INTERNATIONAL JOURNAL OF ADVANCED BIOLOGICAL RESEARCH

www.scienceandnature.org

MICROPROPAGATION OF ANTHURIUM (*Anthurium andreanum* Lind.)

[*]Rocky Thokchom, [a]Soumen Maitra, [b]Subhendu Shekhar Gantait, [c]Arup Sarkar and [d]Sanasam Sanjay Singh
[*]Department of Horticulture, Pandit Deen Dayal Upadhyay Institute of Agricultural Sciences, Utlou, Bishnupur, Manipur 795134
[a]Department of Floriculture, Medicinal and Aromatic Plants, Faculty of Horticulture, Uttar Banga Krishi Viswavidyalaya, Pundibari, Coochbehar, West Bengal-736165.
[b]Department of Floriculture and Landscaping, Faculty of Horticulture, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal-741252
[c]Department of Genetics and Plant Breeding, Faculty of Agriculture, Uttar Banga Krishi Viswavidyalaya, Pundibari, Coochbehar, West Bengal-736165.
[d]ICAR, NEH Region, Lamphelpat, Manipur- 795004
*Corresponding author's email: *rockythokchomaau@gmail.com*

ABSTRACT
Anthurium (*Anthurium andreanum* Lind.), one of the most valued ornamentals is propagated conventionally through vegetative means which is very slow and need attention to develop elite, genuine, true-to-type quality planting materials at a faster rate. The present study attempts the micropropagation of Anthurium through callus culture followed by organogenesis and rhizogenesis *in vitro* including subsequent acclimatization using leaf, petiole and inflorescence explants at Uttar Banga Krishi Viswavidyalaya, Pundibari, Cooch Behar, West Bengal, India during 2013-16. Results revealed that immature coppery leaf lamina of Anthurium were highly responsive towards callus production than that of petiole and inflorescence explants. Culture of leaf explants in MS medium supplemented with 2,4-D (2.0 mg/l) and TDZ (0.1 mg/l) showed better response in respect of earliness in callusing (52.28 days), percentage of callusing explants [78.67% (62.50%)] and weight of produced calli at 15 (0.404 g) and 30 days (2.944 g) after culture. Young growing calli when cultured in MS medium containing Kinetin (3.0 mg/l) + BAP (1.0 mg/l) developed *in vitro* regeneration in greater percentage of explants [98.07% (83.67%)] with higher number of micro shoots (17.27) per culture. Earlier regeneration (26.93 days) was observed with MS culture medium having Kinetin (2.0 mg/l) and BAP (1.0 mg/l). Length of microshoot (9.00 cm) and leaf production (20.67) were found higher with the culture medium MS + BAP (1.0 mg/l) whereas, higher leaf length (2.07 cm) with greater width (1.52 cm) was obtained from the culture media MS + Kin (2.0 mg/l) + BAP (1.0 mg/l). *In vitro* regenerated microshoots when cultured in rooting medium containing MS + IBA (1.0 mg/l) + NAA (1.0 mg/l) initiated earlier (32.98 days) *in vitro* rooting in cent percent of microshoots with higher number of roots per shoot (7.36) having greater length (5.29 cm) and diameter (1.225 mm). Coconut husk was found as the effective hardening media showing earlier acclimatization (11.77 days) with greater survivability [92.75% (74.89%)].

KEY WORDS: *Anthurium andreanum*, micropropagation, explants.

INTRODUCTION

Anthurium, belonging to plant family Araceae and order Spathiflorae, is one of the most important ornamental plants consisting of 108 genera and approximately 3750 monocotyledonous species. Anthuriums are commonly cultivated for its long-lasting and unusually attractive heart shaped spathe with finger like spadix borne on a long stalk. Among the several commercially important Anthurium species, *Anthurium andreanum* is one of the ten most cultivated ornamental plants for cut flowers in the world (Jahan *et al.*, 2009) and is valued next to next to orchids among tropical flowers. Moreover, potted plants are generally appreciated for export in world market (Ullah, 1995). Anthurium is conventionally propagated by seed and division of suckers (Dufour and Guerin, 2003; Maitra *et al*., 2012). Since the conventional method of propagation is time consuming, micropropagation appears as an alternative to increase the production on a sustainable basis (Hamidah *et al*., 1997; Martin *et al*., 2003). It has been achieved with various tissues including leaf (Farsi *et al*., 2012), petiole (Raad *et al*., 2012), spadix, spathe, seed (Matsumoto *et al*., 1998), lateral bud and shoot tips (Harb *et al.*, 2010). Plantlet regeneration of *Anthurium andreanum* has been achieved through adventitious shoot formation from callus (Jahan *et al*., 2009) and direct shoot regeneration from lamina explants (Martin *et al.*, 2003).

