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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 15 (10): 477-483, 2012
ISSN 1028-8880 / DOI: 10.3923/pjbs.2012.477.483

In vitro Micropropagation of *Desmodium gangeticum* (L.) DC (Fam-Fabaceae): A Medicinal Legume through Axillary Bud Multiplication

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Abstract: Medicinal plants possess unlimited and untapped wealth of chemical compounds with high drug potential which make these plants useful as sources of biomedicines. The rising demand for herbal medicines in the organized manufacturing sector has ruthlessly exploited the wild growing plant population those have bulk use. So for high rate multiplication of different medicinal plants, it is necessary to standardize the protocol for high regeneration. The efficiency of any regeneration is primarily depends on factors like type of explants used, composition of the medium and type of genotype. Here, we have developed a regeneration protocol of *Desmodium gangeticum* (L.) DC (Salparni, Fam- Fabaceae) a medicinal plant through axillary bud multiplication. Nodal explants from *Desmodium gangeticum* plants were cultured on Murashige and Skoog's basal medium with Kn or BA at different concentrations. 0.5 mg L^{-1} BA in the medium, showed shoot multiplication. Regenerated shoots measuring 3 cm or more were excised and planted on semi solid basal medium supplemented with varying concentrations of either IAA or IBA for induction of rooting. IBA treatment at 1.0 mg L^{-1} was the best eliciting 100% rooting response. The *in vitro* propagation protocol standardized can be highly useful in raising quality planting materials of *Desmodium gangeticum* for commercial plantation programmes and germplasm conservation.

Key words: *Desmodium gangeticum*, axillary bud, micropropagation, secondary passage, medicinal plant

INTRODUCTION

From ancient times plants and plant parts are being used as medicines to treat various diseases or other health problems. Medicinal plants constitute an important resource, used by the indigenous medicinal system which is about 3000 years old. The demand for plant based raw materials used predominantly in the pharmaceutical and cosmetic industries have grown enormously during the recent years. About seventy percent of these plant based raw materials for the pharmaceutical and cosmetic industries come from the subtropical and tropical parts of the world (Singh and Chowdhery, 2002).

Recently World Health Organization (WHO) has compiled a list of 20,000 medicinal plants used in different countries. However, approximately 10,000 plants are used for phyto-therapy in Indian system of medicine. According to Biological Conservation Letter (Villa-Lobos, 1994), more than 7,000 species of plants found in various ecosystems are said to be medicinal in the country. The global market for medicinal plant materials and herbal medicines is estimated to be worth several billion dollars a year. International export trade in medicinal plants is dominated by China which exports 1,21,900 tonnes of materials a year and India exports 32,600 tonnes annually (Rajasekharan and Ganeshan, 2002). So, India is one of the world's richest sources of medicinal and aromatic plants. The position of India is tenth among plant rich countries of the world and fourth among the Asian countries.

Medicinal plants possess unlimited and untapped wealth of chemical compounds with high drug potential, which make these plants useful as sources of biomedicines. About 80% of the population in India and other developing countries depend on traditional medicine mostly from plant origin. In India 2,500 species belonging to more than 1000 genera, are regularly used in production of Ayurvedic, Unani, Siddha and Tribal medicines (Rajasekharan and Ganeshan, 2002). The Central Council of Research on Ayurveda and Siddha Medicine has drawn a list of 2430 commonly used medicinal plants having greater demand for manufacture of galenicals, mixtures, compound formations and patent medicines (Gupta, 1998). About 75% of these plants are

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collected from tropical and 25% are from temperate forests. For the preparation of indigenous system of medicine, 30% of materials are roots, 14% bark, 16% whole plants, 5% flowers, 10% fruits, 6% leaves, 7% seeds, 2% wood, 4% rhizomes and 6% stems are used (Singh and Chowdhery, 2002).

Now days, demand for plant-based medicine in both developed and developing countries like India is increasing because of the growing recognition that those herbal medicines are non-narcotic. The rising demand in the organized manufacturing sector has ruthlessly exploited the wild growing plant population those have bulk use (Nayar and Sastry, 1987, 1998).

