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Short Communications

Micropropagation of *Stachytarpheta jamaicensis* (L.) Vahl—A medicinally important plant

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An *in vitro*-protocol for micropropagation of *Stachytarpheta jamaicensis* (L.) Vahl was developed through nodal explants. Murashige and Skoog (MS) medium with BA 0.5 mg/L and IBA 0.5mg/L was found to be the best for multiple shoot formation from nodal explants. The maximum response of shoot induction was observed on a medium containing 1.0 mg/L Kn. The regenerated shoots were excised and transferred to rooting medium containing IBA at different concentrations. Highest of 84% rooting was observed at 0.5 mg/L level of IBA and BA. The *in vitro* derived plantlets were hardened and acclimatized in soil. About 75% of plantlets survived in the field condition.

Keywords: Acclimatization, multiple shoots, nodal culture, *Stachytarpheta jamaicensis*

Stachytarpheta jamaicensis (L.) Vahl (Family: Verbenaceae), commonly known as blue porter weed, is a native to South America, India and West Indies. Porter weed is a small perennial shrub that turns woody towards the base of the stem as it grows to about 1-yr-old. Plants grow 1.3 m tall and 1.6 m wide before stems droop and touch the ground[1]. The plant as a whole is having high medicinal importance for curing various disorders. In herbal medicine systems of many countries, the plant is considered to stimulate and aid digestion, suppress coughs, reduce fever and promote menstruation, and anti diarrheal and anti microbial activities[2]. Earlier work on the aerial parts of the plant revealed that it can be used to clean ulcers, cuts and wounds, and the plant leaf extract has anti inflammatory activity[3]. The plant is also considered to be abortive, laxative, diuretic and sedative. The aerial parts of this plant were found to be rich in chemicals like flavonoids, terpenes, phenols, steroids, γ-amino butyric acid, dopamine, citral and lythyroids[4-6].

Seeds are the only means of propagation of *S. jamaicensis*, however they encounter the problem of low viability and germination. There is an immediate need to propagate this species on commercial scale for its conservation and to meet increasing demands of pharmaceutical firms for its medicinal importance. The present article reports for the first time an efficient method for micropropagation of *S. jamaicensis via* regeneration from nodal explants.

Plant Material was collected from the fields of Warangal city, Andhra Pradesh, India. Young nodal segments (1.5-3.0 cm) were separated from the tender parts of the mature plants. They were washed thoroughly under running tap water for 30 min, followed by a wash with surfactant Tween 20 solution (5% v/v) for 5 min. After repeated (3-4 times) washes in double distilled water, nodal explants were taken to laminar air flow chamber, where they were treated with 0.1% $HgCl_2$ for 2-3 min and then washed in sterile distilled water 4-5 times under aseptic conditions prior to inoculation into MS culture medium[7].

For multiple shoot formation, the MS medium consisted of basal constituents supplemented with different concentrations of growth regulators, such as, BA (0.5, 1.0, 1.5, 2.0 & 2.5 mg/L) and Kn (0.5, 1.0, 1.5, 2.0 & 2.5 mg/L). Sucrose (2%) was added in medium as a carbon source and the *p*H of media was adjusted to 5.8 prior to autoclaving at 15 lbs for 20 min. The *p*H of the medium was adjusted by adding 1 *N* HCl and NaOH. The cultures were incubated at 25±2°C under 2000 lux light intensity provided by white fluorescent lamp for 16 h photoperiod. Cultures (25) in triplicate were raised for each treatment and all the experiments were repeated twice. Sub-culturing was carried out regularly at 4 wk interval. Statistical analysis was done where ever found necessary.

The regenerated shoots (1.5-2.0 cm) were transferred to MS medium with BA 0.5 mg/L and different concentrations of GA_3 (0.5, 1.0 & 2.0 mg/L) for shoot elongation. The elongated shoots were excised (2.0-2.5 cm) within 20 d of inoculation and transferred to MS medium with various

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concentrations of IBA (0.25, 0.50, 0.75, 1.0 & 1.5 mg/L) along with BA 0.5 mg/L. The rooted plantlets were first transferred to the plastic cups containing sterilized soil. The regenerated plantlets were hardened by covering them with a thin perforated transparent polythene bag to maintain humidity. Plantlets were watered with 1/10th strength MS salts solution and maintained in the culture condition. After 2 wk, they were transferred to the medicinal plant garden. The number of root intact plantlets recovered per explant was recorded after 5 wk of culture and the data were analyzed statistically by using "sigmastat" one-way ANOVA and LSD using Scientific Statistical Software.

