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Micropropagation of *Anthurium andraeanum*-An important tool in floriculture

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Abstract
Anthurium andraeanum belongs to Araceae family and its thousands of cultivars have been very popular among the gardeners and florists thus, contributing substantially to the floricultural trade. Anthurium flowers are comparatively costlier as the production cost of planting material. Conventional methods of vegetative propagation are slow and cannot keep pace with increasing demand every year. Micropropagation is the only tool which helps in producing high quality planting material in large quantity. Micropropagation is a sophisticated technique which involves different stages which have to be performed carefully to successfully produce the planting material. Various techniques of micropropagation being followed in different parts of the world have been reviewed in this paper.

Keywords: Floriculture, *Anthurium andraeanum,* Micropropagation, Explant, Acclimatization

1. Introduction
Anthuriumandraeanum is a flowering plant species in the Araceae family. It is a perennial herbaceous plant cultivated for its long-lasting and attractive heart shaped inflorescence. Anthuriums a modified leaf (spathe), bearing numerous small botanical flowers on a pencil-like protrusion (spadix) and has a vase life of 14-28 days. The species is native to Colombia and Ecuador. *Anthurium andraeanum* species are produced for many purposes including cut-flower, flowering potted plants and landscape plants [1]. *Anthurium andraeanum* cultivars have occupied a distinguished position in global floriculture trade.
Anthurium andraeanum may be propagated either sexually or asexually. Anthurium are traditionally propagated by seeds [2], however, vegetative propagation methods applied to these plants have not shown encouraging results and tissue culture techniques appears as an alternative to increase the production [3, 4]. Propagation through seeds is not desired because of cross-pollination and the progenies are heterozygous. Moreover, it is hampered by the poor germination rate and low viability of the seeds [5]. Micropropagation of Anthurium has been achieved with various explants. The tissue culture of Anthurium was first reported by Pierik *et al.* (1974). The regeneration of *Anthurium andraeanum* through adventitious shoot formation from callus [4, 6] and also direct shoot regeneration from lamina explants were achieved [5].
Floriculture is now a widespread activity throughout the world. *Anthurium andraeanum* is a slow-growing perennial that requires shady, humid conditions such as those found in tropical rainforests. Although Anthurium is sold both as a potted plant and as a cut flower, the trade in cut flowers is much larger than the potted plant trade. The traded value of Anthurium is second only to that of spray tropical Orchids among the tropical flowers and the world import market size for Anthurium is estimated to exceed US$ 20 million annually [7].

1.1. Economic importance of Anthodium in floriculture
Floriculture is a fast emerging industry in the world. Today, floriculture is a lucrative profession with higher potential for returns than most of the field and other horticultural crops. The demand for flowers both in India and International markets is increasing at a faster rate owing to the liberalization of economy and globalization of trade. The leading flowers which are in great demand are rose, chrysanthemum, carnation, gladiolus and anthurium.
Anthuriums are gaining popularity due to higher returns per unit area and their beautiful and attractive long lasting flowers. Scientists in the Netherlands and Hawaii have hybridized Anthurium, originally found in South America. Hundreds of varieties now grow well in greenhouses, at sea level and up to 1,200 meters in height. Anthuriums are bred for colour, shelf life, and disease resistance.
High cost of propagation material, land, start-up costs, and capital in some producer countries, as well as continued declines in production in Hawaii, have caused world production

to decline 25 per cent since 1986. Geographic diversification is now taking place, however, and the Asian market especially has shown increasing demand for these flowers.

The world consumption of floriculture products is estimated at 50 billion US dollars and the, trade alone is to the tune of 5.2 billion US dollars [8]. The global trade in flowers has also been on the increase with global inputs of Floriculture products including cut flowers, cut foliage and live plants touching 3,000 million US dollars [9]. Cut flowers account for more than half of this amount. Now the International Floricultural trade is estimated at 750 billion Rupees including India's share of only 2 billion rupees [10]. The largest cut flower exporters include Netherlands, Colombia. Israel, Italy, Sri Lanka, Thailand and Kenya. The developing countries, which supply flowers, are Taiwan, Singapore, Peru, Mexico, Costa Rica, Brazil, Ethiopia, Zimbabwe, Mauritius and Malaysia. Besides these, the world import of cut flowers like Orchids, Anthuriums, foliage, and live plants are also on the increase. But India's share in the cut flower trade is negligible. Malaysia's export of floriculture products in 1984 was 2.39 US dollars while in 1987 it increased to 4.87US dollars [11]. Now the international floriculture trade is estimated at 750 billion rupees including India's meagre share of 2 billion rupees.

