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Effect of Different Concentrations and Combinations of BAP and NAA on Micropropagation of Anthurium (*Anthurium andraeanum* Linn.) Red

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Abstract

Anthurium anderanum from Araneae family, is a plant with high commercial value. Tissue culture technique appears as an alternative way propagation to increase Anthurium production. The recent research intended to establish an efficient regeneration method for *Anthurium andraeanum* tissue culture. Young Anthurium plantlets including young shoots, petioles and leaves were applied for regeneration through in vitro indirect organogenesis method from the undefined plant tissues. Callus proliferation was induced by the use of Morishige and Skoog media along with 0.6 mg/l 2,4- Dichlorophenes acetic acid. Shoot regeneration medium consisted of MS salts with supplemented different combinations of BAP and NAA. The number of shoots per explants was significantly increased in 7 months of culture periods in all different combinations but there were found that 14 shoots/explant and 13.9 shoots/explant in NA^+ BAP (0^+3mg/l) and (0.2^+0mg/l) respectively. The longest shoot length (17.3 cm) was found in NAA^+BAP (0^+0.5mg/l). Rooting was found automatically in shoot proliferation medium. Regenerated plantlets were potted in mixture of broken brick, coco husk and charcoal and successfully acclimatized.

Keywords: Micropropagation, Anthurium, Plant Growth Regulators, BAP, NAA, Callus.

INTRODUCTION

Anthurium is one of the well-known potting plant genera which composed of almost 1500 tropically cultivated species which are one of the commercially important genera in Araneae family [1]. Numerous Anthurium plant species of Anthurium are being cultivated for different intentions such as cut-flower, potted flowers, landscaping etc [2]. The commercially produced ornamental potting plants has a great potential in the international markets both tropically and sub-tropically [3]. The Anthurium cultivars are globally important for huge market because of their valuable flowers. Orchids and Anthuriums are first and second among tropical cut flowers respectively [4]. Floriculture is one of the fast-developing industries all over the countries [5]. Nowadays, flower industries are very popular work with the significantly high potential for the production than that of other ornamental plants [6]. In the international market, the demand for flowers is highly increased due to trade globalization and economic liberalization [7]. Anthurium plants have been popular for the attractive, long vase life flower, higher returns per unit area [8]. Anthurium has hybridized by the scientists in the Netherlands and Hawaii [9]. They are known to be natively grown in South America. Thousand varieties of Anthurium have been recently grown widely under artificial environmental conditions, at sea level and above the height of 1,200 m [10]. They have been bred for beautiful color, long shelf life, and disease resistance activities. Anthurium propagation have been made in the means of sexual or asexual way [11]. They have been propagated traditionally through seed, but propagation rate of this method for Anthurium is very slow due to the inherent heterozygosity [12]. So, seed propagation is not recommended for Anthurium spp. The time between pollination and seed maturity and the time of seed development takes three years in a breeding program [13,14]. Growing plants from the seeds cannot produce a reliable technique for developing a new plantation area, in this way, vegetatively propagative method such as cutting might be the only method for making large scale of individuals [15]. The adaptation of propagation method for certain plant species depending upon their genetically potential and also the use of intention.

In this case, stem cutting method is also not a practical way to propagate a huge number of planting materials. Nowadays, a large number of anthurium can be multiplied by micropropagation technique [16]. Micropropagation is the alternative method of the conventionally propagative method in which the culture of certain plant species such as somatic cells, tissue or organ in an artificial environmental condition may be a reliable way for the production of a huge amount of genetically identical plantlets to the stock plant in a relatively short period of time [17]. Anthuriums have been usually propagated through the use of seed [18]. however, vegetatively propagative techniques used for Anthuriums did not give satisfactory results, therefore plant tissue culture techniques could be another way for the production increasingly [19,20]. Seed propagation method may not worthwhile due to cross-pollination as a result the progeny could be heterozygous [21]. Furthermore, the seeds are hampered due to poor rate of germination and low viability. Moreover, it is hampered by the poor germination rate and low viability of the seeds [22]. Anthurium *in vitro* propagations have been successful using different types of tissues.

Anthurium tissue culture was achieved firstly by Pierid and his colleagues in 1974 [23]. The propagation of *Anthurium andreanum* have been achieved through the use of adventitious shoots formation from callus [24]. The regeneration of direct shoot has been achieved with the use of leaf lamina explants [25].

