



## Micropropagation of *Sphaeranthus amaranthoides* Burm. f. – A multipurpose medicinal herb

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### ABSTRACT

A method for induction of multiple shoots using axillary bud and shoot tips of *Sphaeranthus amaranthoides* Burm. f. was described. The experiment was conducted in which multiple shoot induction was noticed on MS (Murashige and Skoog) medium supplemented with different concentrations of two cytokinins (BAP and Kin). These multiple shoots later developed into normal shoots. The highest rate of shoot proliferation was found to be 90% and 75% for axillary bud and shoot tip explants, respectively, on the medium containing BAP 4.0 mg/l. The regenerated shoots were successfully rooted on MS medium supplemented with 2.0 mg/l IBA, after sequential hardening, survival rate was 90%.  
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**Keywords:** *In vitro*, Axillary bud, Shoot tips, Multiple shoots, Regeneration, *Sphaeranthus amaranthoides* Burm. f.

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### INTRODUCTION

*Sphaeranthus amaranthoides* is an annual medicinal herb, belonging to the family *Asteraceae* (Compositae). Various parts of this plant have been used in Siddha and Ayurvedic system of medicine. In Siddha roots, leaf, flower, seeds are used to cure eczema, skin diseases, diseases of vatam, worm infection, piles. The plant is also used as aphrodisiac, and rejuvenator. In Ayurveda the whole plant used to cure anorexia, jaundice, blood disorders, edema, scrofula, filariasis dysuria, fever and diuretic. The biotechnological tools are important to select, multiply and conserve the critical genotypes of medicinal plants by adopting techniques such as micropropagation, somaclonal variations and genetic transformations. Biotechnological tools can also be harnessed for production of secondary metabolites using

plants as bioreactors. There is indiscriminate harvesting of medicinal and aromatic herbs, which leads to extinction of herbal populations in the bio-resources. Now there is need to domesticate and produce organically grown quality medicinal herbs of high standards for internal consumption as well as export. *Sphaeranthus* contains a variety of bioactive compounds, which play a key role in medicinal and dietary purposes. Research has assessed the immunostimulant activity (Babu et al., 2001), anti microbial activity (Sing et al., 1988), anti-inflammatory activity (Jain and Basal 2003 and Adzu et al., 2003) in *S.indicus* and *S.senegalensis*.

Reports of *in vitro* plant regeneration from tissues of medicinal plants are available (Hiraoka and Oyanagi, 1988; Lu et al., 1995; Gururaj et al., 2004; Vidya et al., 2005; George et al., 1993; Budzianowski et al.,

2004 and Jawahar et al., 2004). However, there is no report in *Sphaeranthus amaranthoides*. Therefore, the present study was undertaken to determine the effect of different growth regulators on shoot formation and multiplication of genetically stable multiple shoots from axillary bud and shoot tips of *Sphaeranthus amaranthoides*.

## MATERIALS AND METHODS

The axillary bud and shoot tip explants were collected from field grown plants of *Sphaeranthus amaranthoides*. These explants were washed under running tap water followed by treatment with a surfactant, Tween 20 (5% v/v) for 5 minutes. After repeated washes in double distilled water, surface sterilization was done with mercuric chloride (0.1% w/v) solution for 3 - 5 minutes. The sterilized explants were then washed thoroughly with double distilled water and cut into an appropriate size (1 cm), and cultured on sterile nutrient medium.

The basal medium for multiple shoot bud induction contained MS salts, vitamins and 30 g/l sucrose solidified with 0.8 % w/v agar. The pH of the medium was adjusted to 5.8 before autoclaving at a pressure of 1.06 kg cm<sup>2</sup> for 20 minutes. All the cultures were incubated at 25 ± 2 °C with 16/8 h photoperiod under white fluorescent tubes (25 μmol m<sup>-2</sup> S<sup>-1</sup>). Various plant growth regulators viz., BAP (1.0-5.0 mg/l), Kin (1.0-5.0 mg/l), IAA (1.0-5.0 mg/l) and IBA (1.0/5.0 mg/l) were tested individually to obtain the most suitable concentration for the proliferation of multiple shoots and root induction. Observations were recorded after an interval of four weeks. Rooted micro-shoots were thoroughly washed to remove the adhering gel and planted in plastic cups containing a mixture of peat moss and organic manure (1:1). Plastic cups were covered with polythene bags to maintain humidity. Plants were kept in culture room for ten days. After the plants were transferred to pots containing organic manure, garden soil and sand (1:1:1), and were maintained in green house. Then, the plants were transplanted to the field. All the experiments were repeated at least three times with 20-24 replicates and data was subjected to statistical analysis (DMRT test). A p value of <0.05 was considered statistically significant.

