

Callus mediated plant regeneration of two cut flower cultivars of *Anthurium andraeanum* Hort.

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Abstract

Plant regeneration of *Anthurium andraeanum* cultivars 'Pumasillo' and 'Corallis' has been achieved through adventitious shoots formation from callus. Young brown leaf lamina was used as explants for indirect organogenesis. These were disinfected using sodium hypochlorite (5%), ethanol (70 %) and HgCl₂ (0.1%). The results showed that both the varieties responded differently to callus induction, callus proliferation and shoot initiation treatments. In 'Corallis', the maximum number of cultures initiated callus on Nitsch medium supplemented with 5 mgL⁻¹ adenine and 2 mgL⁻¹ BAP while in Pumasillo, maximum number of cultures initiated callus on modified MS medium supplemented with 5 mgL⁻¹ adenine and 3 mgL⁻¹ BAP. Maximum shoot regeneration was observed on modified MS supplemented with 0.01 mgL⁻¹ TDZ in both the cultivars. Rooting medium was (modified half-strength MS salts) supplemented with 0.5 mgL⁻¹ IBA and 0.04% active charcoals. The callusing frequency of 'Pumasillo' was higher as compared to 'Corallis'. Rooted plantlets were successfully acclimatized in polythene covered plastic trays containing cocopeat. Later on the hardened plantlets were successfully transplanted to commercial potting medium (1:1:1:1 cocopeat: rice husk: sand: FYM) and were transferred to poly house with survival rate of ninety per cent.

Key words: 6-Benzyl amino purine, adenine, *Anthurium andraeanum* Hort., callus, callus index, *in vitro* regeneration, kinetin, leaf explant, micropropagation, photoperiod, shoot regeneration, thiazuron

Introduction

Anthurium andraeanum Hort. of Araceae is a perennial, herbaceous plant highly valued for its colourful attractive luxurious flowers and exotic foliage. Among about 1500 known tropical ornamental *Anthurium* species, *A. andraeanum* is commercially cultivated as a cut flower and potted plant throughout the world primarily because of its long vase life. In the global market, the Anthuriums are highly valued flowers and traded value of Anthuriums is next to orchids among tropical cut flowers. It is propagated both by seeds and by vegetative means (cuttings and suckers). The seed to seed cycle during sexual propagation is long, tedious and amenable for presence of variation. Conventional vegetative propagation proves to be time consuming and takes years to develop commercial quantities of elite clones (Martin *et al.*, 2003). Moreover devastating diseases like bacterial blight caused by *Xanthomonas campestris* pv. Dieffenbachiae, anthracnose caused by *Gleospodium* sp., root rot caused by *Pythium* and *Phytophthora* sp. are serious threats to Anthurium industry which are spread by infested planting material. Also a rapid method of multiplication is necessary to provide enough planting material of the newly developed varieties that are available in meager amount. The world as well as Indian production of cut Anthurium is far behind the demand due to lack of elite planting material (Nair, 2005). Hence, there is an urgent need to standardize a quicker method of propagation.

Tissue culture techniques appear as an attractive alternative to increase the plant material production. Micropropagation of Anthurium is commercially used (Martin *et al.*, 2003). Plant regeneration of *A. andraeanum* has been achieved directly

from lamina explants (Martin *et al.*, 2003), root explant (Chen *et al.*, 1997) as well as from axillary bud (Kunisaki, 1980; Mahanta and Paswan, 2001). The major problems in micropropagation of a *Anthurium* is slow growth rate of plants in culture coupled with the time required for weaving, which push up the cost of production. Moreover, *in vitro* response is genotype dependent and it varies from explant to explant within the genotypes. Thus, standard protocol is needed to be established for each commercial cultivar separately according to different agro-climatic conditions in which they are cultivated. Reliable proliferation of callus and subsequent plant regeneration are important for massive plant propagation, and also for studies on genetic transformation and development of transgenic plants with new traits. The improvement of the micropropagation system for this species could be important for its commercial applicability. This paper describes the preliminary results for the establishment of an alternative method for regeneration of *A. andraeanum* cv. 'Pumasillo' and 'Corallis' plants from callus tissue through organogenesis.