Pierik *et al*. in 1974 first reported the tissue culture of *Anthurium* where they used liquid culture to proliferate callus (Martin *et al*., 2003) while Teng (1997) used the liquid or raft culture instead of solid medium to regenerate into adventitious shoots from leaf explants. Vargas *et al*. (2004) obtained *in vitro* plants from germinated seed and plantlets obtained from culture of micro-cuttings. These plantlets showed callus at the stem base. Micropropagation of Anthurium *in vitro* through indirect method is a difficult step and time consuming. However, for *en masse* multiplication of Anthurium, credible proliferation of callus and subsequent plant regeneration is important. This article describes the detailed protocol of *Anthurium andreanum* for establishment of rapid method for regeneration of *Anthurium andreanum* from callus tissue through organogenesis.

MATERIALS AND METHODS

The experiment was conducted at plant tissue culture laboratory, Department of Floriculture, Medicinal and Aromatic Plants, UBKV, Cooch Behar during the year 2013-2016. Immature coppery-brown coloured leaf, immature petiole and young inflorescence explants of *Anthurium andreanum* was collected from the net house of the department. Explants were previously subjected to running tap water for about half an hour using two to three drops of Tween-20 to remove excess dirt from the leaf surface. They were later treated with fungicides and bactericides (1% each) consecutively for 10-20 minutes to minimize fungal and bacterial contamination. Inside the laminar flow, the explants were sterilized with 0.1 per cent ($^{v}/_{v}$) $HgCl_2$ for 2-3 minutes followed by 70 per cent ($^{v}/_{v}$) ethyl alcohol for 30 seconds. Explants were then rinsed thoroughly with sterile double distilled water to remove the toxic residues from the surface. Well sterilized explants were reduced to the size of about 0.5 to 1.0 cm^2 pieces for inoculation.

Media

For indirect organogenesis, MS basal medium (Murashige and Skoog, 1962) fortified with 30 g/L sucrose was used adjusting the pH to 5.7 before autoclaving.

Callus initiation

Explants were transferred into the culture tubes containing 30 g/L sucrose, with or without or either 2,4-D alone or in combination with TDZ. The pH was adjusted to 5.7 before autoclaving. The media differed in concentrations of plant growth regulators and light conditions of culture.

Shoot regeneration

For *in vitro* shoot regeneration, calli of leaf explants were transferred to the basal medium fortified with or without or different concentrations of Kinetin and BAP either alone or in combination which was kept at 16/8 light and darkness photoperiod.

Rooting

The regenerated shoots longer than 3 cm with a pair of leaves were transferred to the MS medium containing with or without plant growth regulators or different concentrations of IBA either alone or in combination with NAA.

Hardening

The well rooted anthurium plantlets were later transferred to various hardening medium for acclimatization and were maintained at 90 per cent relative humidity in the hardening chamber for proper establishment of the plantlets.

Statistical analysis

The experiment was laid out under factorial completely randomized design for various explants callusing and completely randomized design for the rest of the experiments. The data generated were analyzed by Fisher's analysis of variance (ANOVA) technique at 5 per cent level of significance. Ten explants were used in each treatment for plant organogenesis while five treatments were given for plantlet acclimatization. Each treatment was done in 3 replications for *in vitro* plant regeneration and 5 replications were given in case of plantlets acclimatization. All experiments were repeated three times. The percentile data of the experiments were assumed and subjected to square root transformations.

