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A MICROPROPAGATION TECHNIQUE OF *HEMIDESMUS INDICUS* (ASCLEPIADACEAE), A VALUABLE MEDICINAL PLANT

SUSANTA KUMAR MAITY

Department of Botany,
Govt. General Degree College, KESHIARY,
PASCHIM MEDINIPUR-721135 (WEST BENGAL) INDIA
Email : smaity.bot@gmail.com

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ABSTRACT

An efficient protocol was established for *in vitro* clonal propagation of *Hemidesmus indicus* (Anantamul) belongs to the family Asclepiadaceae, a widely used medicinal plant through callus culture in using nodal segment. Yellowish nodular callus was observed from nodal segments on MS basal medium supplemented with 0.5 mg/L BAP + 0.2 mg/L NAA within four weeks of culture. Large number of shoots (11.4±0.2) and roots (8.2±0.4) were obtained when the callus was sub cultured on MS medium with 0.2 mg/L BAP. The regenerated plantlets were acclimatized by transferring them to soil. The survival rate of plantlets was found to be 90%. Regenerated plants were morphologically comparable having normal leaf shape and growth.

Figures : 05 References : 16 Tables : 02
KEY WORDS : Callus culture; *Hemidesmus indicus*; Medicinal plant; Regeneration; Shoot proliferation;

Introduction

Hemidesmus indicus belongs to the family Asclepiadaceae and it is commonly known as Anantamul (*endless root*). The stem and branches of *H. indicus* are twine anticlockwise and are profusely laticiferous, elongate with the surface slightly ridged at the nodes. Roots are woody, slender and aromatic. Roots smell similar to camphor hence the plant is also known as *Kapoori*. The plant is used to cure leprosy, leucoderma, itching and skin disease, asthma, bronchitis, leucorrhoea, dysentery, piles, syphilis and paralysis, promotes health and cures all kinds of diseases caused by vitiated blood[9]. This plant is found throughout India growing under mesophytic to semi dry conditions in the plains and up to an altitude of 600 m. It is highly recommended for the treatment of snake bite by blending with other drugs. The root of this plant is sweet in taste due to presence of essential oil. The other phytoconstituents present in the plant are â -sitosterol, á and â amyrins, lupeol, tetracyclic triterpenes, fatty acids, tannins and glycosides. The overexploitation of *Hemidesmus indicus* is becoming rare and getting endangered[4,13]. The roots are harvested in autumn and dried for later use. Huge quantities of plant materials were imported for the manufacture of Ayurvedic, Unani and Homeopathic medicines. It is hoped that a standard protocol for *in vitro* propagation of this plant by using different explants for the production of secondary metabolites. The conventional propagation is through seeds and stems cuttings and is not adequate to meet the demand of the plant. Therefore *in vitro* propagation methods through plant tissue culture can be an alternate way of producing large scale plantlets of this medicinally important plant species. A standard protocol to induce multiple shoots in culture may provide a more homogenous source of plants. The protocol developed in the present study for shoot regeneration from indirect organogenesis is a simple, economical and effective in induction of multiple shoots by using nodal explants for potential application in large scale propagation and conservation.

Hence, it is felt that there is a great need of large-scale propagation of this medicinal plant. Micro

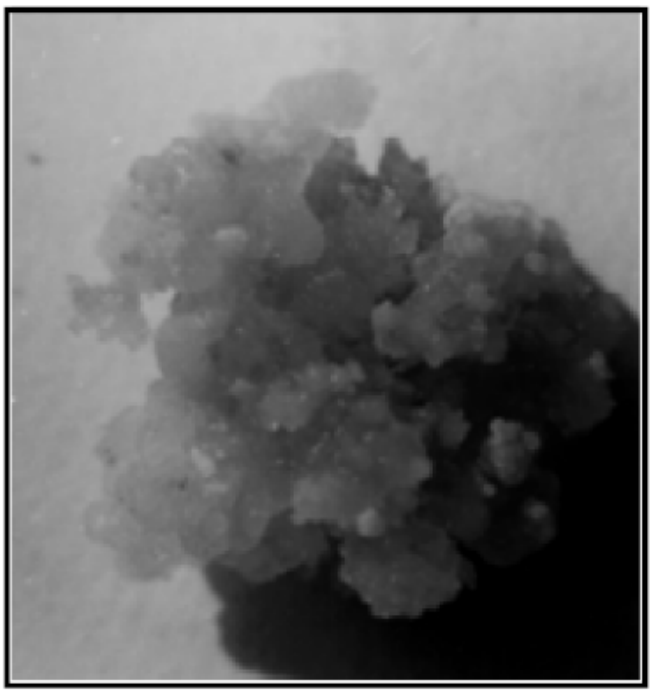


Fig. 1: Profuse growth of callus on MS medium containing BAP (0.5 mg/l)+ NAA (0.2 mg/l) within 4 weeks of culture

