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Micropopagation of *Anthurium andreanum* cv. Jewel from leaf explants

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ABSTRACT

A protocol for the mass propagation of Anthurium andreanum cv. Jewel has been developed through callus induction and organogenesis using leaf lamina as explant. MS basal medium containing 3 mg L^{-1} 2,4-D + 0.5 mg L^{-1} BAP + 0.4 mg L^{-1} TDZ took minimum number of days (37.68) to initiate callus. However, maximum callus production was observed in the media containing 2 mg L^{-1} NAA (77.33%). Young leaves exhibited better responses and the calli were compact and dark green. The shoots were initiated later from the calluses by inoculating on MS media containing 3.0 mg L^{-1} BAP + 0.5 mg L^{-1} NAA which took a minimum of 27.83 days while MS + 2 mg L^{-1} BAP + 0.5 mg L^{-1} NAA gave highest regeneration percentage (98.89%) as well as shoot length of 2.23 cm. Maximum number of shoots per callus (5.83) and highest number of shoots following second subculturing (22.60) were observed when MS basal medium was fortified with 2 mg L^{-1} BAP singly. For root induction, 1/2MS media supplemented with 2.0 mg L^{-1} NAA took the least days (33.55). Maximum root induction per plant (5.83) and length (4.28 cm) of root were recorded in 1/2MS + 1.0 mg L^{-1} NAA. The well rooted Anthurium plantlets were successfully hardened in coconut husk with highest survival percentage of 96.17 per cent at vermiculite + coconut husk (1:1, w/w).

Keywords : acclimatization, Anthurium andraeanum, callus induction, leaf explants, micropropagation

Anthurium andreanum, one of the most important cut flower species in the tropical and subtropical countries belongs to the family Araceae. It consists of 108 genera and approximately 3750 monocotyledonous species. *Anthurium* is commonly cultivated for their highly prized elegant flowers. Conventionally, it is propagated by three basic propagation methods *viz.*, by seeds, traditional vegetative method and tissue culture (Atak and Celik, 2009). Conventional methods of *Anthurium* propagation by separating the newly developing plants is time consuming and produce a low number of new plantlets from its base. It takes years to develop commercial quantities of elite clones. Propagation through seed produces heterozygous progenies due to cross-pollination. Moreover, it is hindered by the poor germination rate and low viability of the seeds. Micropropagation technique appears as an alternative for *en masse* multiplication of anthuriums at faster rates than conventional methods (Martin *et al*., 2003). Micropropagation of *Anthurium* has been achieved with various tissues including leaf, petiole, spadix, spathe, seeds, lateral buds and shoot tips (Martin *et al*.,2003; Viegas *et al*., 2007; Atak and Celik, 2009; Jahan *et al*., 2012; Raad *et al*., 2012).

The tissue culture of *Anthurium* was first reported by Pierik *et al*. (1974) through callus culture and they successfully regenerated the callus through adventitious shoot formation. Martin *et al*. (2003) achieved direct shoot regeneration of anthurium from lamina explants while the liquid or raft culture for regeneration of adventitious shoots from leaf explant was demonstrated by Teng (1997). Vargas *et al*. (2004) obtained *in vitro* plants from germinated seeds and plantlets from micro-cutting culture. The plantlets showed callus formation at the base of the stem. Kuehnle and Sugii (1991) established a regeneration system from leaves and petioles of *Anthurium* Hawaiian cultivars through callus cultures, and Kunisaki (1980) established the micropropagation of *Anthurium* from axillary buds. Chen *et al*. (1997) successfully regenerated *Anthurium* plants from the roots explants while Yi-xun *et al*. (2009) induced calli from proto-corm-like bodies of *A. andreanum*.

Micropropagation by callus induction from leaf explants is a difficult step and time consuming. Multiplying Anthuriums by callus induction implies a long explant-plant cycle (12 months) (Geier, 1986). However, credible proliferation of callus and subsequent plant regeneration is important for enormous plant propagation. This article describes a detailed protocol of *Anthurium andreanum* cv. Jewel for the rapid establishment and indirect organogenesis of *Anthurium andreanum* cv. Jewel through callus intervention.