1.2. Indian Contribution

The andraeanum variety is grown mainly as a cut flower Small-scale cultivation of Anthuriums as cut flowers begun in India during 1980's and thereafter it has spread to many regions in the country on a commercial basis. Anthurium cultivation has gained a momentum in Kerala during 1990's and during recent years it has gained wide popularity in the state similar to that of Orchids. At present anthuriums are mostly grown in some small gardens and nurseries. However, some progressive farmers started growing anthurium under protected condition around Bangalore, Belgaum, Goa, Sirsi etc. The important states cultivating anthuriums are Assam, Kerala, Tamilnadu (Salem) and Karnataka (Coorg) where the favourable climate exists [12].

1.3. Process of Micropropagation

Propagation of anthurium is usually done in the following ways:

(1) **By seeds:** Seeds are scattered on a finely shredded medium and kept under 75% shade. Transplantation can be done within 4 to 6 months after germination. It takes 3 years for a plant to grow from seed to bloom. Homogeneity cannot be ensured.

(2) **Vegetative:** A common method of increasing a popular cultivar is to allow a plant to grow side shoots or suckers, then, they are rooted and developed as individual plants. The mother plant is allowed to grow and throw more suckers.

(3) **Tissue culture:** This technique is becoming increasingly popular as a means for rapid large-scale clonal propagation [13].

Micropropagation begins with the selection of plant material to be propagated. The plant tissues are removed from an intact plant under a sterile condition. Clean stock materials that are free of viruses and fungi are important in the production of the healthiest plants. Once the plant material is chosen for culture, the collection of explant(s) begins and is dependent on the type of tissue to be used; including stem tips, anthers, petals, pollen and other plant tissues. The explant material is then surface sterilized, usually in multiple courses of bleach and alcohol washes, and finally rinsed in sterilized water. This small portion of plant tissue, sometimes only a single cell, is placed on a growth medium, typically containing sucrose as an energy source and one or more plant growth regulators (plant hormones). Usually the medium is thickened with agar to create a gel which supports the explant during growth. Some plants are easily grown on simple media, but others require more complicated media for successful growth; the plant tissue grows and differentiates into new tissues depending on the medium. For example, media containing cytokines are used to create branched shoots from plant buds.

1.4. Explant selection

The tissue obtained from a plant to be cultured is called an explant. It has often been claimed that a totipotent explant can be taken from any part of a plant including portions of shoots, leaves, stems, flowers, roots and single, undifferentiated cells. However, this is not true for all plants [14].

2. Factors affecting explant selection

2.1. Age of explant

The age of the explant can be very important, as physiologically younger tissue is generally much more responsive *in vitro.* In many cases, older tissue will not form callus that is capable of regeneration. Moreover, younger tissue is usually the newest formed and is generally easier to surface disinfect and establish clean cultures [15].

2.2. Size of explant

The explant size has an effect on the response of the tissue. Generally, the smaller the explant, the harder it is to culture. The culture medium usually has to have additional components. The larger explants probably contain more nutrient reserves and plant growth regulators to sustain the culture. Plants have different hormonal balances throughout the plant and depending on the location of the explant; the explant can have a different endogenous level of plant growth regulators. Internal differences in hormone balance in the tissue can result in varying *in vitro* responses [16]. It is advisable to obtain explants from plants which are healthy as compared to plants under nutritional or water stress or plants which are exhibiting disease symptoms.