RESULTS AND DISCUSSION

A regeneration protocol for *Anthurium andreanum* was established using three different explant sources *viz.*, leaf, petiole and inflorescence in thirteen aseptic MS basal media containing different concentrations of either 2,4-D alone or combination with different doses of TDZ for callus induction. Results pertaining to Table 1 revealed that amongst all explant sources, the leaf explant performed the best by producing the maximum callusing percentage [43.40% (41.21%)] in shortest time duration (52.28 days) and yielded the heaviest calli (2.944g). The reason might be due to the choice explants that have adequate morpho-genetical plasticity leading to higher division of cells and rapid callus induction. Morphogenetic plasticity dependent response to callus initiation was also observed by Bejoy *et al.* (2008) in Anthurium; Trejgell *et al*. (2009) in *Carlina acaulis*; Taha *et al*. (2011) in ornamental fern. Among the different plant hormones used, the best callusing ability was observed in the MS basal medium containing 2.0 mg/l 2,4-D + 0.1 mg/l TDZ [61.78% (52.05%)] producing callus at the earliest (42.38 days) with maximum weight (3.653g) at 30 days after explant inoculation. The observations recorded from interaction effect on the callusing ability showed that leaf explants cultured in MS+1.0 mg/l 2,4-D + 0.1 mg/l TDZ yielded calli in maximum percentage of explants [77.78% (62.00%)] taking the minimum duration (43.78 days) to callus initiation. However, MS basal medium containing 1.0 mg/l 2,4-D +0.1 mg/l TDZ generated heaviest calli (5.004 g). It is obvious that the hormonal combination in callusing medium determines the callus formation which varies from species to species and explant to explant (Tsay *et al*., 2006; Gitonga *et al*., 2010; Atak and Celik, 2015). Hence, optimization of phytohormonal combination in callusing media is an important aspect for any conclusive opinion for understanding the mode of action at the molecular level (Grozeva *et al.*, 2006; Arab *et al.*, 2014). For regeneration of Anthurium, both Kinetin and BAP was found suitable. Data depicted in Table 2 revealed that culture of calli in MS basal medium fortified with 2.0 mg/l Kin +1.0 mg/l BAP recorded the earliest regeneration (26.93 days), producing the longest (2.07 cm) and broadest leaf (1.52 cm) while MS basal medium containing only 1.0 mg/l BAP regenerated the tallest microshoots (9.00 cm) and highest number of leaves per microshoot (20.67). However, MS medium supplemented with 3.0 mg/l Kin + 1.0 mg/l BAP recorded the maximum percentage of regeneration[98.07% (83.67%)].

TABLE 1. Effect of culture media on callusing characteristics of *Anthurium andreanum* Lind. through various explant sources

