

In Vitro Multiplication of Indian Sarsaparilla, *Hemidesmus indicus* (L.) R. Br.–An Important Medicinal Herb through Shoot Tip Explants

Shivani Verma¹, B. D. Vashistha²

Research Scholar, Department of Botany, Kurukshetra University, Kurukshetra, Haryana, India¹

Professor, Department of Botany, Kurukshetra University, Kurukshetra, Haryana, India²

ABSTRACT: Large scale multiplication of Indian Sarsaparilla, *Hemidesmus indicus*-an important and rare medicinal herb has been achieved from shoot tip explants using *in vitro* culture technique. Multiple shoots were induced on Murashige and Skoog's medium supplemented with varying concentrations of auxins (IAA, NAA, 2,4-D, IBA) and cytokinins (BAP, KN) individually as well as in various combinations. The maximum number of shoots were regenerated on MS medium fortified with BAP (2.0 mg/l) and IAA (1.0 mg/l) in combination. The maximum average shoot length (5.0cm) was observed on KN (2.0 mg/l) with IAA (1.0 mg/l). Root induction on *in vitro* generated shoots was achieved on half-strength MS medium supplemented with IBA (0.5 mg/l). The *in vitro* raised plantlets were acclimatized successfully to pots containing a mixture of autoclaved soil, sand and compost in 2:1:1 ratio with 80% survival.

Abbreviations: BAP, 6-Benzyl aminopurine; KN, Kinetin, IAA, Indole-3-acetic acid; IBA, Indole-3-butyric acid; NAA, Naphthalene acetic acid; 2, 4-D, 2, 4-Dichlorophenoxy acetic acid.

KEYWORDS: Indian Sarsaparilla, *Hemidesmus indicus*, Asclepiadaceae, Multiple shoots, Auxins, Cytokinins.

I. INTRODUCTION

Hemidesmus indicus(L.) R. Br. (family-Asclepiadaceae) commonly known as Indian Sarsaparilla is a slender, laticiferous, twining, sometimes prostrate or semi-erect ethno medicinal shrub. It is distributed in moist localities throughout India. Roots are aromatic, emitting a sweet scent reminiscent of a combination of vanilla, cinnamon and almond [1]. The plant has been referred to as a significant medicine in ancient Indian literature and was included in the British Pharmacopoeia in 1864. It forms an ingredient of about 46 Ayurvedic preparations either alone or in combination with other drugs [2]. *Hemidesmus indicus* is effective as diuretic, antipyretic, astringent, blood purifier, diaphoretic, refrigerant and tonic [3] [4] [5]. The milky latex of the plant is used for relieving inflammation in the eye. *Hemidesmus indicus* can be used effectively as a treatment for high blood pressure, immune disorders and high stress. The plant is rare and getting endangered due to over exploitation [6]. Since conventional methods of propagation are very slow and inadequate to provide the planting material required for large scale cultivation. Therefore, there is an urgent need to develop alternative means for the propagation of genetically stable and true-to-type progeny of this species [7]. Plant propagation by means of tissue culture represents one of the possible approaches to overcome these problems. So, the present study has been designed to develop a suitable *in vitro* technique for rapid and large scale multiplication of this important endangered, medicinal plant species.

International Journal of Innovative Research in Science, Engineering and Technology

(A High Impact Factor, Monthly Peer Reviewed Journal)

Vol. 5, Issue 2, February 2016

II. MATERIALS AND METHODS

1. Plant material

The young plants were collected from “Dr.Sushila Tiwari Herbal Garden” Rishikesh (Uttarakhand) India. These were planted in the botanical garden of Kurukshetra University, Kurukshetra to provide explant source. Shoot tip explants (0.5-1.0cm) were excised from actively growing shoots and then washed in running tap water to remove dust particles. All the explants were washed with liquid detergent Teepol solution for 5 min. and again explants were washed several times with tap water followed by washing with distilled water to remove all the traces of detergent. Surface sterilization was carried out in Laminar Air Flow chamber by treating these explants with 0.1% (w/v) mercuric chloride solution for 2-3 minutes and subsequently washed with sterilized double distilled water to remove all the traces of mercuric chloride. The explants were trimmed aseptically to smaller segments and used. The surface sterilized shoot tip explants were inoculated on MS medium.

