EFFECT OF SEED HARVESTING SEASON AND STERILIZATION TREATMENTS ON GERMINATION AND *IN VITRO* PROPAGATION OF *Albizia lebbeck* (L.) BENTH.

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Abstract. This study was undertaken with the objective of standardizing the protocol for the micropropagation of *Albizia lebbeck* (L.). Seeds were collected from three different colored pods (yellow, dark yellow and brownish yellow) harvested from field grown *Albizia* tree from November to January and were surface sterilized using different concentration of mercuric chloride (0.05-0.15 % w/v) for different exposure durations (2, 5 and 8 min). Seeds collected from dark-yellow colored pod showed the highest germination (83.3 %) and short germination time (4.29 days), while those collected from brownish yellow colored pod showed relatively less germination (53.3%) and longer germination time (6.0 days). Success of seed germination increased with maturity up to a certain level after which it declines significantly with increasing harvesting time. A five-minute dip of $HgCl_2$ (0.1%) was found to be the optimum duration for giving the maximum (83.3%) germinated and healthy seedlings. Increasing time and concentration of $HgCl_2$ significantly reduced the contamination, but on the other hand it also effected the germination of seeds. Best shoot regeneration response from *in vitro* nodal segment was obtained on MS medium supplemented with BAP (2.0 mg/l) + NAA (0.5 mg/l). The highest percentage of callus induction (85%) was also observed in the above same media from *in vitro* internodal segment. Proliferated micro-shoots showed 60% rooting on half strength medium supplemented with IAA (2.0 mg/l) after 4 weeks of culturing. The well rooted micropropagated plantlets were acclimatized and successfully established in pots containing sterilized soil and sand mixture (1:1) with 60% survival rate under field conditions.

Keywords: *Albizia lebbeck,* callus, multiple shoots, nodal segment, germination.

Abbreviations: BAP - 6 Benzyl amino purine; **2,4-D -** 2,4-Dichlorophenoxyacetic acid; **MS -** Murashige and Skoog (1962) basal medium; **NAA -** *a*-naphthalene acetic acid; **IBA -** Indole-3-butyric acid; $HgCl_2$: Mercuric chloride

INTRODUCTION

The leguminous trees are one of the most significant component of forest vegetation due to their economic and ecological importance [6].

The regeneration rate of leguminous trees in natural habitats is low [6]. As stated by Nanda *et al.* [24], due to poor germination and death of young seedlings under natural conditions, propagation through seeds, as with most leguminous trees is unreliable. Micropropagation offers a rapid means for afforestation, multiplying woody biomass and of conserving elite and rare germplasm [42]. Some of the advantages of this technique are heterozygous materials may be perpetuated without much alteration, easier, faster, dormancy problem eliminated and juvenile stage reduced. It is also a mean for perpetuating clones that do not produce viable seeds or that do not produce seeds at all [35].

Albizia lebbeck (L.) Benth (Fabaceae) commonly known as *shirish,* is a medicinally important multipurpose leguminous tree, native to tropical southern Asia, and widely cultivated and neutralized in other tropical and sub-tropical regions. It is large, erect, unarmed deciduous, mimosoid legume [1].

The plant contains saponins, flavanoids, alkaloids, glycosides and proteins. It is reported to be Antiallergic, Anti-inflammatory, Analgesic, Nootropic, Antispermatogenic, and Antimicrobial [15]. This tree is an important source of chemicals of melacacidin, D-catechin, β-Sitosterol, Albiziahexoside, betulnic acid which are effective as antiseptic, anti-dysenteric, anti-tubercular and used in bronchitis, leprosy, paralysis, helmenth infection, etc. [29].

In vitro plant regeneration of *A. lebbeck* through seedling explants have been studied by many researchers, such as Gharyal and Maheshwari [9]; Mamun *et al.* [21]. The detailed review of earlier studies reveals that there are no published data on effect of seed collection time and different sterilization methods on *in vitro* germination for micropropagation of *A. lebbeck.* As seed collection season and suitable surface sterilization procedure is the initial and vital step of micropropagation, minute error can lead to loss of whole culture with waste of time and labor [39]. So, much attention is needed while dealing with the *in vitro* studies of such a valuable and multipurpose medicinal tree. Therefore, the objectives of the present study were to evaluate the effect seed collection season, concentration and exposure time of surface sterlizants to improve on seed germination of *A. lebbeck* and develop a protocol for its *in vitro* regeneration.