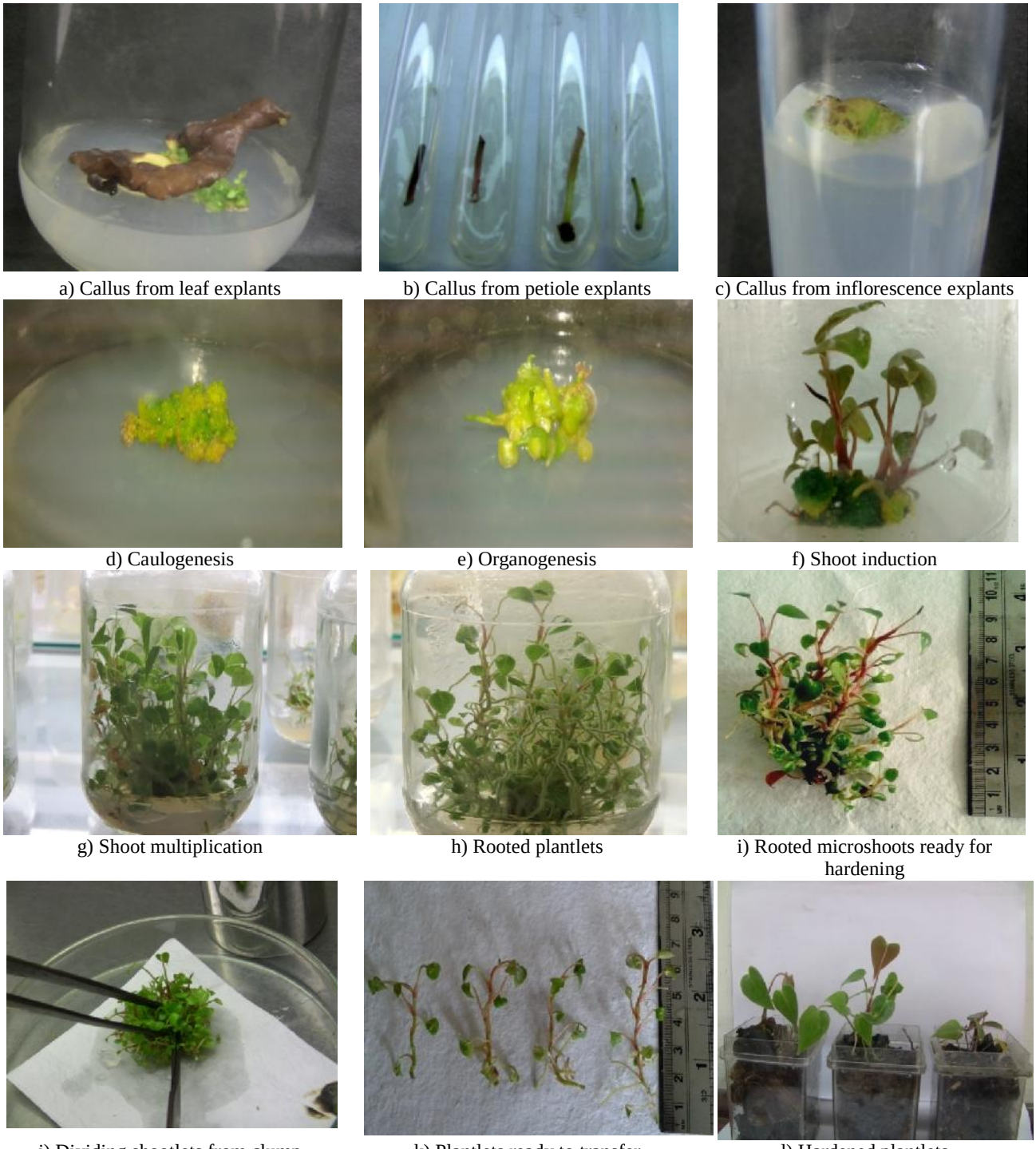
Explants	Percentage of callusing (%)	Days to callus initiation (days)	Weight of callus after 30 days of initiation (g)
Leaf	43.40 (41.21)	58.28	2.944
Petiole	37.16 (37.56)	64.82	2.242
Inflorescence	21.80 (27.83)	73.66	1.409
S. Em ±	***0.15***	***0.42***	***0.018***
CD at 5%	**0.43**	**1.18**	**0.053**
Culture Media			
MS	10.17 (18.46)	142.42	0.239
MS+1.0 mg/l 2,4-D	35.41 (36.17)	54.06	2.505
MS+2.0 mg/l 2,4-D	42.02 (40.13)	52.72	2.684
MS+3.0 mg/l 2,4-D	26.22 (30.38)	67.74	1.619
MS+4.0 mg/l 2,4-D	15.60 (23.22)	84.26	0.887
MS+1.0 mg/l 2,4-D + 0.1 mg/l TDZ	51.26 (45.84)	47.18	3.303
MS+1.0 mg/l 2,4-D + 0.2 mg/l TDZ	55.46 (47.89)	46.22	3.514
MS+2.0 mg/l 2,4-D + 0.1 mg/l TDZ	61.78 (52.05)	42.38	3.653
MS+2.0 mg/l 2,4-D + 0.2 mg/l TDZ	53.04 (46.78)	46.46	3.255
MS+3.0 mg/l 2,4-D + 0.1 mg/l TDZ	36.44 (37.42)	53.16	2.789
MS+3.0 mg/l 2,4-D + 0.2 mg/l TDZ	27.31 (31.46)	63.31	1.907
MS+4.0 mg/l 2,4-D + 0.1 mg/l TDZ	21.33 (27.50)	73.04	1.285
MS+4.0 mg/l 2,4-D + 0.2 mg/l TDZ	17.53 (24.72)	79.67	0.937
S. Em ±	***0.32***	***0.87***	***0.039***
CD at 5%	**0.90**	**2.45**	**0.110**
Explant x Culture Media			
Leaf x MS	12.89 (21.00)	122.91	0.381
Leaf x MS+1.0 mg/l 2,4-D	55.11 (47.94)	43.07	3.915
Leaf x MS+2.0 mg/l 2,4-D	47.11 (43.34)	42.71	3.014
Leaf x MS+3.0 mg/l 2,4-D	40.44 (39.49)	48.89	2.773
Leaf x MS+4.0 mg/l 2,4-D	16.59 (24.02)	81.00	0.669
Leaf x MS+1.0 mg/l 2,4-D + 0.1 mg/l TDZ	77.78 (62.00)	32.00	5.004
Leaf x MS+1.0 mg/l 2,4-D + 0.2 mg/l TDZ	70.16 (56.52)	38.13	4.692
Leaf x MS+2.0 mg/l 2,4-D + 0.1 mg/l TDZ	69.48 (56.50)	40.22	4.797
Leaf x MS+2.0 mg/l 2,4-D + 0.2 mg/l TDZ	61.19 (51.59)	41.24	4.571
Leaf x MS+3.0 mg/l 2,4-D + 0.1 mg/l TDZ	45.04 (42.15)	44.98	3.647
Leaf x MS+3.0 mg/l 2,4-D + 0.2 mg/l TDZ	38.67 (38.00)	58.24	2.930
Leaf x MS+4.0 mg/l 2,4-D + 0.1 mg/l TDZ	21.19 (27.39)	80.73	1.025
Leaf x MS+4.0 mg/l 2,4-D + 0.2 mg/l TDZ	18.96 (25.81)	83.49	0.854
Petiole x MS	8.89 (17.22)	138.51	0.153
Petiole x MS+1.0 mg/l 2,4-D	29.63 (32.970	59.69	2.002
Petiole x MS+2.0 mg/l 2,4-D	56.15 (48.54)	50.44	3.074
Petiole x MS+3.0 mg/l 2,4-D	22.37 (28.20)	69.04	1.441
Petiole x MS+4.0 mg/l 2,4-D	16.00 (23.55)	70.84	1.224
Petiole x MS+1.0 mg/l 2,4-D + 0.1 mg/l TDZ	51.56 (45.89)	53.40	3.016
Petiole x MS+1.0 mg/l 2,4-D + 0.2 mg/l TDZ	66.37 (54.55)	46.73	3.797
Petiole x MS+2.0 mg/l 2,4-D + 0.1 mg/l TDZ	74.07 (59.40)	44.78	3.992
Petiole x MS+2.0 mg/l 2,4-D + 0.2 mg/l TDZ	63.70 (52.96)	49.18	3.297
Petiole x MS+3.0 mg/l 2,4-D + 0.1 mg/l TDZ	46.80 (43. 29)	57.38	2.858
Petiole x MS+3.0 mg/l 2,4-D + 0.2 mg/l TDZ	24.74 (29.82)	62.18	1.694
Petiole x MS+4.0 mg/l 2,4-D + 0.1 mg/l TDZ	21.33 (27.50)	68.82	1.525
Petiole x MS+4.0 mg/l 2,4-D + 0.2 mg/l TDZ	17.33 (24.58)	71.67	1.067
Inflorescence x MS	8.74 (17.15)	165.84	0.180
Inflorescence x MS+1.0 mg/l 2,4-D	21.48 (27.60)	59.42	1.598
Inflorescence x MS+2.0 mg/l 2,4-D	22.81 (28.50)	65.01	1.965
Inflorescence x MS+3.0 mg/l 2,4-D	15.85 (23.44)	85.29	0.643
Inflorescence x MS+4.0 mg/l 2,4-D	14.22 (22.09)	100.93	0.770
Inflorescence x MS+1.0 mg/l 2,4-D + 0.1 mg/l TDZ	24.44 (29.62)	56.13	1.884
Inflorescence x MS+1.0 mg/l 2,4-D + 0.2 mg/l TDZ	29.04 (32.59)	53.80	2.054
Inflorescence x MS+2.0 mg/l 2,4-D + 0.1 mg/l TDZ	41.78 (40.26)	42.13	2.170
Inflorescence x MS+2.0 mg/l 2,4-D + 0.2 mg/l TDZ	34.22 (35.79)	48.96	1.898
Inflorescence x MS+3.0 mg/l 2,4-D + 0.1 mg/l TDZ	20.00 (26.81)	57.11	1.861
Inflorescence x MS+3.0 mg/l 2,4-D + 0.2 mg/l TDZ	19.50 (26.58)	69.51	1.098
Inflorescence x MS+4.0 mg/l 2,4-D + 0.1 mg/l TDZ	21.48 (27.59)	69.56	1.305
Inflorescence x MS+4.0 mg/l 2,4-D + 0.2 mg/l TDZ	16.30 (23.77)	83.87	0.889
S. Em ±	***0.55***	***1.51***	***0.068***
CD at 5%	**1.56**	**4.25**	**0.191**

