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Micropropagation of Woody Legume (*Albizia lebbeck*) Through Tissue Culture

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Abstract: The woody legume, *Albizia lebbeck*, commonly cultivated for social forestry in rural area of Bangladesh was selected for micropropagation by tissue culture technique. Cotyledon, nodal segment of *in vitro* grown seedlings and nodal segments of field grown mature tree were used as explants. With proper manipulation of cytokinin and auxin combinations and concentrations, it was possible to induce callus from three types of explants of *Albizia lebbeck*. Different combinations of BA with NAA and KIN with NAA were proved efficient media formulation for callus induction. Among all the hormonal supplements used BA-NAA combination with MS medium was proved best in all respect of callusing response in *Albizia lebbeck*. Among the explants the *in vitro* internodal segments were the best for callus induction. The highest 100% callus induction was observed in media having MS+2.0 mg L^{-1} BA+0.2 mg L^{-1} NAA and the fresh weight of callus was 1.3 g. Among these three explants of *Albizia lebbeck* only calli derived from cotyledon regenerated best shoots in MS media supplemented with BA singly or in combination with NAA and KIN. In respect of direct shoot regeneration nodal explants of *Albizia lebbeck* produced highest range of regenerated shoots.

Key words: Woody legumes, micropropagation, callus and regeneration

INTRODUCTION

In the present investigation the important woody legume, *Albizia lebbeck*, was selected as the experimental material. Liguminoseae is one of the most important families of the plant kingdom with the novel characteristics of nitrogen fixation and thereby enriching the fertility of the soil. This woody legume is being used as important tree spp. in massive plantation program in social forestry in Bangladesh. This is the fast growing plant and being widely cultivated in the country predominantly for durable timber and fuel. This tree can play an important role in environment conservation and ecosystem balance and can be planted to protect the desertification of northern zone of Bangladesh. Tree legumes with timber value are considered as the important plant species suitable for plantation in almost all types of marginal lands in the country. One of the most important problems in the woody legumes is the establishment of propagules of high quality of regeneration as *A. lebbeck* has long seed dormancy[1]. Under tissue culture approach the plant *spp* belonging to the family leguminoseae are in general considered to be recalcitrant to *in vitro* regeneration[2-4]. Under these efforts *A. lebbeck* was subjected to tissue culture technique for developing the avenues for mass production and development of elite genotypes of *A. lebbeck* from different explants.

MATERIALS AND METHODS

Mature seeds, cotyledons and internodal segments from field grown mature trees as well as *in vitro* grown internodal segment of *A. Lebbeck* were used in this present experiment. All the materials were collected from Rajshahi University campus, Rajshahi, Bangladesh. The field grown explants were washed thoroughly under running tap water for 2 h and taken in a flask containing distilled water supplemented with 2 drops of tween-80 for 2 h and washed properly. Then the explants were surface sterilized with 0.1% $HgCl_2$ for 8-11 min. MS[5] media was used for callus induction, direct regeneration and shoot proliferation. Three auxins α-Naphthalene acetic acid (NAA), Indole acetic acid (IAA), 2, 4-Dichlorophenoxy acetic acid (2, 4-D) and two cytokinins 6- Benzyl aminopurine (BAP) and Kinetin were used in various concentrations and combinations. pH of the media was adjusted to 5.6 before solidified with 0.6% agar supplemented with 3% sucrose and were autoclaved for 20 min at 121°C. All the explants were inoculated in the media and were stored at 27±2°C under light intensity of

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2000-3000 lux with a photoperiod of 16 h in light and 8 h in dark. Data was collected after 28 days of culture.

RESULTS AND DISCUSSION

Direct regeneration: Direct regeneration is the useful means of production of plantlets from young or mature trees with a lower risk of genetic instability than by the other routes[6]. In the present study efforts have been intensified to initiate direct regeneration. A good number of shoots were regenerated directly from the inoculated explants (cotyledon, *in vitro* nodal segment and *in vivo* nodal segment) of *A. lebbeck* with or without formation of base callusing.