MATERIALS AND METHODS

The plant materials and explant sources were collected from Pyrin Oo Lwin, Mandalay Division, Myanmar. The collected Anthurium plants were placed in the Nursery house at Department of Biotechnology Research. The explant materials were stored in the earthen potting materials filling with the soil from the collective place under screen house conditions. This research was carried out at the Plant Tissue Culture Lab. under the Department of Biotechnology Research, Kyaukse Township, Mandalay Region, Myanmar.

Shoot tips, leaf segments and petiole segments were excised from the mother plant (*Anthurium andraeanum* L.) and then used as explants for the initiation of culture.

The excised plant materials were thoroughly washed with running water. Then these explant materials were thoroughly soaked using liquid detergent for about one min. The excised plant materials were rinsed in ten ml/l of antifungal agent for about 2 hrs. And then, these explants were rinsed in antibiotic for about 1 hr. After rinsing the explant segments with the sterilized distilled H_2O, the planting materials were then cut into smaller pieces and discarded the unwanted parts using sterilized scapples to get the pieces of 1 to 2 centimeter in diameter. After all the above steps, the planting materials were then put into laminar air-flow clean bench and they were sterilized with 20% Concore bleach solution for 10 mins, 70% ethyl alcohol for 5 mins and 0.1% mercuric chloride for 2 mins and then thoroughly washed with three to four times using sterilized DW after each sterilizing agents.

After the end of explant sterilization processes, the planting materials were then inoculated in to callus induction media containing basal Morishige and Skoog media comprising of sucrose or table sugar (30 g/l), gelling agent (6 g/l), 0.6mg/L 2,4-D for callus induction. The pH of the culture media was adjusted using with either 1N NaOH or 1N HCl to 5.8 at 121℃, 1 atm for 20 minutes in HICLAVE autoclave [26]. The plant growth regulator 6-benzylaminopurine (BAP), alone or in combination with naphthalene acetic acid (NAA), was added to the same basal medium for shoot induction and proliferation; the control treatment was the same basal medium without any plant growth regulator [27].

The culture bottles were kept cool and white fluorescent tube light (1500 Lux) were kept at a constant temperature of 27±1℃ using an air-conditioning system [28]. Observations were recorded on the callus induction and the shoot proliferation after certain cultural period. The number of shoots was observed and counted at 2 months 4 months and 7 months of culture period. All of the cultural materials were kept at a constant temperature of 271℃, and the number of developed and rooted plants was counted for each treatment.

The *in vitro* plantlets were transferred to a tray with sterile potting material (coconut husk, broken brick, charcoal) and kept in a plastic-covered environment with semi-controlled light and temperature conditions for adaptation to the environment [29]. Fungicidal sprayings were used to keep fungi at distance, and the in vitro plantlets were fully exposed to changes in the environment after two months and hydrated once a week.

Table 1: Different Concentrations of Plant Growth Regulators used in Anthurium Shoot Proliferation

Treatments	BAP (mg/l)	NAA (mg/l)
1	0	0
2	0.5	0
3	1	0
4	1.5	0
5	2	0
6	2.5	0
7	3	0
8	3.5	0
9	4	0
10	0.5	0
11	0	0
12	0.5	0
13	1	0
14	1.5	0
15	0	0.5
16	0.5	0.5
17	1	0.5
18	1.5	0.5
19	2	0.5
20	0	1
21	0.5	1
22	1	1
23	1.5	1
24	2	1
25	0	1.5
26	0.5	1.5
27	1	1.5
28	1.5	1.5
29	2	1.5

RESULTS AND DISCUSSIONS

This experiment was conducted with *Anthurium andreanum* Linn. accomplished with shoot tip, leaf segments and petiole segments of mature plant for callus proliferation, shoot formation, root formation and then full-grown plantlet regeneration on MS media supplemented with different concentrations and combinations of BAP and NAA on *in vitro* condition in the lab. The results of the different developmental stages described along with discussions under the following headings.

Shoot Formation and Proliferation

Shoots were transplanted on MS media supplemented with varied combinations of BAP and NAA, which were begun by organogenesis from different cultivated explants of *Anthurium andreanum* Linn. At 1 month, 4 months, and 7 months after shoot initiation, there was substantial heterogeneity in the interplay of medium composition for shoot development, number of shoots/explants, and length of the largest shoot.