Unfortunately, it is observed that, besides other factors like urbanization, industrialization, development projects, etc., many such useful plants run the risk of extinction, due to over-exploitation. It was reported that out of 2,800 plants, used in traditional medicines practiced in different parts of our country, the drug potential of only 5% of these plants have been studied chemically or pharmaceutically (Sabnis and Daniel, 1990). Of great concern is the fact that before their medicinal values are fully known, many plants may be lost from the local floras. The urgency and seriousness of the problem has rightly deserved and drawn worldwide attention.

Legumes have a long history of use in agriculture. The family includes herbs, shrubs, trees and vines distributed throughout the world, especially the tropical rain forest. Along with uses in agriculture, human food and animal forage, timber and dye legumes are having a greatest value of medicines and can be utilized and explored for its medicinal value. Worldwide, there are a total of 790 genera and 17,600 species of the legumes, of these, there are 163 genera and 1,252 species that are used as sources of medicinal plants. Among the sources of Oriental herbal medicines, the Leguminosae is the fourth largest family in terms of numbers of medicinal genera and species that are used, following the Gramineae (grasses, grains), Compositae (daisies, dandelions) and Orchidacea (orchids). Very few number of medicinal legumes are encouraged for cultivation, still there is large number of plants (wild and endemic) are now unexplored and unattended (Hu, 1980; Wee and Keng, 1992; Ling, 1995).

Desmodium gangeticum (L.) DC (Syn. *Hedyserum gangeticum*) of Fabaceae is known as Salaparni or Prishniparni in Hindi and Sanskrit is a medicinal legume. It has multiple uses in the Ayurveda system. Three pterocarpenoids namely gangetin, gangetinin and desmodin were isolated from hexane extract of roots of *D. gangeticum* (Purushothaman *et al*., 1971). During routine pharmacological screening gangetin exhibited significant anti-inflammatory and anti-fertility activity in albino rats (Ghosh *et al*., 1983; Pillai *et al*., 1981). The plant is commonly used as bitter tonic, digestive, anti-catarrhal, anti-emetic, in inflammatory conditions of the chest and others (Nayar *et al*., 1956). The plant also shows anthelmintic, aphrodisiac, astringent, diuretic properties. Roots are chewed daily for the cure of typhoid and pneumonia. The plant parts form part of the compositions of popular Ayurvedic medicines such as Dasamula Kwatha (M/s Zandu Pharmaceuticals), Dashmularishta (Dabur, Himalayan Drugs).

In vitro propagation system proved to be highly useful for high rate multiplication than the clonal propagation by conventional means (Hunter, 1986). Now, methods for micropropagation of high alkaloid producing lines were established and routinely followed (Schoner and Reinhard, 1986) as in other medicinal plants like *Atropa* (Staba and Chung, 1981) and *Dioscorea* (Heble and Staba, 1980). It was found that *Desmodium gangeticum* grows mainly in South East Asia and Northern Australia, is a rare species, mostly found in Sal forest, shows annual seed set (in the month of March-May) and low rate of multiplication in the natural condition. Due to its high medicinal value *D. gangeticum*, there is an urgency to develop appropriate technique for mass propagation of this valuable species and to domesticate it for future use. In the present study a protocol for regeneration and mass propagation of *D. gangeticum* has been established using nodal explants.

MATERIALS AND METHODS

Plant material and surface sterilization: Healthy plants of *Desmodium gangeticum* (L) DC were collected, grown in the nursery beds of the experimental gardens. For the purpose of micropropagation young shoots were harvested from 3-6 months green house grown plants. These shoots were defoliated. The first 2-3 nodes from the apical region and 1-2 nodes from the basal region of these shoots/branches were discarded. The stem node segments (0.8-1.2 cm) were cut with the help of a clean razor/blade/scalpel so that each contained a dormant axillary bud. These nodal explants were washed under running tap water for another 5-10 min. The explants were surface-sterilized in batches of 15-20 in an aqueous solution of 0.1% (w/v) mercuric chloride ($HgCl_2$) for about 7-10 min. Following surface sterilization all the explants were rinsed 3-4 times in sterile distilled water and inoculated on to the surface of sterilized nutrient agar media.