## RESULTS

Proliferation of multiple shoots was obtained with high frequency from axillary bud and shoot tips. These explants were capable of directly developing multiple shoots on MS medium containing different concentrations of cytokinins. Multiple shoot initiation from both explants was observed after seven days of inoculation (Plate 1&2 Fig. 1) and multiple shoot proliferation was obtained within 20-25 days without subculture. Between the two cytokinin tested, multiple shoot proliferation was observed to be the best on MS medium supplemented with BAP. The highest number of shoots (23/explant) was observed in the medium containing 4.0 mg/l BAP followed by 4.0 mg/l Kin with 12 shoots per explant. Of the two cytokinins (BAP and Kin), BAP was found to be more suitable than Kin for initiation and proliferation of multiple shoot buds (Table 1). The multiple shoot bud frequency was higher in axillary bud (23.70 ± 0.88) than shoot tips (17.39 ± 0.71) (Plate 1&2 Fig. 2). The elongation of shoots and nodes were achieved on the same parental medium (Plate 1&2 Fig. 3). Shoots were harvested every 30-35 days and new shootlets were harvested periodically. Well-developed shootlets then transferred to rooting medium containing different concentration of IBA (1.0-4.0 mg/l) and IAA (1.0-4.0 mg/l) for root induction. Ninety six percent of the plantlets produced roots on the rooting medium containing 2.0 mg/l IBA after a week of inoculation (Plate 1&2 Fig. 4). The higher frequency of root induction was observed on MS medium containing 2.0 mg/l IBA (96% and 14 roots/shoot) and 2.0 mg/l IAA (90% and 10 roots/shoot). The rooted plantlets, after sequential hardening were transferred to green house where 90% of them survived.

## DISCUSSION

In the present study, the relative effectiveness of BAP and Kin varied for *in vitro* multiple shoot regeneration from axillary bud and shoot tips. BAP 4.0 mg/l was found to be the best concentration for generation of maximum number of shoots. When concentrations of BAP and Kin increased percentage of explants response, number of shoots per culture, number of nodes per shoot and shoot length were found to decrease.

**Table 1:** Effect of BAP and Kin for *in vitro* shoot multiplication from the axillary bud and shoot tip explants of *Sphaeranthus amaranthoides* Burm.f.