Materials and methods

Two popular red coloured cultivars, 'Pumasillo' and 'Corallis' (KF Bioplants Pvt. Ltd) having good demand in floriculture industry were chosen as the experimental materials. The mother plants were maintained in Hi-tech green house at Department of Floriculture and Landscaping. Newly opened, young brown leaves were used as source of explants. The explants were subjected to pre-treatment, using a fungicide Bavistin (0.25 %) and a bactericide 8-HQC (0.20 %) for 2 hrs with constant

shaking at 80 rpm on orbital shaker followed by treatment with solution of detergent (Teepol BDH) (10%) for 10 minutes and finally repeated washing in double glass distilled water. Further, sterilization procedure was carried out under aseptic conditions in a laminar air flow cabinet. The pre treated explants were treated with 5% sodium hypochlorite followed by wiping with ethyl alcohol (70%) followed by treating with 0.1 % mercuric chloride solution for 2-5 minutes. The treated leaf pieces were then rinsed four times with sterile distilled water.

The decontaminated explants were cultured on Modified MS (Murashige and Skoog, 1962) medium with macronutrients at half strength and full strength micronutrients (MaMS), modified MS medium with reduced NH_4NO_3 (200 mgL^{-1}) (MMS) strength, Half strength MS medium, ($\frac{1}{2}$ MS) and Modified Nitsch medium (Nitsch, 1969). All the media contained 30 g of sucrose, 8.0 gL^{-1} agar and for callus induction different combinations of growth hormones including adenine (3-5 mgL^{-1}), BAP (0.5-5 mgL^{-1}), 2,4-D (0.2-2 mgL^{-1}), kin (0.2-3 mgL^{-1}) and TDZ (0.1-0.5 mgL^{-1}) were tested. Medium pH was adjusted to 5.8 with NaOH before adding agar. Media were autoclaved for 15 minutes at 121°C and dispensed as 25mL aliquots into 125 mL glass jars. Ten explants were inoculated on each treatment and replicated thrice. For callus induction, explants were grown in a culture environment at 25 ± 2 °C with continuous darkness. For regeneration experiments, the calli were grown at 25 ± 2 °C with a 16 hr photoperiod provided by 40 W cool white fluorescent tubes kept 50 cm above bench surface (approx. 1000 lux light intensity). Growth of the callus was assessed based on a visual rating. The mean score was expressed as the growth score G. Callus index (CI) was computed by multiplying per cent explants initiating callus with the growth score.

After 8 weeks of incubation, the calli were transferred on MS medium supplemented with various growth hormone combinations viz., BAP (0.5-5 mgL^{-1}), adenine (2-5 mgL^{-1}), NAA (0.25-1 mgL^{-1}), TDZ (0.01-0.08 mgL^{-1}) and GA_3 (0.1-0.2 mgL^{-1}) for further growth and regeneration of shoots. Subculture was done every four weeks as and when sufficient callus growth was noticed. To enhance the rooting process shoots were transferred to $\frac{1}{2}$ MS medium supplemented with IBA (0.5-1 mgL^{-1}) and activated charcoal (0.0-0.04%).

Well rooted plantlets were maintained on the same culture media for two months. Later fully grown plantlets were removed from the culture vessels. The roots were washed in tap water, dipped in 0.1% bavistin solution and transferred to plastic tray with polythene cover, containing sterile cocopeat. These were kept under high humidity and low light intensity for 15 days and finally these were transferred to hi tech greenhouse.

Results and discussion

An efficient regeneration system for *A. andraeanum* from callus tissues was achieved in this research. Previous reports have shown that the vegetative propagation of several *Anthurium* species is a very difficult task (Hamidah *et al.*, 1997; Pierik and Steegmans, 1976), moreover, the application of culture techniques, have been also difficult to establish because of the high contamination indexes observed in these culture (Brunner *et al.*, 1995).