Average Shoot Number of the Explant

The shoot number of the explants promoted with time in every media composition. There was not significant difference in 1 month 2 months culture period. The increase in the number of shoots was gradual but noticeable when the culturing period reached 7 months, which was more pronounced as the highest number of shoots (14/explant) in MS media containing 0 mg/L NAA^+ 3 mg/L BAP then 0.5 mg/L NAA^+ 0 mg/L BAP (13.9/explant) at 7 months of culture period as shown in figure (5) and figure (7). The lowest average shoot number was observed in MS media supplemented with 1 mg/L NAA^+ 1 mg/L BAP (2/explant) and this was lower shoots in control medium (without any growth regulator) as shown in figure (9). Martin and his colleagues discovered that *Anthurium andraeanum* Hort. cv. Tinora Red and Senator was established on ½ strength MS medium containing 1.11 µM BA, 1.14 µM IAA, and 0.46 µM kn. Shoot induction and cv were the most effective. In terms of the number of shoots per explant, Tinora Red outperformed Senator [30].

Average length of shoot

The length of shoot was steadily increased and sharp after 7 months of cultural incubation. At 7 months of culture incubation, which was more pronounced in MS medium containing 0 mg/L NAA^+ 1.5 mg/L BAP (17.3 cm), followed by MS medium supplemented 1.5 mg/L NAA^+ 0 mg/L BAP (15.6 cm), and was followed by MS medium containing 1 mg/L NAA + 2 mg/L BAP (15.5 cm) and 1 mg/L NAA^+ 0 mg/L BAP (15.4 cm), which were not considerably different. (Figure 6, 10 and 13). The shortest length of shoots was noted as 3.9 cm in MS media supplemented with 0.5 mg/L NAA^+ 1.5 mg/L BAP which was shorter than control at 7 months of culture incubation. The seedlings were placed on MS media enriched with 2 mg/L BA and 0.2 mg/L NAA to enhance the number of shoots, which then extended to a length of 2.3 cm, according to Atta. which shown that the results are partially supported with findings of us [31].

Rooting

Root development is an important part of the *in vitro* regeneration of any plant species [32]. In some reports, the root formation procedure was carried out in basal MS media without any plant hormone [33]. In this study, even before the individualization, the rooting process began naturally without the use of any growth regulator (Fig. 3). Differentiation and transference to the medium resulted in improved growth and development of the rooted plantlets, with roots appearing in 90% of the in vitro plants derived from elongated shoots [34].

The root formation occurrence lasted between 30 days and 45 days, and most of the developing plantlets produced 4-7 roots; 3 months after individualization all of the plantlets produced roots. Viegas *et al*., (2007), Liendo and Mogollon, (2009) reported that rooting in anthurium did not require the use of any plant growth regulator and other authors for this genus has already been reported the occurrence of spontaneous roots [35]. Our research was therefore in agreement with this finding. So, the formation of this organ is not necessary any specific medium.

Acclimatization

The final and the most difficult stage in micropropagation studies is the hardening of the fully grown plantlets [36,37]. During the hardening or acclimatization stage, loss of most of the young plantlets may be occurred because of several causes. Several environmental factors including temperature, lighting intensity, humidity needed to be modified to improve survival rate of the micro propagated plantlets [38,40]. The findings related with the adaption of hardened plantlets revealed that the remarkable easy acclimatization of *Anthurium andraeanum* plants to the environmental situations with the use of broken bricks, coco-husks and charcoals and controlling the appearance of fungi (Fig. 4 and Fig 5).

