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MICROPROPAGATION FROM *AGERATUM CONYZOIDES* L.

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ABSTRACT

An *in vitro* micropropagation protocol has been standardized from shoot tip, nodal and leaf explants of *Ageratum conyzoides* L. belongs to family Cucurbitaceae is an endemic medicinal plant used for the treatment of hepatitis, eczema, epilepsy, dizziness diarrhea, and fever. Murashige and Skoog, (1962) medium along with the various hormone compositions were used. Callus culture was initiated and established from leaf explants, on MS medium fortified with NAA (2.0 mg/L) 2, 4 - D (2.5 mg/L). Multiple shoots were regenerated from shoot tip explants of NAA (0.5 mg/L) and BAP (1.0 mg/L) and nodal explants of NAA (1.0 mg/L) and BAP (1.5 mg/L). Shoots were transferred to rooting MS medium supplemented with NAA (2.5 mg/L) and IBA (3.0 mg/L). Acclimatization was carried out on artificial soil survival rate.

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INTRODUCTION

The traditional approach towards standardization of insufficient for current herbal market and hence there is a need for more advanced techniques. India is known for its traditional system of medicine such as Ayurveda, Siddha, and Unani. The medical systems are found mentioned even in the ancient Vedas and other scriptures (Lahlou, 2004). It has been an ancient practice and an important component of the health care system in India. The secondary compounds are often produced only in small quantities in a particular type of cells of rare plant species (Endre, 1994). In addition, a selection of high-producing cell lines for a particular secondary metabolite is carried out using callus tissues of either small-aggregate or single cell origin (Yamamoto *et al.,* 1982). The tissue culture allows the production and propagation of genetically homogeneous, disease free plant species, through providing the plants needed to meet ever increasing the world
demand (Hossain *et al.,* 2013).

Medicinal plants constitute a very important bioresoure in India because it has one of richest plant bases Ethonomedical traditions in the world (Naresh Singh Gill *et al.,* 2011). There are about 45,000 medicinal plant species in India, with concentrated spots in the region of Eastern Himalayas, Western Ghats and Andaman and Nicobar Island (Adam *et al.,* 2001; Padmapriya *et al.,* 2011). *In vitro* propagation is a promising tool for the rapid multiplication of threatened and endangered medicinal plants either through micropropagation method (Sivanandhan *et al.,* 2011). In traditional medicine, a decoction or infusion of *Ageratum conyzoides*, belongs to family Cucurbitaceae, an endemic medicinal plant is used for the treatment of hepatitis, eczema, epilepsy, dizziness diarrhea, fever, intestinal worms and filariasis (Jadhav *et al.,* 2010; Anitha and Miruthula, 2014). Because of the importance of *Ageratum conyzoides* in herbal medicine and lack of adequate knowledge of regarding its *in vitro* conservation, a study was initiated (Vespasiano *et al.,* 2003).

MATERIALS AND METHOD

Plant collection and identification

The plant *Ageratum conyzoides* L. was collected from Veeramalai Kavandam Patty at Karur District (Figure - 1). The identification and authentication of the freshly collected plant was done by Dr. S. Soosai Raj, Department of Botany, St. Joseph's College (Autonomous), Tiruchirappalli - 620 002.

Explants source

The healthy and young field grown explants of *Ageratum conyzoides* were thoroughly washed with running tap water for 15 - 20 minutes to remove any extraneous materials followed by immersion in detergent solution for 5 minutes. After washing with distilled water, explants were again washed in 70% alcohol for few seconds and rinsed 3 times with distilled water. The explants were brought to the inoculation chamber and surface sterilized with 0.1% $HgCl_2$ for 3 - 5 min and again washed with sterile distilled water for 5 - 7 times.

Culture medium

Basal Murashige and Skoog, (1962) medium along with the various hormone compositions were used. The pH of the medium was adjusted to 5.7 before adding agar (8.0 gm/L).