MATERIALS AND METHODS

Explant source and sterilization study

The seeds of *A. lebbeck* were collected from a 14 year-old tree growing in Herbal Garden of Botany Department of Kurukshetra University, India. Uniform and healthy seeds were selected and surface sterilized by washing with liquid detergent (Tween-20) and then kept under running tap water for 15 min. Then they were disinfected using different concentrations (0.05-0.15 % w/v) of mercuric chloride ($HgCl_2$) with three selected exposure timings (2, 5 and 8 min) to standardize the best sterilization protocol for *in vitro* germination (Table 1). Finally the seeds were rinsed with sterilized distilled water for three times and cultured aseptically on MS hormone free Murashige

and Skoog [23] basal medium to evaluate the response. Numbers of seeds contaminated and germinated were recorded regularly till 30 days.

Seed collection and germination study

To study the effect of seed collection time on *in vitro* germination, pods were harvested from the tree in three collections from last week of November to last week of January at an interval of one month. At the time of first harvesting (November), the colour of most of the pods (about 70%) was yellow and later they turned from dark yellow to brownish yellow at the time of their second (December) and third (January) harvesting time respectively. After harvesting, the healthy seeds were extracted from three different colored pods and were surface sterilized using $HgCl_2$ (0.1 %) for 5 minutes and cultured aseptically on MS hormone free basal medium to evaluate the response. Time taken for the germination and number of germinated seeds were observed regularly till 30 days.

Selection of explants and in vitro propagation

Nodal and intermodal segments of 15 days old *in vitro* raised seedling were used as explant source and were cultured on MS basal medium with various concentrations (0.5-3.0 mg/l) of auxins (IAA, NAA, 2,4-D and IBA) and cytokinins (BAP and Kn) alone and in various combinations for shoot and callus induction.

Culture conditions

The cultures were incubated at a temperature of 25±2ºC and a photoperiod of 16hrs light (20 µmol m^{-2} s^{-1} photosynthetic photon flux intensity) and 8hrs of dark.

Induction of rooting

The *in vitro* raised elongated shoots (2-3 cm) were excised and implanted in culture tubes containing half strength MS medium with IAA (0.5-2.0 mg/l) under aseptic conditions for rooting. The cultures were maintained under similar physical culture conditions as mentioned earlier.

Acclimatization

The plantlets with well developed roots were thoroughly washed under tap water to remove the traces of agar from the roots and transferred to plastic cups containing sterilize soil and sand mixture (1:1). Each cup was covered with a polythene bag containing small holes, to maintain high humidity around the plants and was kept in the culture room. The pots were irrigated with 1/4 MS salt solution on alternate days. The polythene bags were removed after two weeks for 3-4h daily to expose the plants to the conditions of natural humidity. After one more week, the plants were transferred to larger pots containing sand and garden soil in 1:3 ratio, and were maintained under field conditions.

Statistical analysis

Each treatment consisted of 10 replicates and all experiments were repeated thrice. The data were analyzed statistically using one-way analysis of variance (ANOVA) and the significant differences between means were assessed by Duncan's multiple range test at $P \leq 0.05$. All statistical analyses were performed with Statistical Package for Social Sciences (SPSS, version 11.5).

RESULTS

Among all the different concentration and time of exposure of $HgCl_2$ tested, dipping the seed in 0.1% (w/v) for 5 min. gave the maximum (83.3%, $p \leq 0.05$) germinated and healthy seedlings while less germination and more percentage of contamination was observed with 0.05% (w/v). Increasing exposure time and concentration significantly reduced the contamination, but on the other hand it also effected the germination of seeds (Table 1).

The comparison among the three harvesting seasons is presented in Table 2. The harvesting time of pods also showed a significant effect on *in vitro* germination of seeds. The highest germination (83.3%) was recorded for seeds extracted from dark-yellow colored pods. When pods were harvested beyond a particular

Table 1. Effect of different concentrations with varying time of exposure of $HgCl_2$ on *in vitro* germination and contamination percentage on seeds of *A. lebbeck* recorded after 4 weeks of culture on MS medium.

