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RAPID *IN VITRO* PROPAGATION OF MEDICINALLY IMPORTANT *HEMIDESMUS INDICUS* (L.) R.BR. THROUGH NODAL EXPLANTS

***M. PRASHANTI[1], K.KALPANA[1], P.SHIVAPRASAD[2], SWATHI SINGH[2], A.ROJARANI [2] AND K. JANARDHAN REDDY[1]**

[1] *Department of Botany, Osmania University, Hyderabad, Telangana - 500007*
[2] *Department of Genetics, Osmania University, Hyderabad, Telangana - 500007*
Email:prashantilavu@gmail.com
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The present study deals with a new and an efficient protocol for *in vitro* propagation of medicinally important *Hemidesmus indicus* (L.) R.Br. from nodal explants. Among different cytokinins with varied concentrations of adenine sulphate along with BAP showed the best response for multiple shoot induction. Multiple shoot regeneration was established on MS medium supplemented with 4.0 mg/l adenine sulphate and 2.0 mg/l BAP. The regenerated shoots developed roots on MS medium supplemented with 4.0 mg/l IAA. About 78% of *in vitro* grown plants were successfully acclimatized in the field without any phenotypic changes.

Keywords: *Hemidesmus indicus* (L.) R.Br, micropropagation, nodal explants, adenine sulphate, shoot multiplication, root induction.

Hemidesmus indicus (L.) R.Br, commonly known as Indian Sarsaparilla, belonging to the family Asclepiadaceae is a perennial climber seen mostly in the eastern and southern parts of India and medicinally well known. Since centuries a wide variety of traditional ayurvedic medicines are prepared from its root and they have been validated by modern science. This taxon contains major chemical constituents such as 2-hydroxy 4-methoxy benzaldehyde, 4-hydroxy 3-methoxy benzaldehyde (Chatterjee and Bhattacharya 1955; Nagarajan *et al*. 2001) besides being rich in wide variety of biologically active compounds of essential oils, glycosides, resins, saponins, steroids and tannins. The roots possess antipyretic, antimicrobial as well as antidiarrhoeal activities (Anonymous 1986, 1997, Das *et al*. 2003). It is often called 'Sugandha' because of the wonderful fragrance of its roots.

Due to slow conventional propagation, low seed viability and limited propagation through seeds, this medicinal plant needs an effective conservation method for micropropagation. Micropropagation work was earlier reported by using BAP (Malathy and Pai 1998, Sreekumar *et al.* 2000, Ramulu *et al.* 2003, Saha *et al.* 2003, Arumugam *et al.* 2011, Saryam *et al.* 2012, Purohit *et al.* 2014, Rama Devi *et al.* 2014, Kamraj Singh *et al.* 2015, Shekawat *et al.* 2016). So far only a single report for improvement in clonal propagation of *H.indicus* through adenine sulphate has been reported (Neetha *et al*. 2003). Since no work on the micropropagation of *H.indicus* using adenine sulphate in combination with BAP has been reported, presently an attempt was made using this combination to achieve a higher frequency of regeneration from nodal explants of *H.indicus.*

MATERIALS AND METHODS

Plant material and sterilization

Healthy explants (Leaf, node and internode) were collected from mature plants growing in the Herbal garden, Department of Botany, Osmania University. They were washed thoroughly under running tap water for 10-15 minutes, followed by few drops of Tween 20 (2 drops in 100ml of water) for 1 min and rinsed with distilled water. The explants were again sterilized with 0.1% $Hgcl_2$ for 6-8 min followed by rinsing with distilled water and finally with 5% $NaOHCl_2$ for 15-20 min and washed 4-5 times with distilled water to remove the traces of $NaOHCl_2$.

Culture Conditions

The surface sterilized explants were cultured on full strength MS medium (Murashige and Skoog 1962) containing different growth regulators [6-benzyladenine purine (BAP), kinetin (Kn), adenine sulphate (AS)] with

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varied concentrations either alone or in combination. The pH of the medium was adjusted to 5.8 with 1N NaOH or HCl before adding 0.8% (w/v) agar (Hi Media) 2% (w/v) sucrose and autoclaved at 121^{0}C at 15 psi pressure for 20 min. Medium was poured into Borosil glass tubes (25×150 mm) and explants were inoculated vertically in culture tubes containing 20 ml of culture medium and maintained at 25±2^{0}C under 16 h photoperiod.

Induction of multiple shoots

For shoot induction, explants were cultured on MS medium supplemented with various plant growth regulators at different concentrations (1.0-8.0mg/l) either singly or in combination (Tables 1&2). All of the cultures were sub cultured onto the fresh medium after every two weeks. The frequency with which each explant produced shoots, the number of shoots per explant and the shoot length were recorded after 6 weeks of culture.