2. Culture media and culture conditions

Culture medium contains MS media salts, 3% sucrose and gelled with 0.8% (w/v) agar [Himedia laboratories, India]. The pH of the medium was adjusted to 5.8 by 1N NaOH and 1N HCl. The media were sterilized in an autoclave at 121°C temperature and 15 psi for 20 min. The cultures were incubated at a temperature of 25±2°C and a photoperiod of 16 hours (light intensity 3500 lux) and 8 hours of dark period.

3. Shoot initiation and multiplication

MS basal medium supplemented with various concentrations (0.5-4.0 mg/l) of auxins (IAA, NAA, 2, 4-D and IBA) and cytokinins (BAP, KN) alone and in combinations were used for shoot initiation and multiplication. All the cultures were transferred to fresh medium after 2-3 weeks to maximize shoot multiplication.

4. Root formation

The *in vitro* raised shoots (3.0-4.0cm long) were aseptically excised and implanted on the half strength MS medium supplemented with IBA and NAA (0.5-4.0 mg/l) individually for root development. Data on the number of roots were recorded after 4 weeks.

5. Hardening and acclimatization

Complete regenerated plantlets with sufficient roots were taken out from the medium and washed in sterile distilled water to remove the traces of agar. The plants were then transferred to small plastic cups (6cm diameter) containing sterilized soil, sand and vermiculite mixture (2:1:1). The cups were covered with polythene bags with small holes to maintain high humidity (80-90%) and kept in the culture room (Fig. 1F). The plantlets were irrigated with MS ¼ strength salt solution on alternate days. These plants were taken out from the culture room after 2 weeks and polythene bags were removed for 3-4 hours daily to expose the plants to natural conditions for acclimatization. After about one month, the surviving plants were shifted to the pots containing sterilized garden soil, sand and compost (2:1:1) and maintained in glasshouse to get acclimatized (Fig. 1G). After 4 weeks, the plants were transferred to the soil under natural conditions.

6. Statistical analysis

Each experiment was repeated three times and each treatment had 10 replicates. The number of explants exhibiting regeneration was identified and the number of shoots, shoot length, number of roots and root length per explant was determined. The data were analysed using one-way analysis of variance (ANOVA) and means were compared using Duncan's Multiple Range Test at 5% level of significance ($p < 0.05$).

International Journal of Innovative Research in Science, Engineering and Technology

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III. RESULTS AND DISCUSSION

Shoot tip explants cultured on MS medium devoid of growth regulators (control) failed to stimulate bud break and remained fresh and green for about 2 weeks but after that started to blacken and dry up. Medium supplemented individually with auxins and cytokinins induced formation of shoots and among the various concentrations, BAP (2.0 mg/l) was proved to be optimum for initiation of shoots in which about 4.8 shoots were produced with 80.5% response (Fig. 2a) and an average shoot length of 2.4cm (Table 1, Fig. 1B). As the concentration of BAP increases from 2.0 mg/l rate of shoot regeneration decreases. Although, shoot number was maximum at BAP (2.0 mg/l) but the maximum shoot length (4.2cm) was observed on IAA (2.0 mg/l) (Table 1, Fig. 1A).

