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MICROPROPAGATION OF *ANTHURIUM ANDRAEANUM* FROM LEAF EXPLANTS

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Abstract

Anthurium propagation by tissue culture techniques appears as an alternative to increase the production. Plant regeneration of *Anthurium andraeanum* cultivars "Arizona" and "Sumi" (Anthura) has been achieved through adventitious shoots formation from callus. The leaf explants were used for the propagation by indirect organogenesis. Callus induction medium consisted of half-strength MS basal salt with 0.6 mg/l 2,4-D, 1 mg/l BA. Shoot regeneration medium consisted of modified half-strength MS salts with NH_4NO_3 lowered to 250 mg/l, 0.1 mg/l 2, 4-D and 1 mg/l BA. Rooting medium was (modified half-strength MS salts) supplemented with 1mg/l IBA and 0.04%active charcoals. The callus induction rate of Arizona variety was higher as compared to Sumi variety and this increase was found significant. The number of shoots per explants for Arizona variety also significantly increased as compared to Sumi variety. Arizona and Sumi root percentages were high and these percentages were 98% and 95% respectively. The results showed that Arizona variety had different responses as compared to Sumi variety for callus induction and the number of shoots per explants. Shoot multiplication for two varieties increased in the multiplication stages and this increase in number of shoots per explants were found important ($p<0.05$).

Introduction

Anthurium is a kind of major cut flower species in the tropical and subtropical countries and economically important genera in the family Araceae. Numerous species of flowering potted *Anthurium* cultivars and landscape plants have been grown. In the global market, the *Anthuriums* are valued flowers and traded value of Orchids and *Anthuriums* among tropical cut flowers are first and second respectively (Dufour & Guerin 2003; Chen *et al*., 2003).

The three basic propagation methods for *Anthurium*, propagation by seed, traditional vegetative and tissue culture are used for conventional production. Propagation by tissue culture techniques appears as an alternative to increase the production. Micropropagation of *Anthurium* is commercially used (Hamidah *et al*., 1997; Martin *et al*., 2003).

Micropropagation of *Anthurium* has been achieved with various tissues including leaf, petiole, spadix, spathe, seed, lateral bud and shoot tips. Plant regeneration of *Anthurium andraeanum* has been achieved through adventitious shoots formation from callus and direct shoot regeneration from lamina explants. The tissue culture of *Anthurium* was first reported by Pierik *et al*., (1974) and they used liquid culture to proliferate callus (Martin *et al*., 2003). In *Anthurium*, the liquid or raft culture instead of solid medium was used on the regeneration of adventitious shoots from leaf explants (Teng, 1997). Vargas *et al*., (2004) established an alternative method for regeneration of *Anthurium* plants. They obtained *In vitro* plants from germinated seed and plantlets obtained from micro-cuttings culture. These plantlets showed callus at the stem base.

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Micropropagation by callus induction from leaf culture was difficult step for *In vitro* propagation. Various physical and biological factors including media play role during *Anthurium In vitro* propagation (Silva *et al*., 2005). One of the important factors was genotype for the *In vitro* multiplication. Nhut *et al*., (2006) showed that genotypes of 10 *Anthurium* cultivars had different response to callus induction and shoot regeneration. Bejoy *et al*., (2008) used 1-1.5 cm^2 lamine segments of *Anthurium andraeanum* cv. Agnihothri on half-strength MS basal medium. They compared the regeneration efficiencies of different concentrations of plant growth regulators.

This paper describes the results for the establishment of an alternative and rapid method for regeneration of two commercial cultivars of *Anthurium andraeanum.*

Materials and Methods

Plant material: Commercially obtained *Anthurium andraeanum* cultivars "Arizona" and "Sumi" (Anthura) were selected. The leaf explants from two varieties were used as plant material. Leaf segments of *A. andraeanum* cultivars at the young brown and green stages were collected from these plants (Martin *et al*., 2003).

Surface sterilization: To minimize the contamination caused by fungus, endogen and exogen bacteria, leaf explants were surface sterilized 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 $1cm^2$ pieces.

Media: For the propagation by indirect organogenesis, Medium A, B and C were used. Medium A was used for callus induction, medium B was used for shoot regeneration induction and medium C was used for rooting.