MATERIALS AND METHODS

The experiment was conducted at plant tissue culture laboratory, Department of Floriculture, Medicinal and Aromatic Plants, UBKV, Cooch Behar during March 2014 to February 2015. Immature leaf explants were collected from the net house of the department. To minimize fungal and bacterial contamination, the explants were sterilized with 0.1 per cent (v/v) $HgCl_2$ for 3 minutes followed by 70 per cent (*v/v*) ethyl alcohol for 30 seconds. Leaves were then rinsed three times in sterile distilled water. Sterile leaves were cut to about 0.5 to 1.0 cm^2 pieces which were used as explants.

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Table 1: Influence of different plant growth regulator compositions on the induction from leaf explant of *Anthurium andreanum* cv. Jewel

Treatment	Days to callus induction	Percent Callusing (%)	Callus morphology
MS + 1 mg L^{-1} 2,4-D	66.17	55.78 (48.32)	Greenish Compact
MS + 2 mg L^{-1} 2,4-D	57.50	46.67 (43.09)	Greenish White Compact
MS + 3 mg L^{-1} 2,4-D	59.68	40.44 (39.66)	Greenish white Compact
MS + 1 mg L^{-1} NAA	108.00	16.44 (23.90)	Dark green compact
MS + 2 mg L^{-1} NAA	110.50	77.33 (61.59)	Dark green compact
MS + 3 mg L^{-1} NAA	109.17	70.67 (55.88)	Dark green compact
MS + 1 mg L^{-1} 2,4-D + 0.5 mg L^{-1} BAP + 0.4 mg L^{-1} TDZ	43.68	68.00 (55.56)	Greenish White Compact
MS + 2 mg L^{-1} 2,4-D + 0.5 mg L^{-1} BAP + 0.4 mg L^{-1} TDZ	40.68	60.67 (51.17)	Greenish white Compact
MS + 3 mg L^{-1} 2,4-D + 0.5 mg L^{-1} BAP + 0.4 mg L^{-1} TDZ	37.68	44.22 (41.68)	Yellowish brown nodular
MS + 1 mg L^{-1} NAA + 0.5 mg L^{-1} BAP + 0.4 mg L^{-1} TDZ	63.83	38.22 (37.52)	Greenish White Compact
MS + 2 mg L^{-1} NAA + 0.5 mg L^{-1} BAP + 0.4 mg L^{-1} TDZ	61.50	20.67 (27.03)	Yellowish compact
MS + 3 mg L^{-1} NAA + 0.5 mg L^{-1} BAP + 0.4 mg L^{-1} TDZ	58.33	18.89 (25.75)	Yellowish White fragile
SEm (±)	**2.57**	**0.56**	
LSD (0.05)	**7.29**	**1.58**	

Table 2: Influence of different plant growth regulator compositions on the shoot regeneration from the calli of *Anthurium andreanum* cv. Jewel

