro* plants were transplanted to a pro-tray with sterile media (perlite, cocopeat, vermicompost and sand in ratio of 1:1:1:1; v/v), maintained in covered and with semi-controlled conditions of light and temperature. After 60 days the *in-vitro* plants were totally exposed to environmental conditions and watered once a week to maintain sufficient moisture in the media.

**Statistical analysis:** The results are presented as mean values ± standard errors. All experiments were repeated five times. The data on callus induction rate and number of shoots per explant were subjected to analysis of variance (ANOVA) with the means separation ( $p < 0.05$ ) by Duncan's multiple range test (Duncan, 1955; Mize and Chun, 1988).

## RESULTS AND DISCUSSION

Different explants of anthurium were tried for micro-propagation, however response was observed only in leaf and bud explants. This is in accordance with findings that the success of the protocols depends on the variety of anthurium, type of explants, age of the explants, the components of the media used for shoot and root regenerations (George *et al.*, 2008; Nhut *et al.*, 2006). Both the leaf and bud explants of anthurium showed callus formation in modified MS media. Among the different combination of growth regulators, when 2,4-D was used alone, callus induction was not observed. When 2, 4-D was used in combination with BAP and NAA callus formation was found. The results are in accordance with Silva *et al.* (2005) who reported that various physical and biological factors including media play important roles in propagation of *Anthurium spp.* He also opined that different combinations of plant growth regulators and additives used increase the regeneration potential of explants which is similar to our findings. Explants were transferred to 16 h light and 8 h dark photoperiod to enhance organogenesis in calli. After six weeks, organs which were then sub cultured. Maximum percentage of callus formation was observed in MS media supplemented with 2, 4 D (0.25 mg/l), BAP (2 mg/l) and NAA (1 mg/l) in leaf explant (Table 2). In the two explants used, apical shoot bud showed early callus formation (63 days), maximum callus formation percentage (48.42%), number of shoots per plant (18.64 ± 1.89) and

percentage of rooted shoots (95%) in MS media supplemented with 2, 4 D (0.25 mg/l), BAP (2 mg/l) and NAA (1 mg/l) when compared with leaf as explant.

Shoot multiplication for two explants in our study with three sub-cultures showed the increasing trend in every sub-culturing as  $14 \pm 1.38$ ;  $19.98 \pm 1.56$ ,

$25.82 \pm 1.67$  (for leaves) and  $18.64 \pm 1.89$ ;  $26.70 \pm 1.68$ ;  $35.76 \pm 1.50$  (for apical shoot buds), respectively. Similar trends were observed in two anthurium varieties viz., Arizona and Sumi, as 15.64 and 12.24 (shoots per explants in initial culture) and 33.70 and 26.96 (shoots per explants in final culture) respectively (Atak and Celik, 2009).

