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IN VITRO REGENERATION OF *Anthurium andreanum* cv. NITTA

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Abstract

The present study was undertaken to investigate the effect of different combinations of NAA, IBA, and BAP on *in vitro* organogenesis of *Anthurium andreanum* cv. "Nitta" at the DEBTEC (Development of Biotechnology & Environmental Conservation Centre) Laboratory, Dhaka. Organogenesis from leaf mid rib to shoot initiation required 9.00 days and the best survivality was 80.00% percent at 30 DAI (Day after initiation) with the combination of 1 mg/L NAA and 1 mg/L BAP in MS media. Among the 20 hormone supplements, MS media containing 1 mg/L NAA and 1 mg/L BAP showed the highest shoot formation (68.30%), number of shoots/explant (3.37) and the longest shoot (4.65 cm) at 60 DAI. MS media without any hormone (control) showed the poorest performance in regeneration of shoots. On the other hand, MS media containing 1 mg/L IBA + 1 mg/L BAP showed the best performance in rooting of shoots (83.85%), highest number of roots (4.29/plantlet), root elongation (5.50 cm) were recorded at 60 DAI.

Keywords : *In vitro* regeneration, *Anthurium andreanum*.

Introduction

Anthurium andreanum, belonging to the family Araceae and Order- Spathiflorae. It is a perennial herbaceous plant cultivated for its long-lasting and attractive heart shaped inflorescence. *Anthurium* is a modified leaf (spathe), bearing numerous small botanical flowers on a pencil-like protrusion (spadix) and has a vase life of 14-28 days. Different variant *of Anthurium* are grown for both foliage and brightly coloured attractive waxy spathes (George, 1951). *Anthurium andreanum* is commonly grown for cut flower and sometimes adaptable to pot culture. Reisch (1998) reviewed that among tropical flowers, its trade value is next to orchids and its commercial value has increased in recent years in Asian countries as well as in Europe. Moreover, pot plants are valued standard for export in world market (Ullah, 1995). Bangladesh has a scope of large scale production of *Anthurium* and other tropical ornamental plants through micropropagation method for quality production to meet the demand of internal and external market. Our tropical conditions are ideal for the cultivation of *Anthurium*, which grow best with day temperatures of 25-32°C and night

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temperatures of 21-24°C (Nakasome and Kamemoto, 1962). *Anthurium* produce flowers throughout the year and an average of six to eight flowers per plant per year (Poole and Greaves, 1969).

Anthurium andreanum may be propagated either sexually or asexually. Most of the cultivated *Anthurium* are found to be self-sterile and always remains as a rare species. Therefore, to get true to type plant, micropropagation is the only means presently involved in exploitation as a major trade worldwide (Healy *et al.*, 1980; Sagawa and Kunisaki, 1982; Goh *et al.*, 1992). This is because commercially mass propagation is possible by producing millions of plantlets using tissue culture techniques (Lim-Ho *et al.*, 1985). The shoot tip culture of *Anthurium andreanum* entails the sacrifice of the whole plant or an entire new growth. Using axillary buds, inflorescence axis and other parts of the plant body would also involve damaging or sacrificing certain plant parts. By using the leaf petioles of *Anthurium andreanum,* it would be possible to save the mother plant from such damage. Considering the above condition, this study was undertaken to standardize the media with NAA and BAP for rapid multiplication of *Anthurium andreanum* cv. 'Nitta' through organogenesis from the mid rib of young leaf (red colour) and to regenerate shoot and root was multiplied by the media with IBA and BAP.

Materials and Method

The present study was carried out at the DEBTEC (Development of Biotechnology & Environmental Conservation Centre) Laboratory, Dhaka during December, 2004 to April, 2005. *Anthurium andreanum* cv. Nitta (red colour) was collected from the DEBTEC Nursery; project site: South Panishile, Gazipur. The mid ribs of young leaves were used as explants to study the shoot regeneration, shoot multiplication and rooting in MS medium supplemented with following hormones –

Part 1: For determination of survivality and development of organogenesis of leaf mid rib in MS (Murashige & Skoog, 1962) medium was used with hormone combination NAA @ 0.5, 1 and 1.5 mg/L each with BAP @ 0.5, 1, 1.5 and 2 mg/L, respectively.