Callus induction: Fifty leaf explants from each *Anthurium andraeanum* cultivars "Arizona" and "Sumi" were transplanted onto the Petri dishes containing callus induction medium (Medium A). This medium consisted of half-strength MS (Murashige & Shoog, 1962) basal salt and vitamins supplemented with 30 g/l sucrose, 6 g/l agar, 0.6 mg/l 2,4-D (2,4-dichlorophenoxyacetic acid), 1 mg/l BA (benzylaminopurine). All cultures were placed in the dark at 27°C for one month for callus initiation (Puchooa & Sookun 2003; Nhut *et al*., 2006; Te-chato *et al*., 2006).

Shoot regeneration: White calli formed in medium A after one month and were transferred to Vitro-Vent plant tissue culture containers (Duchefa) containing modified half-strength MS salts with NH_4NO_3 lowered to 250 mg/l (Hamidah *et al*., 1997; Poddar *et al*., 1997; Dufour & Guerin 2005), 100 mg/l myo-inositol, 5 mg/l nicotinic acid, 0.5 mg/l thiamin, 0.5 mg/l folic acid, 0.05 mg/l biotin, 30 mg/l Fe-NaEDTA, 20 g/l sucrose and 6 g/l agar supplemented with 0.1 mg/l 2, 4-D and 1 mg/l BA (Medium B). Shoot regeneration was carried out in growth chamber at 27°C under 16/8 h light and dark photoperiod. One month later, the plantlets were formed.

Rooting: The regenerated shoots longer than 2 cm and with a pair of true leaves were transferred to the medium C. It consisted of Medium B supplemented with 1mg/l IBA (indole-3 butyric acid) and %0.04 active charcoals to initiate roots (Puchooa & Sookun 2003).

Shoot micropropagation of *Anthurium*: Micropropagation of *Anthurium* was established with shoots of *Anthurium andraeanum* cultivars "Arizona" and "Sumi" regeneration through callus culture. Node explants were cultured on the medium B supplemented with 0.1 mg/l 2, 4-D and 1 mg/l BA. Shoots were subcultured at 30 day intervals. Va_1 and Vs_1 was named for shoot regeneration from callus cultures of *Anthurium* cultivars and Va_2, Va_3 and Va_4, for subcultures of Arizona and Vs_2, Vs_3 and Vs_4 for subcultures of Sumi.

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 shoot 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 & Chun, 1988).

Results

Callus formation of *Anthurium* was observed on the leaf explants from green and brown leaves in medium A. Table 1 show callus formation time and the percentage of explants that induced callus formation from the leaves of Arizona variety. Explants from green coloured leaves showed callus formation at 65 days of culture in medium with 31.25% low induction rate and the colour of callus formed was yellow. Callus formation period for brown leaf explants decreased to 30 days of culture with a high rate of 81.25%. Based on results of preliminary experiments, brown leaves we cultured in medium for micropropagation of two *Anthurium andraeanum* cultivars.

Callus induction rate, number of shoots per explants and percentage of rooted shoots for Arizona and Sumi varieties are given in Table 2. While callus induction rate for Arizona variety was 80%, this rate was 70% for Sumi variety. The number of shoots per explants for Arizona variety were more relative to Sumi variety. Callus induction and the number of shoots of explants for Arizona variety increased significantly ($p<0.05$) with respect to Sumi variety. Plantlets 125 of Arizona and 100 of Sumi longer than 2 cm were transferred into medium C supplemented with 1mg/l IBA and % 0.04 active charcoals to initiate roots. The roots emerged from the base of the regenerated shoots. The results showed 98 and 95% rooting rates in Arizona and Sumi varieties in medium C respectively (Table 2).

For *In vitro* propagation of *Anthurium andraeanum* cultivars "Arizona" and "Sumi", shoots were subcultured at 30 day intervals. The number of adventitious shoots per shoot explant is shown in Table 3. The number of shoots of Arizona and Sumi were increased subsequent subcultures (Fig. 1). The increase in number of shoots per explants were found significantly important ($p<0.05$).

Discussions

Ornamental industry has applied immensely *in vitro* propagation approach for large-scale plant multiplication of elite superior varieties (Martin *et al*., 2003; Silva *et al*., 2005). Micropropagation is an alternative method of vegetative propagation, which is well-suited for the multiplication of elite clones (Silva *et al*., 2005). In this study we were interested in regeneration and propagation systems of two *Anthurium andraeanum* cultivars.