**Table 1.** Sterilization methods used for *Anthurium* tissue culture under study

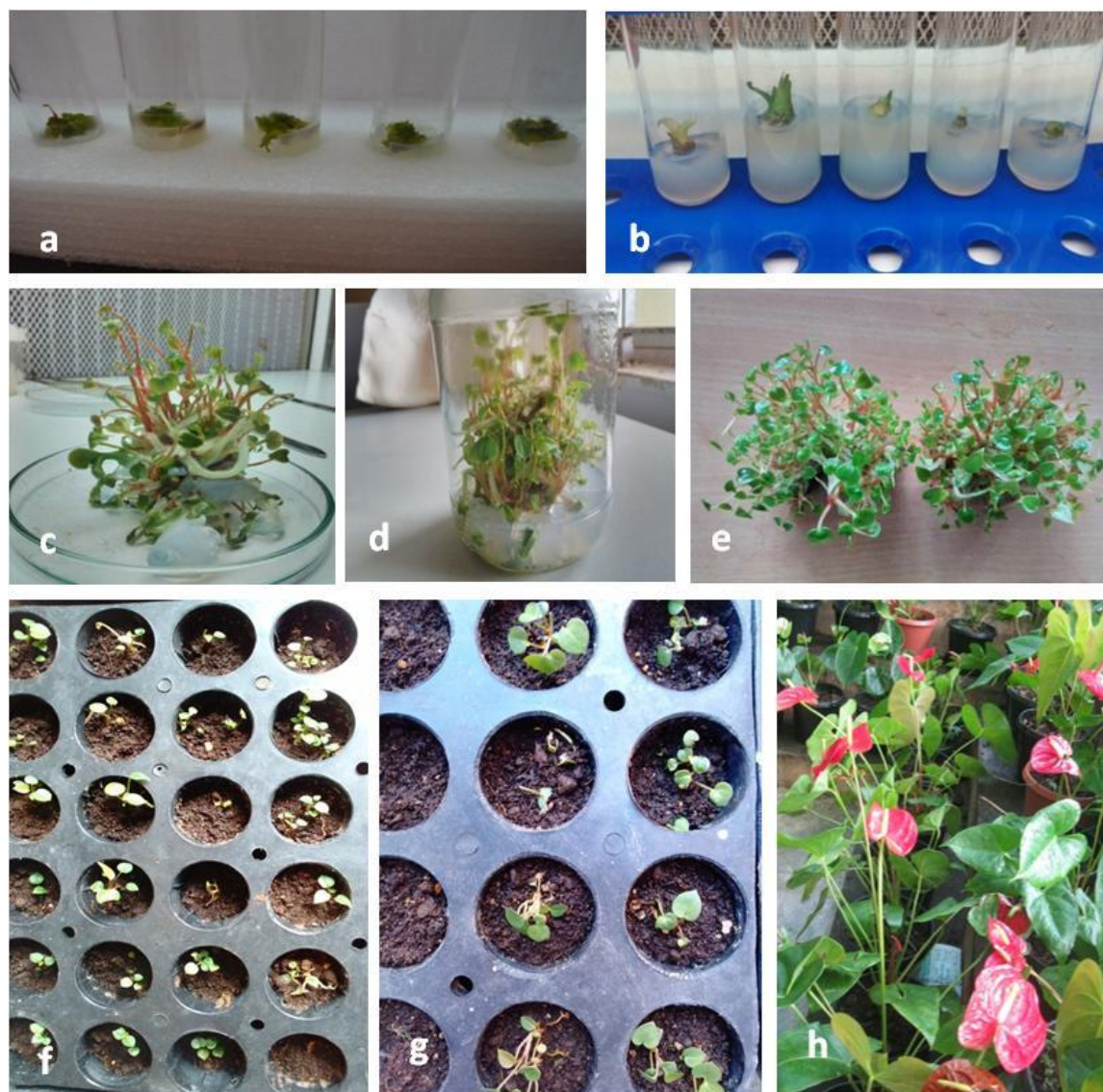
| A. species                 | Explant Source           | Sterilization method   |
|----------------------------|--------------------------|--|
| <i>Anthurium andreanum</i> | Leaf & Apical shoot buds | <ol style="list-style-type: none"> <li>1. Washing under running tap water [30-60 min]</li> <li>2. Teepol+ 0.1% bavistin antifungal solution [5min.]</li> <li>3. Washing under running tap water [10-15 min] till detergent removes</li> <li>4. Washed with distilled water several times</li> <li>5. Shifted under laminar air flow</li> <li>6. 0.1% [w/v] <math>\text{HgCl}_2</math> [5min.]</li> <li>7. Washed with sterile distilled water three times</li> <li>8. Air dried and inoculated on medium.</li> </ol> |

**Table 2.** Callus formation times and percentages of *Anthurium* cultivars along with shoot and root multiplication

| Explant           | Number of Explants | Modified MS media with diff. hormones       | Callus formation (days) | Callus formation percentage (%) | Number of shoots per explant (mean $\pm$ se) | Percentage of rooted shoots (%) |
|-------------------|--------------------|---|-------------------------|---------------------------------|--|---------------------------------|
| Young leaves      | 100                | 2, 4-D 0.25 mg/l<br>BAP-2mg/l+<br>NAA 1mg/l | 75                      | 35.25                           | $14.24 \pm 1.38$                             | 63                              |
| Apical shoot buds | 100                | 2, 4-D 0.25 mg/l<br>BAP-2mg/l+<br>NAA 1mg/l | 63                      | 48.42                           | $18.64 \pm 1.89$                             | 95                              |

**Table 3.** Shoot multiplication of *Anthurium* cultivars for explants types (Brown leaves and Apical shoot buds)

| Brown leaves |                    |  | Apical shoot buds  |  |
|--------------|--------------------|--|--------------------|--|
| Sub-culture  | Number of explants | Number of shoots per explant (mean $\pm$ se) | Number of explants | Number of shoots per explant (mean $\pm$ se) |
| 1            | 50                 | $14.24 \pm 1.38$                             | 55                 | $18.64 \pm 1.89$                             |
| 2            | 50                 | $19.98 \pm 1.56$                             |                    | $26.70 \pm 1.48$                             |
| 3            | 50                 | $25.82 \pm 1.67$                             |                    | $35.76 \pm 1.50$                             |



**Fig. 1.** Stages of micro-propagation of *Anthurium andreanum*

Inoculation of explant in modified MS media b. Callus initiation c-e. Shoot and root induction f-g. hardening of micro-propagated plants h. Field establishment of anthurium

In present study, the explants prepared from young leaves showed higher callus formation rates in a shorter time compare to old leaf explants. Therefore, selection and using the right leaf explants at the appropriate leaf stage is the first step of establishing a successful tissue culture because stages of the leaves show different response to propagation by indirect organogenesis. The effectiveness of young leaves over green leaf on callus induction rate and number of shoots per explants proved by earlier workers (Martin et al., 2003; Viégas et al., 2007; Atak and Çelik, 2009) was once again confirmed in our study also.

The results show the use of suitable explant with use of appropriate growth regulators in modified MS media for rapid and successful mass multiplication of *Anthurium andreanum*.

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