Culture medium: Murashige and Skoog (1962) basal medium was supplemented with various concentrations of Benzyladenine (BA) and Kinetin (Kn) with 2% (w/v)

sucrose and gelled with 0.8% (w/v) agar (Bacteriological Grade, Himedia, Mumbai, India). The pH of the medium was adjusted to 5.8 by addition of 0.1 N KOH/NaOH or 0.1 N HCl.). Three to five explants were transferred onto each culture flask containing 30 mL of medium. For each treatment at least 15 replicates were maintained.

All the cultures were incubated in a growth room with a 16 h photoperiod (cool, white fluorescent light (30 μmol m sec^{-1}) and the temperature maintained at 25±2°C, with 50-80% relative humidity.

Proliferated microshoots were separated and those measuring 2-3 cm and above were individually planted onto the basal MS medium with or without the supplementation of auxins (0.25-1 mg L^{-1}) like IAA (indole-3-acetic acid; Sigma, USA) or IBA (indole-3-butyric acid, Sigma, USA) for rooting.

Plantlets with healthy root systems were washed free of the agar gelled medium (especially the root portions) under running tap water, dipped for 4-5 min in an antifungal solution (0.1% w/v `Bavistin', Bayer India) and transplanted in small (5 cm diameter) plastic tea cups, earthen pots or poly-bags containing autoclaved sand:soil:compost/farmyard manure (1:1:1). The pots were watered with 5 mL of ¼ strength MS major and minor salts on alternate days for the first week and hardened under a polyethylene tent in the greenhouse for at least one week. Relative humidity was 85% at 28-30°C under daylight conditions (during May-July). After hardening for 7-10 days the plants were transferred to bigger pots (25 cm diam.) containing a non-sterile sand: soil: compost mixture (1:3:1) and shifted to field conditions.

Scoring of data: All the data observed, collected and documented on axillary bud multiplication, organogenesis and somatic embryogenesis were statistically analyzed by mean, standard error and some of the results were compared by DMRT (Duncan, 1955). Results are presented in Mean±SE of three independent experiments each with 20 replicates in order to find out the accuracy of results.

RESULTS

Response of nodal explants of *Desmodium gangeticum*: The response of nodal explants of *Desmodium gangeticum* on different culture medium has been studied through establishments of culture and bud break response of nodal explants with axillary bud. Data were recorded on multiplication and proliferation of shoot buds, shoot growth, quality of shoots, rooting and plantlets establishment.

Culture establishment and bud break response through nodal explants with axillary bud multiplication and proliferation of shoots: The response of *Desmodium gangeticum* nodal explants cultured on different shoot multiplication media during the first initiation passage over a period of three weeks has been presented in Table 1. The data in Table 1 include the percentage of explant response, days required to bud break response, number of shoots formed per responsive explant and the shoot growth in terms of shoot length and number of nodes formed per such developed shoot.

Culture medium devoid of growth regulators (control) failed to stimulate the bud break response in the cultured explants even when the cultures were maintained beyond the normal observation period of three to four weeks. Higher levels of the cytokinin (0.25-1.5 mg L^{-1} of Kn or BA) elicited bud break response in varying percentage of the cultured explants. Depending upon the concentrations of Kn or BA tested, the proportion of explants showing bud break varied from 41±0.577 to 100% and the number of days required for such a response also varied from 4-10 days (Table 1). The best response was obtained

Table 1: Shoot formation on stem node explants of *Desmodium gangeticum* cultured on semi solid in the first passage after second week

