An Optimizied Protocol for In Vitro Propagation of Anthurium

Zainab Aamer Saheb Mohammad Omar h. Obaid

Department of Plant Production Technologies, Al-Musayyab Technical College, Al-Furat Al-Awsat Technical University, Babylon, Iraq.

Email: com.ame2@atu.edu.iq , zainab.aamer.tcm31@student.atu.edu.iq

Abstract:

This research was conducted in the Plant Tissue Culture Laboratory of the Department of Plant Production Technologies at Al-Musaiub Technical College, Al-Furat Al-Awsat Technical University. A 1 cm square leaf was cultivated on MS medium supplemented with different concentrations of growth regulators BA (0.0, 1.0, 2.0, and 3.0 mg L^{-1}) and NAA (0.0, 0.1, and 0.5 mg L^{-1}) during the leaf differentiation stage. Different concentrations of BA (0, 1, 1.5, and 2 mg L^{-1}) were used along with NAA concentrations (0, 0.5, 1, and 1.5 mg L^{-1}) to compare the effect of these concentrations on the number and length of shoots and the number and length of leaves. NAA concentrations (0, 0.5, 1, 1.5, and 2 mg L^{-1}) were also used, with the 1.5 concentration yielding the highest percentage of root number and length.

-

1- Introduction

Anthurium is a well-known genus of ornamental plants, consisting of approximately 1,500 tropical cultivated species. It is one of the commercially important genera in the Araceae family. [17] Many species of Anthurium are cultivated for various purposes, such as cut flowers, potted flowers, landscaping, etc. [26] Commercially cultivated ornamentals have great potential in international markets, both tropical and subtropical. [23] Anthurium cultivars are globally important for a huge market due to their valuable flowers. Orchids and anthuriums rank first and second among tropical cut flowers, respectively. [12] Floriculture is one of the rapidly developing industries across the country. [22] Currently, the flower industry is very popular, with significantly higher production potential compared to other ornamental plants. [11] In the international market, the demand for flowers is increasing dramatically due to trade globalization and economic liberalization. [31] Anthuriums are known for the beauty of their flowers, their long lifespan in the vase, and their high yields per unit area. [34,35] Scientists in the Netherlands and Hawaii have hybridized anthuriums, [12] and they are known to grow natively in South America. Thousands of anthurium species have recently been widely cultivated under artificial environmental conditions, both at sea level and above 1,200 meters in altitude. [10] They have been bred for their beautiful color, long lifespan, and disease resistance. Anthurium has been propagated by both sexual and asexual methods [14]. It has traditionally been propagated by seeds, but the propagation rate of this method for Anthurium is very slow due to inherent genetic heterogeneity [12]. Therefore, seed propagation is not recommended for Anthurium spp. The time between pollination and seed maturation and the time of seed development takes three years in a breeding program [13,14]. Growing plants from seeds cannot produce a reliable technique for developing a new culture area, and in this way, vegetative propagation methods such as cuttings may be the only way to produce large quantities of individuals [32]. Adapting the

ISSN 2072-3857

propagation method for a particular plant species depends on its genetic potential as well as the use of the intention. In this case, stem cuttings are also not a practical method for propagating a large number of culture material. At present, a large number of Anthurium can be multiplied using micropropagation techniques. [13] Micropropagation is an alternative method to conventional propagation, whereby culturing certain plant species, such as somatic cells, tissues, or organs, under artificial environmental conditions can be a reliable way to produce large numbers of plants genetically identical to the parent plant in a relatively short period of time. [6] Anthurium is usually propagated through the use of seeds. [12] However, vegetative propagation techniques used for Anthurium have not By giving satisfactory results, and therefore, plant tissue culture techniques could increasingly be another method for production.[28,7] Seed propagation may not be feasible due to cross-pollination, and as a result, the offspring may be heterozygous. [36] Furthermore, the seeds are hampered by poor germination rates and low seed viability. [22] In vitro propagation of Anthurium has been successful using various tissue types. Anthurium tissue culture was first achieved by Pierid and colleagues in 1974 [27] The research aimed to produce better plants directly from leaf explant The research aimed to produce better plants directly from leaf explant and Employing the in vitro culture technique in tissue propagation of anthurium and obtaining plants in large numbers in a short time and determining the optimal concentration of growth regulators added to the nutrient medium in the formation, multiplication, rooting and acclimatization of tubes..

