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Morphogenetic Responses In *Anthurium Andreanum* (Hort) Cultivars

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ABSTRACT

In this study, *in vitro* morphogenetic responses have been studied in popular Anthurium andreanum cultivars Tropical Red, Acropolis, Sun Glow, Esmeralda, Chaco, Pistachio, Neveda and Safari employing explants of various types and growth stages, through indirect organogenesis. Creamy and compact callus initiated in cut ends along the veins of leaf lamina with mid rib (LLMR) and petiole with leaf (PL) explants of various cultivars in 30-120 days of inoculation. The callus was sub cultured and incubated in dark for further proliferation. The leaf tip, candle and petiole explants did not show any kind of response, they turned brown and died subsequently. Explants of young pale green leaves with smooth structure showed early response in all cultivars. Callus proliferation rate was 3.0-5.0 times in 30-35 days of incubation in various cultivars. Shoot bud initiation and elongation was observed after 45-60 days of incubation in dark. The number of shoot buds ranged from 30-60 per explants and 8-10 buds of them attained 1.5-2.5 cm in height. Later, shoots were separated, inoculated in shoot elongation medium and incubated in light and remaining callus was sub cultured in callus multiplication medium and incubated in dark. The callus and shoot multiplication ratio was 1-2 and 2.5-4.2 times, respectively. Shoots attained 4-5.3 cm in height in 45-55 days along with rooting and ready for hardening in 65-80 days. Rooted plantlets were hardened in 1:1 ratio soil rite and coco-peat mix in portrays, transferred to beds prepared with coconut coir-pith, 95% of plants survived, appeared normal and flowered. The present results are useful in in vitro production of good quality planting material as well as in standardizing high through put protocols for commercial production.

Keywords: Tropical Red; *in vitro*; callusing; dark incubation; shoot elongation.

INTRODUCTION

Anthurium, a genus of the family *Araceae*, is indigenous to tropical America. It is popular cut flower in tropical and subtropical countries. Numerous *Anthurium* species are produced and traded internationally as cut-flowers, flowering potted plants and landscape plants. Obviously there is huge demand for quality planting material. The three basic propagation methods for *Anthurium*, propagation by seed, traditional vegetative and tissue culture are used for conventional production. However, as cross pollinating species, the off springs show poor uniformity. Besides poor germination and short viability span make seed propagation difficult. Vegetative propagation is slow and time consuming to achieve large scale production of propagules. Further, *Anthurium* plants grow slowly and would have little commercial potential without multiplication of elite cultivars through plant tissue culture. Micropropagation of *Anthurium* has been achieved through adventitious shoots formation from callus and direct shoot regeneration[1,2,3,4,5] with various tissues including leaf, petiole, spadix, spathe, seed, lateral bud and shoot tips. However, it appears early callusing response, fast and high multiplication rate, fast shoot elongation and high survival rate of plantlets in hardening are factors

to be improved through further research. In this view, in the present study, *in vitro* morphogenetic responses such as culture establishment, callusing response, callus proliferation and shoot multiplication rate been researched employing different explant type and stage/age of explants in elite *Anthurium* cv. Tropical Red, Acropolis, Sun Glow, Esmeralda, Chaco, Pistachio, Neveda and Safari.

MATERIALS AND METHODS

Media preparation and sterilization

Media compositions for callus induction, proliferation, shoot bud induction, shoot elongation and rooting were standardized in preliminary studies conducted in our laboratory.

Callusing, proliferation and shoot initiation medium (A1)

It was prepared by adding MS salts6, 100 mg/ L inositol, 0.4mg/ L Thymine Hcl, 30gm/ L sucrose, 0.2gm/L 2,4-D, 1mg/l BAP and pH was adjusted to 5.8±0.05

Shoot elongation and rooting medium (A2)

It was prepared by adding MS salts, 100 mg/l inositol, 0.4mg/ L thymine HCL, 1mg/ L pyridoxine HCL, 1mg/ L calcium pentothenate, 1mg/ L Nicotinic acid, 1.5 mg/ L BAP, 0.3mg/l IAA and pH was adjusted to 5.8±0.05.Medium was boiled with 6.5gm/ L agar, dispensed 30-35ml into pre sterilized 250ml glass culture bottles, autoclaved in steam sterilizer (Nat steel Pvt. Ltd., India) at 121oC and 18lbs for 18 minutes and stored in media storage room at ambient temperature for 7-10 days before usage.