TABLE 2. Effect of culture media on the shooting parameters of *Anthurium andreanum* Lind. *in vitro.*

Treatments	Days to regeneration from callus (days)	Regeneration Percent from callus (%)	Number of microshoots/ culture	Length of microshoot (cm)	Number of leaves/microshoot	Leaf length (cm)	Leaf diameter (cm)
MS_0	80.51	31.85 (33.96)	2.56	2.92	3.33	0.45	0.28
MS+0.5 mg/l Kin	37.73	81.33 (64.43)	9.98	5.64	6.47	0.82	0.56
MS+1.0 mg/l Kin	30.38	87.26 (69.12)	14.29	6.48	8.27	1.38	1.02
MS+2.0 mg/l Kin	33.36	96.89 (80.19)	9.91	5.63	7.18	1.10	0.78
MS+0.5 mg/l BAP	32.27	77.19 (61.52)	12.42	6.29	9.09	0.90	0.60
MS+1.0 mg/l BAP	28.73	82.52 (65.35)	17.07	9.00	20.67	1.65	1.27
MS+2.0 mg/l BAP	33.22	75.85 (60.57)	13.80	7.68	16.38	1.46	1.10
MS+2.0 mg/l Kin + 1.0 mg/l BAP	26.93	98.22 (83.37)	8.20	7.91	18.18	2.07	1.52
MS+2.0 mg/l Kin + 2.0 mg/l BAP	29.56	97.33 (82.62)	12.53	7.32	14.53	1.82	1.40
MS+2.0 mg/l kin + 3.0 mg/l BAP	34.27	87.41 (69.27)	11.20	7.07	13.80	0.60	0.34
MS+3.0 mg/l Kin + 1.0 mg/l BAP	32.80	98.07 (83.67)	8.73	6.42	11.02	0.75	0.45
MS+3.0 mg/l Kin + 2.0 mg/l BAP	41.24	94.22 (76.57)	7.27	5.98	7.60	0.69	0.41
MS+3.0 mg/l Kin + 3.0 mg/l BAP	44.89	78.22 (62.23)	5.51	5.61	5.44	0.55	0.32
S.E(m)±	***0.43***	***1.79***	***0.24***	***0.06***	***0.21***	***0.02***	***0.02***
C.D. at 5%	**2.10**	**8.67**	**1.15**	**0.30**	**1.04**	**0.09**	**0.09**