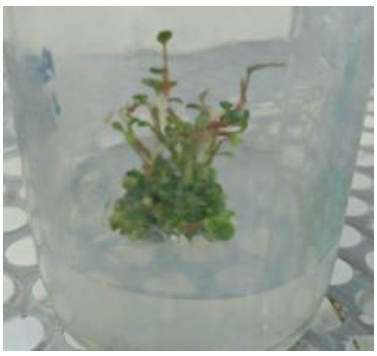

Fig 1: Shoot initiation from the proembryo Nic Calli

Fig 2: Shoot proliferation in MS media supplemented with BAP and NAA

Fig 3: Root formation from the elongated shoots

Fig 4: Primary hardening in the growth chamber

Fig 5: Secondary hardening in the outdoor environment

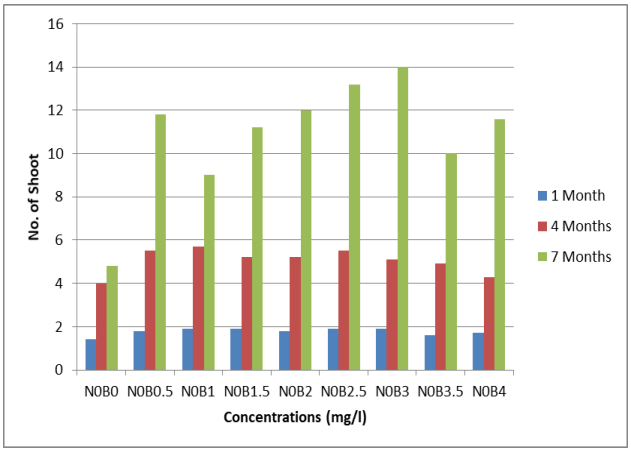

Fig 6: Effect of different combinations of BAP and NAA on number of shoots after 1 month, 4 months and 7 months of culture

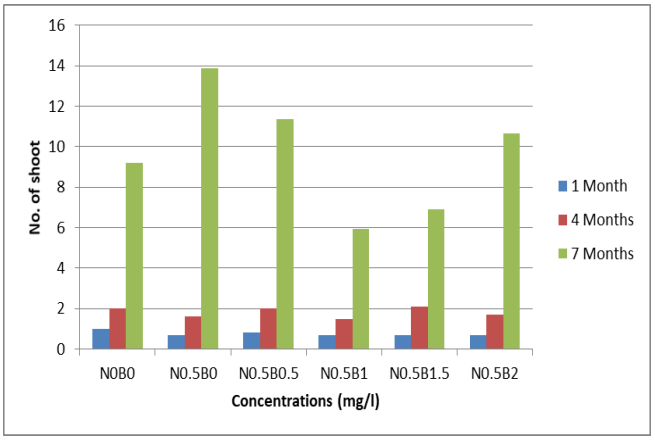

Fig 8: Effect of different combinations of BAP and NAA on number of shoots after 1 month, 4 month and 7 months of culture

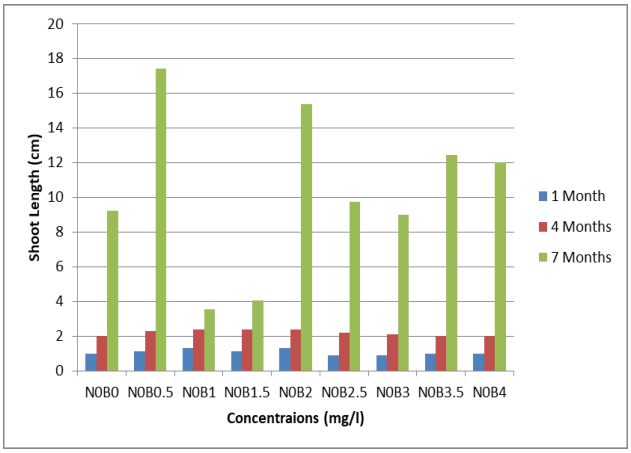

Fig 7: Effect of different combinations of BAP and NAA on number of shoots after 1 month, 4 month and 7 months of culture

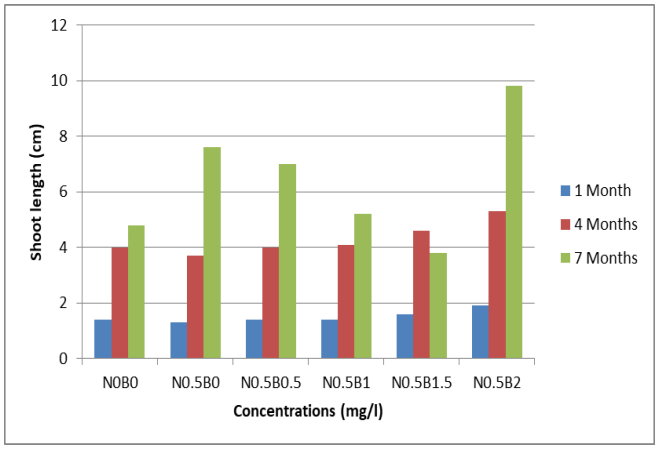

Fig 9: Effect of different combinations of BAP and NAA on number of shoots after 1 month, 4 month and 7 months of culture