2- Materials and Methods:

Anthrum seedlings are obtained from nurseries at the flowering stage or before flowering to obtain plant parts of the anther and petal, in addition to young leaves. The plants are kept in a greenhouse until the explant is harvested. The plant parts (young leaves, petals, and anthers) are taken and placed in a 250 ml laboratory flask. They are washed with water and liquid soap to remove dust and suspended matter. They are then transferred to a laminar air flow cabinet for sterilization. The plant parts (young leaves, petals, and anthers) are taken to the laminar air flow cabinet, and different concentrations of sodium hypochlorite (NaOCl) are added to sterilise the plant parts at concentrations of (2%) for periods. (15) minutes, then the samples were transferred to a 250 ml glass container containing 70% ethanol for one minute and then washed with distilled water three times to remove any remaining sterilizing agents. After that, they were placed in Petri dishes that were previously sterilized with alcohol and flame. After that, the leaf explant was taken and cut into small square pieces of 1 cm. They were cut using a previously sterilized blade and the small pieces were taken using sterilized tweezers and planted on BA medium at concentrations of (0.0, 1.0, 2.0, 3.0) with NAA at concentrations of 0.0, 0.1, 0.5) at the stage of producing plants directly from the leaf Ten tubes per transaction.. Different concentrations of BA (0, 1, 1.5, 2) mg L-1 were used with NAA concentrations (0, 0.5, 1, 1.5) mg L^{-1} to compare the effect of concentrations on the number and length of shoots and the number and length of leaves Ten tubes per transaction.. Concentrations of NAA (0, 0.5, 1, 1.5, 2) in Anthurium rooting Ten tubes per transaction.. Samples were incubated in a growth chamber at 25∓C and 1000 lux light intensity for 16 hours, followed by 8 hours of alternating darkness. The results were observed after one month of culture. (4.49) g of ready-mixed culture medium powder was weighed to prepare one liter for plant tissue culture. 7 grams of agar were added per liter as a solidifying agent, with the addition of 3% sucrose and BA, 2,4-D, NAA according to the requirements of the experiment. The pH of the medium was reduced to (5.6) by adding hydrochloric acid solution HCI or sodium hydroxide solution NaOH. The medium was placed on a hot magnetic stirrer and then poured into test tubes at a rate of 10 ml per

ISSN 2072-3857

tube. After closing them tightly, they were placed in an autoclave at a temperature of 121°C and a pressure of 1.04 kg/g cm2 for 15 minutes. After a minute, the culture was removed from the autoclave and left to cool until the medium solidified at room temperature, making it ready for culture. This experiment was carried out according to a completely randomized design (CRD) with ten replications. Its significance was tested using the LSD test at a probability level of 0.05 using the GENSTAT program [2].

-3 Results and Discussion:

3-1- The effect of BA, NAA and their interaction on the number of differentiated plants directly from a 1 cm leaf piece cultivated on MS medium after 45 days of culture.

The results of Table (1) showed that using a concentration of 2.0 mg L^{-1} of the growth regulator BA resulted in a significant increase in the average number of differentiated plants from leaf piece, reaching 11.17 differentiated plants As it mentioned at fig.no1 a, compared to the control treatment (0 mg L^{-1}), which did not record any differentiation. The results also showed a significant effect of the growth regulator NAA, with the 0.1 mg/L concentration achieving the highest number of differentiated plants, reaching 8.44 plants, significantly excelled on the control treatment, which recorded 3.44 plants.