TABLE 3. Effect of culture media on the rooting parameters of *Anthurium andreanum* Lind. *in vitro*.

Treatments	Days to root initiation (days)	Percentage of rooted microshoots (%)	Number of roots per microshoot	Length of root (cm)	Root diameter (mm)
MS_0	82.78	27.70 (31.74)	2.00	1.88	0.684
MS+1.0 mg/l IBA	33.27	100.00 (90.00)	5.60	4.22	1.090
MS+1.5 mg/l IBA	36.71	100.00 (90.00)	4.24	3.79	1.070
MS+2.0 mg/l IBA	42.09	100.00 (90.00)	4.16	3.35	1.025
MS+2.5 mg/l IBA	45.91	98.67 (84.66)	3.73	2.92	0.965
MS+3.0 mg/l IBA	52.49	98.37 (83.183)	2.80	2.47	0.910
MS+3.5 mg/l IBA	56.16	97.04 (80.23)	2.44	1.88	0.856
MS+1.0 mg/l IBA + 1.0 mg/l NAA	32.98	100.00 (90.00)	7.36	5.29	1.225
MS+1.5 mg/l IBA + 1.0 mg/l NAA	34.16	100.00 (90.00)	6.09	5.03	1.144
MS+2.0 mg/l IBA + 1.0 mg/l NAA	37.24	100.00 (90.00)	5.22	4.79	1.052
MS+2.5 mg/l IBA + 1.0 mg/l NAA	42.33	99.85 (89.26)	4.49	4.58	0.969
MS+3.0 mg/l IBA + 1.0 mg/l NAA	45.89	98.96 (85.27)	3.69	4.06	0.933
MS+3.5 mg/l IBA + 1.0 mg/l NAA	52.87	96.74 (80.03)	2.73	2.81	0.856
S.E(m)±	***0.79***	***0.72***	***0.14***	***0.08***	***0.01***
C.D. at 5%	**3.83**	**3.48**	**0.68**	**0.40**	**0.05**



a) Callus from leaf explants b) Callus from petiole explants c) Callus from inflorescence explants

d) Caulogenesis e) Organogenesis f) Shoot induction

g) Shoot multiplication h) Rooted plantlets i) Rooted microshoots ready for hardening

j) Dividing shootlets from clump k) Plantlets ready to transfer l) Hardened plantlets

FIGURE 1. Different phases of micropropagation of Flowering Anthurium (*Anthurium andreanum* Lind.)

The pivotal factors that governing the regeneration of callus like auxin: cytokinin ratio, presence of meristematic tissue, absence of apical dominance, nature of totipotency were also observed by Su *et al*., (2011). Higher cytokinin requirement during regeneration from callus was observed by Maitra *et al.* (2012) while enhancement of shoot growth and development *in vitro* of anthurium using kinetin and BAP was also examined by Bejoy *et al.* (2008); Atak and Celik (2009); Murillo-Gómez *et al.* (2014).

Rhizogenesis occurred satisfactorily when the cultures were allowed to stand for long period under artificial illumination. Isolation of microshoots from a mass resulted rapid and consistent rooting when cultured in specified rooting medium *in vitro*. For this purpose a supplementation with IBA and NAA in MS medium was found essential. Data presented in Table 3 revealed that MS + 1.0 mg/l IBA + 1.0 mg/l NAA took the minimum days (32.98 days) to initiate roots *in vitro*, maximum number of roots per microshoot (7.36), longest root (5.29

cm) and maximum root diameter (1.225 mm). Cent percent rooting were observed in almost all the basal medium fortified with either IBA alone or the combination of both IBA and NAA. Hormone free MS basal medium developed least percentage to root initiation [27.70% (31.74%)] of microshoots. It has been observed in several crops that NAA when used in the rooting medium along with IAA or IBA induced *in vitro* rooting with higher magnitude as compared to application of IAA or IBA simply (Raad *et al.*, 2012). This may be due to the enhancement of auxin environment inside the regenerated microshoot and auxin transport in the basipetal manner leading to higher auxin accumulation at the lower end of microshoots results higher, rapid and consistent rooting *in vitro*. Use of a combination of IBA and NAA fortified in basal MS medium for *in vitro* rooting was also observed by Zhang *et al*. (2001); Joseph *et al*. (2003).