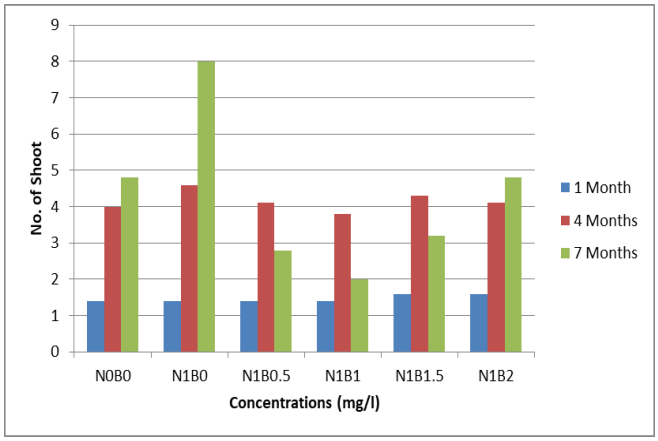

Fig 10: Effect of different combinations of BAP and NAA on number of shoots after 1 month, 4 month and 7 months of culture

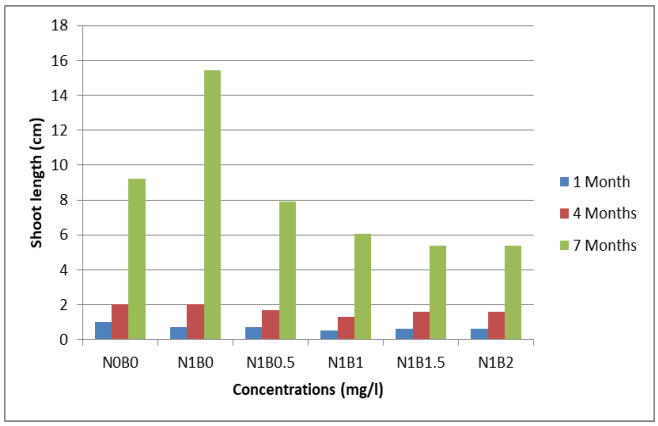

Fig 11: Effect of different combinations of BAP and NAA on number of shoots after 1 month, 4 month and 7 months of culture

CONCLUSION

This research work develops an efficient protocol for in vitro propagation of Anthurium Red. We discovered the fact that types of the plant materials used as explants may be important in addition to basal media compositions and plant hormones for the successful work of any plant tissue culture. In this study, different segments of *Anthurium andreanum* explant were inoculated in basal media composed of different concentrations of NAA and BAP. The regeneration of shoot gave better results on MS media without any hormone supplement than MS in supplementation with certain combination of NAA and BAP. The procedures raised from the current work could be quite applicable for the development of large number of disease-free, healthy *Anthurium andraeanum* plantlet formation commercially.

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Conflict of Interest

None declared.

Financial Support

None declared.

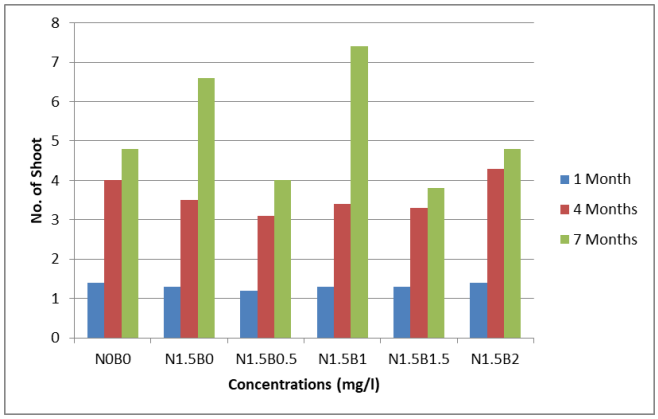

Fig 12: Effect of different combinations of BAP and NAA on number of shoots after 1 month, 4 month and 7 months of culture

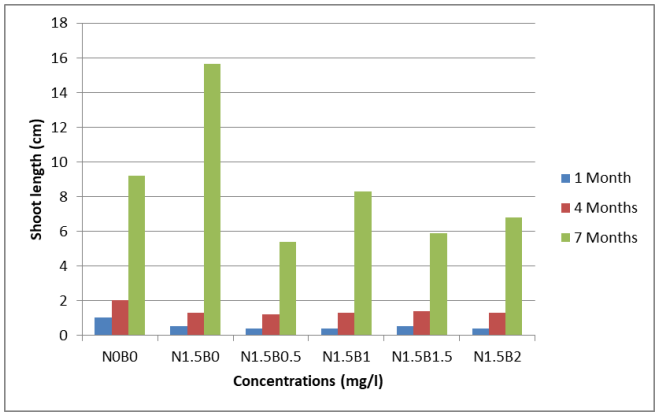

Fig 13: Effect of different combinations of BAP and NAA on number of shoots after 1 month, 4 month and 7 months of culture