Cotyledon: Regeneration of cotyledonary explants of *A. lebbeck* were greatly influenced by BA either used singly or in combination with auxin. The cytokinin BAP ranging from 2.0-2.5 mg L^{-1} showed the best performance in direct regeneration in cotyledonary explant of *A. lebbeck* (Table 1). The highest percentage (83.33%) of explants developed shoots in 2 mg L^{-1} BAP media composition.

***In vitro* nodal segment:** Nodal segments were taken from one week old *in vitro* seedlings and have been widely used for regeneration of multiple shoots without intervention of callus. In the present investigation *in vitro* nodal segments were cultured in MS media with different hormonal combinations and concentrations. *In vitro* nodal segments of *A. lebbeck*, maximum 75% of explants regenerated shoots in the MS media supplemented with 2.0 mg L^{-1} BA+0.2 mg L^{-1} NAA (Table 1). The highest mean number of shoots per culture was 7.3 in media containing 2.5 mg L^{-1} BA+0.2 mg L^{-1} NAA (Table 1). Effectiveness of cytokinin BA for shoot multiplication in trees has been well demonstrated in the seedling tissue of *Calophyllum*, *Eugenia*[6], *Swietenia*[6]. Other

Table 1: Effect of different concentrations and combinations of growth regulators in MS media on direct proliferation of shoots from three types of explants of *Albizia lebbeck* (Twelve test tubes were cultured in each combinations. Data collect after 42 days of culture)

	Cotyledon			*In vitro* nodal segments			*In vivo* nodal segments		
Supplements (mg L^{-1})	% of explants regenerated shoots	Mean number of shoot explant^{-1}	Mean length of the longest shoot	% of explants regenerated shoots	Mean number of shoot explant^{-1}	Mean length of the longest shoot	% of explants regenerated shoots	Mean number of shoot explant^{-1}	Mean length of the longest shoot
BA									
0.1	-	-	-	-	-	-	-	-	-
0.2	16.66	2.3	3.7	25.00	1.8	3.1	-	-	-
0.5	25.00	2.1	3.6	25.00	2.2	3.4	-	-	-
1.0	33.33	3.2	4.1	41.66	2.3	4.1	-	-	-
2.0	75.00	4.2	4.2	50.00	3.7	4.7	-	-	-
2.5	83.33	9.0	5.1	50.00	3.7	4.7	-	-	-
3.0	41.66	3.4	3.1	33.33	2.0	3.2	16.66	1.0	2.0
4.0	16.66	1.7	2.9	16.66	1.7	3.3	-	-	-
BA+NAA									
1.0+0.1	25.00	1.6	3.4	25.00	2.7	3.4	-	-	-
1.0+0.5	25.00	2.3	4.1	33.33	2.1	3.5	-	-	-
2.0+0.2	50.00	5.4	5.6	75.00	4.6	4.3	16.66	1.0	2.5
2.0+0.5	50.00	5.2	6.0	50.00	3.4	3.9	-	-	-
2.5+0.2	66.66	6.0	5.7	66.66	7.3	4.1	16.66	1.0	2.0
2.5+0.5	50.00	5.1	3.1	50.00	3.2	3.8	25.00	1.5	2.7
3.0+0.2	25.00	2.6	3.2	25.00	1.8	3.2	8.33	1.0	2.6
3.0+0.5	16.66	2.1	2.9	16.66	1.0	3.3	-	-	-
KIN+NAA									
1.0+.01	33.33	1.2	2.7	16.66	1.2	2.5	-	-	-
1.0+0.5	41.66	1.5	2.6	25.00	1.5	2.4	-	-	-
2.0+0.2	50.00	2.7	3.5	33.33	1.8	3.0	-	-	-
2.0+0.5	41.66	2.3	2.9	41.66	2.4	3.4	16.66	2.0	2.4
2.5+0.2	50.00	2.6	3.7	33.33	2.1	3.1	8.33	1.0	2.1
2.5+0.5	33.33	1.4	2.5	25.00	1.0	1.8	-	-	-
3.0+0.2	-	-	-	-	-	-	-	-	-
3.0+0.5	-	-	-	-	-	-	-	-	-
KIN+IAA									
1.0+.01	-	-	-	-	-	-	-	-	-
1.0+0.5	16.66	1.0	2.0	8.33	1.0	2.5	-	-	-
2.0+0.2	25.00	1.5	2.5	16.66	1.5	2.4	-	-	-
2.0+0.5	33.33	1.8	2.8	25.00	1.6	2.4	-	-	-
0.1+1.0	50.00	2.7	2.7	33.33	2.1	2.6	8.33	1.0	2.5
0.5+1.0	41.66	2.3	2.5	41.66	2.3	2.7	16.66	1.0	2.8
0.2+2.0	50.00	3.4	3.1	50.00	2.3	2.9	-	-	-
0.5+2.0	41.66	2.2	2.4	41.66	1.5	2.3	-	-	-