Concentration of Mercuric chloride ($HgCl_2$ w/v)	Exposure time (min)	Germination %	Contamination %
0.05 %	2	20^{c}	86.6^{a}
	5	33.3^{b}	66.6^{ab}
	8	46.6^{b}	40^{b}
0.1 %	2	36.6^{b}	73.3^{ab}
	5	83.3^{a}	30^{b}
	8	76.6^{ab}	20^{bc}
0.15 %	2	40^{b}	30^{b}
	5	70^{ab}	13.3^{bc}
	8	60^{ab}	3.3^{c}
LSD ($p \leq 0.05$)		2.34375	2.1064
ANOVA ($F_{8,18}$)		7.023*	14.565*

Each value is a mean of thirty replicates.
Mean value followed by different alphabet/s within a colum do not differ significantly over one other at p≤0.05 lead by Duncan's Multiple Range Test.
**Significant at p≤0.05*

Table 2. Effect of seed harvesting (collection) time on *in vitro* growth and germination of *A. lebbeck* seeds recorded after 4 weeks of culture on MS medium.

Time of harvesting (last week)	Pod Colour	Germination %	Germination time (days)
November	Yellow	70^{ab}	5.1^{a}
December	Dark yellow	83.3^{a}	4.29^{a}
January	Brownish yellow	53.3^{b}	6.06^{b}
LSD ($p \leq 0.05$)		2.30385	1.2911
ANOVA ($F_{2,6}$)		4.356*	0.616*

Each value is a mean of thirty replicates.
Mean value followed by different alphabet/s within a colum do not differ significantly over one other at p≤0.05 lead by Duncan's Multiple Range Test.
** Significant at p≤0.05*

stage, germination ability gradually declined. Roughly it may be because the seeds in dark-yellow colored pods were approximately progressed to 1/2 to 1/3 in their development stage. This clearly indicates that seed germination in *A. lebbeck* increase with maturity upto a certain level after which it declines significantly. It may be due to with increasing maturity there is a rapid accumulation of reserve food material which tends to hardness in seed. Here, the pod color served as satisfactory means indicating the physiological maturity of seeds. Besides it development of hardseedness resulted in lower germination percent.

Time taken for seed germination also increased significantly ($p \leq 0.05$) with increasing harvesting time. In the seeds cultured from dark yellow colored pods, seed germination was observed within 4.2 days, while for those from brownish yellow pods, seeds took more than 6 days to germinate.

Out of the two cytokinins tested, BAP was the most effective for inducing bud break. The maximum bud break (60%) was observed in nodal explants cultured on MS medium supplemented with BAP (2.0 mg/l) + NAA (0.5 mg/l) (Table 3). This combination also produced the highest (4.6) mean number of shoots per culture (Fig. 1d). Good level of shoot regeneration was also noticed in different combination of BAP alone (Fig. 1e).

For callus induction, the *in vitro* internodal segments were culture on media supplemented with different concentrations of BAP and 2, 4-D alone and in various combinations with NAA (0.5 mg/l) (Table 4). The internodal segments inoculated on media containing 2.0 mg/l BAP and 0.5 mg/l NAA showed eighty-five per cent callus induction (Fig. 1b-c) followed by 2.0 mg/l BAP (Fig. 1a). The callus so obtained was friable greenish white.

The well developed regenerated shoots were isolated and transferred on half strength basal MS medium fortified with IAA (0.5-2.0 mg/l) for root induction. The highest sixty per cent micro shoots initiated root in media having IAA (2.0 mg/l) after twenty days of inoculation (Fig. 1f).

DISCUSSIONS

In fact according to losses due to contamination under *in vitro* conditions average between 3-15% at every subculture in the majority of commercial and scientific plant tissue culture laboratories, the majority of which is caused by fungal and bacterial contaminant [19]. Therefore, to ensure the reduction of the contaminants as well as high survival rate of explants it requires efficient aseptic techniques in tandem with effective sterilization methods before subjecting them for tissue culture study [39].

Table 3. Effect of cytokinins and auxins supplemented individually and in various combinations on *in vitro* nodal segments of *A. lebbeck* recorded after 4 weeks.