Part 2: For shoot differentiation, hormone combinations of NAA @ 0.5, 1 and 1.5 mg/L each with BAP @ 0.5, 1, 1.5 and 2 mg/L used in MS media for shoot multiplication.

Part 3: For root induction, different combinations of IBA @ 0.5, 1 and 1.5 mg/L and BAP @ 0.5, 1, 1.5 and 2 mg/L used in MS media for rooting of regenerated shoots of *Anthurium andreanum.*

The unfold leaf segment were collected and washed thoroughly under running tap water and dead tissues were carefully removed with the help of a

sharp stainless steel knife (Plate 1). The leaf segment was chopped into small pieces and sterilized by 70% ethanol for 30 seconds then washed 3 times with double distilled water. Afterwards, it was sterilized with 0.1% Mercuric chloride ($HgCl_2$) solution for 10 minutes and washed several times then inoculated into vials containing 25 ml MS media with different hormone concentrations. Each treatment was conducted with four replications. The culture medium, glasswares and instruments were sterilized by autoclaved at 15-psi pressure at 121°C for 20 minutes and the culture room was surface disinfected with 70% ethyl alcohol to ensure aseptic condition under *in vitro* propagation. Cultures were maintained in an incubator at 25°C temperature in complete dark condition for organogenesis. The organogenesis placed on MS medium supplemented with NAA and BAP for shoot regeneration was maintained within 25±2°C by air conditioner at 16 hours light period by the illumination from white florescent tube light (Phillips). When the shoots grew about 3-4 cm in length with 4-5 well developed leaves they were rescued aseptically from the culture tubes and were separated from each other and again cultured on freshly prepared MS medium containing different concentrations of IBA and BAP for root induction. The effect of different treatments on survivability (%), days required for organogenesis, percent of shoot induction, shoots per explant, shoot length (cm), percent of root development, roots per plantlet and root length were collected and statistically analyzed. The experiment was conducted in growth room and arranged in completely randomized design (CRD). The analysis of variances for different characters was performed and the means were compared by least significant difference (LSD) test for interpretation of results (Gomez and Gomez, 1984).

Results and Discussion

The experiment was conducted with *Anthurium andreanum* cv. Nitta accomplished with mid rib of leaf for shoot induction, rooting and finally plantlet regeneration on MS medium with different concentrations of auxins (NAA and IBA) and cytokinin (BAP) on *in vitro* condition. Results of different steps described along with discussions under the following heads.

Part 1. Survivality percent (%) and Organogenesis from explant

Anthurium was cultured on MS medium supplemented with 20 different hormone combinations for survivability percent and number of days required for shoot initiation. The analysis of variance revealed that the media supplemented with different hormone concentrations had conspicuous effect on shoot induction ability.

Survivability percentage

Mean squares due to different hormone supplementation were highly significant for survivability % (Plates 2). Highest survivality (80%) was noticed in

combination with MS medium containing 1 mg/L NAA + 1 mg/L BAP followed by medium with 0.5 mg/L NAA + 1 mg/L BAP (76.66%) and 1 mg/L NAA + 1.5 mg/L BAP (75%) which were significantly different at 1 % level of significance at 30 days after initiation (Table 1). The present findings were partially supported by Hamidah (1997) where he observed that leaf pieces from micropropagated plants of *Anthurium scherzerianum* Schott were the best explant and somatic embryos converted to entire plants on a medium with 0.46 mμ Kinetin.

Days required for organogenesis

Mean squares due to different hormone supplementations were highly significant for shoot initiation. MS medium containing 1 mg/L NAA + 1 mg/L BAP took minimum time (9 days) for shoot initiation followed by 1.5 mg/L NAA + 2 mg/L BAP MS medium (Table 1). Control treatment take highest time (14.75 days) for shoot induction.