Sterilization of culture media

The sterilized MS medium with various concentrations of growth hormones was dispensed in culture tubes (20) ml. The culture tubes were plugged with cotton and autoclaved at 121^{o}C for 15 min. After autoclaving, the culture tubes were left undisturbed until the medium was solidified. Then culture tubes were transferred to the inoculation chamber.

Inoculation

Before starting inoculation, all the required equipment's such as sterilized forceps, petriplates, the sterile blade, scalpel, sterile distilled water and spirit lamp were transferred to laminar air flow chamber. The surface and two sides of the chamber were wiped with alcohol and the door was tightly closed. Then the UV light was switched on 15 min. After that, the equipment's were sterilized by dipping in 95% alcohol followed by flaming and cooling. Before starting the inoculation, hands were cleaned with alcohol and the inoculation was carried out in the vicinity of the flame. The sterilized explants were placed on the medium at the center of culture tubes.

Incubation

Inoculated culture containing vials were marked with necessary information regarding media, explants, date of inoculation *etc.,* incubated on the culture room. Cultures were maintained at 25 ± 2°C with a photoperiod of 16 hrs light and 8 hrs dark per day of fluorescent light 3000 lux for all treatments. Subcultures were made once in 15 - 20 days.

Hardening

The rooted plantlets were removed from the culture tubes and washed in sterilized distilled water. Then they were transplanted into cups containing sterilized vermiculite, sand and soil (1:1:1) ratio. The plants need 95 - 100% humidity and therefore they were covered with plastic bags with perforation or holes. After 15 days, the plantlets in the cups were transferred to a shadow for about 30 days and then transfer to the field.

RESULTS & DISCUSSION

Callus induction from leaf explants

Ageratum conyzoides leaf explants were taken from healthy mother plants were cultured on MS medium fortified with various concentrations of NAA and 2, 4-D. The callus development from leaf explants was observed after 15 days of culture media tested. The leaf calli appeared in systematically from cut margins of the explants after 4 weeks of cultures were distinguished morphologically as light green callus, yellow callus and brown callus as given in Figure - 2. The highest leaf callus induction frequency of 86% from leaf explants were obtained on MS medium supplemented with the combination of NAA (2.0 mg/L) and 2, 4-D (2.5 mg/L) as depicted in Table - 13. The results similarly reported that species in *Celosia argentea* by Abu Bakar *et al.*, 2014.

Micropropagation from shoot tip explants

The shoots were regenerated from each auxiliary meristem on MS medium supplemented with the different combination of NAA (0.5 mg/L) and BAP (1.0 mg/L) was shown in Figure - 3. The shoot buds were initiated after 15 days of inoculation. The percentage of response of micro shoots per explants was 67% (2.70 ± 1.05) as shown in Table - 2. Shoots were transferred to rooting on MS medium supplemented with various combination of NAA (2.5 mg/L) and IBA (3.0 mg/L). The rooted plantlets were transferred to small cups containing sterilized soil, sand and vermiculite (1:1:1) for hardening and then successfully transferred to soil.

It can be concluded that the maximum number of explants was responded with plant regeneration and positive efficacy in the growth hormones of NAA (0.5 mg/L) and BAP (1.0 mg/L) a suitable phytohormones for shoot proliferation and elongation from shoot tip explants of *Ageratum conyzoides.* The results similarly reported that species in *Cucumis sativus* by Ahmad and Anis, 2005.