Media Composition	Concentration of growth regulators (mg/l)	Percentage of bud break	Number of days required for bud break	Number of shoots (Mean±SE)
MS (Control)	-	-	-	-
BAP	0.5	23.3^{bc}	11^{bc}	2.28 ± 0.48^{bc}
	1.0	43.3^{ab}	09^{a}	2.76 ± 0.72^{ab}
	2.0	50^{ab}	08^{a}	3.46 ± 0.74^{ab}
	3.0	30^{b}	10^{b}	2.33 ± 0.50^{b}
Kn	0.5	-	-	-
	1.0	16.6^{c}	11.4^{c}	1.60 ± 0.54^{c}
	2.0	30^{b}	10.2^{b}	1.77 ± 0.44^{bc}
	3.0	-	-	-
BAP + NAA	1.0 +0.5	46.6^{ab}	8.9^{a}	2.78 ± 0.42^{ab}
	2.0 +0.5	60^{a}	7.1^{a}	4.33 ± 0.84^{a}
	3.0 +0.5	-	-	-
Kn + NAA	1.0 +0.5	20^{bc}	10.6^{bc}	1.66 ± 0.51^{c}
	2.0 +0.5	36.6^{b}	10^{ab}	2.18 ± 0.40^{b}
	3.0 +0.5	-	-	-
LSD ($p \leq 0.05$)		2.37675	2.2783	0.67835
ANOVA ($F_{14,30}$)		2.745*	1.192*	8.934*

(–) No Response.
Data shown are Mean ± SE of thirty replicates.
Mean value followed by different alphabet/s within a colum do not differ significantly over one other at p≤0.05 lead by Duncan's Multiple Range Test.
** Significant at p≤0.05*