Table 1. Combined effect of NAA and BAP on percent survivability and days required for organogenesis of *Anthurium andreanum.*

NAA (mg/L)	BAP (mg/L)	Explants initiated shoot (out of 30 explants)	Survivability %	Days required for shoot initiation
	0	11	36.66 r	14.25i
	0.5	14	46.66 o	13.75h
0	1.0	16	53.331	13.25fg
	1.5	18.5	61.66 h	13.50gh
	2.0	15.5	51.66m	13.50gh
	0	19	63.33g	13.00f
	0.5	21	70.00d	13.75h
0.5	1.0	23	76.66b	13.25 fg
	1.5	18	60.00i	13.00f
	2.0	16.5	55.00 k	13.25 fg
	0	13.5	45.00 p	10.75de
	0.5	20.5	68.33e	10.75de
1.0	1.0	24	80.00 a	9.00 a
	1.5	22.5	75.00 c	10.00b
	2.0	19.5	65.00 f	10.25 bc
	0	13.5	45.00 p	11.00e
	0.5	16	53.33 1	10.50 cd
1.5	1.0	17.5	58.33j	10.00b
	1.5	15	50.00r	10.00b
	2.0	12	40.00 q	9.75 b

Figures in a column followed by different letters differ significantly, whereas, with common letter(s) do not differ significantly at 1% level of significance.

Part 2. Shoot development

Shoot initiated by organogenosis from cultured mid rib of *Anthurium andreanum* cv. Nitta was transplanted on MS medium supplemented with NAA and BAP in different combinations. There was significant variation between the interaction of media composition for shoot development, number of shoots/explanf and length of the largest shoot at 20, 40, and 60 days after initiation (Plate 3).

Shoot induction percent (%)

Effect of media with different hormone supplementations on shoots induced (%) was found highly significant. The result showed that the percent of shoot induction was maximum 68.30% with MS medium containing 1 mg/L NAA + 1 mg/L BAP followed by 61.65% with medium containing 1 mg/L NAA + 1.5 mg/L BAP at 60 DAI and same trend was observed at 40 DAI and 20 DAI,, respectively (Table 2). The minimum percent of explants induced shoot (22.3 0%) at 60 DAI was obtained with MS medium without any hormone (control). The results of present experiment agree with the findings of Atta (1998) where he found that the decontaminated germinated seedlings were placed onto a multiplication MS media containing 2 mg/L BA and 0.2 mg/L NAA.

Table 2. Effect of different hormone concentrations on shoot formation.