Table 2: Effect of different concentrations and combinations of cytokinin with auxin on MS medium on morphogenic response of different types of explants of *Albizia lebbeck*. (Data collected after 28 days of culture)

		Explants induced callus (%)					
Supplements (mg L^{-1})	Number of explants inoculated	Cotyledon	Degree of developing g shoot bud	Internodal segments (*in vitro* grown)	Degree of developing g shoot bud	Internodal segments field grown	Degree of developing g shoot bud plants
1	2	3					
BA+NAA							
1.0+ 0.1	11	63.63	-	72.72	+	27.27	-
1.0+0.2	11	63.63	+	81.81	+	36.36	-
2.0+0.2	11	90.90	+++	100.00	+++	45.45	-
2.0+0.5	11	90.90	+++	100.00	+++	36.36	-
3.0+0.2	11	81.81	++	90.90	++	-	-
KIN+NAA							
1.0+ 0.1	11	-	-	18.18	+	18.18	-
1.0+0.2	11	27.27	+	36.36	+	36.36	-
2.0+0.2	11	54.54	+	54.54	+	27.27	+
2.0+0.5	11	45.45	+	63.63	++	-	-
3.0+0.2	11	36.36	+	45.45	+	-	-
2,4-D							
0.1	11	-	-	-		-	-
0.5	11	-	-	-		-	-
1.0	11	18.18	-	-		-	-
2.0	11	36.36	-	27.27		18.18	-
3.0	11	18.18	-	27.27		18.18	-

Table 3: Proliferated shoots (No.) per callus cultured in different concentrations and combinations of growth regulators in MS medium

Supplements (mg L^{-1})	Callus derived from cotyledon	Callus derived from nodal segment (*in vivo*)	Callus derived from nodal segment (*in vitro*)
BA			
0.1	2.23	-	-
0.2	4.45	1.25	-
2.0	5.17	4.75	2.34
2.5	2.50	2.30	2.16
3.0	1.30	-	-
5.0	-	-	-
BA+NAA			
1.0+.01	2.12	-	-
1.0+0.2	2.75	-	-
2.0+0.2	3.40	3.12	-
2.0+0.5	4.10	3.25	-
2.5+0.2	5.12	3.75	2.75
3.0+0.3	2.75	1.25	1.12
3.0+0.5	1.50	-	-
BA+KIN			
0.1+0.1	-	-	-
0.1+1.0	2.12	-	-
2.0+0.2	2.50	1.25	1.25
0.2+2.0	3.75	3.12	2.12
2.0+0.5	2.50	3.50	-
0.5+2.0	4.75	3.25	2.50

combinations and concentrations of KIN with NAA and IAA showed relatively lower result.

***In vivo* nodal segments:** *In vivo* juvenile nodal segments, harvested from mature tree of *A. lebbeck* were cultured on MS media. *In vivo* nodal segment could not exert efficient regeneration as obtained from *in vitro* nodal segments. *A. lebbeck* showed maximum 25% regeneration in MS medium supplemented with 2.5 mg L^{-1} BA+0.5 mg L^{-1} NAA (Table 1). The highest 90% of microshoots of *A. lebbeck* regenerated roots in MS medium containing 1.0 mg L^{-1} IBA.