REFERENCES

1. Harb EM, Talaat NB, Waheeda BM, El-Shamy M, Omira GA. Micropropagation of Anthurium andreanum from shoot tip explants. Journal of Applied Sciences Research. 2010;6(8):927-31.
2. Nowbuth P, Khittoo J, Bahorun T, Venkata Amy S. Assessing genetic diversity of some Anthurium andraeanum Hort. Cut-flower cultivars using RAPD markers. Afr. J Biotechnology. 2005;4(10):1189-94.
3. Maira O, Alexander M, Vargas TE. Micropropagation and organogenesis of Anthurium andreanum Lind cv Rub run. In Protocols for In Vitro Propagation of Ornamental Plants. 2010: 3-14.
4. Dufour L, Guérin V. Main environmental factors affecting flowering of Anthurium andreanum Lind. Soilless cultivation in tropical conditions. Floriculture, ornamental and plant biotechnology. 2006:172-82.
5. Mahanta S, Paswan L. In-Vitro propagation of Anthurium from axillary buds. J Ornamental Hurt, New Seri. 2001;4:17-21.
6. Dhal NK, Sahu SC, Atak Ç, Çelik Ö. Micropropagation of Anthurium spp. In: Plant science. In Tech Press, Rijeka, Croatia. 2012:241-45.
7. Rout GR, Mohapatra A, Jain SM. Tissue culture of ornamental pot plant: A critical review on present scenario and future prospects. Biotechnology Advances. 2006;24 531-60.
8. Gantait S, Sinniah UR. Morphology, flow cytometry and molecular assessment of ex vitro grown micro propagated anthurium in comparison with seed germinated plants. African Journal of Biotechnology. 2011;10(64):13991-98.
9. Bejoy M, Sumitha VR, Anish NP. Foliar regeneration in Anthurium andraeanum Hort. cv. Agnihotri. Biotechnology. 2008;7:134-38.
10. Yi-xun Y, Ling L, Juan-xu L, Jing W. Plant regeneration by callus-mediated proto-corm-like body induction of Anthurium andraeanum Hort. China. J. Agric. Sci. 2009;8(5):572-77.
11. Cimen A, Ozge C. Micropropagation of Anthurium andreanum from leaf explants. Pak, J. Bot. 2009;41(3):1155-61.
12. Jahan MT, Islam MR, Khan R, Mamun AF, Ahmed G, Hakim L, *et al*. In vitro clonal propagations of Anthurium (andreanum an L.) Using callus culture. Plant Tissue Cult Biotech. 2009;19:61-69.
13. Hamidah M, Karim AG, Debergh P. Somatic embryogenesis and plant regeneration in Anthurium Scherzer Anum. Plant Cell, Tissue and Organ Culture. 1997; 48:189-93.