MS media with diff. hormones		Shoot induction percent			No. of shoots per explant			Length of shoots (cm)		
NAA	BAP	20 DAI	40 DAI	60 DAI	20 DAI	40 DAI	60 DAI	20 DAI	40 DAI	60 DAI
	0	8.30h	18.29 p	22.30n	0.00i	0.01 s	0.11 mn	1.32f	l.59m	1.931
	0.5	1l.60f	26.80j	38.14k	0.23gh	0.57 r	0.95m	1.47d-f	1.681	2.08k
0	1.0	10.00 g	26.80j	41.65j	0.32 ef	0.68 p	1.201	1.55 d-f	1.91 k	2.85j
	1.5	9.99g	23.29m	41.60j	0.36e	0.76n	1.201	1.59de	2.12i	3.55 e-g
	2.0	14.96 d	21.56 n	43.30 i	0.19 h	0.63 q	0.90 m	1.49 d-f	2.01	3.13 h
	0	8.30 h	24.941	33.00 m	0.28 fg	0.700	1.221	1.44 ef	1.991	3.04 hi
	0.5	14.95 d	33.30 f	48.30 g	0.38 e	0.88 m	2.03 i	1.56 d-f	2.28 fg	3.74 d
0.5	1.0	20.00b	43.30a	54.95c	0.49d	1.08h	2.41 f	1.68b-f	2.53d	3.72d
	1.5	18.30 c	34.30 c	61.65 b	0.53 d	1.16 g	2.52 e	1.64 c-e	2.52 d	3.98 c
	2.0	18.30 c	29.94 i	44.95 h	0.48 d	0.96k	1.69 j	1.45 d-f	2.25 gh	3.44 g
	0	13.30 e	23.30 m	33.46 m	0.65 c	1.02 i	2.09 h	1.50 b-f	2.23 gh	2.92 ij
	0.5	18.30c	31.65g	48.30g	0.85a	1.58d	2.97b	1.84a-c	2.77b	4.13c
1.0	1.0	23.30 a	43.30 a	68.30 a	0.86 a	1.91 a	3.37 a	2.04 a	3.15 a	4.65 a
	1.5	20.00 b	41.59 b	61.65 b	0.87 a	1.75 b	3.01 b	1.91 ab	2.60 c	3.98 c
	2.0	18.30 c	36.65 d	53.30 d	0.76 b	1.64 c	2.75 d	1.70 b-d	2.32 f	3.64 de
	0	8.10 i	19.80 o	34.951	0.53 d	0.93 1	1.49k	1.44 ef	1.99j	3.11 h
	0.5	11.65f	26.65k	43.15 i	0.62 c	1.53 e	2.55 e	1.62 c-e	2.21 h	3.61 df
1.5	1.0	13.25 e	35.00 e	51.60 e	0.69 c	1.58 d	2.86 c	1.65 c-e	2.50 d	4.53 b
	1.5	11.59 f	31.50 h	50.00 f	0.61 c	1.26 f	2.55 e	1.60 c-e	2.41 f	3.66 de
	2.0	13.20 e	26.65 k	43.15 i	0.51 d	0.99j	2.33 g	1.51 d-f	2.12 i	3.46 fg

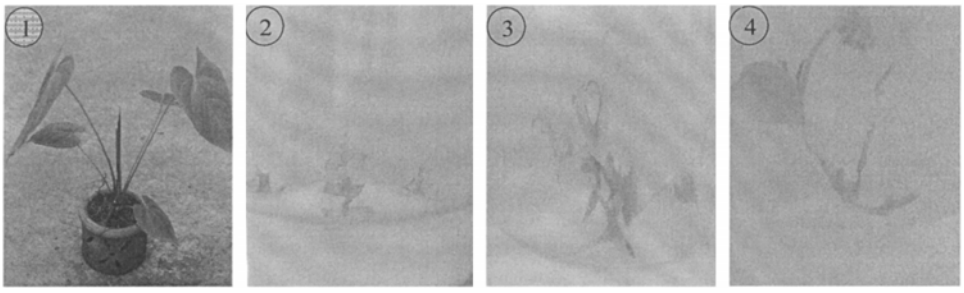
Figures in a column followed by different letters differ significantly, whereas, with common letter(s) do not differ significantly at 1% level of significance.

Number of shoots/explant

The number of shoots/explant significantly increased with time in all media. The increase in number of shoots was steady and sharp, which was more pronounced (3.37/explant) in MS medium containing 1 mg/L NAA + 1 mg/L BAP followed by medium containing 1 mg/L NAA + 1.5 mg/L BAP (3.01/explant) at 60 DAI and same trend was found at 40 DAI, but at 20 DAI, the highest number of shoots/explant was found in MS medium containing 1 mg/L NAA + 1.5 mg/L BAP (0.87/explant) which is insignificant with medium containing 1 mg/L NAA + 1.0 mg/L BAP (0.86/explant) and 1 mg/L NAA + 0.5 mg/L BAP (0.85/explant) shown in Table 2. The minimum number of shoots per explant (0.11/explant) was obtained in control at 60 DAI. Martin *et al.* (2003) observed that *Anthurium andraeanum* Hort. cultivars Tinora Red and Senator was established on the half-strength MS medium containing 1.11 µM BA, 1.14 µM IAA, and 0.46 µM kn at pH 5.5 was most effective for induction of shoot formation and cv. Tinora Red was more regenerative than Senator in terms of number of shoots per explant. The use of a lower BA concentration (0.44 µM) was essential for callus-free shoot multiplication during subculture.