The success of tissue culture is related to the correct choice of explants material (George *et al.*, 2008). In our preliminary study,

explants from brown lamina were found better for callus induction. Similar results were reported by Martin *et al.* (2003). Bejoy *et al.* (2008) reported that pale green leaf explants were better and showed good callus development in the initial medium.

Probably the most critical factor in *Anthurium* tissue culture is the genotype (Nhut *et al.*, 2006; Geier, 1987). This fact is also highlighted from the results of present study as it was found that both the cultivars used had different media requirement at all the stages of plant regeneration. 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).

As with most of the plant species, the higher percentage of explants survival reported in the present study depended on the duration of treatment and surface sterilant used. Sterilization procedures reported in the literature were found less effective and new procedure suitable to local conditions were developed. Significant difference among different surface sterilization treatments was observed for the control of rate of contamination and survival of cultured explants (Table 1). Surface sterilization with a combination of three sterilants, sodium hypochlorite (0.5 %), ethanol (70%) and mercuric chlorite (0.1 %) was found to be most effective for controlling contamination of the leaf explants and also showed maximum survival (83.43 %) of the cultures. The combination treatment involving 3 sterilants might have helped in controlling the infection of explants by virus, systemic bacteria or fungal contaminations, which resulted in less contamination and better survival of explants.

The basal medium requirement is highly tissue dependent and varies from one variety to another (Aswath and Biswas, 2001). Also, callus induction depends on the plant genotype (Nhut *et al.*, 2006). Results of present study are in line with these earlier

Table 1. Effect of various sterilization treatments on contamination and establishment of *Anthurium* leaf explant

Sl. no.	Surface sterilant (%)	Time (min)	Contamination (%)	Death of explant (%)	Culture established (%)
1.	NaOCl (0.5)	15	100 ^a (90.00)*	0 ^e (00.00)	0.00 ⁱ (0.00)
2.	NaOCl (1.0)	15	45 ^c (42.12)	16.6 ^d (24.05)	38.32 ^e (38.24)
3.	NaOCl (1.5)	15	28.1 ^d (32.02)	41.7 ^b (40.20)	26.63 ^f (31.07)
4.	HgCl ₂ (0.05)	5	95.5 ^b (77.71)	0.56 ^f (04.31)	3.29 ^h (10.45)
5.	HgCl ₂ (0.1)	5	21.6 ^e (27.71)	14.8 ^d (22.60)	63.33 ^c (52.73)
6.	HgCl ₂ (0.1)	8	10 ^g (18.43)	33.3 ^c (35.25)	56.66 ^d (48.83)
7.	Ethyl alcohol (70) wipe + HgCl ₂ (0.1)	5	13 ^f (21.14)	14.8 ^d (22.60)	71.69 ^b (57.85)
8.	Ethyl alcohol (70) wipe + NaOCl (1.0)	15	31.6 ^d (34.23)	51.7 ^a (45.96)	16.60 ^g (24.04)
9.	NaOCl (0.5) + Ehtanol (70) + HgCl ₂ (0.1)	10+3	6.49 ^g (14.76)	10 ^e (18.43)	83.43 ^a (65.98)
LSD (P=0.05)			4.48	3.33	3.52

*Data shown in parenthesis is arcsine transformed data. Column values with different superscript are significantly different LSD (P=0.05)