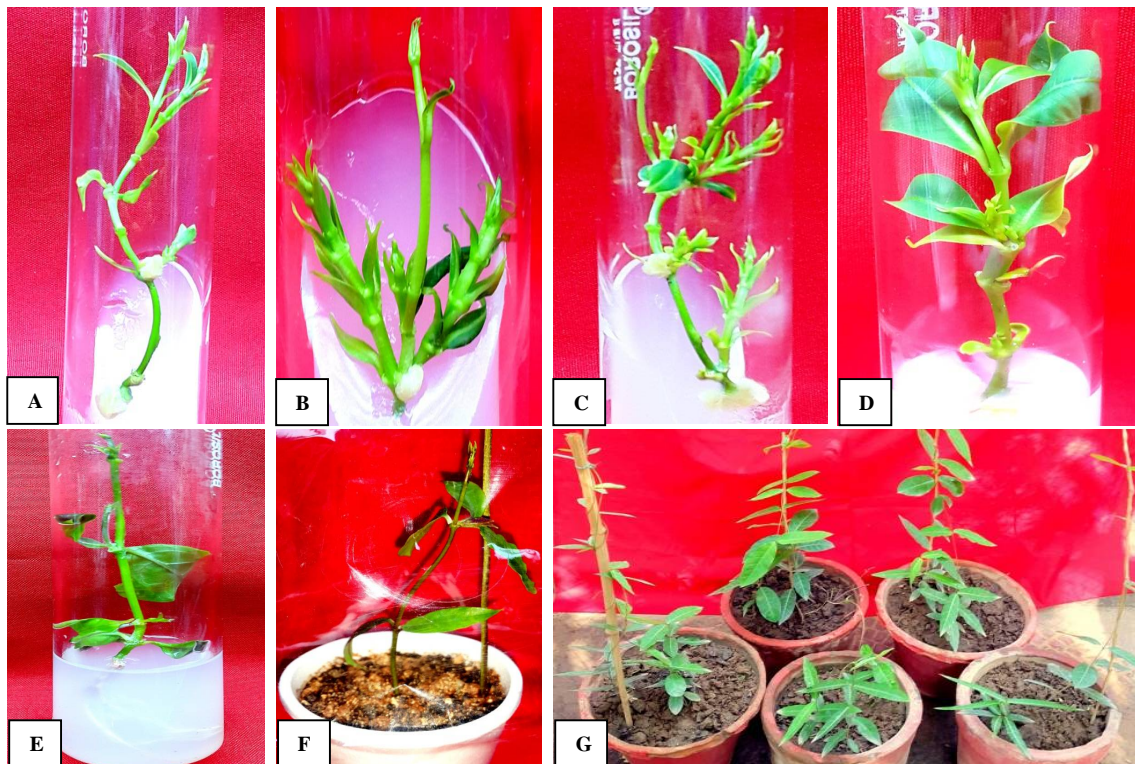


Fig. 1: Direct regeneration of shoots from shoot tip on MS medium fortified with auxins and cytokinins individually and in combinations.

(A) Shoot regeneration on MS+IAA 2.0 mg/l. (B) Shoot regeneration on MS+BAP 2.0 mg/l. (C) Shoot multiplication on MS+BAP 2.0 mg/l+IAA 1.0 mg/l after 5 weeks of culture in 2nd sub culture. (D) Shoot regeneration on MS+KN 2.0 mg/l+ IAA 2.0 mg/l. (E) *In vitro* regenerated shoots with well-developed roots on MS(half strength)+IBA 0.5mg/l. (F) Establishment and acclimatization of *in vitro* regenerated plantlets. (G) Hardening of plantlets in pots.

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Table-1 Effect of auxins and cytokinins on shoot tip explants of *Hemidesmus indicus* cultured on MS medium.

Medium and conc. of growth regulators (mg/l)	Days required for bud initiation (mean value)	% Response*	No. of shoots per explants*	Shoot length* (cm)	Callus growth
MS Medium	0.00	0.00	0.00±0.00 ^k	0.00±0.00 ^l	-
MS+IAA(0.5)	6.0	70.8	1.8±0.16 ^{ghi}	3.1±0.13 ^b	+
(1.0)	5.0	68.7	2.8±0.13 ^{cd}	2.7±0.12 ^c	+
(2.0)	5.4	77.7	3.0±0.05 ^c	4.2±0.16 ^a	+
(4.0)	4.0	72.2	2.5±0.12 ^{de}	2.2±0.10 ^{de}	+
MS+NAA(0.5)	8.2	72.2	1.4±0.09 ^j	1.5±0.09 ^{ij}	+
(1.0)	8.0	75.6	1.6±0.17 ^{hij}	1.8±0.11 ^{gh}	+
(2.0)	7.6	77.7	1.8±0.08 ^{ghi}	3.2±0.15 ^b	+
(4.0)	6.8	80.5	1.9±0.06 ^{gh}	2.1±0.08 ^{ef}	+
MS+2,4D(0.5)	0.00	0.00	0.00±0.00 ^k	0.00±0.00 ^l	-
(1.0)	0.00	0.00	0.00±0.00 ^k	0.00±0.00 ^l	-
(2.0)	0.00	0.00	0.00±0.00 ^k	0.00±0.00 ^l	-
(4.0)	0.00	0.00	0.00±0.00 ^k	0.00±0.00 ^l	-
MS+BAP(0.5)	6.5	83.3	2.3±0.13 ^{ef}	1.7±0.07 ^{hi}	-
(1.0)	6.0	84.7	3.5±0.15 ^b	2.0±0.13 ^{efg}	+
(2.0)	5.3	80.5	4.8±0.23 ^a	2.4±0.12 ^d	+
(4.0)	5.0	88.8	3.0±0.13 ^c	1.5±0.05 ^{ij}	+
MS+KN (0.5)	6.3	66.6	1.7±0.08 ^{ghij}	1.0±0.04 ^k	++
(1.0)	6.0	65.2	2.0±0.11 ^{fg}	1.4±0.06 ^j	++
(2.0)	5.5	72.2	2.5±0.13 ^{de}	1.9±0.09 ^{fgh}	++
(4.0)	5.5	70.8	1.5±0.09 ^{ij}	1.5±0.02 ^{ij}	++
LSD ($P<0.05$)			0.30	0.24	
ANOVA			$F_{20,42}=143.47$	$F_{20,42}=182.88$	