Cytokinin	Callus response (%)	Days to bud break	Mean No. of shoots	Shoot length	No. of nodes
Control	NR	NR	NR	NR	NR
Kinetin					
0.1	**41±0.577^{e}**	**9.33±0.666^{a}**	**1.00±0.00^{f}**	**1.43±0.035^{d}**	**2.26±0.005^{d}**
0.25	71±0.577^{c}	7.0±0.577^{b,c}	1.09±0.005^{bc}	1.52±0.005^{b,c,d}	2.38±0.005^{d}
0.5	100±0.00^{a}	6.00±0.577^{c}	1.20±0.057^{bc}	1.72±0.005^{b}	4.66±0.881^{a,b}
1.0	100±0.000^{a}	6.00±0.577^{c}	1.53±0.033^{a}	1.71±0.005^{b}	2.41±0.005^{d}
1.5	100±0.000^{*a}	8.66±0.881^{a,b}	1.49±0.057^{a}	1.63±0.006b^{c}	2.67±0.005^{c,d}
Benzyladenine					
0.1	50.46±0.260^{d}	5.33±0.881^{c}	1.00±0.00^{f}	1.54±0.176^{b,c,d}	2.90±0.057^{b,c,d}
0.25	91±0.577^{b}	5.50±1.040^{c}	1.26±0.033^{b}	1.55±0.026^{b,c,d}	3.00±0.577^{b,c,d}
0.5	100±0.00^{a}	5.00±0.577^{c}	1.56±0.181^{a}	2.9±0.057^{a}	5.50±0.288^{a}
1.0	100±0.00^{*a}	5.00±0.577^{c}	1.56±0.033^{a}	1.59±0.005^{b,c,d}	4.33±1.201^{a,b,c}
1.5	**100±0.00^{*a}**	**5.66±0.333^{c}**	**1.50±0.057^{a}**	**1.48±0.005^{c,d}**	**3.00±0.577^{b,c,d}**

Data (Mean±SE) of three independent experiments each with 20 replicates, NR: Not responded, Means followed by the same letter within the column are not significantly different at $p<0.05$ as tested by the multiple range test of Duncan (1955) *Callus formation Shoots measuring <0.5 cm not taken into account for calculation of shoot length, Highest and least response are presented in bold

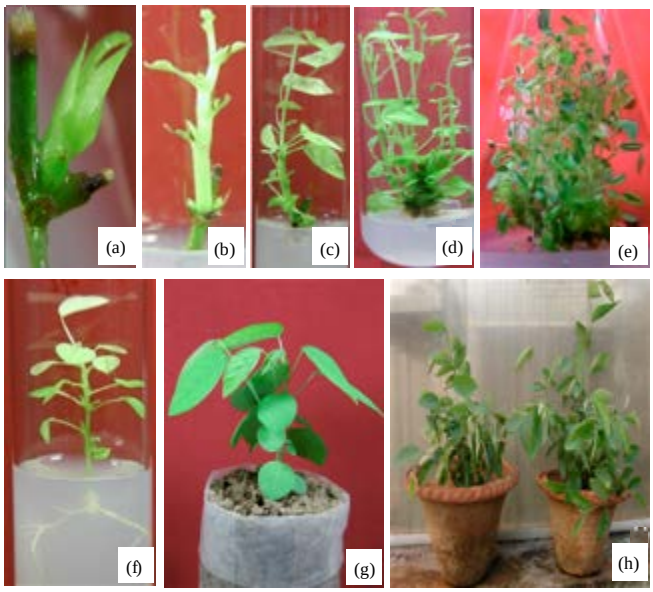

Fig. 1(a-h): *In vitro* morphogenesis of *Desmodium gangeticum* and their derived plants, (a) Axillary shoot bud from nodal explant after 1 week, (b-c) Nodal explant with well developed shoots, (d) Nodal explant with multiple well developed shoots, (e) Nodal explant with multiple shoots after second passage, (f) Excised shoots showing well developed roots, (g) Fully developed plantlets after 6 week of hardening on sand:soil (1:1) on poly bag and (h) Fully developed plantlets after 12 weeks of hardening on sand:soil (1:1) on pots

when the basal medium was supplemented with 0.5 mg L^{-1} of BA (Fig. 1a). In this medium, an average of 5.00±0.577 days required for bud break and 1.56±0.181 mean number of shoots having 2.9±0.057 cm shoots length and 5.50±0.288 number of nodes were produced (Fig. 1b). The least response obtained in Kn 0.1 mg L^{-1}. The response was 41±0.577% and 9.33±0.66 number of days required for bud break (Table 1). The explants from the control set when transferred any time within the second week of incubation on to fresh medium containing either Kn or BA also exhibited bud break response.