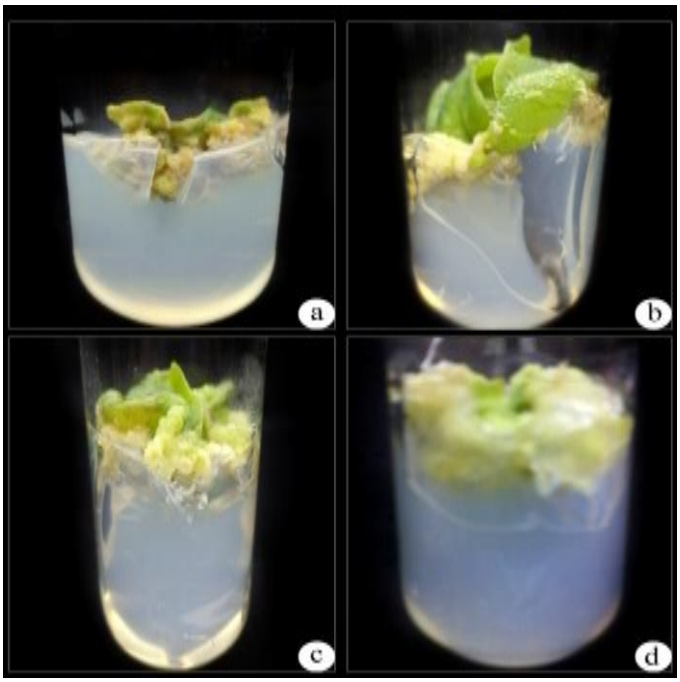
Micropropagation from nodal explants

The explants were responded with combination of NAA (1.0 mg/L) and BAP (1.5 mg/L) as shown in Figure - 3. The shoots were regenerated from the nodal region and the response number of micro shoots per explants was 86% (2.16 ± 0.75) as given in Table - 3. *In vitro* rooting was induced with the auxin tested in the present investigation, auxin treatments generally stimulated adventitious root formation and they were found to have a significant effect on number of roots and root length. The results were observed on MS medium fortified with NAA (2.5 mg/L) and IBA (3.0 mg/L) for root formation with 86% response (6.5 ± 1.04) as shown in Table - 4. The rooted plantlets were transferred to small cups containing sterilized soil, sand and vermiculite (1:1:1) for hardening and then successfully transferred to soil.

It can be concluded that the maximum number of explants were responded with the growth hormones of NAA (1.0 mg/L) and BAP (1.5 mg/L). This is concordance with the results similarly reported earlier in same species such as Laxmikant Sharma, 2008.

a) Habit b) Young flower c) Mature flower d) Collection of plant

Figure – 1: Habit of *Ageratum conyzoides* L.

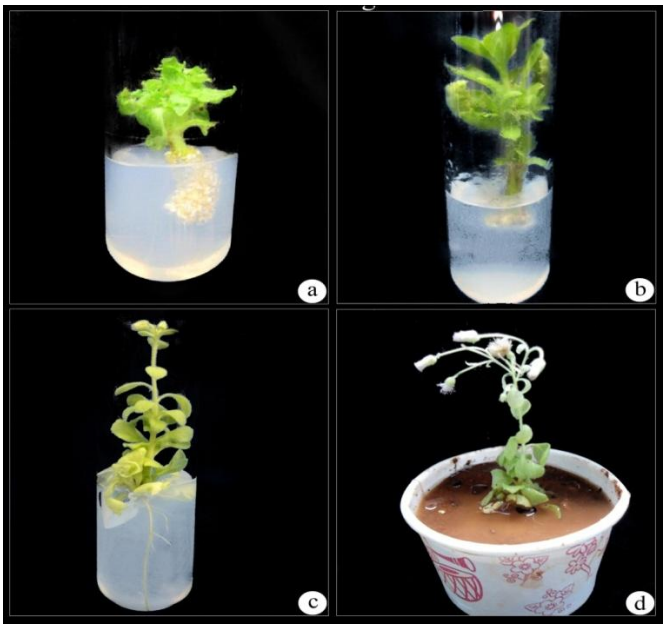


a) Brown compact callus. b) Yellow compact callus.
c) Yellowish white Callus. d) Light green compact callus.

Figure – 2: Different types of callus induction from leaf explants of *Ageratum conyzoides* L.

a) Initiation of explants. b) Shoot formation of NAA (0.5 mg/L) and BAP (1.0 mg/L).
c) Root formation of NAA (2.5 mg/L) and IBA (3.0 mg/L). d) Hardening.