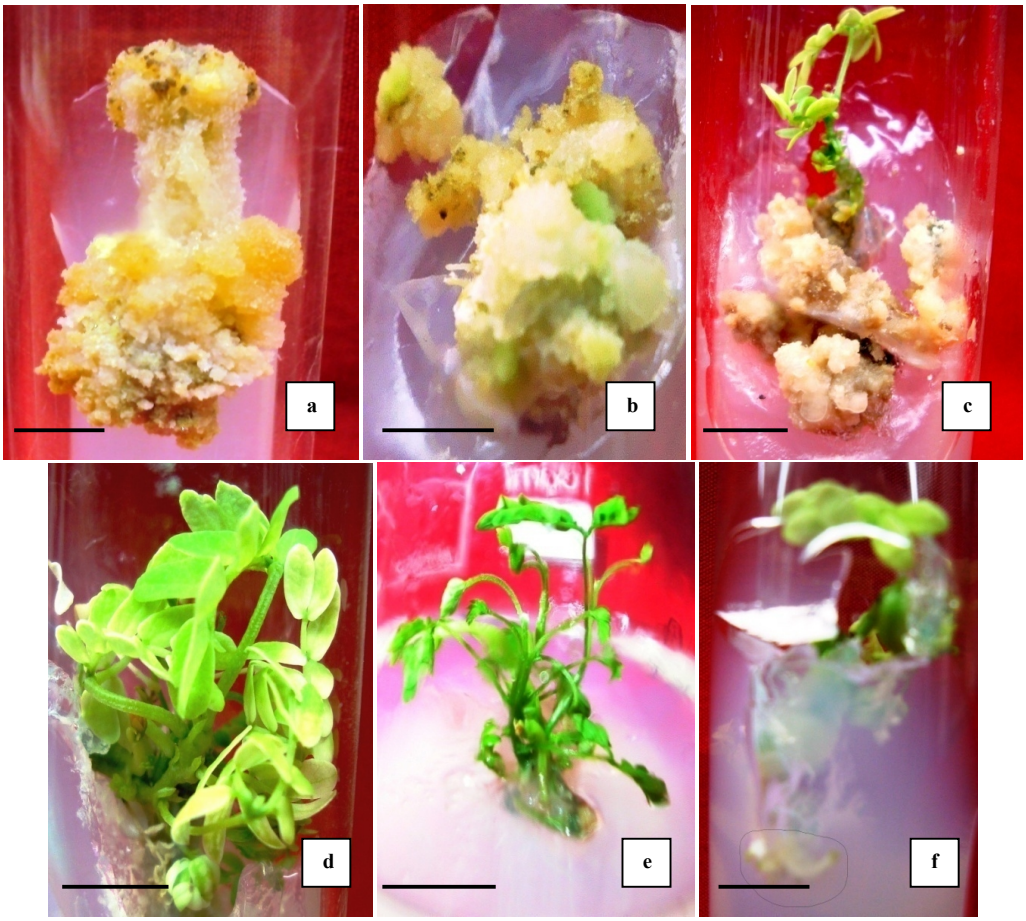

Figure 1. Micropropagation of *A. lebbeck*. **(a)** Callus induction from internodal segment on MS medium with BAP (2.0mg/l); **(b)** Appearance of green patches on callus inducted on MS + BAP (2.0 mg/l) + NAA (0.5 mg/l); **(c)** Shoot proliferation along with callus formation from nodal segment; **(d)** Multiple shoots formation along with callus formation on node cultured on BAP (2.0mg/l) + NAA (0.5mg/l); **(e)** Shoot formation from nodal explant on MS medium with BAP (2.0mg/l); **(f)** Induction of rooting from micro shoots cultured on ½ MS + IAA (2.0 mg/l) after two weeks of culture (Bar = 1.0 cm and it represents the length of the plant).

Table 4. Effect of cytokinins and auxins supplemented individually and in various combinations on callus formation on *in vitro* internodal explants of *A. lebbeck* recorded after 4 weeks.

Media Composition	Concentration of growth regulators (mg/l)	No. of days required for callus induction	Percentage of callus induction	Nature of callus	Callus growth
MS (control)	–	–	–	–	–
2,4-D	0.5	–	–	–	–
	1.0	–	–	–	–
	2.0	10.28^{c}	23.3^{d}	Greenish white, friable	C+
	3.0	12.5^{c}	20^{d}	Greenish white, friable	C+
BAP	0.5	–	–	–	–
	1.0	8.33^{a}	60^{b}	Greenish white, friable	C++
	2.0	7.12^{a}	80^{a}	Greenish white, friable	C+++
	3.0	7.0^{b}	60^{b}	Greenish white, friable	C++
BAP + NAA	1.0 +0.5	8.3^{a}	66.6^{b}	Greenish white, friable	C++
	2.0 +0.5	6.9^{a}	83.3^{a}	Greenish white, friable	C+++
	3.0 +0.5	7.0^{a}	80^{a}	Greenish white, friable	C++
2,4-D +NAA	1.0 +0.5	11.8^{b}	36.6^{c}	Greenish white, friable	C+
	2.0 +0.5	10^{a}	50^{bc}	Greenish white, friable	C+
	3.0 +0.5	10^{b}	40^{c}	Greenish white, friable	C++
LSD ($p \leq 0.05$)		2.1839	2.32225		
ANOVA ($F_{14,30}$)		2.379*	7.250*		

(–) No Response, (C+) Poor growth, (C++) Moderate growth, (C+++) Good growth.
Each value is a mean of thirty replicates.
Mean value followed by different alphabet/s within a colum do not differ significantly over one other at $p \leq 0.05$ lead by Duncan's Multiple Range Test.
** Significant at $p \leq 0.05$*

Mercuric chloride is a very effective sterilant and extensively used to stimulate seed germination and reduce contamination in cultures [10]. According to Narayanaswami [25], with an increasing exposure time, embryo was damaged because $HgCl_2$ may have been toxic to the embryo due to longer exposure time. Therefore, the explants should be thoroughly washed in order to completely remove all traces of mercury still

adhering to the material. Stimulatory effects of $HgCl_2$ for different explants have been reported in various medicinal plants *viz., Acacia sinuata, Pterocarpus santalinus*, *Balanites aegyptiaca*, *Cicer arietinum*, *Plumbago zeylanica*, *Aconitum heterophyllum, Solanum tuberosum* etc. [2, 5, 11, 37, 39, 40, 41]. Same concentration of $HgCl_2$ was effective in case of *Inula racemosa* Hook.f. [14] and *Picrorhiza kurroa* [38] but the time of exposure was comparatively less, 2 min. and 30 sec. respectively.

The growth pattern is greatly influenced by the seasonal changes. The level of maturity of seeds/pods at harvesting is an important factor of seed germination. Towards the end of the dry season growth continues and flowering begins. In India, flowering occurs in April-June and pods fruits mature in November-January. The pods become mature when they have turned yellow [35]. Mature pods remain on the tree for 3-4 months. It is important that collection is not delayed as the seed maturity significantly influences seed germination in this species. Prasad and Chaturvedi [30] also reported that the explant collection season was a critical factor for successful establishment of cultures.

Immature seeds with 1/3 mature embryos germinated better than fully mature ones because of their ability to germinate well has been attributed to the protein mobilization that occurs during rehydration *in vitro* and an undeveloped embryonic envelope that may repel water [31]. So, in some species, seed germination may increase during early stages of maturity and decrease as full maturity is achieved at the time of seed fall [3]. But generally, the most mature seed possesses greatest vigour and potential to become seedling [26]. Also according to Kumar and Kumar [18], generally, more juvenile the plant material, greater the chances of success in culture initiation.