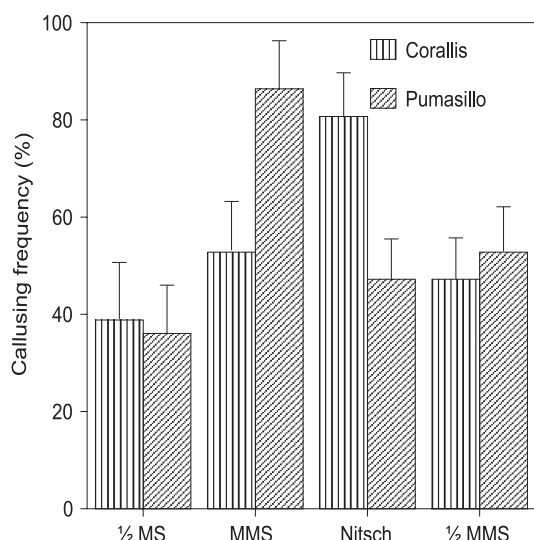


Fig. 1. Effect of different culture media on callus induction and proliferation of anthurium cv. 'Corallis' and 'Pumasillo'

findings, both the varieties tried, responded differently to the basal media composition. Variety 'Corallis' performed better on Nitsch medium while 'Pumasillo' performed well on MS medium (Fig. 1). The importance of the ammonium level was first demonstrated by Pierik and Steegmans (1976). The results of present experiment are in line with the earlier findings that a reduced NH_4NO_3 concentration was essential for callus induction in both the cultivar. Similar results have been reported by Sreelatha *et al.* (1998) and Mini Balachandran (1998).

During the present investigations cytokinins were found to be

essential for callus induction from leaf explant. In this study the growth regulator adenine in combination with BAP was found to be good in callus as well as shoot induction. These findings are supported by that of Prasad *et al.* (1999) who used adenine and BAP for callus induction in *A. andraeanum*. For 'Corallis' the maximum number of cultures initiating callus (88.89 %) was recorded on Nitsch basal medium supplemented with 5 mgL^{-1} adenine and 2 mgL^{-1} BAP, this treatment also took minimum number of days for callus induction (23.33 days), while in 'Pumasillo', MMS medium supplemented with 5 mgL^{-1} adenine and 3 mgL^{-1} BAP recorded highest per cent (88.89 %) of cultures initiating callus (Table 2). 'Corallis' recorded intense callusing on Nitsch medium supplemented with 5 mgL^{-1} adenine and 2 mgL^{-1} BAP. The callus was pale green in colour and globular in appearance whereas 'Pumasillo' recorded intense callusing on MMS medium supplemented with 5 mgL^{-1} adenine and 3 mgL^{-1} BAP yielding pale yellow callus.

In the present study, MMS medium fortified with 2, 4-D 0.5 mgL^{-1} showed highest callus multiplication rate, giving a pinkish white, nodular callus with organogenic potential. Treatment combination MMS medium supplemented with 3 mgL^{-1} adenine and 2 mgL^{-1} BAP also recorded good callus growth yielding pale yellow nodular callus (Fig. 2).

Excellent proliferation of callus occurred on same culture media tried, but the callus failed to regenerate. Callus showing exuberant growth is less conducive for regeneration and organ neof ormation generally follows cessation of unlimited proliferation. Regeneration was noticed in these calli on addition

Table 2. Effect of different treatments on callus induction from leaf explant in *A. andraeanum* cv. 'Pumasillo' and 'Corallis'