Table 1. Callus formation times and percentages of *Anthurium adreanum* cultivars "Arizona".

Leaf stage	Number of explants	Callus formation times in medium a (day)	Callus formation percentage in medium a (%)
Yellow leaves	48	65	31.25
Brown leaves	48	30	81.25

Table 2. Shoot and root regeneration from callus cultures of *Anthurium andreanum* cultivars.

Anthurium andreanum cultivars	Number of explants	Callus induction rate (%)	Number of shoots per explant (mean ± SE)	Percentage of rooted shoots
Arizona	50	80[a]	15.64 ± 1.69[a]	98
Sumi	50	70[b]	12.24 ± 1.18[b]	95

Data presented as means with different letters within a column indicating significant differences at $p<0.05$ according to Duncan's Multiple range test. Each mean represented 5 replications.

Table 3.Shoot multiplication of *Anthurium andreanum* cultivars.

Subculture	Arizona		Subculture	Sumi	
	Number of explants	Number of shoots per explant (mean ± se)		Number of explants	Number of shoots per explant (mean ± se)
Va_1	50	15. 64 ± 1.69[a]	**Vs_1**	50	12.24 ± 1.18[a]
Va_2	50	22.70 ± 1.46[b]	**Vs_2**	50	15.98 ± 1.36[b]
Va_3	50	26.76 ± 1.30[C]	**Vs_3**	50	21.82 ± 1.87[c]
Va_4	50	33.70 ± 1.09[d]	**Vs_4**	50	26.96 ± 1.46[d]

Data presented as means with different letters within a column indicating significant differences at 0.05 according to Duncan's Multiple range test. Each mean represented 5 replications.
Abbreviations: **Va_1** =initial shoot (Shoot regeneration from callus cultures of Arizona variety) **Va_2**, **Va_3**, **Va_4**,= subcultures of Arizona variety, **Vs_1** = initial shoot (Shoot regeneration from callus cultures of Sumi variety), **Vs_2**, **Vs_3**, **Vs_4**= subcultures of Sumi variety.

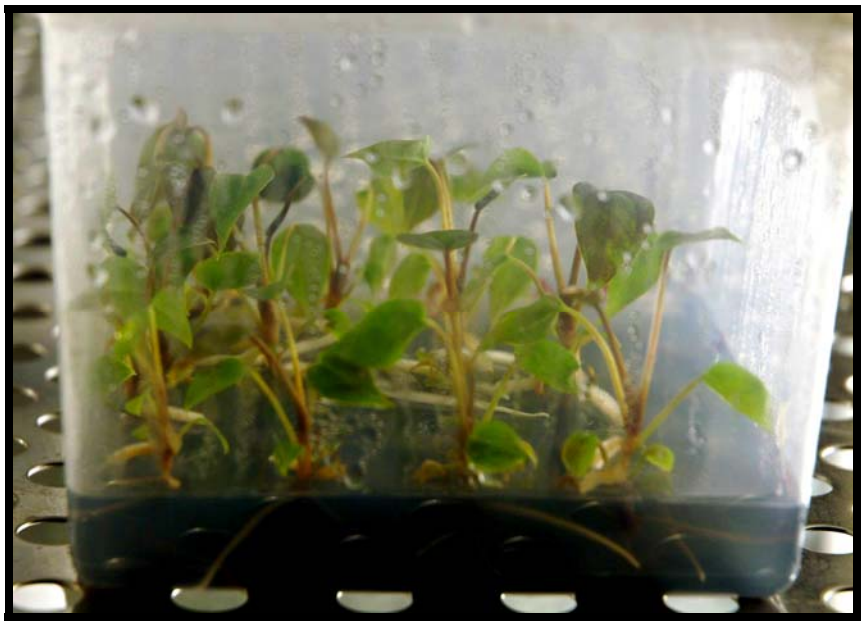

Fig. 1. *In vitro* propagation of Anthurium cultivars (Arizona). The shoots with root were grown in plant tissue culture container.

The success of tissue culture is related to the correct choice of explants material (George *et al*., 2008). The explants from lamina segments of *Anthurium* at the brown and green stage showed different response to propagation by indirect organogenesis. In our preliminary study, explants from brown lamina were better for callus induction rates and callus formation duration.