Effectiveness of BAP was also observed in *Leucaena leucocephala* [36], *Acacia chundra* [32], *Prosopis cineraria* [17], *Acorus calamus* [46], *Aegle marmelos* [44], *Spilanthes acmella* [43, 45]. Kinetin alone or in combination did not show much satisfactory response on bud break. Similar results were reported by Dhar and Upreti [7] in *Bauhinia valii* Wight & Arnott.

Callus initiated from the cut ends of the explants and finally whole surface of the explant was involved. Similar observations have been made by Guo *et al*. [12] in *Saussurea involucrate* and also by Singh and Lal [36] in *Leucaena leucocephala.* This may be obviously due to the production of endogenous auxins from the damaged cells of cut surface which triggered the cell division as found in *Ornithogallum* [13] where active cell division was observed at cut ends of tissue. Induction of callus in response to BAP has also been reported in *Spilanthes acmella* [43]. The interaction of higher cytokinin with lower auxin enhancing callus induction has been reported by other authors also [22, 28]. Here the auxin, 2,4-D alone was found to be less effective in case of callus induction. Similar observations were observed in *Bellevalia romana* [20], *Gymnema sylvestre* [16]. This indicates the importance of nature, combination and concentration of various growth regulators on callus induction of woody legume species.

Micropropagation is meaningless without successful establishment of plantlets in the soil [4]. Similar results were observed in *Ormacarpum sennoides* and *Withania somnifera* for root induction [33, 34]. Rooted plantlets were initially hardened under culture conditions. After about a week these acclimatized plants were shifted to the greenhouse where they grew normally with 60% survival rate (data not shown). Similar results were obtained in *Dalbergia sissoo* by Obidulla [27].

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REFERENCES

[1] Anonymous, (1985): The wealth of India, (Publication & Information Directorate, CSIR, New Delhi), pp. 126.

[2] Badoni, A., Chauhan, J.S., (2010): *In Vitro* Sterilization Protocol for Micropropagation of *Solanum tuberosum* cv. 'Kufri Himalini'. Academia Arena, 2(4): 24-27.

[3] Baily, C.V., (1961): Early collection and immediate sowing increase germination of bassoowood (*Tilia Americana*) seed. Tree Planter's Notes, 46: 27-28.

[4] Bari, M.A., Ferdaus, K.M.K.B., Hossain, M.J., (2008): Callus induction and plantlet regeneration from *in vivo* nodal and internodal segments and shoot tip of *Dalbergia sissoo* Roxb. Journal of Biological Sciences, 16: 41-48.

[5] Chaturani, G.D.G., Subasinghe, S., Jayatilleke, M.P., (2006): *In vitro* establishment, germination and growth performance of Red Sandalwood (*Pterocarpus santalinus* L.). Tropical Agricultural Research & Extension, 9: 116-130.

[6] Dewan, A., Nanda, K., Gupta, S.C., (1992): *In vitro* micropropagation of *Acacia nilotica* subsp, Indica Brenan via cotyledonary nodes. Plant Cell Reports, 12: 18-21.

[7] Dhar, U., Upreti, J., (1999): *In vitro* regeneration of a mature leguminous liana *(Bauhinia vahlii* Wight and Arn.). Plant Cell Reports, 18: 664-669.

[8] Falkiner, F.R., (1990): The criteria for choosing an antibiotic for control of bacteria in plant tissue culture. IAPT, Newsletter, 60: 13- 22.

[9] Gharyal, P.K., Maheshwari, S.C., (1983): *In vitro* differentiation of plantlets from tissue cultures of *Albizia lebbeck* L.. Plant Cell Tissue and Organ Culture, 2: 49-53.

[10] Gopal, J., Minocha, J.L., Dhaliwal, H.S., (1998): Microtuberization in potato (*Solanum tuberosum* L.). Plant Cell Reports, 17: 794-798.