TABLE-1 : Effect of different concentrations and combinations of growth regulators on MS medium for the callus initiation and embryo formation from the nodal explants of *Hemidesmus indicus*. Results are the mean of 6 replicates±SE.

MS basal medium		% of explants producing callus	Total no. of embryoids
BAP(mg/l)	NAA(mg/l)		
0.2	0.2	89	102.5±0.6
0.5	0.2	91	135.2±0.4
1.0	0.2	52	62.3±0.6
0.2	0.5	66	31.3±0.2
0.5	0.5	61	-
1.0	0.5	61	-
0.2		12	-
0.5		09	-
1.0		09	

propagation of a pharmaceutically superior *H. indicus* would provide large amount of highly uniform plantlets suitable for further propagation in the field. *In vitro* propagation has proved as a potential technology for mass scale production of plant species[2,8]. The present study was, therefore, undertaken to develop a suitable protocol for *in vitro* propagation of this important medicinal herb through callus culture.

Materials and Methods

1. Plant Material

The plant material of *Hemidesmus indicus* was collected from the experimental garden. The tender twigs with 3-4 nodes cuttings were excised and the plant samples were treated with Tween 20 solution for 10 min. The plant material was kept under running tap water for 30 min and subsequently was washed with distilled water. The explants were finally surface sterilized with 0.1% $HgCl_2$ for 5 min. Excised explants for 1.0-1.5 cm were inoculated on MS medium containing various growth regulators.

2. Media preparation:

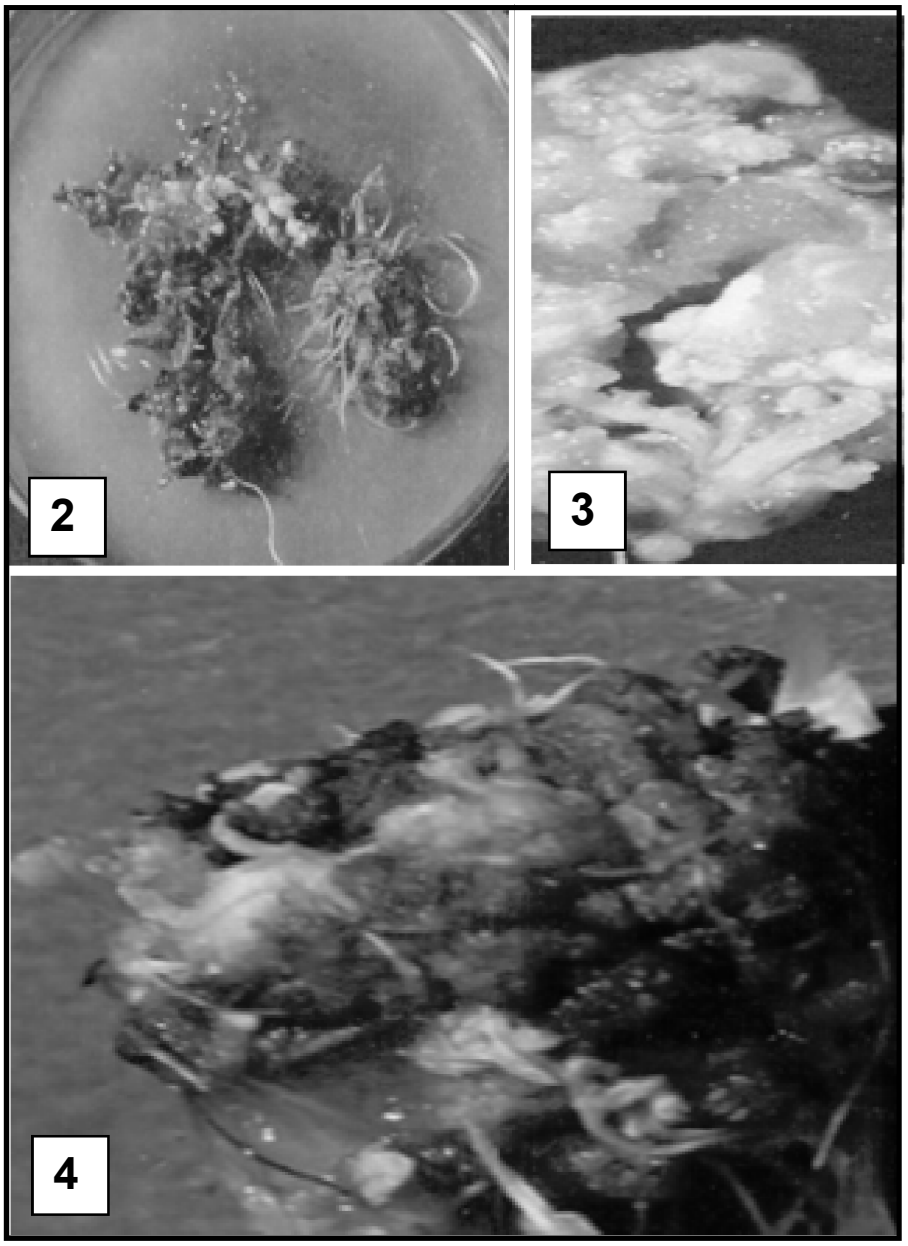
The explants were cultured in the MS medium supplemented with different combinations and concentrations of growth regulators such as 6-benzyl amino purine (BAP) and á-naphthalene acetic acid (NAA). The MS medium also contained 3% sucrose and 0.5% (w/v) agar. The pH of the media was adjusted to 5.8 ± 0.1 before autoclaving. The culture vessels containing the media were autoclaved at 121ºC and 15 pound per square inch pressure for 17 minutes. Cultures were grown at 22-24^{0}C with a relative humidity of 50-60% and a photoperiod for 16h per day provided by fluorescent tube (12000 lux).