Shoot multiplication: The potential for shoot multiplication in *Desmodium gangeticum* appeared to be very strong in the presence of a cytokinin like Kn or BA in the culture medium. In all cultures where Kn or BA supplement was used at least one shoot emerged per axillary bud soon after the bud break (Fig. 1b, c). During the third week more number of shoot buds (about 1-5 buds) appeared around the main shoot on some explant depending on the concentration of the cytokinin used. Normally, when the cytokinin used was Kn, the shoots formed in the cultures were healthy with thicker shoots and well-formed leaves (Fig. 1d). On the other hand, shoots formed in medium containing BA were thinner with smaller leaves. Although the mean number of shoots obtained per explant with BA treatment was slightly higher than that with Kn there was not much difference in the overall shoot growth i.e., both in terms of mean shoot length and the mean number of nodes per shoot (Table 1). Treatment wise both the cytokinins BA and Kn appeared to be most effective when used singly at 0.5 mg L^{-1} concentration. At higher concentrations of BA and Kn the explants occasionally formed small mass of callus.

After the primary culture period of three weeks the shoots attained a length of about 1.5 to 3 cm. At this stage the main shoot was aseptically excised/removed and used for rooting experiments. The explants following removal of the shoots were recultured on fresh media supplemented with either 0.5 mg L^{-1} of Kn or BA and maintained for about 4 weeks for the second passage. During the second passage the shoot forming potentiality of these residual explants appeared to be much stronger than that of the fresh explants during the primary culture passage. The response of these explants has been presented in Table 2. Explants recultured on simple basal medium devoid of cytokinin produced 3-5 shoot buds within the first week. The shoot multiplication rate

Table 2: Shoot formation on stem node explants of *Desmodium gangeticum* in the second passage after excision of shoots

Treatment (mg L^{-1})	No. of shoots	Shoot length	No. of nodes
Control	2.80±0.057^{c}	3.02±0.005^{b}	4.05±0.028^{a}
MS with 0.5 Kn	5.76±0.088^{b}	2.38±0.004^{c}	3.22±0.005^{c}
MS with 0.5 BA	33.80±0.057*^{a}	3.85±0.071^{a}	3.97±0.005^{b}

Data (Mean±SE) of three independent experiments each with 20 replicates, Means followed by the same letter within the column are not significantly different at $p<0.05$ as tested by the multiple range test of Duncan (1955), *Callus formation. Shoots measuring <0.5 cm not taken into account for calculation of shoot length

remarkably improved as soon as the basal medium was supplemented with 0.5 mg L^{-1} of Kn or BA. Thus after a 4-week period of second passage, with Kn, the shoot multiplication rate increased up to about 5.76±0.088 shoots per explant (Fig. 1e) and to as high as 33.8±0.057 shoots per explant with BA (Table 2). Unlike in the cultures with Kn treatment, invariably there was the formation of callus at the cut shoot base prior to the formation of multiple shoot buds in all cultures with BA treatment. In general, after culture period of 5 weeks the shoot multiplication as well as the shoot growth was more pronounced with BA treatment than Kn treatment.

Shoot growth and quality: The overall growth response of the shoots regenerated on various media during both the primary passage and the second passage was recorded in terms of mean shoot length and mean number of nodes per shoot (Table 1, 2).

Shoot growth on nutrient media supplemented with lower levels of cytokinins (0.1 mg L^{-1} of BA or Kn) was visibly poor during the primary passage, where the mean shoot length did not exceed 1.5 to 2 cm after three weeks of observation period. These shoots had only 1-3 nodes on an average. Growth of the regenerated shoots was optimal at a concentration of 0.5 mg L^{-1} of BA (2.9±0.057 cm length with 5.50±0.288 nodes) followed by that of 0.5 mg L^{-1} of Kn (1.72±0.005 cm length with 4.66±0.881 nodes) in the nutrient medium (Table 1). The appearance of the shoots was also more or less normal.