TABLE 4. Effect of hardening media on plantlets acclimatization of *Anthurium andreanum* Lind. *ex-vitro*.

Treatments	Days required for acclimatization (days)	Survivability percentage (%)
Vermiculite + Sand (1:1)	14.20	63.08 (52.63)
Sand + Sawdust (1:1)	18.47	55.42 (48.12)
Coconut husk	11.77	92.75 (74.89)
Vermiculite + coconut husk (1:1)	18.98	73.67 (59.23)
Vermiculite	21.32	68.92 (56.21)
S.E(m)±	***0.62***	***1.05***
C.D. at 5%	**3.05**	**3.05**

The success of micropropagation lies in acclimatization of plantlets and subsequently establishment. Use of Coconut husk only as hardening substrate took the least number of days (11.77 days) for acclimatization of Anthurium plantlets *ex vitro* with greater rate of plantlet survivability [92.75% (74.89%)] *ex vitro*. Greater success in hardening of anthurium explants was achieved with coconut husk might be due to its light texture and extremely porous nature allowed greater aeration and helped better root respiration as compared to the other combinations. Success in Anthurium hardening using coconut husk as a medium was also reported by Keatmetha and Suska-Ard (2004); Thokchom and Maitra (2017).

CONCLUSION

The study demonstrated that among the different plant tissues employed for successful caulogenesis, immature leaf lamina explants were found as the best followed by petiole and inflorescences explants. Optimal use of 2, 4-D and TDZ in MS basal medium gave rapid response when the immature coppery leaf explants were inoculated in it. The same hormonal mixture yielded good quality callus as well. The calli responded better when cultured in MS basal medium containing a combination of Kinetin and BAP at different concentrations which resulted in higher percent of callus regeneration and morphogenic characters of microshoots. Inclusion of an optimal combination of IBA and NAA or IBA alone in the basal medium was found effective for early response as well as growth characteristics of the roots. Well sterilized coconut husks proved as the best medium for successful acclimatization of the *in vitro* regenerated plantlets.

ACKNOWLEDGEMENT

The authors gratefully acknowledge Mr. Kamal Das, Laboratory technician for his hard work and dedication in carrying out the present research work and the financial assistance provided by the Department of Biotechnology, Ministry of Science and Technology, Government of India, for conducting the research work.

REFERENCES

Arab, M.M., Yadollahi, A., Shojaeiyan, A., Shokri, S., Ghojah, S.M. (2014) Effects of nutrient media, different cytokinin types and their concentrations on *in vitro* multiplication of G N15 (hybrid of almond x peach) vegetative rootstock. Journal of Genetic Engineering and Biotechnology, 12, 81–87.

Atak, C. and Celik, O. (2009) Micropropagation of *Anthurium andraeanum* from leaf explants. Pakistan Journal of Botany, 41, 1155-1161.

Bejoy, M., Sumitha, V.R. and Anish, N.P. (2008) Foliar regeneration in *Anthurium andraeanum* Hort. cv. Agnihotthri. Biotechnology 7(1): 134-138.

Dufour, L. and Guérin, V. (2003) Growth, developmental features and flower production of *Anthurium andraeanum* Lind. in tropical condition. Scientia Horticulturae, 98, 25-35.

Farsi, M., Yazdi, T.M.E. and Qasemiomran, V. (2012) Micropropagation of *Anthurium andraeanum* cv. Terra. African Journal of Biotechnology, 11(68), 13162-13166.

Gitonga, L.N., Gichuki, S.T., Ngamau, K., Muigai, A.W.T, Kahangi, E.M., Wasilwa, L.A., Wepukhulu, S. and Njogu, N. (2010) Effect of explant type, source and genotype on *in vitro* shoot regeneration in Macadamia (*Macadamia* spp.) Journal of Agricultural Biotechnology and Sustainable Development, 2, 129–135.

Grozeva, S., Rodev, V. and Danailov Z. (2006) Effect of Genotype, Explant Type and Culture Medium on Shoot Regeneration in Tomato (*Lycopersicon esculentum* Mill.) *in vitro*. Bulgarian Journal of Agricultural Science, 12, 435-439.

Hamidah, M., Karim, A.G.A. and Debergh, P. (1997) Somatic embryogenesis and plant regeneration in *Anthurium scherzerianum*. Plant Cell, Tissue, Organ Culture, 48, 189-193.