The results also revealed significant differences between the growth regulators BA and NAA in stimulating direct differentiation of plants from a 1 cm leaf fragment 45 days after culture. The combination of 2.0 mg L^{-1} BA and 0.1 mg L^{-1} NAA recorded the highest number of differentiated plants, reaching 17.0 plants. These results are consistent with what was indicated by [19], who demonstrated that using low concentrations of the auxin NAA with a lower percentage of the cytokinin BAP enhances the growth and reproduction rate compared to using BAP alone. The reason for the increased shooting rate when BA is added to the nutrient medium is due to its role in breaking apical dominance, which leads to stimulating the growth of lateral buds and increasing the number of vegetative shoots. [22] also indicated that the effectiveness of BA in shoot multiplication is due to the presence of three double bonds in its side chain.On the other hand, [20] indicated that auxin NAA hinders the formation of vascular connections between the tissues of the axillary buds and the stem, which reduces the transfer of nutrients to these buds and leads to poor growth.

Table (1) Effect of BA, NAA, and their interaction on the number of plants differentiated directly from a 1 cm leaf piece grown on MS medium after 45 days of culture.

average BA	0.5	0.1	0.0	NAA / BA
0	0	0	0	0.0
6.25	7.75	8.75	2.25	1.0
11.17	10.25	17.0	6.25	2.0
7.33	8.75	8.00	5.25	3.0
	6.69	8.44	3.44	NAA average
NAA= 0.888	BA=1.026	NAA*BA= 1.777		LSD

ISSN 2072-3857

3-2--Studying the effect of both BA and NAA, as well as their interaction, on the number of shoots produced directly from a 1 cm square leaf 35 days after culture.

The results of Table (2) showed a significant effect of the growth regulator BA, with the 2 mg L^{-1} concentration significantly excelled on the other concentrations in the number of shoots, recording the highest average of 6.80 shoots per plant part, while the control treatment produced the lowest average of only 0.60 shoots.

The table also showed significant differences between NAA concentrations, with the 1.0 mg L^{-1} concentration significantly excelled on all concentrations, recording the highest average of 6.20 shoots. The results of the table showed significant differences due to the interaction of the growth regulators BA and NAA. The concentration of 2 mg L^{-1} of BA and 1.0 mg L^{-1} of NAA achieved the highest number of shoots, reaching 17.20 shoots. In contrast, the lowest number of shoots was achieved with the concentration of 0 mg L^{-1} of BA and 0 mg L^{-1} of NAA, with an average of 0.20 shoots in the control treatment. When used at concentrations appropriate for tissue culture, BA helps break apical dominance by creating attractive zones in the buds, stimulating the transfer of nutrients and growth materials to them, thus enhancing the growth and development of vegetative buds. Regarding the effect of NAA, its addition at moderate concentrations leads to an increase in the number of shoots. However, its use at high concentrations may cause a decrease in the number of vegetative shoots. This is due to its excessive accumulation in plant tissues, which leads to hormonal imbalance and antagonism with the effect of BA, which contributes to inhibiting shooting and promoting apical dominance, thus reducing the number of lateral shoots [21].

ISSN 2072-3857

Table (2) Study of the effect of both BA and NAA, as well as the interaction between them, on the number of shoots produced directly from a 1 cm square leaf 35 days after culture.

average BA	NAA mg.L-1				BA mg.L-1
	1.5	1	0.5	0	
0.60	0.60	1.00	0.60	0.20	0
1.60	3.00	2.00	1.00	0.40	1
3.00	4.20	4.20	2.20	1.40	1.5
6.80	3.60	17.60	3.80	2.20	2
	2.85	6.20	1.90	1.05	average NAA
		interaction= 1.498	NAA= 0.749	BA= 0.749	L.S.D 0.05

3-3 - Studying the effect of both BA and NAA, as well as their interaction, on the length of shoots produced directly from a 1 cm square leaf 35 days after culture.

The results in Table 3 indicate significant differences when BA and NAA are combined at different concentrations. The highest average shoot length was 1.60 cm in the culture medium prepared with a 2 mg L^{-1} concentration of BA, which differed significantly from the other treatments. The lowest average shoot length was 0.45 cm in the control treatment.