Growth regulators (mg/L)	Axillary bud			Shoot tip		
	% of response	No. of shoots per culture	Shoot length (cm)	% of response	No. of shoots per culture	Shoot length (cm)
<b>BAP</b>						
1.0	30	8.80 ± 0.83 <sup>f</sup>	3.07 ± 0.41 <sup>i</sup>	30	6.93 ± 0.69 <sup>h</sup>	2.50 ± 0.16 <sup>h</sup>
2.0	55	12.88 ± 0.78 <sup>c</sup>	4.71 ± 0.54 <sup>e</sup>	50	9.99 ± 0.30 <sup>d</sup>	3.68 ± 0.11 <sup>fg</sup>
3.0	70	20.40 ± 0.57 <sup>b</sup>	6.24 ± 0.15 <sup>b</sup>	65	12.99 ± 1.36 <sup>b</sup>	5.60 ± 0.50 <sup>c</sup>
4.0	90	23.70 ± 0.88 <sup>a</sup>	11.62 ± 0.93 <sup>a</sup>	75	17.39 ± 0.71 <sup>a</sup>	7.50 ± 1.50 <sup>a</sup>
5.0	72	12.50 ± 0.70 <sup>cd</sup>	6.04 ± 0.34 <sup>d</sup>	60	9.63 ± 0.88 <sup>de</sup>	5.25 ± 1.00 <sup>cd</sup>
<b>Kin</b>						
1.0	25	6.76 ± 0.56 <sup>h</sup>	2.50 ± 0.16 <sup>j</sup>	20	6.96 ± 0.48 <sup>h</sup>	2.20 ± 0.06 <sup>j</sup>
2.0	40	8.78 ± 0.40 <sup>fg</sup>	3.98 ± 0.14 <sup>h</sup>	40	7.50 ± 0.40 <sup>fg</sup>	3.86 ± 0.29 <sup>fg</sup>
3.0	60	10.25 ± 1.70 <sup>e</sup>	4.34 ± 0.16 <sup>fg</sup>	55	9.46 ± 0.50 <sup>de</sup>	4.10 ± 0.16 <sup>f</sup>
4.0	75	12.50 ± 1.36 <sup>cd</sup>	6.10 ± 1.40 <sup>cd</sup>	70	11.02 ± 1.14 <sup>c</sup>	6.24 ± 0.15 <sup>b</sup>
5.0	65	8.74 ± 0.76 <sup>fg</sup>	4.40 ± 0.11 <sup>f</sup>	60	7.62 ± 0.46 <sup>f</sup>	4.88 ± 0.30 <sup>c</sup>

Each value represents the mean ± SD of 20-24 replicates and each experiment was repeated at least thrice. Values with the same superscript are not significantly different at the 5% probability level according to DMRT.

**Table 2:** Effect of IBA and IAA on rooting of *in vitro* regenerated shoots of *Sphaeranthus amaranthoides* Burm.f.

Growth regulators (mg/L)	Axillary bud		Shoot tip	
	% of root induction	No. of roots per shoot	% of root induction	No. of roots per shoot
<b>IBA</b>				
1.0	64	6.56 ± 0.37 <sup>de</sup>	60	5.52 ± 0.40 <sup>c</sup>
2.0	96	14.38 ± 1.37 <sup>a</sup>	90	10.76 ± 0.97 <sup>a</sup>
3.0	60	9.46 ± 0.56 <sup>c</sup>	55	7.20 ± 0.24 <sup>c</sup>
4.0	56	6.10 ± 1.50 <sup>f</sup>	40	4.68 ± 0.47 <sup>f</sup>
<b>IAA</b>				
1.0	48	5.02 ± 0.37 <sup>g</sup>	45	4.00 ± 0.14 <sup>gh</sup>
2.0	82	12.68 ± 0.96 <sup>b</sup>	80	10.38 ± 1.16 <sup>ab</sup>
3.0	64	7.70 ± 0.22 <sup>d</sup>	60	6.66 ± 0.38 <sup>cd</sup>
4.0	54	5.00 ± 0.20 <sup>gh</sup>	50	4.10 ± 0.10 <sup>g</sup>

Each value represents the mean ± SD of 20-24 replicates and each experiment was repeated at least thrice. Values with the same superscript are not significantly different at the 5% probability level according to DMRT.

The capacity of shoot bud differentiation and shoot proliferation from axillary bud and shoot tips of *Sphaeranthus amaranthoides* depended on hormonal content of the culture medium. There was good shoot bud initiation and proliferation response only in the presence of cytokinin and absence in the basal medium. Similar observation was made by Pattnaik and Chand (1996), in *Ocimum* species. The potential for shoot multiplication in *Sphaeranthus amaranthoides* appears to be high in the presence of cytokinin alone in the culture medium. The stimulatory effect of singular supplement of BAP on bud burst and multiple shoot formation is similar to that reported in other medicinal plant species by Sahoo and Chand (1998) in *Vitex negundo*, Jawahar et al. (2008) in *Cardiospermum halicacabum*, Deka et al. (1999) in *Withania somnifera*, and Verma and Kant (1996) in *Embllica officinale*. Sharon and Marie (2000) reported that the shoot tip and nodal explants were preferred over meristem to produce large number of genetically identical clones in *Bixa ovellana* L. in the medium containing BAP and Kin alone.