14. Vargas TE, Mejias A, Oropeza M, Garcia E. Plant regeneration of Anthurium andreanum cv Rub run. Electronic Journal of Biotechnology. 2004;7(3):285-89.
15. Silva JA, Nagae S, Tanaka M. Effect of physical factors on micropropagation of Anthurium andraeanum. J. Plant Tissue Cult. 2005;15(1):1-6.
16. Te-Chato S, Susano T, Sontikun Y. Medium inflicting embryo genesis and organogenesis in Anthurium spp. 06. Cultivar, explant type and culture. J. Song klanakarin. Sci. Technol. 2006;28(4):717-22.
17. Chen FC, Chen FC, Kuehnle AR, Sugii N. Anthurium roots for micropropagation and Agrobacterium tumefaciens-mediated gene transfer. J. Plant –cell-tissue-organ-cult. 1997;49:71-74.
18. Dufour L, Guerin V. Nutrient solution effects on the development and yield of Anthurium adreanum Lind. in tropical soilless conditions. Scientia Horticulture. 2005;105:269-82.
19. Pierik RL, Mayes J, Stegman's HH. Vegetative propagation of Anthurium andraeanum in propagating tubes. J Vaklad- Voor. De-Bloomstein. 1974;29:12-15.
20. Chen FC, Kuehnle AR, Sugii N. Anthurium roots for micropropagation and Agrobacterium tumefaciens mediated gene transfer. J Plant –cell –tissue –organ -cult. 1997;49:71-74.
21. Devinder-Prakash N, Choudhary ML, Prasad KV, Nagesh N, Prakash DD. Regeneration of plantlets from petiole explants of Anthurium andraeanum Lind. Cv. Mauritius Orange. Phyto morph. 2001;51:83-85.
22. Nhut DT, Duy N, Vy NN, Khue CD, Khiem DV, Vinh DN, *et al*. Impact of Anthurium spp. genotype on callus induction derived from leaf explants and shoot and root regeneration capacity from callus. Journal of Applied Horticulture. 2006;8(2):135-37.
23. Pierik RL, Stegman's HH. Vegetative propagation of Anthurium Scherzer Anum Schott through callus cultures. J. Sci. Hort. 1976;4:291-92.
24. Zhao Q, Jing J, Wang G, Wang JH, Feng YY, Xing HW, *et al*. Optimization in Agrobacterium-medium transformation of Anthurium andreanum using GFP as a reporter. Electronic Journal of Biotechnology. 2010;13(5)1-11.
25. Farsi M, Taghavizadeh YM, Qasemiomran V. Micropropagation of Anthurium andreanum cv. Terra. African Journal of Biotechnology. 2012;11(68):13162-66.
26. Vargas TE, Mejías A, Oropeza M, De García E. Plant regeneration of Anthurium andreanum cv Rub run. Electronic Journal of Biotechnology. 2004 ;7(3):10-1.
27. Yu CH, Jin LH, Zhou MG. Effect of azoxystrobin on oxygen consumption and cyt b gene expression of Colletotrichum capsica from chilli fruits. Agricultural Sciences in China. 2009;8(5):628-31.
28. Budiarto K, Handayati W. In vitro propagation of several Anthurium accessions using leaf explants. Agri Vita. 2007;39(3):261-68.
29. Vargas TE, Mejias A, Oropeza M, Garia de E. Plant regeneration of Anthurium andraeanum cv. Rub run. Electron. J Biotechnology. 2004; 7:282-86.
30. Martin KP, Joseph D, Madassery J, Philip VJ. Direct shoot regeneration from lamina explants of two commercial cut flower cultivars of Anthurium anderanum Hort. J in Vitro Cell. Dev. Biol. – Plant. 2003; 39:500-04.
31. Atta-AIIa H1, Mc Aiister2, J. van Staden2*, In vitro culture and establishment of Anthurium parvis Pathum, S. Mr. J. Bot. 1998;64(5):296-98.
32. Mutanda O, Kumar L. Google earth engine applications. Remote Sensing. 2019;11(5):591.
33. Gantait S, Mandal N. Tissue culture of Anthurium andreanum: A significant review and future prospective. İnternational Journal of Botany. 2010;6(3):207-19.
34. Bejoy M, Sumitha VR, Anish NP. Foliar Regeneration in Anthurium andreanum Hort. cv. Agnihotri. Biotechnology. 2008;7(1)134-38.
35. Viegas J, Rosa da Rocha MT, Ferreira-Moura I, Leiria da Rosa D, Almeida de Souza J, Correa MG, *et al*. Anthurium andraeanum [Linden ex Andre] culture: in vitro and ex vitro. Floriculture. Ornamental Biotechnology. 2007;1:61-65.
36. Puchooa D. In vitro mutation breeding of Anthurium by gamma radation. International Journal of Agricultural Biology. 2005;7:11-20.
37. Winarto B, Rachmawati F, Silva JA. New basal media for half –anther culture of Anthurium andreanum Linden ex Andre cv. Tropical. Plant Growth Regulation. 2011;65:513-29.
38. Jahan MT, Islam MR, Khan R, Mamun AN, Ahmed G, Hakim L, *et al*. In vitro clonal propagation of Anthurium [Anthurium andreanum L.] using callus culture. Plant Tissue Cult. Biotech. 2009;19:61-69.
39. Joseph M, Martin KP, Madassery J, Philip VJ. In vitro propagation of three commercial cut flower cultivars of Anthurium andreanum. Horti. Indian Journal of Experimental Biology. 2003;41:154-59.
40. Wang KH, Kuehnle AR, Sipes BS. In vitro tolerance and resistance to burrowing nematode, Rhacophorus similes, in Anthurium species. Euphotic. 1998;103:23-28.