MS medium supplemented with two different cytokinins (BA & Kn) separately was used to initiate shoot development from nodal explants (Table 1; Fig. 1A). The type and concentration of the cytokinins were determining factor for multiple shoot induction in *S. jamaicensis.* Morphogenic response demonstrated that amongst the two cytokinins (BA & Kn) incorporated into the medium, BA at concentration of 0.5 mg/L initiated high frequency regeneration and induced multiple shoots (3.13) with an average shoot length of (3.9 cm) from nodal explants (Table 1). On the other hand, the explants cultured on medium containing Kn 1.0 mg/L developed maximum number of shoots (4.1) with mean shoot length of 3.4 cm (Fig. 1B). Kn was found to be effective than BA in inducing multiple shoots. BA and Kn at higher concentration tested also induced shoot formation. However, their number remained low as compared to optimal levels of BA 0.5 mg/L and Kn 1.0 mg/L. Repeated sub-culturing of nodal explants isolated from *in vitro* regenerated shoots on medium supplemented with BA 0.5 mg/L resulted in continuous production of normal and healthy shoots. Moreover, the augmentation of auxin GA_3 at 1.0 mg/L with BA (0.5 mg/L) showed more shoot elongation and percentage of response (Table 2; Fig 1C). The statistical analyses performed by analysis of variance test with one factor and mean separation test, Fisher's least significance difference, indicated the significance difference in pair of means.

The elongated shoots were excised from the shoot clumps and inoculated on MS medium containing different concentrations of IBA (0.25, 0.50, 0.75, 1.0 &1.5 mg/L) along with BA (0.5 mg/L). The root initiation was observed after 14 d of inoculation. The

Table 1—Response of nodal explants to different concentrations of BA and Kn in *S. jamaicensis*

Growth regulator	(mgL)	% response	Shoot/ explant (mean±SE)	Shoot length (mean±SE)
BA	0.5	88	3.13±0.16	3.90±0.15
	1.0	76	2.36±0.22	3.33±0.16
	1.5	68	2.11±0.20	3.63±0.08
	2.0	72	2.66±0.15	3.60±0.10
	2.5	68	2.29±0.21	3.51±0.11
Kn	0.5	72	3.33±0.21	3.55±0.09
	1.0	84	4.14±0.18	3.42±0.02
	1.5	68	3.23±0.17	3.31±0.10
	2.0	68	2.94±0.18	3.21±0.14
	2.5	64	2.31±0.19	3.14±0.10

Observations were made after 20 d of inoculation
Data represents mean±SE of 25 replicates

Fig. 1 (A-E)—Micropropagation of *S. jamaicensis* from nodal explants: A. Shoot induction on MS+0.5 mg/L BA after 4 wk; B. Multiple shoots formed at basal region induced from explants; C. Shoot elongation on MS+1.0 mg/L GA_3 in addition to BA (0.5 mg/L); D. Root induction on MS supplemented with 0.5 mg/L IBA in addition to BA (0.5 mg/L); & E. Acclimatized plantlets of 6 wk.

Table 2—Effects of different concentrations (0.5, 1.0 & 2.0 mg/L) of GA_3 on elongation of regenerated shoots of *S. jamaicensis*

Growth regulators (mg/L)	% response	Shoot length (mean±SE)
BA+GA_3		
0.5+0.5	64	4.96±0.11
0.5+1.0	76	5.54±0.11
0.5+2.0	56	4.16±0.08

Observations were made after 20 d of inoculation
Data represent mean±SE of 25 replicates

Table 3—*In vitro* root induction to *S. jamaicensis* plantlets

Growth regulators (mg/L)	% response	Roots (mean±SE)	Root length (mean±SE)
BA+IBA			
0.5+0.25	76	3.36±0.15	2.02±0.06
0.5+0.50	84	4.42±0.17	2.31±0.07
0.5+0.75	76	4.31±0.13	1.76±0.04
0.5+1.0	72	4.00±0.24	1.80±0.03
0.5+1.5	72	3.44±0.26	1.98±0.03

Observations were made after 20 d of inoculation
Data represent mean±SE of 25 replicates

maximum percentage (84%) of rooting was observed at 0.5 mg/L of IBA (Table 3). The roots were long, pale white, linear and robust with root hairs with an average of 1.5-3.0 cm length (Fig. 1D). After 30 d of inoculation in rooting medium, the rooted plantlets were removed from the culture tubes and washed with distilled water. These *in vitro*-derived plantlets were subjected to hardening and finally transferred to clay pots (Fig. 1E). About 75% of plantlets were hardened successfully and survived in the field condition. Similar rapid and efficient protocol for shoot regeneration was also reported from other medicinally important plants[8-11].

Thus, an *in vitro*-protocol has been developed for micropropagation of *S. jamaicensis*, a plant of several medicinal values. The study shows that through tissue culture, mass production of plantlets can be achieved in a short span of time. This may help in continuous supply of medicinal products to herbal industries and also in conservation of depleting population in nature.

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