The same table shows that the NAA concentration had a non-significant effect at some concentrations on increasing the average length of vegetative shoots. The 1.5 mg L^{-1} concentration of NAA achieved the highest average shoot length of 1.35 cm, while the lowest average was 0.35 cm in the control treatment. The results of the same table show that the concentration of 2 mg L^{-1} of BA and 1.5 mg L^{-1} of NAA By giving the highest shoot length, reaching 2.60 cm, while the control treatment yield no results.

This may be due to the fact that the movement and activity of cytokinin in the shoot are

ISSN 2072-3857

activated by the addition of auxin, leading to the emergence of cytokinin's effect. Its activity in the shoot is activated by the addition of auxin, leading to a clear emergence of cytokinin's effect on the growth of lateral shoots and the transport of nutrients [29]. The addition of BA during the vegetative multiplication stage of many plant species is due to its effectiveness in liberating axillary buds from the dominance of the terminal bud without the need to cut them. The importance of adding cytokinin lies in stimulating cell division and stimulating the formation and growth of axillary lateral shoots [30].

Table (3) Effect of both BA and NAA, as well as their interaction, on the length of shoots produced directly from a 1 cm square leaf 35 days after culture.

BA average	mg.L-1 NAA				BA mg.L-1
	1.5	1	0.5	0	
0.45	0.80	0.60	0.40	0.0	0
0.65	1.0	0.60	0.60	0.40	1
0.95	1.0	1.20	1.20	0.40	1.5
1.60	2.60	1.60	1.60	0.60	2
	1.35	1.00	0.95	0.35	NAA average
		0.811=interaction	NAA=0.405	BA=0.405	L.S.D 0.05

3-4- The effect of both BA and NAA, as well as their interaction, on the number of leaves produced directly from a 1 cm square leaf 35 days after culture.

The results in Table 4 show that the use of BA had a significant effect on the average number of leaves, which increased with increasing concentrations. The 1.5 mg L^{-1} concentration outperformed the other concentrations, yield the highest average of 7.10 leaves. The control treatment By giving the lowest average number of leaves, at 0.65 leaves. The same table also showed a significant effect of the growth regulator NAA. The 1 mg L^{-1} concentration significantly excelled on the other treatments, yielding the highest average number of leaves, at 6.05 leaves. The control treatment By giving the lowest average, at 1.15 leaves. Regarding the interaction between BA and NAA, significant differences were observed between the interactions. The interaction between 1.5 mg L-1 of BA and 1 mg L-1 of NAA by giving the highest value, reaching 17.40 leaves.

ISSN 2072-3857

The positive effect of NAA on increasing leaf number is due to this slow-moving auxin, on the one hand, and its stability and inability to be degraded by the enzymes that degrade the natural auxin IAA, on the other hand, increasing its effectiveness. Furthermore, NAA is one of the auxins that move within the tissue and have side effects. [15] reported that NAA and D-2,4 move from the treatment site to the growth zones and influence them in a way that encourages mutations. The reason for the increased leaf number may be due to the increased number of shoots.

Table 4: Effect of both BA and NAA, as well as their interaction, on the number of leaves produced directly from a 1 cm square leaf 35 days after culture.

average BA	NAA mg.L-1				BA mg.L-1
	1.5	1	0.5	0	
0.65	0.80	1.00	0.80	0.0	0
1.85	3.20	2.20	1.40	0.60	1
7.10	3.60	17.40	5.20	2.20	1.5
3.75	4.40	3.60	5.20	1.80	2
	3.00	6.05	3.15	1.15	average NAA
		interaction= 1.983	NAA= 0.991	BA= 0.991	L.S.D 0.05

3-5- Studying the effect of both BA and NAA, as well as their interaction, on leaf length produced directly from a 1 cm square leaf 35 days after culture.