Basal medium	Treatment combinations	'Pumasillo'		'Corallis'	
		culture initiating callus (%)	days for callus induction	culture initiating callus (%)	days for callus induction
MMS	5 mgL^{-1} A+ 3 mgL^{-1} BAP	88.89 ^a (70.53)*	24.00 ^p	51.86 ^b (46.06)*	45.33 ^b
	4 mgL^{-1} A+ 3 mgL^{-1} BAP	66.67 ^d (54.74)	27.67 ⁿ	-	-
	5 mgL^{-1} A+ 2 mgL^{-1} BAP	69.32 ^{cd} (50.37)	28.67 ⁿ	55.56 ^e (48.19)	42.67 ^c
	0.5 mgL^{-1} BAP+ 1 mgL^{-1} 2,4-D	81.82 ^b (64.76)	34.67 ^l	-	-
	0.5 mgL^{-1} BAP+ 2 mgL^{-1} 2,4-D	63.03 ^c (52.55)	31.00 ^m	59.32 ^f (50.37)	35.67 ^f
	1 mgL^{-1} BAP+ 0.5 mgL^{-1} 2,4-D	70.52 ^c (57.12)	25.33 ^o	55.56 ^e (48.19)	37.67 ^e
MaMS	1 mgL^{-1} Kn+ 5 mgL^{-1} A	25.77 ^m (30.51)	43.00 ^e	-	-
	3 mgL^{-1} Kn+ 5 mgL^{-1} A	48.14 ^h (43.94)	37.00 ^k	-	-
	2 mgL^{-1} Kn+ 1 mgL^{-1} 2,4-D	40.68 ⁱ (39.63)	45.33 ^f	36.97 ^h (37.45)	41.00 ^d
	1 mgL^{-1} Kn+ 2 mgL^{-1} 2,4-D	33.01 ^j (35.07)	46.67 ^e	40.68 ⁱ (39.63)	48.33 ^a
	2 mgL^{-1} BAP+ 5 mgL^{-1} A	-	-	33.33 ^j (35.26)	45.67 ^b
MS/2	5 mgL^{-1} A+ 3 mgL^{-1} BAP	51.86 ^e (46.06)	66.67 ^{ab}	-	-
	0.25 mgL^{-1} BAP+ 0.19 mgL^{-1} IAA+ 0.09 mgL^{-1} Kn	44.44 ⁱ (41.81)	66.00 ^b	-	-
	0.1 mgL^{-1} BAP+ 0.2 mgL^{-1} IAA+ 0.1 mgL^{-1} Kn	33.33 ^j (35.26)	67.33 ^a	-	-
	0.2 mgL^{-1} 2,4-D+ 1 mgL^{-1} BAP	40.68 ⁱ (39.63)	62.33 ^c	-	-
	0.5 mgL^{-1} 2,4-D+ 2 mgL^{-1} BAP	33.33 ^j (35.26)	53.67 ^d	-	-
	Nitsch	4 mgL^{-1} A+ 3 mgL^{-1} BAP	48.14 ^h (43.94)	41.67 ^h	66.67 ^c (54.74)
5 mgL^{-1} A+ 3 mgL^{-1} BAP		51.86 ^e (46.06)	41.67 ^h	77.78 ^c (61.87)	30.00 ^h
5 mgL^{-1} A+ 2 mgL^{-1} BAP		55.56 ^f (48.19)	38.33 ^j	88.89 ^a (70.53)	23.33 ^k
0.5 mgL^{-1} BAP+ 2 mgL^{-1} 2,4-D		51.86 ^e (46.06)	39.67 ⁱ	55.56 ^e (48.19)	30.00 ^h
0.5 mgL^{-1} TDZ+ 0.2 mgL^{-1} BAP		36.97 ^k (37.45)	34.67 ^l	70.52 ^d (57.12)	29.33 ⁱ
0.5 mgL^{-1} TDZ+ 1 mgL^{-1} 2,4-D		48.14 ^h (43.94)	37.67 ^{jk}	77.78 ^c (61.87)	37.33 ^e
1 mgL^{-1} BAP+ 0.1 mgL^{-1} 2,4-D		33.33 ^j (35.26)	40.67 ^{hi}	85.53 ^b (67.64)	34.67 ^g
LSD ($P=0.05$)		3.25	1.04	2.55	0.65

* Data shown in parenthesis is arcsine transformed value. The blank space in table shows that no response was recorded on that particular treatment and data analysis was done considering only the treatments giving response. Column values with similar superscript values are non-significantly different with each other ($P=0.05$)

Table 3a. Effect of various treatments on shoot regeneration and growth of shoots cv. 'Pumasillo'