Explants collection, sterilization and inoculation

Healthy mother plant of various cultivars free from pest and disease were selected, leaf, petiole and candles were excised, immediately stalks were dipped into sterile water and brought to laboratory. They were washed twice in sterile, soaked in solution containing Bavistin (200 mg/100 ml sterile water) and citrimide (50mg/100ml sterile water) with 2 drop soap oil, kept in shaker for one hour, water washes 3-4 times. Then were immersed in 2% sodium hypochlorite for four minutes, washed twice in sterile water at two minutes interval, taken to Laminar Air Flow chamber, changed into fresh sterile flask, treated with 0.01% mercuric chloride for five minutes, washed 4 times in sterile water at three minutes interval, dipped for 30 seconds in 70% ethanol and washed twice in sterile water at 2 minutes interval.

Experiments and incubation

Experiments on type of explants

Sterilized explants were excised into shoot tip (ST), petiole, petiole with leaf (PL) leaf lamina with mid rib (LLMR-0.5 cm both side) and candles (0.5 cm), inoculated into A1 medium and incubated in dark conditions at 25±1°C.

Experiments on stage of leaf

Leaves of various growth stage viz., stage 1 (Just opened leaf having brown color and very soft texture), stage 2 (Young leaf having light/pale green color with brown tinge and smooth texture) and stage 3 (Fully matured leaf having dark green color with rough texture) were collected from different cultivars, sterilized, excised, inoculated in A1 medium and incubated in dark at 25±1°C.

Experiments on callus proliferation and shoot multiplication rate

Callus was separated from initiated cultures, five callus clumps of ~0.5 cm^2 size has been inoculated per culture bottle containing A1 medium and incubated at 25±1°C in dark conditions for proliferation and induction of shoots. Shoots of >1.5 cm with a 2-3mm callus at base were separated, eight shoots were inoculated per culture bottle containing A2 medium and incubated in 16/8 hour light by incandescent tubes maintained at 25±1°C for elongation and rooting.

Observations, data collection and analysis

Observations on callus induction and bacterial appearance were recorded after four weeks of inoculation till 10th week. Observations on shoot bud induction and elongation were recorded after 6 weeks of inoculation till 12th week. All experiments were repeated for four times with 5 replications. Percent callusing response and bacterial appearance were calculated. Callus proliferation and shoot multiplication rates were calculated (no. of bottles having 5 clumps of ~0.5 cm obtained from a bottle of culture). The data was subjected to ANOVA where

ever necessary and the results are presented as mean± standard errors.

RESULTS AND DISCUSSION

In the present study, the effect of type of explants, growth stage of explants and genotype on in vitro morphogenetic response has been experimented in Anthurium cv. Tropical Red, Acropolis, Sun Glow, Esmeralda, Chaco, Pistachio, Neveda and Safari. Leaf tip (LT), petiole, petiole with leaf (PL) leaf lamina with mid rib (LLMR-0.5 cm both side) and candles were used as explants. The results revealed that 40-50% and 60-85% of LLMR and PL explant showed callusing response, respectively in 60-100 days in various cultivars (Table 1). Callusing was first observed along the cut ends of leaf lamina especially at the mid rib and vein region of explants. The leaf tip, petiole and candle explants did not show any kind of response. Similarly, foliar explants exhibit more potential for callus induction when they contained midrib or vein, which was in line with results reported earlier[4]. In this study, visual bacteria started appearing in these explants and subsequently they started turning into brown color. The petiole and candle explants were first to show bacteria, browning. All of them died in 15-20 days. The leaf tip explants initially turned yellow, were second to show bacteria, browning and died in 25-30 days (Table 1). Unresponsive LLMR (up to 25%) and PL (up to 25%) explants also showed bacteria, browning and died, but less compared to other explants.