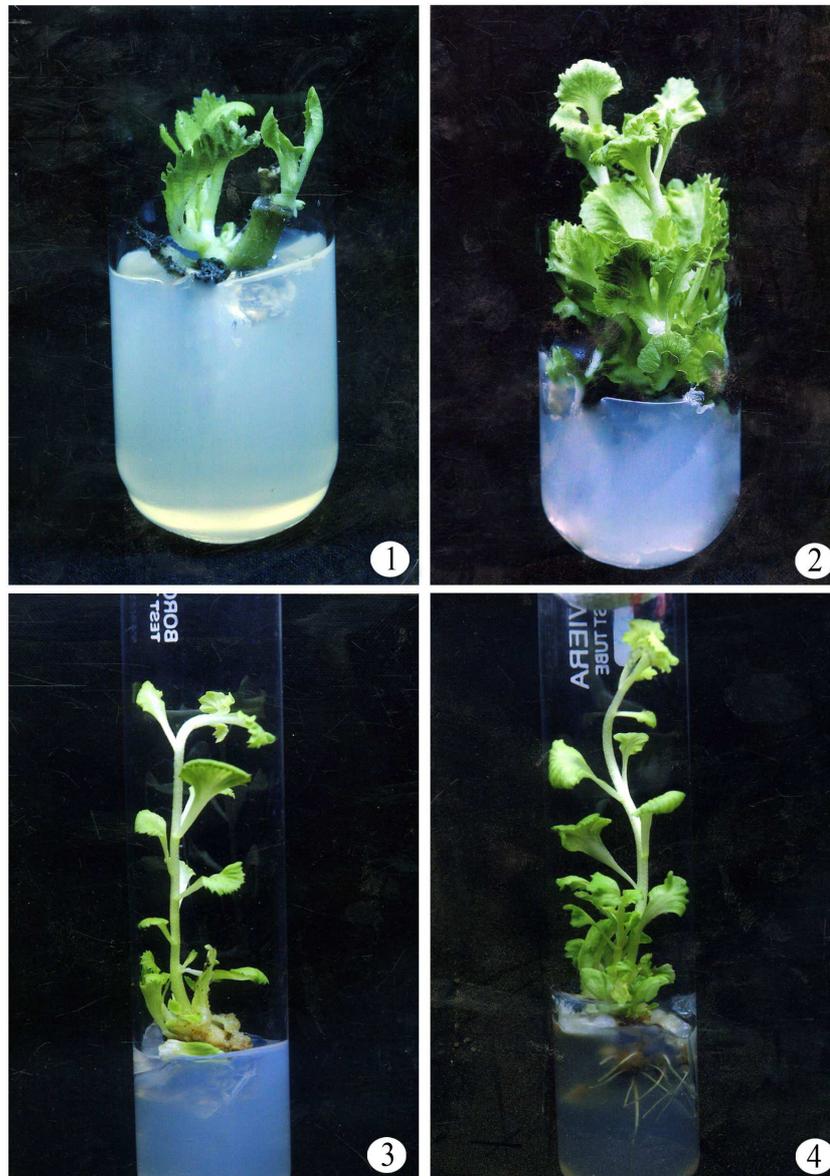
Reddy et al. (1998), Komalavalli and Rao (2000) and Jawahar et al. (2008) reported that the MS medium containing BAP was more effective than Kin for inducing multiple shoot proliferation. From our study it was clear that 4.0 mg/l BAP was significantly

more effective than 4.0 mg/l Kin for inducing shoot organogenesis.

