Recent Research in Science and Technology 2012, 4(4): 01-04
ISSN: 2076-5061
Available Online: http://recent-science.com/

In vitro micropropogation Of *Sphaeranthus amaranthoides* Burm.F

R. Devika , Justin Koil Pillai * and S.Nazareth Arockiamary

Department of Bioinformatics., Aarupadai Veedu Institute of Technology. Paiyanoor.,Tamilnadu, India
*Department of Biotechnology., Satyabama University., Tamilnadu, India

Abstract
Sphaeranthus amaranthoides commonly known as garden Lavender / Kesavardini is used to cure eczema, skin diseases, worm infestation, pile, aphrodisiac etc, from Ancient Era. In present investigation the auxillary buds and shoot tips were used as explants for *invitro* micropropogation. The initiation and best multiple shoots was developed at 4mg/L of BAP with a highest frequency of 70% and good root proliferation was observed at 2.0mg/L of IBA.

Keywords: Micropropogation, Auxillary bud, Initiation, Proliferation, Induction.

INTRODUCTION

Medicinal plants, since times immemorial, have been used as the source of traditional medicine and for the maintenance of good health [1]. About 1400 herbal preparations such as beauty oriented therapeuticals like skin tissue regenerators, anti wrinkling agents, skin tonics and anti age creams were widely used, according to recent survey by Member States of the European Union. Tissue culture techniques are being increasingly exploited for clonal multiplication in *invitro* conservation of valuable indigenous germplasm threatened with extinction. Greater demand for medicinal plants especially for the purpose of food and medicines which is one of the causes for their rapid depletion from primary habitats [2]. *Invitro* micropropogation offers a great potential for large scale multiplication and subsequent exploitation [3].

Sphaeranthus amaranthoides (Garden Lavender/ Kesavardhini) is an annual medicinal herb which belong to Asteraceae family (compositae) known to be the largest family of flowering plants comprising about 1,100 genera with 20,000 species. In Siddha, root, leaf, flower, seeds of *Sphaeranthus amaranthoides* are used to cure eczema, skin diseases, disease of vatam, worm infestation, piles, aphrodisiac etc.[4]. In Ayurveda, the whole plant of *Sphaeranthus amaranthoides* is used to cure anorexia, jaundice, blood disorder, oedema, filariasis, dysuria, diuretic etc. [5],[6] isolated three new endesmanoids from the acetone extract of *Sphaeranthus indicus* and [7] extracted seven carvatacetone derivatives and mixtures of myo inositol esters from the four spcies of *Sphaeranthus* group. An immunostimulant sesquiterpene glycoside, *Sphaeranthanolide* has been isolated from the flowers of *Sphaeranthus indicus* by [8].

MATERIALS AND METHODS

Received: Feb 12, 2012; Revised: March 18, 2012; Accepted: April 15, 2012.

*Corresponding Author
R.Devika
Department of Bioinformatics., Aarupadai Veedu Institute of Technology.
Paiyanoor.,Tamilnadu, India

Tel: +91-9941411106
Email: vineeth_2001@yahoo.com

The auxillary buds and shoot tip explants of *Sphaeranthus amaranthoides* were collected and sterilized as per Standard Methods. The explants were inoculated in MS medium culture tubes in an aseptic condition. The growth regulators such as 1- Napthalene acetic acid (NAA); Indole 3- butyric acid (IBA); Indole 3 acetic acid (IAA); 6- Benzyl amino purine (BA) and Kinetin (KN) were prepared and maintained in aseptic condition. Initiation of multiple shoots was developed in the MS medium supplemented with different concentrations of BAP (1.0-5.0mg/L) and KN (1.0-5.0 mg/L), and in combination of BAP and KN at different concentration ranging from 1.0 to 5.0mg/L of BAP and constant concentration of 2 mg/L of KN, well developed platelets were transferred to MS basal medium supplemented with different concentration of IAA (1.0-5.0 mg/L) and IBA (1.0-5.0 mg/L) for root induction. The percentage of responses of root induction was calculated.

RESULTS AND DISCUSSION

Shoot proliferation

The auxillary bud and shoot tip explants from healthy growing plants were excised and after sterilization, they were inoculated on MS medium supplemented with cytokinin BAP and KN (1.0-5.0 mg/L). In both explants the shoot initiation was observed after seven to ten days of inoculation and multiple shoot proliferation was obtained after 25 days. Comparative study between the two cytokinins revealed that BAP showed multiple shoot organogenisis than KN supplemented MS medium (Table 1). Out of different BAP concentrations tried, the concentration of 4.0mg/L had the maximum proliferation of shoot induction with the highest frequency (70%) and the number of multiple shoot developed was 32.5 ± 5.5, the reason for the above effectiveness may be its ability to stimulate the plant tissues to metabolize the natural hormone system for the shoot organogenesis induction [9].