Harb, E.M., Talaat, N.B., Weheeda, B.M., El-Shamy, M.A. and Omira, G.A. (2010) Micropropagation of *Anthurium andraeanum* from Shoot Tip Explants. Journal of Applied Sciences Research, 6(8), 927-931.

Jahan, M.T., Islam, M.R., Khan, R., Mamun, A.N.K., Ahmed, G. and Hakim, L. (2009) *In-vitro* clonal propagation of Anthurium (*Anthurium andraeanum* L.) using callus culture. Plant Tissue Culture and Biotechnology, 19(1), 61-69.

Joseph, M., Martin, K.P., Mundassery, J. and Philip, V.J. (2003) *In vitro* propagation of three commercial cut flower cultivars of *Anthurium andreanum*. Indian Journal of Experimental Biology, 41, 154-159.

Keatmetha, W. and Suska-Ard, P. (2004) Effect of rooting substrates on *in vitro* rooting of *Anthurium andreanum*. Walailak Journal of Science and Technology, 1(2), 49-55.

Maitra, S., Ghosh, P.D., Roychowdhury, N. and Satya, P. (2012) Effect of culture media on *in-vitro* regeneration of anthurium (*Anthurium andraeanum* Lind.) from axillary bud explants. International Journal of Bio-resource and Stress Management, 3(1), 035-039.

Martin, K.P., Joseph, D., Madassery, J. and Philip, V.J. (2003) Direct shoot regeneration from lamina explants of two commercial cut flower cultivars of *Anthurium andraeanum* Hort. In-vitro Cellular and Developmental Biology – Plant, 39, 500-504.

Matsumoto, T.K., Kuehnle, A.R. and Webb, D.T. (1998) Zygotic embryogenesis in *Anthurium* (Araceae). *American Journal of Botany* 85, 1560-1568.

Murashige T and Skoog F. (1962) A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiologiae Plantarum, 15, 473-497.

Murillo-Gómez, P.A., Naranjo, E., Callejas, R., Atehortúa, L. and Urrea, A. (2014) Micropropagation of the native species *Anthurium antioquiense* Engl. for conservation purposes. Agronomía Colombiana, 32(3), 334-340.

Raad, M.K., Zanjani, S.B., Shoor, M., Hamidoghli, Y., Sayyad, A.R., Masouleh, A.K. and Kaviani, B. (2012) Callus induction and organogenesis capacity from lamina and petiole explants of *Anthurium andraeanum* Linden (Casino and Antadra). Australian Journal of Crop Science, 6(5), 928-937.

Su, Y.H., Liu, Y.B. and Zhang, X.S. (2011) Auxin–cytokinin interaction regulates meristem development. Molecular Plant, 4(4), 616–625.

Taha, R.M., Haron, N.W. and Wafa, S.N. (2011) Morphological and tissue culture studies of *Platycerium coronarium*, a rare ornamental fern species from Malaysia. American Fern Journal, 101(4), 241-251.

Teng, W.L. (1997) Regeneration of *Anthurium* adventitious shoots using liquid or raft culture. Plant Cell, Tissue and Organ Culture, 49, 153-156.

Thokchom, R. and Maitra, S. (2017) Micropopagation of *Anthurium andreanum* cv. Jewel from leaf explants. Journal of Crop and Weed, 13(1), 23-27.

Trejgell, A., Dąbrowska, G. and Tretyn, A. (2009) *In vitro* regeneration of *Carlina acaulis* subsp. *simplex* from seed ling explants. Acta Physiologiae Plantarum, 31(3), 445–453.

Tsay, H.S., Lee, C.Y., Agrawal, D.C. and Basker, S. (2006) Influence of ventilation closure, gelling agent and explant type on shoot bud proliferation and hyperhydricity in *Scrophularia yoshimure-* a medicinal plant. In Vitro Cellular and Developmental Biology – Plant, 42, 445-449.

Ullah, M.H. (1995) 2nd Plant Tissue Culture Conference, Dhaka, Bangladesh. Organized by Bangladesh Association for Plant Tissue Culture and Biotechnology, December 12-14, 1995.

Vargas, T.E., Mejias, A., Oropeza, M. and Garcia, E. (2004) Plant regeneration of *Anthurium andraeanum* cv. Rubrun. Electronic Journal of Biotechnology, 7(3), 282-286.

Zhang, G.H., Xu, B.Y., Peng, C.Z. and Lu, L. (2001) Shoot cutting tissue culture and propagation *in vitro* of *Anthurium andreanum* Lind. Acta Agric. Shanghai, 17, 13-16.