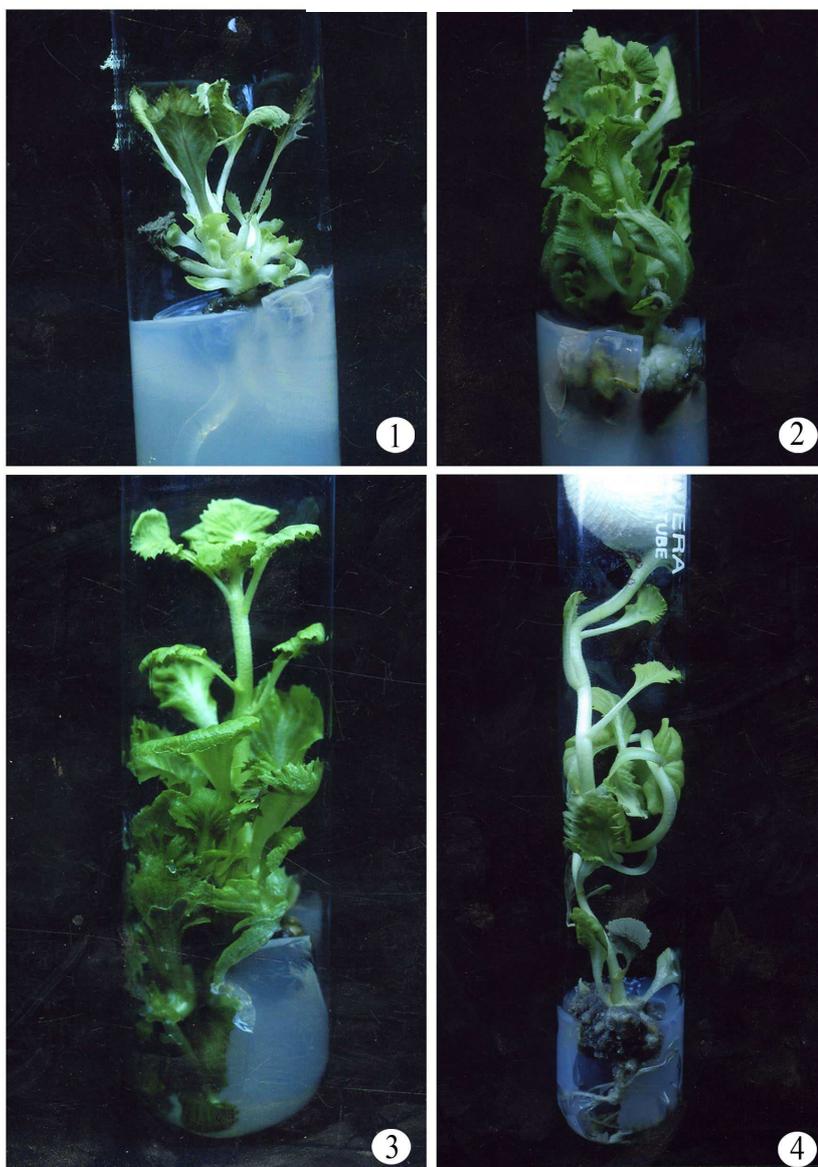
Kulkarni and Rao (1999) reported that Kin did not support the proliferation of multiple shoots in *Acorous calamus*. This result was in contrast to the present study where Kin was found to increase the frequency and the number of shoots. Pawar et al. (2002) reported that BAP and Kin individually and combination induced a higher frequency of adventitious shoots from a single explants of *Solanum xanthocarpum*. This result was similar to that recorded in the present study.

Pande et al. (2000) reported that IBA was effective for root induction in *Ammi majus*. Similar effects of IBA were reported by Pattnaik and Chand (1996) in *Ocimum* sps., Wakhulu and Sharma (1999) in *Heracleum candicans*, Jawahar et al. (2008) in *Cardiospermum helicacabum* and Karuppusamy and Pullaiah (2007) in *Bupleurum distichophyllum*.

From our experimental data, it is evident that BAP and Kin are best suited for inducing multiple shoots and IBA for rooting. The axillary bud showed better response compared to shoot tips. In conclusion, this micropropagation and shoot multiplication system is suitable for conservation of germplasm of this highly prized medicinal plant.



**Plate 1:** Fig. 1: Shoot bud initiation from axillary bud  
Fig. 2: Shoot bud proliferation  
Fig. 3: Shoot Elongation  
Fig. 4: Rooting



**Plate 2:** Fig. 1: Shoot bud initiation from shoot tip  
Fig. 2: Shoot bud proliferation  
Fig. 3: Shoot Elongation  
Fig. 4: Rooting

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