An attempt was made with the combination of BAP and KN at different concentration ranging from 1.0 to 5.0mg/L of BAP and constant concentration of 2 mg/L of KN along with MS culture medium, both auxillary buds and shoot tip explants were sterilized and inoculated in various culture tubes (Table 2).A maximum shoot induction was observed at 4.0 mg/L of BAP and 2.0 mg/L of KN with multiple proliferation of (22.5± 5.5 shoots / explants) of auxillary buds. Simultaneously a maximum shoot induction was observed at

4.0mg/L of BAP and 2.0mg/L of KN (19±0.7 number of shoots/ explant) in the shoot tip explants culture tubes. Similarly [10] reported that BAP (5.0 mg/L) showed multiple shoot proliferation in the compositae plant *Spilanthes acmella* from auxillary bud as explants.

Table 1.Effect of various concentrations of BAP / KN on multiple shoot formation from auxillary buds (AB) and shoot tip (ST) explants of *Sphaeranthus amaranthoides*

BAP (mg/l)AB	Percentage Of Response (%)	No of Shoots / Explants (Mean+SE)
1.0	30	1.75±0.8
2.0	50	5.00±0.7
3.0	60	12.50±4.5
4.0	70	32.50±5.5
5.0	60	19.00±3.3
KN(mg/L) AB	**Percentage of Response (%)**	**No of Shoots / Explants (Mean+SE)**
1.0	20	1.25±0.4
2.0	40	1.75±0.4
3.0	50	5.25±1.0
4.0	60	10.25±1.7
5.0	50	6.00±1.5
BAP (mg/l)ST	**Percentage Of Response (%)**	**No of Shoots / Explants (Mean+SE)**
1.0	0	0
2.0	10	1.25±0.4
3.0	20	1.50±0.5
4.0	30	3.00±0.7
5.0	30	1.25±0.4
KN(mg/L) ST	**Percentage of Response (%)**	**No of Shoots / Explants (Mean+SE)**
1.0	0.0	0.0
2.0	0.0	0.0
3.0	10	2.50±0.5
4.0	20	2.75±0.8
5.0	20	2.25±0.4

Table 2.Effect of various concentration of BAP+ KN on multiple shoot formation from auxillary buds (AB) and shoot tip (ST) explants of *Sphaeranthus amaranthoides*

BAP+KN (mg/l)AB	Percentage Of Response (%)	No of Shoots / Explants (Mean+SD)
1.0+2.0	50	5.50±2.0
2.0+2.0	70	6.50±1.1
3.0+2.0	80	10.50±2.6
4.0+2.0	90	22.50±5.5
5.0+2.0	80	19.25±3.6
BAP+KN (mg/l)ST	**Percentage Of Response (%)**	**No of Shoots / Explants (Mean+SD)**
1.0+2.0	30	3.00±0.7
2.0+2.0	40	3.25±0.8
3.0+2.0	50	11.00±2.2
4.0+2.0	60	19.00±0.7
5.0+2.0	50	13.75±3.7

Root induction

After 6 weeks, well grown shoot culture of 8 cm height were transferred to MS medium supplemented with IBA (1.0-5.0 mg/L) for rooting. After 15-20 days of inoculation, very good root proliferation was observed at 2.0 mg/L and it was observed to be the best rooting response (70%) with higher number of roots (17.25±5.7) (Table 3). Complete plantlets were observed after 5-6 weeks of inoculation has proved that IBA was the best root inducting auxin for root induction in two generas such as *Fibigia triquerta* and *Centaurea ragusina*.

The rooted plantlets were hardened under green house condition for a germplasm conservation followed by field transfer for multiple production and growth. The survival rate was 60% and appeared normal after successful acclimatization.

Table 3.Effect of various concentration of IBA on root induction from shoot tip and auxillary bud regenerates of *Sphaeranthus amaranthoides*

IBA (mg/l)	Percentage Of Response (%)	No of roots/Shoots (Mean+SD)
1.0	40	8.75±2.5
2.0	70	17.25±5.7
3.0	60	10.00±1.8
4.0	50	11.25±2.3
5.0	50	5.75±1.4

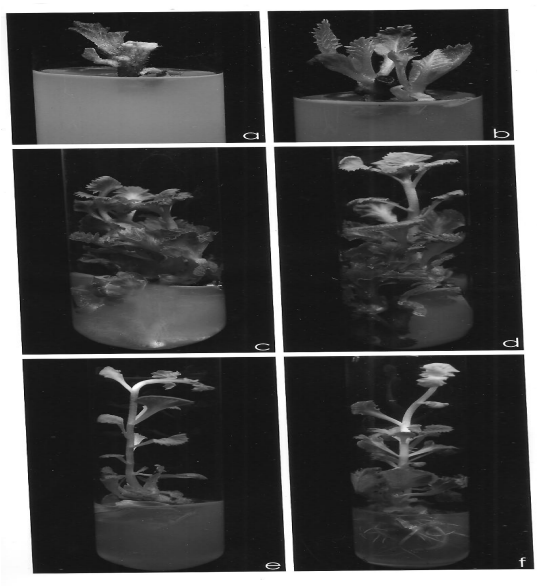

Plate 1. Micropropogation from auxillary bud explants of *Sphaeranthus amaranthoides*
Fig. a- Auxillary bud, b- Shoot initiation, c- Shoot multiplication after 20 days, d- Shoot multiplication after 30 days, e- Shoot elongation, f- Root formation