Medium code	Days to shoot induction	Shoot regeneration (%)	Shoot length (cm)	No. of shoots per callus at first subculturing	No. of shoots per callus after 2nd subculturing
MS + 1 mg L^{-1} BAP	41.67	50.00 (33.65)	1.42	4.00	17.33
MS + 2 mg L^{-1} BAP	35.17	81.78 (64.10)	1.82	5.83	22.60
MS + 3 mg L^{-1} BAP	33.67	88.44 (68.99)	1.77	4.83	18.53
MS + 1 mg L^{-1} Kn	43.50	94.44 (80.86)	1.08	2.17	9.67
MS + 2 mg L^{-1} Kn	38.33	76.22 (60.39)	1.37	3.67	16.97
MS + 3 mg L^{-1} Kn	35.67	82.00 (65.82)	1.37	4.33	20.07
MS + 1 mg L^{-1} BAP + 0.5 mg L^{-1} NAA	33.67	79.56 (60.67)	1.73	2.83	13.70
MS + 2 mg L^{-1} BAP + 0.5 mg L^{-1} NAA	33.50	98.89 (84.66)	2.23	4.00	12.60
MS + 3 mg L^{-1} BAP + 0.5 mg L^{-1} NAA	27.83	95.78 (82.25)	1.93	2.50	9.67
MS + 1 mg L^{-1} Kn + 0.5 mg L^{-1} NAA	37.00	89.56 (69.98)	1.32	2.67	7.63
MS + 2 mg L^{-1} Kn + 0.5 mg L^{-1} NAA	34.00	97.78 (83.64)	2.08	3.50	9.07
MS + 3 mg L^{-1} Kn + 0.5 mg L^{-1} NAA	32.83	90.89 (75.57)	1.98	2.33	8.63
SEm (±)	**1.09**	**1.93**	**0.11**	**0.35**	**0.55**
LSD (0.05)	**3.08**	**5.46**	**0.32**	**1.00**	**1.55**

Table 3: Influence of different plant growth regulator compositions on the root regeneration of the *in vitro* grown shoots of *Anthurium andreanum* cv. Jewel

Treatment	Root regeneration (%)	Days to rooting	No. of roots shoot^{-1}	Length of root (cm)
½ MS + 1.0 mg L^{-1} NAA	98.50 (83.51)	56.13	5.83	4.28
½ MS + 2.0 mg L^{-1} NAA	97.33 (81.36)	33.55	4.33	3.76
½ MS + 3.0 mg L^{-1} NAA	100.00 (90.00)	36.78	3.95	3.29
½ MS + 1.0 mg L^{-1} IBA	100.00 (90.00)	41.88	3.73	2.91
½ MS + 2.0 mg L^{-1} IBA	100.00 (90.00)	45.93	2.85	2.46
½ MS + 3.0 mg L^{-1} IBA	99.67 (88.34)	52.45	2.48	1.91
SEm (±)	**0.85**	**0.64**	**0.09**	**0.09**
LCD (0.05)	**2.46**	**1.86**	**0.26**	**0.25**

Table 4: Influence of hardening media on the survivability of *Anthurium andreanum* cv. Jewel *in vitro*-raised plantlets.

Hardening media	Survival percentage (%)
Vermiculite + Sand (1:1)	62.83 (52.45)
Sand + Sawdust (1:1)	54.00 (47.30)
Coconut husk	96.17 (78.85)
Vermiculite + coconut husk (1:1)	75.33 (60.23)
Vermiculite	69.33 (56.38)
SEm (±)	**0.392**
LCD (0.05)	**1.602**

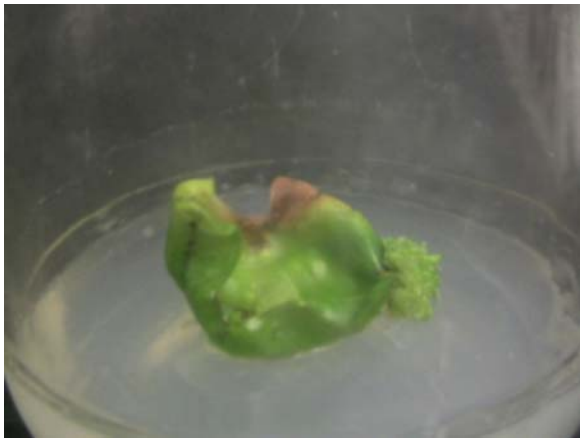
For the propagation by indirect organogenesis, MS medium (Murashige and Skoog, 1962) and 1/2MS were used. For callus induction, the leaf explants were transplanted into the MS medium supplemented with 30 g/L sucrose and different concentrations and combinations of 2,4-D, NAA, BAP and TDZ. The pH was adjusted to 5.7 before autoclaving. For shoot regeneration, the calli obtained from the leaf explants were transferred to the MS basal medium supplemented with different concentrations and combinations of BAP, Kin and NAA. The regenerated shoots longer than 3 cm with a pair of leaves or more were transferred to 1/2MS medium fortified with different concentrations of either NAA or IBA. All the cultures incubated at 25±2-^{0}C with a 16 h light/8 h dark photoperiod and 3500 lux of light intensity. The data generated was subjected to ANOVA in complete randomized design at 5 per cent level of significance. Ten explants were used in each treatment and each treatment was done in 3 replications. The percentile data of the experiments were assumed and subjected to square root transformations. 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.