Length of the shoot

The increase of length of shoot was steady and sharp which was more pronounced in MS medium containing 1 mg/L NAA + 1 mg/L BAP (4.65 cm) followed by medium containing 1.5 mg/L NAA + 1 mg L' BAP (4.53 cm) at 60 DAI and same trend was shown at 40 DAI and 20 DAI, respectively, which were significantly different (Table 2). The lowest length of shoots 1.93 cm was noted in control at 60 DAI. The results are partially supported with findings of Atta (1998) who reported that the seedlings were placed onto a multiplication MS media containing 2 mg/L BA and 0.2 mg/L NAA to increase in the number of shoots and shoots elongated to a length of 2.3 cm.



Plates
1. Plant with folded young leaf of *Anthurium andreanum* cv. Nifla (red colour),
2. Shoot initiation in MS medium containing 1.0 mg/L NAA + 1.0 mg/L BAP at 30 DAI,
3. Shoot induction in MS medium containing 1.0 mg/L NAA + 1.0 mg/L BAP at 60 DAI,
4. Rooting on MS medium supplemented with 1.0 mg/L IBA + 1.0 mg/L BAP at 60 DAI

Part 3. Root formation

Root initiation is an important part of *in vitro* regeneration. Shoots derived via organogenosis was transferred to MS medium supplemented with IBA and BAP combinations. Data of root regeneration was recorded in percentage of shoots developed root, number of roots/plantlet and length of roots at 20, 40, and 60 DAI and analyzed (Plate 4).

Table 3. Effect of different hormone concentrations on root development.

MS media with diff. hormones		Percent of root induction			No. of roots per plantlet			Length of root (cm)		
IBA	BAP	20 DAI	40 DAI	60 DAI	20 DAI	40 DAI	60 DAI	20 DAI	40 DAI	60 DAI
	0	5.17 o	8.73t	11.911	0.70p	0.91 m	l.07s	1.l0 q	l.84.p	2.23 q
	0.5	7.24 n	14.34 s	17.94k	0.97 n	1.07 kl	1.39 g	1.30 o	1.89 o	2.43 p
0	1	11.86 k	21.43o	26.95ij	l.37f	1.77de	2.49i	l.49m	2.29m	3.30 o
	1.5	16.86 h	29.501	35.32 gh	1.42 e	1.70f	1.93 k	1.69i	2.36 1	4.05 i
	2	22.68 e	39.13 i	49.22 f	0.67 p	1.11k	l.42,p	1.28 p	2.14n	3.53 n
	0	8.47m	14.74r	18.83k	0.94o	1.031	1.31 r	1.4ln	2.371	3.98j
	0.5	9.76 1	19.55 p	23.86j	1.21 j	1.47 h	2.88 f	1.66 j	2.49 k	3.89k
0.5	1	10.641	1916 q	23.33j	1.52 c	2.45 b	3.92 b	1.85f	2.91 e	4.40 e
	1.5	15.75 i	30.56k	49.11 g	1.24i	l.46h	2.76h	1.77g	2.70g	4.40e
	2	19.16 g	37.51 j	46.35 f	1.18k	1.37 i	1.75 m	1.571	2.52 ij	4.05 i
	0	13.03 j	25.78 m	34.37 h	1.08 1	1.26j	1.53 n	1.71 h	2.53 i	4.14 g
	0.5	25.67 d	47.79 f	56.74 de	1.34 g	1.81 d	3.23 d	1.93 d	3.20 b	4.97 c
1	1	34.54 a	67.76 a	83.85 a	2.18 a	2.51 a	4.29 a	2.36 a	3.93 a	5.50 a
	1.5	32.32 b	61.26c	73.24b	l.35g	1.58g	3.16e	2.16b	2.93d	5.04b
	2	29.65 c	53.87d	65.75c	1.22j	1.491	2.05j	l.87e	2.49k	4.17f
	0	13.57j	23.73 n	28.85 i	1.04 m	1.24j	1.46 o	1.63 k	2.15 n	3.58 m
	0.5	18.72g	42.36g	55.13e	1.48d	1.74ef	3.14e	1.71 h	2.50jk	4.07h
1.5	1	29.41 c	61.68b	74.04b	1.58b	2.06c	3.51 c	1.98c	3.07c	4.92d
	1.5	26.39 d	52.16 e	64.32 c	1.30 h	1.56 g	2.82 g	1.68j	2.84 f	3.81 1
	2	20.87 f	40.80 h	59.76 d	1.19k	1.48 h	1.841	1.56 1	2.62 h	4.13 g