Root initiation

For root induction, i*n vitro* regenerated shoots were transferred to full strength and half strength MS medium supplemented with different concentrations of IAA and IBA (0.5-7.0 mg/l) (Table-3). The rooting response was recorded every 6 weeks. Data was recorded on percentage of rooting, mean number and length of the roots. Plantlets with well developed shoots and roots were washed thoroughly to remove traces of agar medium and transferred to plastic cups containing sterilized sand and soil (1:2) and acclimatized in the green house.

Statistical analysis

The experiment had three replicates and each of them consisted of 20 culture tubes. Observations were recorded after 6 weeks on the frequency concerning the number of shoots per explant, shoot length, roots per each shoot and root length. The data was analyzed using one-way analysis of variance (ANOVA), means were compared using the Tukey's test (John Tukey) at the 0.05 level of significance. Result of experiments was expressed in terms of mean± standard deviation.

RESULTS AND DISCUSSION

Induction and Multiplication of shoots

Among different explants used (leaf, internode and node), nodal explants responded better by initiating shoot bud formation within a week (Plate 1A). Concentration and the type of the growth regulator played an important role in the percentage of response. Leaf explants developed callus with all the concentrations of growth regulators while internode explants showed very poor or no shoot development (Table-1). The nodal explants failed to develop the shoots when inoculated onto growth regulator free MS medium (control) (Table-1). Further, explants inoculated on MS medium with minimum of all growth regulators (1.0-2.0mg/l) induced only 1–2 shoots after 8 weeks of inoculation (Table-1). However, highest mean number of shoots (8.51±0.39) and mean shoot length (7.78±0.53) with highest (87%) shoot regeneration frequency were recorded with 4.0mg/l adenine sulphate in combination with 2.0mg/l BAP (Table-2) (Plate 1C, D, E and F). Increase in the concentration of BAP & AS more than optimum level tested did not improve any parameters and suppressed regeneration frequency, number of shoots and shoot length (Table-2). Though the nodal segments responded with BAP or AS alone, the percentage of shoot induction and shoot length was less when compared to BAP & AS in combination (Table-2). Sharma Shalini and Vimala (2010) reported that adenine sulphate enhanced *in vitro* shoot regeneration in *Centella asiatica.*

***In vitro* root induction**

Best rooting was observed on half strength MS medium supplemented with 4.0mg/l IAA with highest mean number of roots (7.91±0.54) and mean root length (11.6±0.22) with 80% response (Plate 1G&H). Formation of roots was significantly higher in IAA when compared to IBA (Table-3). As regards to rooting response, similar results were also reported earlier by (Sreekumar *et al.* 2000 and

Table - 1: Effect of growth regulators on shoot regeneration from explants of *H.indicus* on MS medium.

Growth Regulator Concentration (mg/l)			Percentage of response in Nodal explants (%)	Nodal Response (Mean ± SD)*	Leaf Response	Internode Response
Growth regulator free MS medium(control)			--	--	--	--
BAP	**KN**	**AS**				
1.0			19	1.41±0.29	Callus formation	--
2.0			23	4.3 ±0.21	,,	--
3.0			18	2.3 ±0.27	,,	--
4.0			9	1.4 ±0.34	,,	--
	1.0		14	0.6 ±0.29	,,	--
	2.0		17	1.56±0.25	,,	--
	3.0		19	2.4±0.29	,,	--
	4.0		17	3.3±0.19	,,	--
		1.0	20	2.3±0.12	,,	--
		2.0	24	3.4±0.33	,,	--
		3.0	26	4.4±0.33	,,	--
		4.0	34	6.3±0.31	,,	--

*Mean±SD is based on three replicates, each of which consists of 20 individual culture tubes. Values are not significantly different by the Tukey's multiple comparison test (P=0.05).

Table - 2:- Effect of 6'benzyladenine purine (BAP) + Adenine sulphate (AS) on shoot regeneration from nodal explants of *H.indicus* on MS medium.

Growth Regulator concentration (mg/l)	Percentage response of Nodal explants (%)	No. of shoots per explant (Mean ± SD)*	Length of shoot (cm) (Mean ± SD)*
1.0 + 1.0	59	2.43±0.19	1.86±0.17
1.0 + 2.0	63	3.61±0.24	2.35±0.22
1.0 + 3.0	66	4.46±0.29	3.5±0.29
2.0 + 3.0	74	6.53±0.38	4.90±0.32
2.0 + 4.0	**87**	**8.51±0.39**	**7.78±0.53**
2.0 + 6.0	80	6.55±0.28	6.38±0.35
2.0 + 8.0	70	4.43±0.17	3.66±0.16

*Mean±SD is based on three replicates, each of which consists of 20 individual culture tubes. Values are not significantly different by the Tukey's multiple comparison test (P=0.05).

Table - 3:- Effect of IAA (Indole acetic acid) and IBA (Indole butyric acid) on root induction from *in vitro* raised shoots of *H.indicus* on MS medium after 8 weeks of culture.