3. Acclimatization:

Healthy rooted plantlets were taken from the medium and washed several times with sterile distilled water to remove the medium. Plantlets were potted in soil and were kept under controlled temperature at 22-26^{0}C and light conditions in the culture room. After 2 weeks when new leaves emerged from such plantlets, they were taken outside the culture room and kept in a shady place under normal temperature and light.

Results and Discussion

The nodal explants were used for the induction of callus of *Hemidesmus indicus*. Initially callus formation was observed from the cut ends and the epidermal surfaces of the nodal explants as protuberances within 10-12 days. The whole explants were gradually covered by callus within four weeks of culture. BAP in the range of 0.2-1.0 mg/l and NAA 0.2-0.5 mg/l in different combinations were tested for callus induction. Of all the combinations and concentrations of growth regulators tested, MS medium supplemented with BAP (0.5 mg/l) and NAA (0.2 mg/l) proved to be the best with regard to callus formation and their further development into



Figs. 2,3& 4 : Formation of shoots and roots of callus on MS medium containing BAP (0.2 mg/l)

embryoids. In this medium 91% of the explants were responses to induce callus initiation. Higher and lower concentrations of BAP and NAA were not more effective to induction of callus and their subsequent development. However BAP with NAA was found to be more effective than BAP alone for callus induction (Table-1).

Visual identification and selection of embryogenic sectors and removal of non-embryogenic portions maintained the embryoids developed from friable callus during subcultures at two weeks interval. After 6 weeks, the differentiated calli were again subcultured on MS medium supplemented with lower concentrations of BAP (0.2 mg/l) for the development of the embryoid, maturation of embryoids and plant regeneration. The globular structures of embryos appeared on the surface of the pro-embryogenic callus gradually became enlarged to a detectable size within 9-10 weeks of culture. The maximum number of globular embryoids was recorded on the MS medium containing 0.2 mg/l BAP after 10 weeks of culture (Table 1).