Table (5) indicated that there was a significant effect of adding BA concentration, as the 1.5 mg L-1 concentration achieved a significant superiority over the other concentrations, giving the highest average leaf length of 1.40 cm. The results of the same table also revealed significant differences between NAA concentrations, as the highest average leaf length was 1.30 cm at the 0.5 mg L-1 concentration, which differed significantly from the control treatment, and the lowest

ISSN 2072-3857

average was 0.40 cm at the control treatment. The results of the same table showed a significant effect of the interaction of BA and NAA on increasing average leaf length. The combination of BA at a concentration of 1.5 mg L-1 and NAA at a concentration of 0.5 mg L-1 produced the highest average leaf length of 2.80 cm, while the control treatment produced the lowest average of 0.2 cm.

The increased leaf length may be attributed to the role played by auxin in stimulating cell division and elongation, which is reflected in the longitudinal growth of leaves [25,30]. Cytokinins also encourage cell division and differentiation, as well as activating cell enzymes and the synthesis of chlorophyll and proteins, thus leading to organ formation [17].

Table (5) Effect of both BA and NAA, as well as their interaction, on leaf length resulting directly from a 1 cm square leaf 35 days after culture.

average BA	NAA mg.L-1				BA mg.L-1
	1.5	1	0.5	0	
0.45	0.60	0.60	0.40	0.2	0
0.75	1.20	0.80	0.60	0.40	1
1.40	0.80	1.40	2.80	0.60	1.5
0.85	0.60	1.00	1.40	0.40	2
	0.80	0.95	1.30	0.40	average NAA
		interaction= 0.835	NAA= 0.417	BA= 0.417	L.S.D 0.05

3-6- The effect of different concentrations of NAA on the percentage of rooting, number of roots, and length of Anthurium plants 35 days after culture.

The data in Table (6) indicate that using NAA at a concentration of 1.5 mg/L was the most effective, with a rooting percentage of 60%, compared to the medium without auxins and the other concentrations. In contrast, no rooting percentage was recorded in the control

ISSN 2072-3857

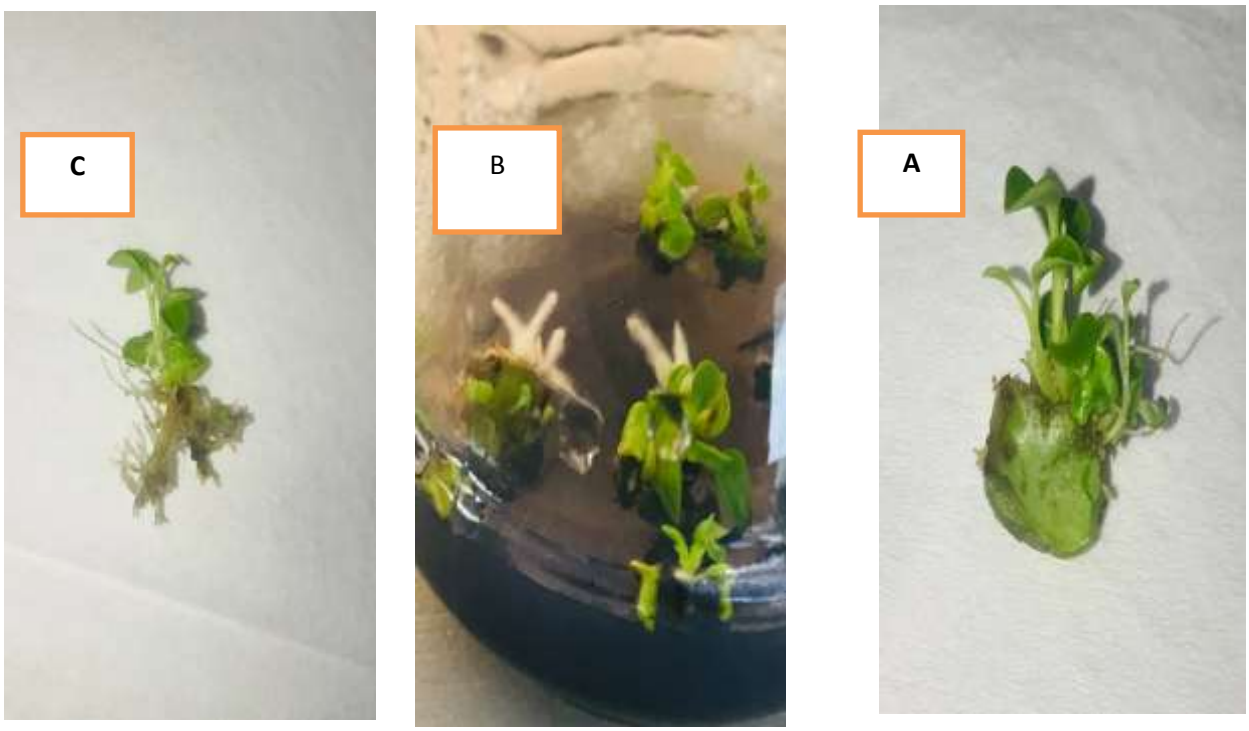
treatment or when using the concentration of 0.5 mg/L, under experimental conditions. The same table also shows the superiority of the 1.5 mg/L concentration in the average number and length of roots, with the number of roots reaching 3.40 As it mentioned at fig.no1 b, with an average length of 2.60 cm As it mentioned at fig.no1 c. The reason for the increased number of roots is due to the optimal concentration of NAA, which provided the best average rooting, because NAA is one of the auxins that plays a direct role in rooting [23]. In addition, auxins work to form roots by increasing cell division and elongation. [9]