During the second passage shoots produced on Kn (0.5 mg L^{-1}) media were had a somewhat stunted appearance with shorter internodes (2.38±0.004 length with 3.22±0.005 nodes) and thinner leaves but when these were sub cultured on media with lower Kn level (0.25 mg L^{-1}) the shoots became normal after about a 2 weeks growth period. On the other hand, shoots produced on BA (0.5 mg L^{-1}) supplemented media were taller and had longer internodes (3.85±0.071 length with 3.97±0.005 number of nodes). But such shoots had a somewhat hyper hydrated or vitrified look. However, when such shoots were transferred to media devoid of cytokinin or with lower levels of BA or Kn (0.1-0.5 mg L^{-1}) the normal growth resumed and the appearance of the shoots improved gradually. Irrespective of the concentration of the cytokinin used in the medium there was a considerable degree of leaf fall from the developing shoots. However, in spite of the leaf fall these shoots did not die and behaved normal during the rooting experiment.

Table 3: Root formation of *Desmodium gangeticum* affected by various concentrations of IAA and IBA

Treatment (mg L^{-1})	Callus response (%)	Days to rooting	No. of root	Length of root
Control	NR	NR	NR	NR
IAA				
0.25	0	0	0	0
0.5	0	0	0	0
1	31±0.577^{c}	14.33±1.201^{a}	1.35±0.0170^{e}	1.68±0.005^{d}
1.5	51±0.577^{b}	14.33±1.452^{a}	1.63±0.0330^{d}	1.57±0.008^{e}
IBA				
0.25	0	0	0	0
0.5	51±0.577^{b}	9.00±0.577^{b}	2.64±0.0260^{c}	1.74±0.026^{c}
1	100±0.000^{a}	7.00±0.577^{b}	4.60±0.0577^{a}	3.37±0.120^{a}
1.5	100±0.000^{a}	7.66±0.881^{b}	4.11±0.0080^{b}	3.31±0.005^{b}

Data (Mean±SE) of three independent experiments each with 20 replicates, NR: Not responded, Means followed by the same letter within the column are not significantly different at $p<0.05$ as tested by the multiple range test of Duncan (1955)

Rooting and plantlets establishment: When regenerated shoots attained a length of about 3 cm or more, they were excised and planted on semi solid basal medium alone or supplemented with varying concentrations (0.25, 0.5, 1.0 or 1.5 mg L^{-1}) of either IAA or IBA for induction of rooting. The rooting responses of shoots on different media which included rooting percentage, days required for root initiation, mean number of roots per shoot and mean root growth over a period of three weeks have been presented in Table 3. There was no rooting in case of shoots planted on basal media devoid of growth regulator (control). Similarly, at lower levels of IAA (0.25 and 0.5 mg L^{-1}) or IBA (0.25 mg L^{-1}) treatments also there were hardly any rooting in the cultured shoots during the 3 weeks observation period. In all cultures with higher levels of IAA (1.0 and 1.5 mg L^{-1}) or IBA (0.5, 1.0 and 1.5 mg L^{-1}) treatments, root primordia emerged from the shoot base starting from day 6 to 16 after shoot inoculation (Fig. 1f) and soon after that the root growth was rapid. IBA was more effective than IAA in induction of rooting as days required to rooting was only 6-8 days as against the 12-16 days required for similar response in case of the latter. Further, the IBA treatments also recorded better percentage of rooting than the IAA treatments (Table 3). IAA always produced fewer (only 2-3) roots than that (4-7 roots) obtained with IBA.

Occasionally, there was leaf drop during rooting but such leaf drop was not a problem for shoot survival because such shoots developed new leaves sooner or later when transplanted in potted soil. During the rooting experiments in all the cultures supplemented with the auxins IAA or IBA there was considerable thickening of the shoot base prior to root initiation. Depending on the concentrations of auxin in the medium there was also in some cultures simultaneous callusing and rooting at the thickened shoot base.

For acclimatization, rooted plantlets were removed from the culture medium, washed free from the agar medium, treated with some antifungal solution and transferred to small poly-cups containing vermiculite and then to sterile potting medium contained in poly-bags or earthen pots following the procedure as described at appropriate section under 'material and methods'. The potting mix consisted of garden soil, farmyard manure (compost) and sand in the ratio of 1:1:1(v: v: v). The pots with the tender plantlets were kept under small polythene tents in a green house for hardening purpose. After about 7-10 days of hardening the polythene tents were removed. Soon the plants developed new leaves and started to grow. The micropropagated plants were maintained in the green house for about two weeks and subsequently transferred to the open for normal growth (Fig. 1g, h).