Growth Regulator concentration (mg/l)	Percentage response of rooting (%)		No. of roots/shoot (Mean ± SD)*		Length of root (cm) (Mean ± SD)*	
	IAA	IBA	IAA	IBA	IAA	IBA
0.5	20	10	1.50±0.34	1.21±0.23	2.78±0.26	1.11±0.50
1.0	44	30	2.43±0.27	1.43±0.27	4.31±0.21	1.83±0.21
2.0	61	54	4.45±0.35	2.96±0.36	5.90±0.23	2.48±0.14
3.0	70	59	5.50±0.35	3.51±0.33	8.28±0.19	3.28±0.26
4.0	80	58	**7.91±0.54**	3.45±0.35	**11.6±0.22**	5.11±0.51
5.0	67	61	5.48±0.31	5.8±0.37	9.28±0.23	4.03±0.46
6.0	60	40	4.01±0.33	4.5±0.61	7.40±0.33	4.23±0.60
7.0	50	30	4.38±0.33	3.5±0.21	6.32±0.23	3.93±0.21

*Mean±SD is based on three replicates, each of which consists of 20 individual culture tubes. Values are not significantly different by the Tukey's multiple comparison test (P=0.05).

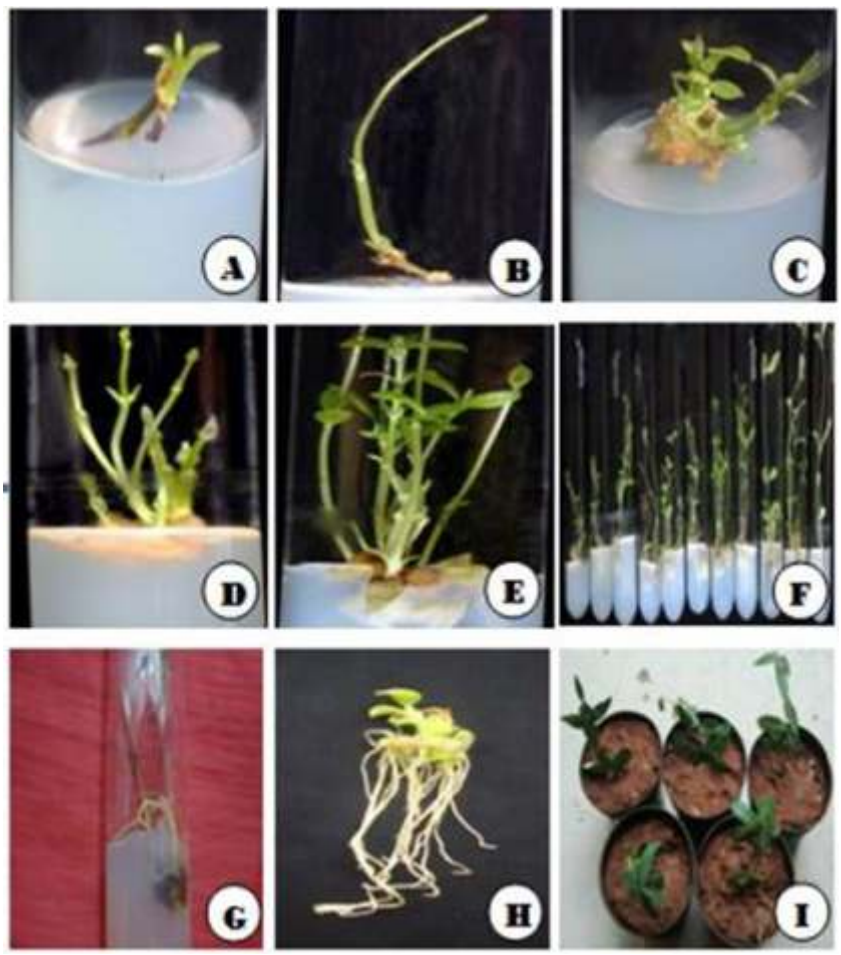

Plate 1(A-I). Various stages of i*n vitro* propagation of *Hemidesmus indicus*: **(A)**: Nodal explant inoculation**, (B&C):** Initiation and elongation of shoot from nodal explant**, (D&E)**: Enhanced shoot multiplication, **(F)**: Multiple shoot regeneration on MS+AS (4.0mg/l) + BAP (2.0mg/l) after 6 weeks of inoculation, **(G):** Initiation of roots on ½ MS+IAA (4.0 mg/l), **(H)**: Shoot with well developed roots, **(I):** *In vitro* grown plantlets prior to field transfer.

Patnaik and Debata 1996). In this investigation, about 78% of the plantlets survived and grew well in the green house without any morphological and phenotypic changes (Plate 1I). Thus the protocol described above is a rapid method for the large scale propagation and multiplication as well as conservation of *H.indicus*.

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