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Shoot Regeneration from Hypocotyl and Shoot Tip Explants of *Sterculia foetida* L. Derived from Seedlings

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ABSTRACT: Hypocotyl and shoot tip explants derived from the seedlings of *Sterculia foetida* were placed on MS medium supplemented with various cytokinin and auxin combinations. Cytokinin-like N^6-benzylaminopurine (BAP), kinetin (KN), thidiazuron (TDZ) and auxin-like indole acetic acid (IAA), naphthalene acetic acid (NAA) and indole butyric acid (IBA) were supplemented either individually or in combinations. The regenerated shoots were separated and placed on the rooting medium augmented with different auxin-like IAA, IBA and NAA (0.1-2 mgL^{-1}). IAA (2 mgL^{-1}) induced 3-4 roots from the cut end of the micro shoots. Rooted plantlets were acclimatized to field conditions by placing them in pots containing sterilized sand, soil and manure mixture (1:1:1) and subsequently transferred to field with 40% survival.

KEY WORDS: ***In vitro*** **propagation, Hypocotyl, Shoot tip explants,** ***Sterculia foetida*****.**

INTRODUCTION

Sterculia foetida L. is a large deciduous tree belonging to Sterculiaceae. It is distributed in Asia, Eastern tropical Africa and Northern Australia. Seed oil of *Sterculia foetida* is used for treating skin diseases and rheumatism. Seeds, commonly known as Stinking beans, are edible and if taken in large quantities act as violent purgative and said to bring about abortion. Bark and leaves have minor medicinal value (Kirtikar and Basu, 1935). Seeds are also used in soap and surface coating industries, while timber is valued for interior decoration. Besides this tree also yields gum and bast fibres.

Natural regeneration has major constraints such as loss of viability with passage of time, long life cycles and seasonal response; therefore *in vitro* regeneration was preferred. This fascinating technique has the advantage to maintain superior, genetically stable, disease free stocks with minimum space and plant material independent of season (Winton, 1970; Mascarenhas and Muralidharan,1989). *In vitro* regeneration also accounts for the *ex situ* conservation of plant diversity (Krogstrup *et al.*, 1992; Fay, 1994). In Sterculiaceae *in vitro* propagation was reported in some members such as *Sterculia urens* (Pandey, 1998; Purohit and Dave, 1996), *Hildegardia populifolia* (Anuradha and Pullaiah, 2001) and *Theobroma cacao* (Wen and Kinsella, 1991). In view of its medicinal, industrial applications and lack of tissue culture reports the present work was undertaken as a means of rapid propagation.

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MATERIALS AND METHODS

Dry follicles of *Sterculia foetida* were collected in the month of September during 1997-1998 from Andhra University campus, Visakhapatnam and Bobbili, Vizayanagaram district, Andhra Pradesh, India. Seeds collected soon after harvest i.e., from first week to fifth month with an interval of 20 days, were washed in an agitated solution of liquid detergent (1% labolene) for 15 min and washed under running tap water for half-an-hour. Seeds were rinsed with sterilized double distilled water and surface sterilized with 70% ethyl alcohol for 1 min followed by $HgCl_2$ (0.1%) treatment for 15 min. Finally the seeds were rinsed 4-5 times with sterilized distilled water and soaked in water for 24 hours. After fifth month onwards there was decrease in viability of seeds slowly (Anitha, 2001). Seeds were later placed on half strength Murashige and Skoog's (1962) (MS) medium fortified with BAP (0.1 mgL^{-1}), 1% sucrose, gelled with 0.5% agar and incubated at 25 ± 2°C under 16hr photoperiod with light intensity of 2,000 lux. After 15 days excised seedling explants like shoot tip (1 cm), epicotyl (0.5 cm), hypocotyl (0.5 cm) and root (0.5 cm) were placed on full strength MS medium supplemented with BAP (2 mgL^{-1}), 2% sucrose and gelled with 0.8% agar. The selected shoot tip and hypocotyl explants were later placed on various hormone incorporated media containing BAP (0.1, 1, 2, 3 and 5 mgL^{-1}), KN (0.1, 1, 2 and 3 mgL^{-1}) and TDZ (0.05 and 0.5 mgL^{-1}) individually, or in combinations of BAP and KN (1 and 2 mgL^{-1}) or BAP (2 mgL^{-1}) along with auxins IAA or IBA or NAA (0.1 and 1 mgL^{-1}). Effect of various carbon sources like cane sugar, sucrose, fructose and dextrose at 2% concentration and influence of different concentrations of sucrose (1, 2, 3, 5 and 7% w/v) was also assessed. Medium was adjusted to pH 5.8 followed by addition of 0.8% agar before dispensed into Borosil glass tubes (25 x 150 mm) with 15 ml volume and then autoclaved at 1.06 kg/cm^2 pressure for 15 min. All culture tubes were incubated at 25 ± 2°C and maintained under 16 hr photoperiod with cool white fluorescent lamps (2,000 lux). For shoot elongation regenerated shoots were placed on MS medium containing IAA or IBA or NAA or GA_3 (0.1 and 1 mgL^{-1}). Elongated de novo shoots of 2-3 cm obtained mostly from shoot tip were separated and placed on rooting medium containing half strength MS medium supplemented with NAA or IAA or IBA (0.1 and 1 mgL^{-1}) individually or in combinations as mentioned in table 3. When roots formed measured 3-4cm (i.e., 35 to 60 days of rooting) plantlets were removed and placed in liquid quarter strength MS basal medium, after cleaning properly with sterile distilled water so as to remove agar traces. After one week these plantlets were again transferred to the pots containing sterilized sand, soil and manure mixture (1:1:1), combination used by Rout *et al.* (1999) and covered with polythene bags to maintain humidity and placed in culture room at 25 ± 2°C. After 14 days of above procedure plants were shifted to the green house placed under shade for 30 days and subsequently planted in the field.