Callus induction: Three types of explants (cotyledon, nodal segment of *in vitro* grown seedlings and nodal segments of field grown trees) of woody legume, *Albizia lebbeck* were conducted with a view to finding out optimum culture media and hormonal combination and concentration for callus induction. Explants of *A. lebbeck* were cultured on MS media supplemented with different combination and concentrations of BA with NAA, KIN with NAA and 2, 4-D alone (Table 2). In case of callus induction, among the three types of explants (cotyledon, *in vitro* nodal segments and *in vivo* nodal segments) of *A. lebbeck,* best callus induction was obtained in internodal segments and 100% callus induction was obtained in media containing 2.0 mg L^{-1} BAP+0.2 mg L^{-1} NAA and 2.0 mg L^{-1} BAP+0.5 mg L^{-1} NAA (Table 3 and Fig. 1). Other explants showed relatively lower result in different hormonal combinations and concentrations. Efficiency of combinations of higher cytokinin and lower auxin in callus induction corroborate with that of Nagmani and Venketeswaran[7]. They observed the callus induction of *Leucaena leucocepa* in MS media supplemented with NAA (0.5 mg L^{-1}) and BA (0.5-5.0 mg L^{-1}) in both hypocotyl and cotyledonary explants. Satisfactory level of callus induction was noticed in different concentrations and combinations of KIN with NAA. Many workers observed 2, 4-D as the best auxin for callus induction in monocot and even in dicot[8-11]. But in this investigation it

Fig. 1: (A) Callus proliferated from cotyledon explants in MS+2.0 mg L^{-1} BA+0.2 mg L^{-1} NAA. (B) A callus proliferated from cotyledon explant in MS+2.0 mg L^{-1} BA+0.5 mg L^{-1} NAA. (C) Bud primordial differentiation from cotyledon callus MS+2.0 mg L^{-1} BA+0.2 mg L^{-1} NAA. (D) Bud primordial differentiation from *in vivo* internodal segments callus MS+2.0 mg L^{-1}. (E) Multiple shoot development from cotyledon callus in MS+2.5 mg L^{-1} BA+0.2 mg L^{-1} NAA. (F) Multiple shoots development from *in vitro* internodal segments callus in MS+2.5 mg L^{-1} BA+0.2 mg L^{-1} NAA

Fig. 2: (A) Multiple shoot development from cotyledon in MS+2.5 mg L^{-1} BA. (B) Multiple shoot development from cotyledon in MS+2.5 mg L^{-1} BA+0.2 mg L^{-1} NAA. (C) Multiple shoot developed from *in vitro* nodal segments in MS+ 2.5 mg L^{-1} KIN+0.2 mg L^{-1} NAA. (D) Multiple shoot developed from *in vitro* nodal segments in MS+2.0 mg L^{-1} BA+0.2 mg L^{-1} NAA. (E) Multiple shoot developed from *in vitro* nodal segments in MS+2.0 mg L^{-1} BA. (F) Multiple shoot developed cotyledon in ½ MS+2.0 mg L^{-1} BA. (G) Multiple shoot developed from *in vitro* nodal segments in ½ MS+2.0 mg L^{-1} BA+0.2 mg L^{-1} NAA. (H) Multiple shoot developed from *in vitro* nodal segments in ½ MS+2.0 mg L^{-1} BA+0.2 mg L^{-1} NAA. (I) Multiple shoot developed from *in vitro* nodal segments in ½ MS+2.0 mg L^{-1} IAA+0.2 mg L^{-1} KIN

was been found that the auxin 2, 4-D alone was not so effective in these three explants of *A. lebbeck*. The analysis of experimental results indicates that the nature, concentrations and combinations of growth regulator as well as the types of explants played significant role in callus induction in the woody legume (*A. lebbeck*). Growth regulators and explants showed their individual and specific effects on callus induction separately on legume species.

Callus regeneration: Callus obtained from three types of explants of *Albizia lebbeck* regenerated shoots. A good number of shoot regeneration observed in calli population derived from cotyledonary explants. For callus regeneration cytokinin BA singly or in combination with NAA and KIN were tried in MS medium. *In vitro* internodal segments derived calli were also good in regeneration but the number of regenerated shoot was less than those of calli derived from cotyledonary explants (Fig. 2). Calli produced by *in vitro* internodal segments were the poorest in respect of shoot regeneration as well as number of regenerated shoots. MS media supplemented with the cytokinin BA alone was the best for regeneration. Different concentration of BA in the range of 0.2-2.5 mg L^{-1} showed different levels of regeneration efficiency. BA in combination with NAA and KIN also stimulated moderate degree of regeneration.

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