Auxins are a group of organic acids with high molecular weights and the ability to influence biological processes within the plant at very low concentrations. They also influence plant growth regulation, as they are involved in cell division and expansion. Their formation sites are in young tissues such as the apical meristem, young leaves, and lateral buds. Auxins increase the flexibility and elasticity of the cell wall by breaking its bonds and returning them to new locations under the influence of turgor pressure. This contributes to increasing cell volume and expansion, and may also influence the function of enzymes responsible for building cell wall components. [33]

Table (6) Effect of different concentrations of NAA on the percentage of rooting in Anthurium plants, as well as the number and length of roots, 35 days after culture.

Average root length (cm)	Average number of roots	Rooting percentage	NAA($mg.L^{-1}$)
0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.5
1.20	2.00	26.0	1.0
2.60	3.60	60.0	1.5
1.40	2.40	37.0	2
0.792	1.265	3.989	L.S.D 0.05

ISSN 2072-3857



A: Anthurium plants differentiated directly from a 1 cm leaf.

B: Rooting of Anthurium plants in the jar with a concentration of 1.5 mg/L of NAA

C: Anthurium roots outside the jar 2.60 cm long

4- Conclusions:

The study demonstrated the effect of growth regulators (BA, NAA) added to MS medium in producing the best method for Anthurium plant differentiation from the leaf. The concentration of 2.0 mg/L-1 outperformed all treatments, providing the highest average plant differentiation. The study also demonstrated the effect of BA and NAA at different concentrations on the number of shoots, shoot length, number of leaves, and leaf length. It also demonstrated the effect of NAA on rooting, where the concentration of 1.5 mg/L-1 outperformed the concentration, providing the highest percentage of root number and root length, reaching 60%.

5- References

1. Al-Khafaji, Makki Alwan (2014). Plant Growth Regulators: Their Horticultural Applications and Uses. University House for Printing, Publishing, and Translation, College of Agriculture, University of Baghdad. Ministry of Higher Education and Scientific Research, Republic of Iraq.
2. Al-Rawi, Khashe' Mahmoud and Abdul Aziz Mohammed Khalaf Allah (2000). Design and Analysis of Agricultural Experiments. Ministry of Education and Scientific Research, Printing and Publishing House, University of Mosul, Republic of Iraq, 488 pages.
3. Atteya, A.K.G., S.K. Al- Taweel, E.A.E. Genaidy, and H.A. Zahran,.(2018). Effect of gibberellic acid and zinc sulphate on vegetative, flowering, seed Yield and chemical consistent of Jojoba plant