- *Values are Means ±S.E. of three independent experiments, each consisted of 10 replicates. Data were recorded after 10 weeks of culture.
- -No response, + Poor growth, ++ Moderate growth, +++ Good growth.
- Means values within a column sharing the same superscript are not significantly different at $P<0.05$ according to Duncan's Multiple Range Test.

The addition of BAP and KN with various concentrations of IAA significantly increases the frequency of shoot formation as compared to BAP and IAA alone, as maximum number of shoot buds was initiated in the BAP and IAA

combinations. Among all the tested growth regulator combinations, BAP (2.0 mg/l) with IAA (1.0 mg/l) was found to be best which promoted maximum shoot number (7.6) with an average shoot length of 4.8cm (Table 2, Fig. 1C).

International Journal of Innovative Research in Science, Engineering and Technology

(A High Impact Factor, Monthly Peer Reviewed Journal)

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Explants raised on KN (2.0 mg/l) with IAA (2.0 mg/l) supplemented medium produced 2.5 average shoots with an average shoot length of 3.3cm (Table 2, Fig. 1D). As the concentration of IAA increases from 1.0 mg/l to 4.0 mg/l in combination with BAP (2.0 mg/l), average number of shoots decreases (Table 2).

Table-2 Effect of auxins and cytokinins in combination on shoot tip explants of *Hemidesmus indicus* cultured on MS medium.

Medium and conc. of growth regulators (mg/ l)	Days required for response (mean value)	Per cent Response*	No. of shoots per explant*	Shoot length* (cm)	Callus growth
MS+BAP(2.0)+IAA(0.5)	5.2	83.3	2.2±0.11 ^d	3.8±0.13 ^b	+
	(1.0) 5.0	87.4	7.6±0.23 ^a	4.8±0.23 ^a	+
	(2.0) 5.3	80.5	5.6±0.09 ^b	2.2±0.07 ^e	+
	(4.0) 5.5	77.7	4.5±0.22 ^c	2.0±0.07 ^e	-
MS+KN(2.0)+IAA(0.5)	5.7	69.4	1.0±0.00 ^f	2.7±0.12 ^d	-
	(1.0) 6.0	66.6	1.5±0.05 ^e	5.0±0.18 ^a	-
	(2.0) 5.5	70.8	2.5±0.12 ^d	3.3±0.13 ^c	-
	(4.0) 5.6	63.8	1.2±0.08 ^{ef}	2.8±0.13 ^d	-
LSD (P<0.05)			0.39	0.41	
ANOVA			F _{7,16} =327.76	F _{7,16} =65.98	

- *Values are Means ±S.E. of three independent experiments, each consisted of 10 replicates per treatment. Data were recorded after 10 weeks of culture.
- -No response, + Poor growth, ++ Moderate growth, +++ Good growth.
- Means values within a column sharing the same superscript are not significantly different at P<0.05 according to Duncan's Multiple Range Test.

In the present study, shoot tips of *Hemidesmus indicus* did not respond on MS basal medium. This was in agreement with the results of *Peganum harmala* [8] and *Crataeva nurvala* [9] on MS basal medium. Application of exogenous growth regulators in present investigation had variable effects which varied with the type of growth regulator and their concentrations. The number and frequency of shoot length were mainly dependent on the combination of auxin and cytokinin used in the culture medium. The potential for shoot multiplication seems to be more in the presence of BAP with IAA in the culture medium. Some workers also suggest that formation of multiple shoots *in vitro* is a function of combination of BAP with IAA as in *Rauwolfia tetraphylla* [10] and *Withania somnifera* [11].