Treatment	Shoot regeneration (%)	Days for shoot induction	No. of shoot primordia/explant	Mean number of shootable explant (>1cm)	Length of longest shoot (cm)
MMS + 0.5 mgL ⁻¹ BAP	73.80 ^{bc} (59.21)*	31.00 ^d	6.67 ^c	3.33 ^b	2.27 ^{bc}
MMS +0.5 mgL ⁻¹ BAP + 0.5 mgL ⁻¹ NAA	60.00 ^{de} (50.77)	41.00 ^a	5.67 ^d	2.67 ^c	2.00 ^d
MMS + 1 mgL ⁻¹ BAP	67.09 ^{cd} (54.99)	36.67 ^b	5.33 ^{de}	2.00 ^d	2.17 ^c
MMS + 1 mgL ⁻¹ BAP + 0.5 NAA	53.35 ^e (46.92)	40.00 ^a	5.00 ^e	1.67 ^d	1.40 ^e
MMS + 5 mgL ⁻¹ adenine + 3 mgL ⁻¹ BAP	67.09 ^{cd} (46.92)	31.33 ^{cd}	5.67 ^d	2.00 ^d	1.09 ^f
MMS + 3 mgL ⁻¹ adenine + 2 mgL ⁻¹ BAP	80.00 ^b (63.43)	20.33 ^f	12.67 ^a	5.67 ^a	2.53 ^a
MMS + 0.01 mgL ⁻¹ TDZ	90.75 ^a (72.29)	28.67 ^c	12.67 ^a	6.00 ^a	2.40 ^{ab}
MMS + 0.02 mgL ⁻¹ TDZ	80.00 ^b (63.43)	32.33 ^c	11.67 ^b	5.67 ^a	2.37 ^{ab}
LSD (P=0.05)	7.31	1.15	0.58	0.45	0.16

* data shown in parenthesis is arcsine transformed value. Values with similar superscript values are non-significantly different with each other (P=0.05)

Table 3b. Effect of various treatments on shoot regeneration and growth of *Anthurium* cv. 'Corallis'

Treatment	Days to shoot initiation	No of shoot primordial/explant	No of shootable shoots/explant	Length of longest shoot	No of leaves/shoot
MMS + 5 mg/L ADN+ 2 mg/L BAP	30.6	5.6	2.6	1.9	3.5
MMS+0.5 mg/L BAP	28.0	7.0	3.5	1.7	3.0
MMS+ 0.01 mg/L TDZ	26.5	11.0	6.0	2.5	3.5
Nitch+0.5 mg/L BAP	28.6	9.6	4.0	2.5	5.0
Nitch+ 3 mg/L ADN+2 mg/L BAP	22.0	12.0	5.0	2.6	7.0
Nitch+1 mg/L 2,4-D+ 0.5 mg/L KIN	25.0	7.5	4.5	1.9	5.6

of cytokinins. TDZ is one of the several substituted ureas such as N-N' diphenyl urea and N-(2-chloro-4-pyridyl)-N' phenyl urea that have been investigated for cytokinin activity (Huetteman and Preece, 1993). TDZ-induced physiological and morphological responses in a wide variety of plant species have been reported. The activity of TDZ varies widely depending on its concentration, exposure time, cultured explant and species. TDZ at higher concentration causes a reduction in shoot formation (Magioli *et al.*, 1998). The results obtained in our experiments with TDZ are also in line with these earlier findings. In both the cultivars, maximum shoot regeneration was observed in the treatment containing low concentration of TDZ (0.01 mgL⁻¹) (Table 3a,b). High levels of cytokinin to auxin favours shoot bud induction whereas an opposite condition *i.e.* an increased concentration of auxin and low cytokinins is root promoting (Skoog and Miller, 1957). In general, the "Skoog-Miller Model" holds good for many species. But in the present investigation cytokinins alone were found to be

more potential in inducing shoots (Fig. 4). Further, shoot regeneration ability decreased at higher concentration of BAP. Addition of adenine released the suppressive effect of higher BAP; incorporation of 3.0 mgL⁻¹ adenine with higher level of BAP (2.0 mgL⁻¹) resulted in 80 % shoot regeneration. However, highest per cent regeneration (90.75 %) was registered with TDZ when used at low concentration (0.01 mgL⁻¹). Both the treatment combinations containing GA₃ resulted in yellowing of the green callus and no regeneration occurred.