Figures in a column followed by different letters differ significantly, whereas, with common letter(s) do not differ significantly at 1% level of significance.

Percentage of root development

Effect of media with different hormone supplementation on percent of shoots induced roots was found highly significant. The result show that the percent of

explants induced root was maximum 83.85% with MS medium containing 1 mg/L IBA + 1 mg/L BAP followed by 73.24% with medium containing 1 mg/L IBA + 1.5 mg/L BAP at 60 DAI and same trend was found at 40 DAI, and 20 DAI, respectively, which were statistically different (Table 3). The minimum percent of shoots induced roots (11.91%) at 60 DAI was obtained with MS medium without any hormone (control). Martin *et al.* (2003) reported that in *Anthurium andraeanum* Hort. cv. Tinora Red and Senator regenerated shoots could induce to form roots on half-strength MS medium supplemented with 0.54 μM NAA and 0.93 μM kn.

Number of roots per plantlet

Number of roots per plantlet increased with time. The increase in number of roots was steady and sharp which was more pronounced in MS medium containing 1 mg/L TBA + 1 BAP mg/L. On this medium, the maximum number of roots was 4.29/explant at 60 DAI followed by 3.92/plantlet in medium, containing 0.5 mg/L TBA + 1 BAP mg/L and same trend was observed at 40 DAI and 20 DAI, respectively (Table 3). The minimum number of roots per plantlet (1.07/plantlet) at 60 DAI was obtained in control. In a research conducted by Atta (1998) found that *in vitro* multiplicated shoots were subsequently placed on different rooting medium contained 0.25 mg/L IBA which produced 3.6 roots per plantlet with a 94% success.

Length of roots

In MS media with different hormone combinations, length of root increased significantly with time. The increase of length of root was steady and sharp, which was more pronounced in MS medium containing 1 mg/L IBA + 1 mg/L BAP. The largest root was recorded 5.50 cm followed by 5.04 cm in medium containing 1 mg/L IBA + 1.5 mg/L BAP at 60 DAI and same result was found at 40 DAI and 20 DAI, respectively, which were significantly different at 1% level of significance (Table 3). The lowest length of root (2.23 cm) was noted in control at 60 DAI. Puchooa (1996) reviewed that the root formation of three varieties of *Anthurium*, achieved from regenerated shoots, rooted readily on medium containing IBA (1.0 mg/L).

Conclusion

Mid rib of *Anthurium andreanum* cv. Nitta were cultured on MS medium supplemented with I mg/L NAA + I mg/L BAP showed the best result for percent survivability (80.00%). Shoot initiation in MS media containing 1 mg/L NAA +

1 mg/L BAP took minimum time of 9.00 days, maximum percentage of shoots induced explant was 68.3 0%, the number of shoots per explant were 3.37 and the length of shoots was highest (4.65 cm) at 60 DAI and similar result was shown at 40 DAI and 20 DAI, respectively. MS media containing 1 mg/L IBA + 1 mg/L BAP showed the best performance in rooting of shoots (83.85%), highest number of roots (4.29/plantlet) and root elongation (5.50 cm) at 60 DAI and same trend was found at 40 DAI and 20 DAI, respectively. Shoot regeneration and rooting was poor on MS media without any hormone supplement. The protocol developed from the experiment may be useful for large-scale production of healthy and disease free planting materials of *Anthurium andreanum* cv. Nitta commercially. Also these findings of the study may be used for genetic transformation for the improvement of *Anthurium andreanurn* cv. Nitta using biotechnological approach.

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