[11] Gour, V.S., Sharma, S.K., Emmanuel, C.J.S.K., Kant, T., (2007): A rapid in vitro morphogenesis and acclimatization protocol for *Balanites aegyptiaca* (L.) Del- a medicinally important xerophytic tree. Journal of Plant Biochemistry and Biotechnology, 16: 151-153

[12] Guo, B., Gao, M., Liu, C.Z., (2007): *In vitro* propagation of an endangered medicinal plant *Saussurea involucrata* Kar. et kir. Plant Cell Reports, 26: 261-265.

[13] Hussey, G., (1976): Planlets regeneration from callus and parent tissue in *Ornithogalum thyrsoides*. Journal of Exerimental Botany, 27: 375-382.

[14] Jabeen, N., Shawl, A.S., Dar, A.H., Jan, A., Sultan, P., (2007): Micropropagation of *Inula racemosa* Hook.f. A High Valuable Medicinal Plant. International Journal of Botany, 3(3): 296-301.

[15] Kirtikar, K.R., Basu, B.D., (1981): Indian Medicinal Plants, (Lalit Mohan Basu, Allahabad), Vol. II. pp: 936.

[16] Komalavalli, N., Rao, M.V., (2000): *In vitro* micropropagation of *Gymnema sylvestre* a multipurpose medicinal plant. Plant Cell Tissue and Organ Culture, 61: 97-105.

[17] Kumar, S., Singh, N., (2009): Micropropagation of *Prosopis ceneria* (L.) Druce - A multiple desert tree. Researcher, 1(3): 28-32.

[18] Kumar, A., Kumar, V., (1998): Clonal tissue culture of important fruit crops. International book distributing company, India. pp. 1-55.

[19] Leifert, C., Waites, W.M., Nicholas, J.R., (1989): Bacterial contaminants of micropropagated plant cultures. Journal of Applied Bacteriology, 67: 353-361.

[20] Lupi, C., Bennici, A., Gennai, D., (1985): *In vitro* culture of *Bellevalia romana* (L.) Rchb. plant regeneration through adventitious shoots and somatic embryos. Protoplasma, 125: 185-189.

[21] Mamun, A.N.K., Matin, M.N., Bari, M.A., Siddique, N.A., Sultana, R.S., Rahman, M.H., Musa, A.S.M., (2004): Micropropagation of woody legume (*Albizia lebbeck*) through tissue culture. Pakistan Journal of Biological Science, 7(7): 1099-1103.

[22] Mamun, A.N.K., (2000): Micropropagation of some woody legumes (*Acacia catechu* wild., *Albizia lebbeck* (Linn.) Benth. and *Dalbergia sissoo* Roxb.) through tissue culture techinique. M. Phil Thesis. Institute of Biological sciences, University of Rajshahi, Bangladesh. In: Bari, M.A., Ferdaus, K.M.K.B., Hossain, M.J., (2008): Callus induction and plantlet regeneration from *in vivo* nodal and internodal segments and shoot tip of *Dalbergia sissoo* Roxb. Journal of Biological Sciences, 16: 42.

[23] Murashige, T., Skoog, F., (1692): A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum, 15: 473-497.

[24] Nanda, R.M., Das, P., Rout, G.R., (2004). *In vitro* clonal propagation of *Acacia mangium* Willd. and its evaluation of genetic stability through RAPD marker. Annals of Forest Science, 61: 381-396.

[25] Narayanaswamy, S., (1999): Plant cell and tissue culture. Tata McGraw hill publishing company limited, India, pp. 1-236.

[26] Negi, A.K., Todaria, N.P., (1995): Effects of seed maturity and development on germination of five species from Garhwal Himalaya, India. Journal of Tropical Forest Science, 8(2): 255-258.

[27] Obidulla, M., (1995): Studies *in vitro* propagation of a legume tree, *Dalbergia sissoo* Roxb M Sc Thesis. University of Rajshahi, Rajshahi, Bangladesh. In: Bari, M.A., Ferdaus, K.M.K.B., Hossain, M.J., (2008): Callus induction and plantlet regeneration from *in vivo* nodal and internodal segments and shoot tip of *Dalbergia sissoo* Roxb. Journal of Biological Sciences, 16: 47.

[28] Pattnaik, S., Pradhan, C., Naik, S.K., Chand, P.K., (2000): Shoot organogenesis and plantlet regeneration from hypocotyl- derived cell suspensions of a tree legume, *Dalbergia sissoo* Roxb. *In vitro* cellular and development biology. Plant, 36(5): 407-410.