DISCUSSION

The dependence of cultured explants on bud break response and shoot multiplication was extensively discussed (George. and Sherrington, 1984). This has also been recently reported in the case of micropropagation of many medicinal plants like *Hemidesmus indicus* (Patnaik and Debata, 1996), *Gmelina arborea* (Thirunavoukkarasu and Debata, 1998) and *Plumbago zeylanica* (Sahoo and Debata, 1998). In many other plants like *Saussurea lappa* by Arora and Bhojwani (1989) while multiple shoots originated from leaf axils in stem node explants in the growth hormone supplemented nutrient media, the stem portion below the node often formed callus. Such situation did not hinder the overall shoot multiplication rate in this species. Premature leaf fall is not an uncommon phenomenon as it is often encountered in *in vitro* micropropagation experiments of many species like *Sassafras randaience* (Wang and Hu, 1984). In these studies the problems of leaf fall were easily overcome by supplementation of certain growth substances.

As observed by Kokate (1995), a large number of medicinal plants that generate multiple shoots in culture of axillary and shoot tip meristems have opened up new avenues for production of pharmaceuticals with preservation and propagation of elite genotypes. Protocol for micro propagation through shoot bud cultures of a number of medicinal plants like *Calotropis procera* (Das *et al*., 2005) and *Houttuynia cordata* (Handique and Bora, 1999) etc., were developed.

In most of the cultures by supplementation of BA in the medium produced more number of shoots produced which agrees with the result obtained by Singh and Tiwari, 2010 in *Clitoria ternatea* The shoots obtained from the multiplication medium were rooted on full strength of MS with supplementation of IBA also reported by Dvin *et al*. (2011).

Starting with a single stem node explant 3-5 plants could be obtained after three weeks of primary culture. Following excision of these shoots from the explant and reculturing on fresh media for four weeks one could get about 35-40 shoots. Each of these shoots offering 3-4 nodes for the next culture cycle, about 105-160 shoots can be obtained after another 2 months. Thus starting with one explant one would expect to obtain such number of plants through only two culture cycles involving shoot multiplication, rooting and hardening in about 8-10 weeks.

REFERENCES

Arora, R. and S.S. Bhojwani, 1989. *In vitro* propagation and low temperature storage of *Saussurea lappa* C.B Clarke, an endangered medicinal plant. Plant Cell Rep., 8: 44-47.

Das, S., P.P. Behera, U. Mohapatra and S.P. Rath, 2005. *In vitro* multiplication of a medicinal plant *Calotropis procera*, (Ait) R. Br. Plant Sci. Res., 27: 55-61.

Duncan, D.B., 1955. Multiple range multiple F test. Biometrics, 11: 1-42.

Dvin, S.R., E.G. Moghadam, E. Neyestani and A. Mokhtarian, 2011. Studies on rooting of azayesh apple cutting using indolebutyric acid and media. Asian J. Applied Sci., 4: 780-786.

George, E.F. and P.D. Sherrington, 1984. Plant Propagation by Tissue Culture: Handbook and Directory of Commercial Laboratories. Exegetics Ltd., London, pp: 102-110.

Ghosh, D., P. Thejomoorthy and P. Veluchamy, 1983. Anti-inflammatory and analgesic activities of oleanolic acid 3-/3-glucoside (RDG-1) from *Randia dumetorum* (Rubiacceae). Indian J. Pharmacol., 15: 331-342.

Gupta, R., 1988. Genetic resources of medicinal plants. Indian J. Plant Genet. Resour., 1: 98-102.

Handique, P.J. and P. Bora, 1999. *In vitro* regeneration of medicinal plant: *Houttuinia cordata* from nodal explants. Curr. Sc., 76: 1245-1247.

Heble, M.R. and E.J. Staba, 1980. Diosgenin synthesis in shoot cultures of *Dioscorea composita*. Planta Med., 35: 120-123.