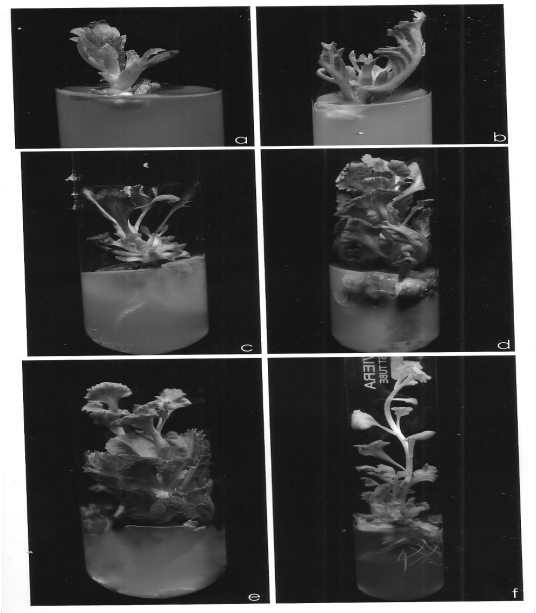

Plate 2. Micropropogation from shoot tip explants of *Sphaeranthus amaranthoides*
Fig. a- Shoot tip, b- Shoot initiation, c- Shoot multiplication after 15 days, d- Shoot multiplication after 30 days, e- Shoot multiplication after 40 days, f- Root formation

Plate 3.
Hardening of the Plantlet

CONCLUSION

The present investigation results proved that *invitro* micropropogation is an efficient means of an *exsitu* conservation of plant diversity and it assists in the sustainable maintenance of germplasm on long term basis. Attempts has been made with combination of BAP and KN at various concentrations for multiple shoot formation from auxillary buds and shoot tip explants of *Sphaeranthus amaranthoides* . The combination of BAP (4.0 mg/L) and KN (2.0mg/L) showed the best responses of 90% with maximum mean number of shoots of 22.5 ± 5.5. The shoot tip explants showed a good result of 60% response at 4.0 mg/L of BAP and 2.0mg/L of KN and the mean number of shoots developed was 19.0 ± 0.7.

REFERENCE

[1] UNESCO.1998. FIT/ 504-RAF-48 terminal report: Promotion of Ethnobotany and the sustainable use of plant resources in Africa. Pg.60

[2] Jothi Basu M, Ramanathan R, Yogananth N, and Baburaj S, 2009. Micropropogation of crataeva religiosa, *curr.Trends in Biotech & Pharmacy.* 3(3): 237-239.

[3] Babu R, Rao RVK, Annapurna A and Babu DRK. 2001. Immunostimulant profile of a polyherbal formulation RVO8. *J. Pharm.*33 (6): 454-455.

[4] Adzu B, Amos S, Kapu D and Gamaniel RS. 2003. Antiinflammatory & antinoceptive effects of *Sphaeranthus senegalensis. J.Ethnopharmocology* 83 (23): 169-73.

[5] Yoganarasimhan .S. 2000. Medical plants of India. *Phytochemistry*.2: 67- 68.

[6] Pujar PP , Swawaikar DD , Rozalkar SR and Nagasampagi BA. 2000. Eudesmanoides from *Sphaeranthus indicus.* 71 (3): 264-268.

[7] Zdero .C, Bohlmann. F and Mungai GM. 1991. Carvotacetone derivatives and other constituents from representatives of the *Sphaeranthus* group. *Phytochemistry.* 30 (10): 3297 -3303.

[8] Sherkhan FR, Kalita MC, Barua CC and Deka A. 2001. Micropropogation of Hydrocotyle rotundifolia. *J.of Medi. Aram. Plant.Sci.22* (1): 59-62.

[9] Suchitra Baneyee, Jyots Tripathi, Praveen, Chandra Verma, Prem Dutt, Suman Preet Singh, Kharuja GP & Bagchi. N.2004. Thiadiazuron induced high frequency shoot proliferation in cineraria maritimia .L. *current Science* .87 (9)

[10] Ang and Chan. 2005. Invitro micropropogation of Spilanthus acmella. *Plant cell Biotech. & Mol. Bio*. 5(8):45-50.