2.3. Sterilization

Modern plant tissue culture is performed under aseptic conditions under HEPA filtered air provided by a lamina flow cabinet. Living plant materials from the natural environment are naturally contaminated on their surfaces (and sometimes interiors) with microorganisms, so surface sterilization of explants in chemical solutions is necessary (usually alcohol and sodium or sodium hypochlorite) [17]. In favour of most of the explants, the universally adopted system involves surface disinfection of initial explants with 70% (v/v) ethanol for 1min followed by 0.01% $HgCl_2$ alone for 7-12 min and rinsing in sterile distilled water or treating with 1-3%

sodium hypochlorite for 15-20 min. However, Gantait *et al,* Sterilized the shoot tips of *Anthurium andraeanum* varieties using antifungal solution cetrimide for 5 min followed by NaOCl and 0.1% $HgCl_2$. Later, Jahan *et al*. effectively used 70% (v/v) ethanol for 1 min, 1.5% NaOCl for 8 min as disinfectant and added 0.01% Tween-20 as surfactant. Most recently, to diminish the contamination caused by fungus, endogenous and exogenous bacteria, Atak and Celik surface sterilized the explants for 1min in 70% (v/v) ethanol, soaked in gentamicin solution for 30min and then again soaked in 20% (v/v) commercial bleach [containing 5% (v/v) NaOCl] for 12 min. (Ref; Table-1).

Table 1: Initiation of Micropropagation

Sr.No	Aseptic culture sterilization	Sources
1	-70% ethanol - 1min -0.1% $HgCl_2$- 2min	Mahanta and Paswan (2001)
2	-0.1% $HgCl_2$ – 7-12min -Rinse with distilled water	Martin *et al.* (2003), Duong *et al.* (2007), Bejoy *et al.* (2008).
3	-1-3% NaOCl – 15-20min	Teng, 1997,Vargas *et al.* (2004)
4	-Shoot tip sterilization -Antifungal (Cetrimide – 5min) followed by NaOCl. - 0.1% $HgCl_2$	Gantait *et al.* (2008)
5	-70% ethanol – 1min -1.5% NaOCl – 8min(Disinfectant) -0.01% Tween-20 (Surfactant)	Jahan *et al*. (2009)
6	-Recently, to diminish the contamination caused by fungus, endogenous and exogenous bacteria. -70% ethanol – 1min - Soaked in gentamicin solution – 30min -Soaked in 20% bleach[Containing 5% (v/v) NaOCl] -12min	Atak and celik (2009)

3. Media

After sterilization explants are placed on the surface of a solid culture medium, but are sometimes placed directly into a liquid medium, particularly when cell suspension cultures are desired. Solid and liquid media are generally composed of inorganic salts plus a few organic nutrients, vitamins and plant hormones. Solid media are prepared from liquid media with the addition of a gelling agent, usually purified agar. The composition of the medium, particularly the plant hormones and the nitrogen source (nitrate versus ammonium salts or amino acids) have profound effects on the morphology of the tissues that grow from the initial explant. For example, an excess of auxin will often result in a proliferation of roots, while an excess of cytokines may yield shoots. A balance of both auxin and cytokine will often produce an unorganised growth of cells, or callus, but the morphology of the outgrowth will depend on the plant species as well as the medium composition. As cultures grow, pieces are typically sliced off and transferred to new media (sub cultured) to allow for growth or to alter the morphology of the culture [22].

4. Organogenesis

Organogenesis is a stage of micropropagation that involves regeneration of adventitious organs or axillary buds directly or indirectly from the explants. The standardization ratio of growth hormones is a key factor in organogenesis (Ref; Table-2).

Table 2: Organogenesis in vitro from *Anthurium andraeanum*

Sr.No	Cultivars	Explants	Media	Hormones	Response	Sources
1.	*Anthurium andraeanum*cv.Terra	Leaf	-Callus Basal MS medium -Shoot Modified MS medium -Rooting Basal MS medium	-Callus -30g/L Sucrose and different treatment of 0.1mg/L 2,4-D and 1.5mg/L BAP -pH-5.7(before autoclaving) -Shoot 0.1mg/L 2,4-D and 1mg/L BAP -Rooting MS medium without plant growth regulator	The best medium for call us prompting from leaf explants	Faris *et al.* (2012)
2.	*Anthurium andraeanum* cv. Nitta(red color)	Leaf mid rib	-Callus and shoot Basal MS medium -Rooting MS Basal medium	-Callus and Shoot MS medium with hormone combination 0.5,1	-1mg/L NAA+1mg/L BAP best for survivability	ISLAM *et al.* (2010)