Callusing response was tested in explants of three growth stages of leaves. In vitro morphogenetic response in explants is expressed in accordance with favorable characteristics, achieved by selection of explants of right growing stage characterized by color, texture, maturity etc. In this study, in general, among explants of three growth stages of leaves employed, explants of stage-2 leaves showed early (30-50 days) callusing response compared to stage-1 (90-120 days) and stage-3 (60-90 days) leaves in various cultivars (Table 2). In particular, using explants of growth stage-2 leaves, Anthurium cv. Tropical Red showed early callusing response (30-35 days), whereas Pistachio and Sun Glow (50-60 days) showed late response and callusing response in other varieties ranged between times taken by these two varieties. Moreover, there was no much difference in time taken for callusing

Table 1: Effect of explants on culture establishment and callusing in Anthurium cultivars

Cultivar	Leaf lamina with mid rib (LLMR)	Leaf tip (LT)	petiole with Leaf (PL)	Candle	petiole
Tropical Red	Callusing	NR/BC	Callusing	NR/BC	NR/BC
Acropolis	Callusing	NR/BC	Callusing	NR/BC	NR/BC
Sun Glow	Callusing	NR/BC	Callusing	NR/BC	NR/BC
Esmeralda	Callusing	NR/BC	Callusing	NR/BC	NR/BC
Chaco	Callusing	NR/BC	Callusing	NR/BC	NR/BC
Pistachio	Callusing	NR/BC	Callusing	NR/BC	NR/BC
Nevada	Callusing	NR/BC	Callusing	NR/BC	NR/BC
Safari	Callusing	NR/BC	Callusing	NR/BC	NR/BC
Callusing response (days)	60-75	--	90-100	--	--
Callusing response (%)	40-50%	--	60-85%	--	
Appearance of bacteria (days)	35-40 (IVth)	25-30 (IIIrd)	35-40 (Vth)	8-10 (Ist)	15-20 (IInd)
Explants shows bacteria (%)	Upto 25%	Upto 100%	Upto 25%	Upto 100%	Upto 100%

Note: NR-no response; BC-Bacteria

response in explants of stage-1 and stage-3 among the mentioned varieties. However, an earlier study reported that superior in vitro morphogenetic response from relatively older explants to younger one4. On the contrary to this, explants from folded brown leaves2 and newly expanded brown leaves exhibited better callusing response in Anthurium cultivars. Also, explants from green leaves showed oxidation and turned necrotic and dead later3,5,7. Further, time taken for callus induction was above 45 days in the mentioned earlier studies2,3,4,5,7. However, in the present study both younger (growth stage-1) and older (growth stage-3) explants showed delayed in vitro response compared to young (growth stage-2) explants.

In this study, rate of callus response was tested in explants of growth stage-2 and stage-3. In general, among the explants used, the rate of callusing response from explants of stage-2 leaves was better (53-87%) compared to explants of growth stage-3 leaves (24-53%) in various cultivars. Further, explants of stage-2 leaves showed good callus growth and development also. In particular, employing explants of growth stage-2 leaves, callusing rate was significantly highest and lowest in cv. Tropical red (87.50±8.65) and cv. Acropolis (53.33±5.43), respectively and the same in other cultivars ranged between responses of these two cultivars. In earlier studies, callus induction rate was 52.9% using pale green leaves4, 81% in newly expended brown leaves5,3,7 and 67% folded brown leaves2 In this study, rate of callusing response was in accordance with those studies used explants from growth stage-1, whereas the response was fast and high in explants of growth stage-2 leaves (young, pale green and smooth texture) in all cultivars. Hence, employing appropriate explants of right growth stage found to be of prime importance to obtain fast as well as high rate of callusing response.