Maximum numbers of germinated embryos were observed at the end of second week after transferring the embryos on the same MS medium. Of the various medium tested, MS medium containing BAP (0.2 mg/l) were more effective for germination of embryos (Table-2). Shoot differentiation and profuse shoot formation was found to be best from callus on the same medium after twelve weeks of culture. The optimal concentration of BAP (0.2 mg/L) showed maximum shoot response with mean of 11.4 shoots per culture and attained their shoot length of 4.2 ± 0.4 cm and maximum root response with mean of 8.2±0.4 roots per culture and attained their root length of 2.4 ± 0.2 cm after 6 weeks of culture. Remarkable decrease in response was observed at higher level of BAP or in combination with NAA (Table-2). The lower concentration of BAP (0.1 mg/L) contributed to response with mean of 5.5±0.3 shoots per culture and mean length of 3.3±0.5 cm. At higher concentration (1.0 mg/l) showed the response with mean of 2.2±0.6 shoots per culture and an average shoot length of 2.4±0.3 cm. The % of response was more at lower level than at higher concentrations of BAP. There were differences in regeneration frequencies, number of shoots per culture and length of shoots per culture in different combination and concentrations of growth regulators. The regenerated plantlets with well developed root systems were removed from the culture medium. They were subsequently hardened and acclimatized. The *in vitro* raised shoots grew vigorously and developed without any visible deformities. An almost 90% survival of the transplanted

TABLE-2 :. Effect of different concentrations and combinations of growth regulators on MS medium for the adventitious shoot and root regeneration from the callus of *Hemidesmus indicus.* Results are the mean of 6 replicates ±SE.

MS basal medium		Mean no. of shoots/culture (±SE)	Mean length of shoots (cm) (±SE)	Mean no of roots/culture culture(±SE)	Mean length of roots (cm) (±SE)
BAP(mg/l)	NAA				
0.1		5.5±0.3	3.3±0.5	5.3±0.3	2.2±0.5
0.2		11.4±0.2	4.2±0.4	8.2±0.4	2.4±0.2
0.4		8.8±0.5	4.6±0.4	6.4±0.3	2.8±0.4
0.6		6.4±0.5	4.1±0.1	5.4±0.3	2.6±0.2
0.8		6.4±0.2	3.6±0.5	4.4±0.3	2.2±0.4
1.0		2.2±0.6	2.4±0.3	4.6±0.2	2.2±0.8
0.2	0.5	3.4±0.2	4.2±0.4	5.8±0.4	2.6±0.7
0.4	0.5	3.1±0.4	4.1±0.6	5.8±0.4	2.6±0.6
0.6	0.5	2.6±0.3	2.2±0.3	5.6±0.4	2.2±0.3
0.8	0.5	-	-	-	-
1.0	0.5	-	-	-	-

Fig: 5. A complete plantlet grown in the culture tube

plantlets of *Hemidesmus indicus* was observed in field.

The MS medium containing and NAA (0.2 mg/l) proved to be the best with regard to callus formation and their further development into embryoids, the concentrations of cytokinin and auxin are also known to be critical in the induction of differentiating callus and their subsequent proliferation. BAP, which has been used as a source of cytokinin in the present study, has also been used successfully to induce embryogenesis in several plants[1,12]. In the present study, it has been found that the higher concentration of cytokinin (0.5 mg/l BAP) was necessary for the initiation of the callus although low concentration (0.2 mg/l BAP) was needed for the proliferation and differentiation of the calli. Mostly auxins or substances having auxin like activity were reported to be effective for inducing embryogenesis in different plants[5,10]. The auxin-cytokinin combination, which was found to be effective in inducing embryogenesis in the present investigation, was also used in some species like *Mussaenda philippica* var. *aurorae* for the induction of embryogenesis[7] and *Mucuna pruriens* var. *utilis*[14]. Three weeks old germinating embryoids when transferred to

germinating medium consisting of MS basal medium were converted to plantlets within five weeks of transfer. This is probably due to restoration of endogenous hormone balance necessary for normal plantlet development. The method of callogenesis and their development were shown in the present investigation demonstrates the potentiality of the nodal explants for generating highly proliferative, embryogenic cultures for *Mentha arvensis*. Since *Mentha* is a medicinal plant, this approach can be useful in maintaining the germplasm of the genus for future conservation. Such indirect organogenesis was reported in many medicinal plant species including P*hellodendron amurense*[2], *Abrus precatorius*[3], *Plumbago zeylanica*[6], *Scoparia dulcis*[8] and *Rotula aquatica*[11].

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