				and 1.5mg/L NAA+0.5,1,1.5and 2 mg/L BAP –Rooting MS medium with 0.5,1 and 1.5 IBA+0.5,1,1.5 and 2 BAP	-1mg/L IBA+1mg/LBAP best for shoot formation	
3.	*Anthurium* (Dual light condition)	Spathe	-Callus Nitsch and Nitsch medium -Shoot Nitsch and Nitsch	-Callus 1mg/L 2,4-D+ 1mg-/L Kinetin+1mg/L BA - Shoot 1 mg/l 2,4 D + (1, 2, and 3) mg/l BA	-Better callus development in dark light after 60 days.	Budirto *et al.*
4.	*Anthurium andraeanum* cv Rubrun	Seed	MS Basal medium with 1.2µM Thaimin+0.6µM myoinositol+0.3% sucrose+0.2% Gelrite at pH 5.8 before autoclaving.	-Callus 8.9 µM BA +2.7 µM NAA and were incubated under continuous fluorescent Light (50 m mol m-2 s-1) at 25 ºC.	80% of plant Acclimatization was obtained**.**	Vargas T. E. *et al.*
5.	*Anthurium andraeanum*Linden cultivars Casino and Antadra	Leminaandpetiole	-Callus MS basal medium	-Callus NAA (0.0, 0.01, 0.1, 0.5, 1 and 2 mg/L) + BA (0.0, 0.5, 1, 2 and 3 mg/L). -Shoot NAA (0.0, 0.005, 0.01 and 0.02 mg/L) + 2, 4-D (0.00, 0.05 and 0.1 mg/L) + KIN (0.0 and 1.0 mg/L) + BA (0.0 and 1.0 mg/L). -Rooting IBA (0.0, 0.5, 1.0 and 2.0 mg/L) + NAA (0.0, 0.05, 0.1 and 0.25 mg/L) + KIN (0.0 and 0.2 mg/L).	- Best callus production in medium containing 0.5 mg/L NAA + 3 mg/L BA in dark conditions. -0.01 mg/L NAA + 1 mg/L BA after 8 weeks in a 16/8 h light and dark cycle under a photoperiod of 50 µmol/m2/s. - Largest no of root medium supplemented with 1 mg/L IBA + 0.2 mg/L KIN. -	Devinder-Prakash*etal.* (2001)
6.	*Anthuriumandraeanum* Lind.	Leaf explants of A. *andraeanum*'Alabama' and 'Sierra'	-Callus MS Basal medium -Shoot MS basal medium -Rooting Modified MS medium	-Callus supplemented with 0.23, 0.91, 1.82, or 2.73 µM TDZ. -Shoot 0.89 µM BA with 2.32 µM KN, 0.98 µM IBA, or 1.07 µM NAA; 0.89 µM BA with 2.32 µM KN and 0.98 µM IBA or with 2.32 µM KN and 1.07 µM NAA as well as 0.93 µM KN with either 0.98 µM IBA or 1.07 µM NAA. -Rooting 0.98 µM IBA only.	The highest callus formation frequency occurred in medium containing 1.82 µM TDZ.	AisuGu *et al.* (2012)

(**TDZ**-Thidiazuron, IBA-Indole-3-butyric acid, **NAA**-Naphthalene acetic acid, **2, 4 –D -2, 4**-Dichloropenoxyacetic acid, **KIN**- Kinetin, **BA**- 6-Benzy laminopurine.)

5. Acclimatization

In micropropagation studies, the last and the critical step is acclimatization of the rooted seedlings. In this stage, plant losses have been due to different reasons [28, 29]. Directly rooted shoots in soil show higher survival rate in the field than rooted under *in vitro* conditions. Many environmental factors have to be modified such as light, temperature, relative humidity to increase the survival rate of *in vitro* rooted shoots. Cultured plants must adapt to low humidity, high light intensities and large temperature fluctuations with ex vitro acclimatization techniques [28, 29, 30]. The growing media ex vitro and supplementing nutrients are also important factors for the survival and healthy growth of plantlets.

6. Conclusion

Anthurium andraeanum cultivars are some of the most popular floricultural crops and contribute significantly in the world trade. India is also showing a considerable progress in cultivating and exporting anthurium cut flowers. Micropropagation is contributing in the steady growth in anthurium trade. Cost of production by micropropagation is comparatively higher and due to this anthurium flowers are not affordable by common man. We feel future investigation on haploid production, another culture and protoplast culture etc. and use of advance biochemical and molecular markers to develop new varieties and strategies which should bring down the cost of production.

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