[29] Perveen, S., Varshney, A., Anis, M., Aref, I.M., (2011): Influence of cytokinins, basal media and pH on adventitious shoot regeneration from excised root cultures of *Albizia lebbeck.* Journal of Forestry Research, 22(1): 47-52.

[30] Prasad, K.N., Chaturvedi, H.C., (1988): Effect of season of collection of explants on micropropagation of *Chrysanthemum morifolium.* Biologia Plantarum, 30: 20-24.

[31] Rasmussen, H.N., (1995): Terrestrial orchids from seed to mycotrophic plants. Cambridge: Cambridge University Press. In: Mweetwa, A.M., Welbaum, G.E., Tay, D., (2008): Effects of development, temperature and calcium hypochlorite treatment on *in vitro* germinability of *Phalaenopsis* seeds. Scientia Horticulturae, 117: 257-262.

[32] Rout, G.R., Senapati, S.K., Aparajeta, S., (2008): Micropropagation of *Acacia chundra* (Roxb.) DC. Horticultural Science, 35(1): 22-26.

[33] Shanti, P., (2008): *In vitro* propagation of *Ormocarpum sennoides* (Willd) DC. Prodr. from shoot tip explant. Indian Journal of Plant Physiology, 13:1.

[34] Shekhawat, S.G., Mathur, S., Batra, A., (2009): Role of phytohormones and nitrogen in somatic embryogenesis induction in cell culture derived from leaflets of *Azadirachta indica.* Biologia Plantarum, 53(4): 707-710.

[35] Singh, B., Prasad, S., Uniyal, A.K., Rawat, J.S., (2008): Effect of seed germination and seedling growth of *Albizia lebbek* (L.) Willd. Bulletin of the National Institute of Ecology, 19: 15-19.

[36] Singh, N., Lal, D., (2007): Growth and organogenesis potential of calli from some explants of *Leucaena leucocephala* (Lam.) de Wit. International Journal of Tropical Agriculture, 25: 389-399.

[37] Sivanesan, I., (2007): Shoot Regeneration and Somaclonal Variation Leaf Callus Cultures of *Plumbago zeylanica* Linn. Asian Journal of Plant Sciences, 6(1): 83-86.

[38] Sood, H., Chauhan, R.S., (2009): Development of Low Cost Micropropagation Technology for an Endangered Medicinal Herb *(Picrohiza kurroa)* of North-Western Himalayas. Journal of Plant Sciences, 2: 21-31.

[39] Srivastava, N., Kamal, B., Sharma, V., Negi, Y.K., Dobriyal, A.K., Gupta, S., Jadon, V.S., (2010): Standardization of Sterilization Protocol for Micropropagation of *Aconitum heterophyllum*- An Endangered Medicinal Herb. Academia Arena, 2(6): 37-42.

[40] Sujatha, G., Jayabalan, N., Ranjitha Kumari, B.D., (2007): Rapid *in vitro* micropropagation of *Cicer arietinum* L. Horticultural Science (Prague), 34: 1-5.

[41] Vengadesan, G., Ganapathi, A., Pream Anand, R., Selvaraj, N., (2003): *In vitro* propagation of *Acacia sinuata* (Lour.) Merr. from nodal segments of a 10-Year-OldTree. *In vitro* cellular and developmental biology. Plant, 39(4): 409-414.

[42] Xie, D., Hong, Y., (2001): *In vitro* regeneration of *Acacia magium* via organogenesis. Plant Cell Tissue Tissue and Organ Culture, 66: 167-173.

[43] Yadav, K., Singh, N., (2010): Micropropagation of *Spilanthes acmella* Murr. – An Important Medicinal Plant. Nature and Science, 8(9): 5-11.

[44] Yadav, K., Singh, N., (2011): *In vitro* propagation and biochemical analysis of field established Wood apple (*Aegle marmelos* L.). Analele Universităţii din Oradea – Fascicula Biologie, 18(1): 23-28.

[45] Yadav, K., Singh, N., (2011): *In vitro* flowering of shoots regenerated from cultured nodal explants of *Spilanthes acmella* Murr. - an ornamental cum medicinal herb. Analele Universităţii din Oradea – Fascicula Biologie, 18(1): 60-64.

[46] Yadav, K., Singh, N., Aggarwal, A., (2011): Influence of arbuscular mycorrhizal (AM) fungi on survival and development of micropropagated *Acorus calamus* L. during acclimatization. International Journal of Agricultural Technology, 7: 775-781.

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