Hu, S., 1980. An Enumeration of Chinese Materia Medica. Chinese University Press, Hong Kong.

Hunter, C.S., 1986. *In vitro* Propagation and Germplasm Storage of Cinchona. In: Plant Tissue Culture and its Agricultural Applications, Plant Tissue Culture and its Agricultural Applications. Withers, L.A. and P.G. Alderson (Eds.). Butterworth, London, pp: 291-303.

Kokate, C.K., 1995. Plant Tissue Culture as a Source of Biomedicinals. In: Advances in Horticulture: Medicinal and Aromatic Plants, Chada, K.L. and R. Gupta (Eds.). Vol. 2. Malhotra Publishing House, New Delhi, India.

Ling, Y., 1995. A New Compendium of Materia Medica. Science Press, Beijing, China, ISBN: 9787030037893, Pages: 326.

Murashige, T. and F. Skoog, 1962. The revised medium for rapid growth and bioassay with tobacco tissue culture. Plant Physiol., 15: 473-479.

Nayar, S.L., R.N. Chopra and I.C. Chopra, 1956. Glossary of Indian Medicinal Plants. Council of Scientific and Industrial Research, New Delhi, India, Pages: 94.

Nayar, M.P. and A.R.K. Sastry, 1987, 1998. Red Data Book on Indian Plants. Botanical Survey of India, Calcutta.

Patnaik, J. and B.K. Debata, 1996. Micropropagation of *Hemidesmus indicus* (L.) R. Br. through axillary bud culture. Plant Cell Rep., 15: 427-430.

Pillai, N.R., M. Alani and K.K. Purushothaman, 1981. Anti implantation studies with Gangetin in Albino rats. Bull. Medico-Ethno-Bot. Res., 2: 285-293.

Purushothaman, K.K., V.M. Kishore, V. Narayanaswami and J.D. Connolly, 1971. The structure and stereochemistry of gangetin, a new pterocarpan from *Desmodium gangeticum* (Leguminosae). J. Chem. Soc., 1971: 2420-2422.

Rajasekharan, P.E. and S. Ganeshan, 2002. Conservation of medicinal plant biodiversity in Indian perspective. J. Med. Arom. Pt. Sci., 24: 132-147.

Sabnis, S.D. and M. Daniel, 1990. Phytochemical Approach to Economic Botany. Kalyani Publishers, New Delhi, India.

Sahoo, S. and B.K. Debata, 1998. Microprogation of *Plumbago zeylanica* Linn. Herbs Spices Med. Plants, 5: 87-93.

Schoner, S. and E. Reinhard, 1986. Long term cultivation of *Digitalis lanata* clones propagated *in vitro*: Cardenolide content of regenerated plants. Planta Med., 6: 478-481.

Singh, J. and K.N. Tiwari, 2010. Evaluation of cotyledonary node of *Clitoria ternatea* L. for high frequency *in vitro* axillary shoot proliferation. Asian J. Plant Sci., 9: 351-357.

Singh, N.P. and H.J. Chowdhery, 2002. Biodiversity Conservation in India. In: Perspective of Plant Biodiversity, Das, A.P. (Ed.). Department of Botany, North Bengal University, West Bengal, India, pp: 501-527.

Staba, E.J. and A.C. Chung, 1981. Quinin and Quinidine production by Cinchona leaf, root and unorganized cultures. Phytochemistry, 20: 2495-2498.

Thirunavoukkarasu, M. and B.K. Debata, 1998. Micropropagation of *Gmelina arborea* through axillary bud culture. J. Plant Physiol., 3: 82-85.

Villa-Lobos, J., 1994. Biological conservation news letter No. 133. Smithsonian Institution, Department of Botany, National Museum of Natural History. http://botany.si.edu/pubs/bcn/issue/133.htm

Wang, P.J. and C.Y. Hu, 1984. *In vitro* cloning of the deciduous timber tree *Sassafras randaience*. Zeitschrift Pflanzenphysiol., 113: 331-335.

Wee, Y.C. and H. Keng, 1992. Illustrated Dictionary of Chinese Medicinal Herbs. CRCS Publications, Sebastopol, CA., USA., ISBN: 9780916360535, Pages: 184.