Martin *et al*., (2003) cultured brown and green lamina explants of *Anthurium andraeanum* cultivars Tinora Red and Senator. And found that the explants from brown young lamina of Senator and Tinora Red showed 60% and 70% callus formation and green lamina explants showed 40% and 45% callus formation respectively. Bejoy *et al*., (2008) reported that pale green leaf explants were better and showed good callus development in the initial medium.

Our investigations showed that the callus induction rate of Arizona variety was increased with respect to Sumi variety and this increase was found significant ($p<0.05$). The number of shoots per explants for Arizona variety was also showed significant increase in respect to Sumi variety. Callus induction rate and shoot numbers per explants showed differences between Arizona and Sumi varieties'. These varieties had different responses to the same medium and culture conditions. Many factors play an important role during *Anthurium In vitro* propagation and genotype is the most important factor.

The behaviour of plant tissues *In vitro* in processes such as callus formation and growth and regeneration often seem to be under an over-riding genetic control with other factors exerting only a minor effect (George *et al*., 2008).

Nhut *et al*., (2006) studied the effects of 10 different *Anthurium* genotypes on callus induction derived from leaf explants. 'Arizona' callus induction rate was 9.8% after 60 days and 10.7% after 100 days. They reported 65.1% explants of 'Pistache' induced calli after 100 days of culture. Also, they realized that this process was best for 'Pistache', not for the other Anthurium cultivars. Callus induction from leaf explants depends strongly on genotype.

Te-chato *et al*., (2006) used leaves, node and internodes of three genotypes of *Anthurium* (Plew Thien Phuket, Sonat, Valantino). They cultured the explants Murashige Skoog medium (MS) (Murashige & Skoog 1962), modified MS (MMS), woody plant medium (WPM) and Nitsch & Nitsch medium (NN) and observed highest callus formation of 86.6% from leaf explants in MS and MMS medium. Callus formation of Valantino was higher according to the others.

Arizona and Sumi shoots formed roots and percentages of rooted shoots were high and root formation percentage were 98% and 95% respectively. These results showed that Arizona variety had different responses according to Sumi variety for callus induction and the number of shoots per explants but there were no differences in root formation of *Anthurium andraeanum* between cultivars "Arizona" and "Sumi".

Martin *et al*., (2003) cultured their regenerated *A. andraeanum* plantlets in half strength MS medium supplemented with 0.54 µM NAA and 0.93 µM Kinetin for *in vitro* rooting. *In vitro* rooting percentage of Tinora Red and Senator variety were 100% and 99%. Feng *et al*., (2006) reported that, among the regenerated Anthurium shoots, approximately 50% had roots in raft culture. Bejoy et al. (2008) achieved about 98% root from the newly formed shoots in 0.5 mg/l NAA in half-strength MS medium in six weeks. In micropropagation study of *Parthenocissus quinquefolia* (L). Feng *et al*., (2006) observed only 76% of plantlets rooted in NAA while over 90% of plantlets rooted in IBA containing rooting medium.

We observed that shoot multiplication for two varieties was increased in the subculture of Arizona and Sumi varieties. Our initial culture of Arizona and Sumi, Va_1 and Vs_1 produced 15.64 and 12,24 shoots per explants respectively. At every subculture, shoot numbers regenerated form nodal explants gradually increased. In the multiplication stage Va_4 and Vs_4 the number shoot per explant for Ariona and Sumi variety were 33.70 and 26.96 respectively. The increase in number of shoots per explants were found significantly important ($p<0.05$).

Vargas *et al*., (2004) achieved regeneration of *A. andraeanum* from callus tissue. It was observed that the subculture of calli generates a higher number of shoots per callus. Four weeks later they observed an average of 3.6 shoots per explants produced and 6 weeks later, the calli increased in its size and 43.8 plantlets per callus were obtained.

Bejoy *et al*., (2008) reported that multiplication was enhanced in the subculture of *Anthurium andraeanum* Hort. cv. Agnihotthri. They succeeded to increase the rate of shoot production in the second multiplication stage with the average of 9.7 shoots per explant in 60 days.

Many genotype dependent effects are caused by interactions between the plant's genotype and the cultural environment (George *et al*., 2008). Because of different genotypes of varieties, different responses for callus induction and shoot formation by leaf explant culture of two *Anthurium* varieties were observed in our study. The suitable method of micropropagation of new variety must be determined by experiment. The present investigation demonstrated the regeneration protocol was good responses for two *Anthurium* varieties.

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