ISSN 2072-3857

(Simmondsia Chinensis). India Journal of Agricultural .547., 52)5(: 542- Research
4. Bejoy M, Sumitha VR, Anish NP. Foliar regeneration in Anthurium andraeanum Hort. cv. Agnihotri. Biotechnology. 2008;7:134-38.
5. Bruyn M.H., D.I. Ferreira and M.H. Debruyn. (1992). In Vitro corm production of Gladiolous dalenii and G. tristis. Plant cell Tissue and Organ Culture, 31:2, 123-128
6. 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.
7. 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.
8. Cimen A, Ozge C. Micropropagation of Anthurium andreanum from leaf explants. Pak, J. Bot. 2009;41(3):1155-61.
9. Danial, G.H., D.A. Ibrahim, A.N. Yousef, S.B. Elyas. (2019). Rapid protocol of Aloe vera in vitro propagation. Iraq Journal of Agricultural sciences, .1377-1382:95(50.(
10. 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.
11. Dhal NK, Sahu SC, Atak Ç, Çelik Ö. Micropropagation of Anthurium spp. In: Plant science. In Tech Press, Rijeka, Croatia. 2012:241-45.
12. Dufour L, Guérin V. Main environmental factors affecting flowering of Anthurium andreanum Lind. Soilless culture in tropical conditions. Floriculture, ornamental and plant biotechnology. 2006:172-82.
13. 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.
14. 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.
15. Gianfagna; T.J. 1987. Natural and synthetic growth regulators and thei use inhorticulture and agronomic crops .In: plant Hormones and Their Role in Plant Growth and Development Davies P.J. ed. PP .61 Martinus Njhoff Publisher ,Dorhrecht.
16. 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.
17. 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.
18. 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.
19. Khaleghi, A., A. Khaleghi, P. Azadi, and M. Mii (2008). Induction of embryogenic callus and plant regeneration from nodes of greenhouse-grown plants of Alstroemeria cv. Fuego. Journal of food, agriculture, and environment Vol. 6(3&4): 374-377
20. Kim, K W., MS. Kang, and DH. Goo (1991). The external and histological characteristics of organogenesis from Gladiolus callus Korean Soc. Hort. Sci. 32:124-129
21. Kim, K. W. and S.Y. Han. (1993). Corm let formation in gladiolus shoot base by growth retardants in Vitro .Journal-of-the-Korean-Society-for Horticulture Science-34:2,/ 36-144; 23 ref.,3 p
22. Mahanta S, Paswan L. In-Vitro propagation of Anthurium from axillary buds. J Ornamental Hurt, New Seri. 2001;4:17-21.
23. 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.
24. Mohsen, Mohammed Mahdi (2009). Propagation of Gladiolus bulbs (Gladiolus sp.) using tissue culture technology. Master's

ISSN 2072-3857

thesis, Al-Musayyab Technical College, Al-Furat Al-Awsat Technical University, Republic of Iraq.

25. 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.
26. 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.
27. Pathania, N.S., Misra, R.L. and S.P.S. Raghava,(2001).Precocious shoot proliferation and microform production in gladiolus through tissue culture .J. Onramp .Hort. (NS),4: 69-73.
28. Pierik RL, Mayes J, Stegman's HH. Vegetative propagation of Anthurium andraeanum in propagating tubes. J Vaklad-Voor. De- Bloomstein. 1974;29:12-15.
29. Pierik RL, Stegman's HH. Vegetative propagation of Anthurium Scherzer Anum Schott through callus cultures. J. Sci. Hort. 1976;4:291-92.
30. Razdan, M. K. 2003 . Introduction to Plant Tissue Culture . 2nd Ed. Science Publishers. Inc.
31. 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.
32. Silva JA, Nagae S, Tanaka M. Effect of physical factors on micropropagation of Anthurium andraeanum. J. Plant Tissue Cult. 2005;15(1):1-6.
33. Taiz, L. and Zeiger, E. (2010) Plant Physiology. 5th Edition, Sinauer Associates Inc., Sunderland, 782 p.
34. 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.
35. 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.
36. 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.

ISSN 2072-3857