In this study Callus obtained was creamy, compact, and slow growing and five callus clumps of ~0.5 cm^2 size was subcultured per bottle containing A1 medium and incubated in dark for proliferation. About 3-5 fold increase in volume of calli clumps was achieved after 30-35 days of incubation in various cultivars. On proliferated calli, shoot buds originated as protuberances, which ranged from 30-60nos in various cultivars. *Anthurium* cv. Tropical Red, Sun Glow, Safari and Nevada showed fast and high callus proliferation rate (45-50 days and >4.5 folds; Table 2), cv. Esmeralda, Chaco, Pistachio showed fast and medium callus proliferation rate (45-50 days and <4.0 folds) and cv. Acropolis, showed slow and less callus proliferation rate (60 days and <3 folds). A previous study reported that 2-3 fold increase in volume of callus[4].

Table 2: Effect of growth stage of leaf on in vitro morphogenetic response in Anthurium cultivars

Cultivar	Stage 1 leaf (Days)	Stage 2 leaf (Days)	Stage 3 leaf (Days)	Stage1 leaf: Response (%)	Stage2 leaf: Response (%)	Callus Multiplication rate	Shoot multiplication rate
Tropical Red	90-100	30-35	60-75	40.29±12.32	87.50±8.65	1:5.0	1:4.2
Acropolis	90-100	40-45	60-65	24.28±8.34	53.33±5.43	1:2.9	1:2.5
Sun Glow	100-120	50-60	75-80	35.23±7.97	80.20±6.47	1:5.0	1:4.0
Esmeralda	90-100	40-45	60-65	53.34±10.36	66.66±6.72	1:4.0	1:3.0
Chaco	100-120	45-50	75-80	33.30±9.64	60.20±5.75	1:3.5	1:2.7
Pistachio	100-120	50-60	75-80	40.20±8.93	72.38±5.32	1:4.0	1:3.8
Nevada	100-120	40-45	75-90	35.26±10.43	59.63±6.31	1:4.5	1:3.6
Safari	100-120	40-45	75-90	30.30±9.12	55.97±6.12	1:4.9	1:3.3
General Response	Very late	Early	Late response	Low	High	2.9-5.0	2.5-4.2
SEM				1.11	0.93		
CD				3.27	2.87		

Fig. 1: Morphogenetic responses in Anthurium cv. Tropical Red

In this study, incubation of callus cultures for 15-20 days more after 35 days (callus proliferation period) of regular incubation period resulted in elongation of shoots. Among sprouted buds, 8-10 shoots of 1.5-2.5 cm in height per clump has been recorded. Shoots were slender and etiolated under dark incubation. However, upon shifted into light, they started turning green and become sturdy. Similarly, the etiolated shoots produced in dark were transformed into green shoots when incubated under light4. Eight shoots of >1.5 cm were inoculated per bottle containing A2 medium and incubated in light for further shoot elongation and rooting. The results showed that, the shoot multiplication rate was high in Anthurium cv. Tropical Red, and Sun Glow (>4 folds), medium in cv. Esmeralda, Pistachio, Chaco, Safari and Nevada (3-4 folds) and low in Acropolis (<3folds). In few of earlier studies, eight shoots sprouts per explants4,7 and in few of other studies 12-15 shoots per explants5 has been reported. In this study, simultaneously rooting appeared in third week of inoculation, shoots have grown up to 4.0-5.3 cm in height in 45-55 days (Figure1) and rooted plantlets were ready in 65-80 days after inoculating into A2 medium in various cultivars. Incubation period for elongation and rooting appears as 2-3 weeks more in our study compared to earlier studies4,5,7. In this study, the plantlets were ready with sufficient height (growth) by 7-8 weeks after inoculation into A2 medium, however, extending incubation in same medium for another 3-4 weeks found to help in higher plantlet survival in hardening. Overall results shows genotype has also got strong impact on in vitro morphogenetic responses in Anthurium. The in vitro rooted plantlets were washed thoroughly in running tap water, hardened in 1:1 ratio soil rite and coco-peat mix in portrays, transferred to beds prepared with coconut coir-pith, 95% of plants survived, appeared normal and flowered.

In conclusion, LLMR and PL explants and explants of young pale green leaves with smooth texture showed fast and high *in vitro* morphogenetic responses in all eight elite cultivars of *Anthurium*. These results would help in standardizing high throughput protocols for commercial production of quality planting material.

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