RESULTS AND DISCUSSION

The experiment was conducted with *Anthurium andreanum* cv. Jewel accomplished with newly emerging brown coloured young leaves for callus induction, shoot initiation, rooting and finally plantlet hardening on MS basal medium with different concentrations of auxins (2, 4-D and NAA) and cytokinin (BAP) on *in vitro* condition. Results pertaining to table 1 revealed that the MS basal medium when supplemented with 3 mg L^{-1} 2,4-D + 0.5 mg L^{-1} BAP + 0.4 mg L^{-1} TDZ induced calli after 37.68 days producing yellowish brown nodular calli, whereas the highest percentage (77.33%) of callus induction was observed in MS medium fortified with 2 mg L^{-1} NAA, producing dark green and compact calli. This might be due to the fact that the young lamina explants possessed more potentiality for callus induction as they contained midrib. The results are in agreement with the findings reported by Bejoy *et al*. (2008) and Jahan *et al*. (2009).

The six to eight weeks old calli were then transferred to the proliferation medium consisting of solidified MS media supplemented with different plant growth regulator compositions and combinations of BAP, Kinetin and NAA to produce shoot. Results from Table 2 showed that MS basal medium fortified with 3 mg L^{-1} BAP + 0.5 mg L^{-1} NAA took the least number of days (33.50) to induce shoots from calli while MS + 2 mg L^{-1} BAP + 0.5 mg L^{-1} NAA gave the highest (98.89%) shoot regeneration percentage as compared to the rest of the treatments. This composition was also found to

Fig. *In vitro* indirect organogenesis of *Anthurium andreanum* cv. Jewel using foliar explants



A

Fig. (A) Formation of callus from the leaf explan

B

Fig. (B) Callus proliferation after 60 days of subculturing

C

Fig. (C) Shoot regeneration from callus after 8 weeks of incubation

D

Fig. (D) Shoots with well developed roots after 12 weeks of incubation

E

Fig.(E) Plantlets were harvested for *ex vitro* transfer

F

Fig. (F) Well acclimatized plantlets after 60 days of *ex vitro* transfer

be the best treatment for producing longest shoots (2.23 cm). MS basal medium supplemented with 2 mg L^{-1} BAP produced the maximum number of shoots per callus both at first subculturing (5.83) as well as in the second subculturing (22.60). Vargas *et al*., (2004) and Bejoy *et al*., (2008) achieved regeneration of *A. andraeanum* from callus tissue and obtained a higher number of shoots per callus after the subculture of calli.

For root induction and elongation, 1/2MS basal media were used in all the treatments supplemented with different concentrations of NAA and IBA. Cent percent root regeneration were observed in 1/2MS basal medium fortified with 3.0 mg L^{-1} NAA, 1.0 mg L^{-1} IBA and 2.0 mg L^{-1} IBA respectively (Table 3). Half strength MS media when supplemented with 2.0 mg L^{-1} NAA produced roots in shortest duration (33.55 days) as compared to the rest of the treatments, while highest number of roots per shoot (5.83) and longest roots (4.28 cm) were observed in half MS fortified with 1.0 mg L^{-1} NAA. Martin *et al*. (2003) cultured their regenerated *A. andraeanum* plantlets in half strength MS medium supplemented with 0.54 ìM NAA and 0.93 ìM Kinetin for *in vitro* rooting. Bejoy *et al*. (2008) and Atak and Celik (2009) achieved about 95 to 98 per cent root from the newly formed shoots in 0.5 mg/l NAA in half-strength MS medium in six weeks.