Shoot proliferation was generally achieved by cultivating isolated shoots under low illumination on medium supplemented with cytokinins as sole hormonal compounds. The lower levels of cytokinins were observed to regenerate more number of shoots per explant, and when the concentration of cytokinins was increased, reduced or no regeneration at all took place. Similar results have been reported by Pierik and Steegmans (1976) and Kunisaki (1980). On contrary, some other workers (Sreelatha *et al.*, 1998) have found a combination of auxin and cytokinins more suitable for shoot regeneration.

Spontaneous rooting was observed when the regenerated shoots were left in the regeneration medium for prolonged period. The high level of endogenous auxin and the prolonged exposure to light (for shoot proliferation) might have enhanced spontaneous rooting of shoots. However, the process was slow. Addition of IBA to the culture medium enhanced rooting. The root induction ability of IBA is universally accepted. Similar results have been reported by Ajitkumar (1993) and Malhotra *et al.* (1998) in anthurium. Addition of active charcoal to the rooting medium was found to improve rooting. The positive response of active charcoal on rooting may be attributed to the known fact that active charcoal absorbs phenolic compounds and extra concentration of growth hormones from the medium thus reducing their inhibitory effect on rooting processes.

Transplanting was taken up when the plantlets had developed 4-5 leaves and 2-3 well developed roots (Fig. 5). Such plantlets showed nearly cent per cent survival. These observations are supported by various earlier workers (Yu and Peak, 1995; Ajitkumar and Nair, 1998). The roots were washed in tap water and plantlets were transferred to polythene covered plastic tray containing cocopeat drenched with 0.05% Bavistin. Plastic covering was gradually removed and within 15 days the plantlets were completely uncovered. The plants were kept in chambers with high relative humidity and low light intensity. One month later, when the plants showed growth, they were transferred to the greenhouse.

The study revealed that the plant regeneration of *A. andraeanum* cultivars 'Pumasillo' and 'Corallis' can be achieved through adventitious shoots formation from callus and better shoot regeneration can be obtained on modified MS supplemented with 0.01 mgL⁻¹ TDZ in both the cultivars.



Fig. 2. Callus induction in (a) cv. 'Corallis' on Nitsch's medium+5 mgL⁻¹ Adenine+2mgL⁻¹ BAP. (b) cv. 'Pumasillo' on MMS+5 mgL⁻¹ Adenine+3 mgL⁻¹ BAP



Fig. 3. Shoot regeneration in cv. (a) 'Corallis' on MMS + 0.01 mgL⁻¹ TDZ (b) 'Pumasillo' on MMS + 0.01 mgL⁻¹ TDZ

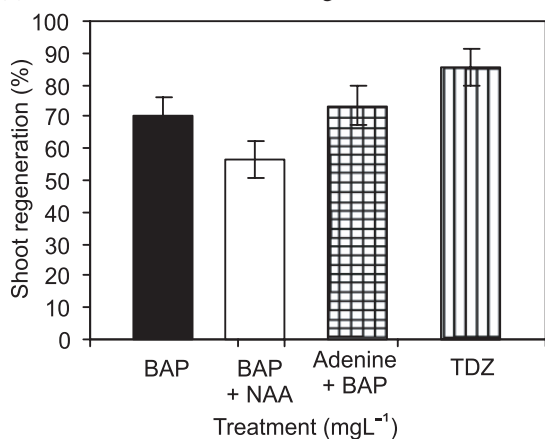


Fig. 4. Effect of various treatments on shoot regeneration of *Anthurium* cv. 'Pumasillo'

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Fig. 5. Rooted shoot of cv. 'Pumasillo' on ½ MS + 0.5 mgL⁻¹ BAP+0.04% activated charcoal

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