Figure – 3: Micropropagation from shoot tip explants of *Ageratum conyzoides* L.



a) Initiation of explants. b) Shoot formation of NAA (1.0 mg/L) and BAP (1.5 mg/L). c) Root formation of NAA (2.5 mg/L) and IBA (3.0 mg/L). d) Hardening.

Figure – 4: Micropropagation from nodal explants of *Ageratum conyzoides* L.

Table – 1: Different types of callus induction from leaf explants of *Ageratum conyzoides* L.

S. No.	Plant Growth Regulators (PGRs)		No. of explants Inoculated / Response	% of explants response	Type of callus formation
	NAA (mg/L)	2, 4-D (mg/L)			
1.	0.5	1.0	3/7	43	Brown compact callus
2.	1.0	1.5	3/7	43	Yellow compact callus
3.	1.5	2.0	5/7	71	Yellow compact callus
4.	**2.0**	**2.5**	**6/7**	**86**	Light green compact callus
5.	2.5	3.0	4/7	57	Yellowish white Callus

Table – 2: Micropropagation from shoot tip explants of *Ageratum conyzoides* L.

S. No.	Plant Growth Regulators (PGRs)		No. of explants Inoculated / Response	% of explants response	No. of shoots per explants Mean ± SD
	NAA (mg/L)	BAP (mg/L)			
1.	**0.5**	**1.0**	**10/15**	**67**	**2.70 ± 1.05**
2.	1.0	1.5	8/15	53	2.25 ± 0.88
3.	1.5	2.0	6/15	40	2.16 ± 0.75
4.	2.0	2.5	5/15	33	2.0 ± 0.70
5.	2.5	3.0	3/15	20	2.33 ± 0.57

Table – 3: Micropropagation from nodal explants of *Ageratum conyzoides* L.

S. No.	Plant Growth Regulators (PGRs)		No. of explants Inoculated/ Response	% of explants response	No. of shoots per explants Mean ± SD
	NAA (mg/L)	BAP (mg/L)			
1.	0.5	1.0	4/7	57	2.0 ± 0.81
2.	**1.0**	**1.5**	**6/7**	**86**	**2.16 ± 0.75**
3.	1.5	2.0	4/7	57	2.5 ± 0.57
4.	2.0	2.5	3/7	43	2.33 ± 0.75
5.	2.5	3.0	3/7	43	1.66 ± 0.57

Table – 4: Root induction from *in vitro* cultured explants of *Ageratum conyzoides* L.

S. No.	Plant Growth Regulators (PGRs)		No. of explants Inoculated/ Response	% of explants response	No. of shoots per explants Mean ± SD
	NAA (mg/L)	IBA (mg/L)			
1.	0.5	1.0	3/7	43	5.0 ± 1.0
2.	1.0	1.5	3/7	43	5.33 ± 0.57
3.	1.5	2.0	4/7	57	5.66 ± 1.52
4.	2.0	2.5	5/7	71	5.4 ± 1.81
5.	**2.5**	**3.0**	**6/7**	**86**	**6.5 ± 1.04**

CONCLUSION

A suitable protocol for callus induction and micropropagation of the valuable endemic medicinal plant *Ageratum conyzoides* L. has been developed. Combination of NAA (2.0 mg/L) 2, 4 - D (2.5 mg/L) was suitable for callus induction. The multiple shoot proliferation from shoot tip explants of NAA (0.5 mg/L) and BAP (1.0 mg/L) and NAA (1.0 mg/L) and BAP (1.5 mg/L) for nodal explants. However MS medium supplemented with NAA (2.5 mg/L) and IBA (3.0 mg/L) was found to be suitable for root development. The above protocol can be exploited for the micropropagation of this valuable germplasm and for *in vitro* conservation.

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