The well rooted plantlets were later removed from culture vessels and washed thoroughly with tap water to remove the adhering medium completely without causing damage to the roots. The plantlets were then transferred to the plastic pots containing different hardened media and placed into the greenhouse at 27 ± 1°C, light density of 4000 lux and 70 per cent relative humidity. Among the different hardening media used, coconut husk showed the best result for plantlet survivability (96.17%) (Table 4).

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REFERENCES

Atak, C. and Çelik, O. 2009. Micropropagation of *Anthurium andraeanum* from leaf explant. *Pak. J. Bot.* **41** (3): 1155-61.

Bejoy, M., Sumitha, V.R. and Anish, N.P. 2008. Foliar regeneration in *Anthurium andraeanum* Hort. cv. *Agnihotthri. Biotechnology*, **7(1)** : 134-38.

Chen, F.C., Kuehnle, A. and Sugii, N. 1997. *Anthurium* roots for micropropagation and *Agrobacterium tumefaciens*-mediated gene transfer. *Plant Cell Tiss. Organ Cult.* **49**: 71 -74.

Geier, T. 1986. Factors affecting plant regeneration from leaf segments of *Anthurium scherzerianum* Schott cultured *in vitro*. *Plant Cell Tiss. Org. Cult.* **6**:115-25.

Jahan, M.T., Islam, M.R., Khan, R., Mamun, A.N.K., Ahmed, G. and Hakim, H. 2009. *In vitro* clonal propagation of Anthurium (*Anthurium andraeanum* Lind) using callus culture. *Plant Tiss. Cult. Biotech.*, **19**(1): 61-69.

Kuehnle, A.R. and Sugii, N. 1991. Callus induction and plantlet regeneration in tissue cultures of Hawaiian *Anthurium*. *Hort. Sci.,* **26**: 919-21.

Martin, K.P., Joseph, D., Madassery, J. and Philip, V.J. 2003. Direct shoot regeneration from lamina explants of two commercial cut flower cltivars of *Anthurium andraeanum* Hort. *In Vitro Cell. Dev. Biol. Plant*, **39**: 500-504.

Pierik, R.L.M., Steegmans, H.H.M. and Van der Meys, J.A.J. 1974. Plantlet formation in callus tissues of *Anthurium andreanum* Lind. *Scientia Horticulturae*, **2 :**193-98.

Pierik, R.L.M. and Steegmans, H.H.M. 1976. Vegetative propagation of *Anthurium scherzerianum* Schott through callus cultures. *J. Sci. Hort.* **4 :** 291-92

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 J. Crop Sci.*, **6**(5): 928-37.

Teng, W.L. 1997. Regeneration of *Anthurium* adventitious shoots using liquid or raft culture. *Plant Cell, Tiss. Organ Cult.,* **49**: 153-56.

Vargas, T.E., A. Mejias, M. Oropeza and E. Garcia. 2004. Plant regeneration of *Anthurium andraeanum* cv. Rubrun. *Electronic J. Biotech.,* **7**(3): 282-86.

Viégas, J., Da Rocha, M.T.R., Ferreira-Moura, I., Da Rosa, D.L., De Souza, J.A., Corrêa, M.G.S. and Da Silva, J.A.T. 2007. *Anthurium andreanum* (Linden ex André) culture: *In vitro* and *Ex vitro*. *Floriculture Ornamental Biotech.* **1**(1): 61-65.

Yi-xun, Y., Ling, L., Juan-xu, L. and Jing, W. 2009. Plant regeneration by callus-mediated proto-corm-like body induction of *Anthurium andraeanum* Hort. *China. J. Agric. Sci.* **8**(5):572-77.