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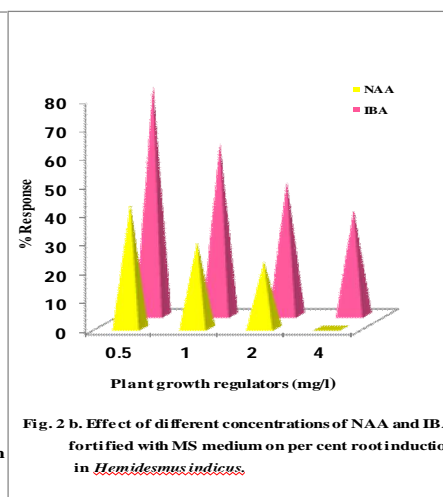
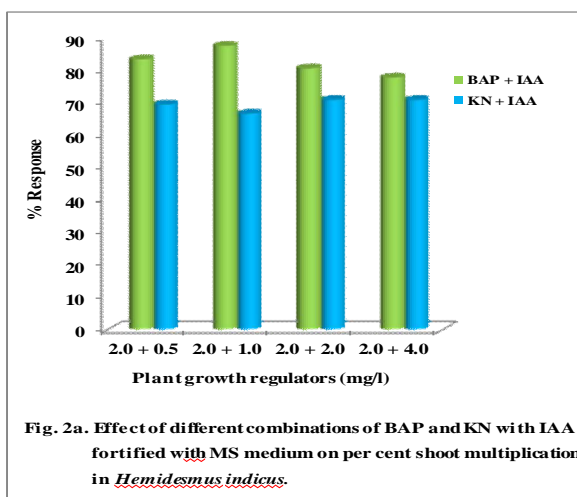
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Table-3 Effect of auxins on root induction of *in vitro* regenerated shoots of *Hemidesmus indicus* cultured on MS medium.

Medium and conc. of growth regulators (mg/l)	Days required for root induction (mean value)	Per cent Response*	No. of roots per shoot*	Root length* (cm)
MS Medium	0.00	0.00	0.00±0.00 ^f	0.00±0.00 ^g
MS Medium+NAA(0.5) (Half strength)	(1.0)	43.3	2.8±0.12 ^c	2.0±0.11 ^{de}
	(2.0)	30.0	2.2±0.09 ^d	1.8±0.10 ^{ef}
	(4.0)	23.3	1.5±0.08 ^e	1.5±0.06 ^f
	(4.0)	0.00	0.00±0.00 ^f	0.00±0.00 ^g
MS Medium+IBA(0.5) (Half strength)	(1.0)	80.0	5.0±0.18 ^a	4.5±0.16 ^a
	(2.0)	60.0	3.8±0.13 ^b	3.5±0.15 ^b
	(4.0)	46.7	2.6±0.11 ^c	2.9±0.12 ^c
	(4.0)	36.7	2.0±0.09 ^d	2.3±0.12 ^d
LSD ($P<0.05$)			0.325	0.329
ANOVA			$F_{7,16}=189.67$	$F_{7,16}=152.40$

- *Values are Means ±S.E of three independent experiments, each consisted of 10 replicates. Data were recorded after 10 weeks of culture.
- Means values within a column sharing the same superscript are not significantly different at $P<0.05$ according to Duncan's Multiple Range Test.



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In the present study, *in vitro* regenerated shoots rooted best on the medium fortified with IBA (Table 3, Fig. 1E, 2b). Similar observations have also been reported in *Bacopa monnieri* [12] and *Plectranthus amboinicus* [13].

IV. CONCLUSION

The present micropropagation protocol provides a method of rapid proliferation of shoots and roots of this species from shoot tip explants. The plantlets were easily acclimatized to the external environment and undergoing normal physiological development. This protocol ensures a successful and rapid technique which can be used for propagation of *Hemidesmus indicus* to minimize the pressure on wild populations and contribute to the conservation of valuable species in India.

V. ACKNOWLEDGEMENTS

Authors are thankful to Dr. Ashok Kumar Sharma, Head of the Non Wood Forest Product (NWFP) Division, Forest Research Institute (FRI) Dehradun, Uttarakhand for helping in procuring the plant material.

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