Ten replicates were used per treatment and each experiment was repeated thrice. All cultures were examined periodically and visible morphological changes were recorded. The data pertaining to the mean number and mean length of shoots/roots formed were statistically analyzed and standard deviation was noted for twenty replicates.

RESULTS AND DISCUSSION

Among different seedling explants tested only shoot tip (1 cm) and hypocotyl (0.5 cm) fragments responded with shoot bud formation while other explants developed callus slightly on BAP (2 mgL^{-1}) supplemented full strength MS medium. Shoot tip explants responded after 20 days of culture with the formation of new set of leaves, while hypocotyl segments responded with callus formation towards cut end and developed shoot buds only after 35 days of culturing. Among different carbon sources tested, i.e. cane sugar, fructose, dextrose and sucrose, sucrose was preferred and optimum sucrose concentration was found to be 2-3% (Fig. 1). Further increase in sucrose concentration (5 and 7%) decreased shoot number and increased callus formation towards basal end. In the present investigation cane sugar was tested for developing a cost effective protocol. Sucrose was found to be suitable even in earlier reports (Purohit and Dave, 1996; Shailaja *et al.*, 1997).

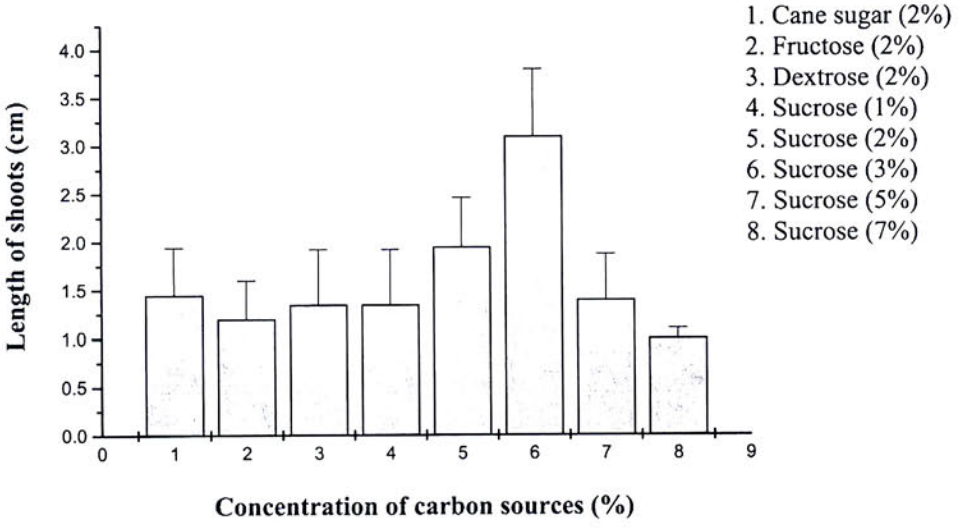

Fig. 1. Influence of various carbon sources and sucrose concentrations on shoot formation using shoot tip explant.

When different cytokinins were fortified in the medium, BAP (2 mgL^{-1}) induced 4-5 shoots from shoot tip explants followed by 3-4 shoots from hypocotyl explants (Fig. 2 A-C). KN and TDZ at different concentrations induced 1-2 shoots from shoot tip explants, while hypocotyl explants developed only white friable callus towards cut end (Table 1). Shoot bud formation from hypocotyl explants is an uncommon phenomenon and reported only in a limited number of woody species (Subbaiah and Minocha, 1990; Bansal and Pandey, 1993; Sankhla *et al.*, 1993). BAP elicited best response among three tested cytokinins. Superiority of BAP was also reported in earlier studies on members of Sterculiaceae (Sunnichan *et al.*, 1998; Purohit and Dave, 1996; Anuradha and Pullaiah, 2001). Action of different cytokinins may vary with the type and size of the explant used. BAP in combination with KN and auxins like IAA, NAA and IBA was not effective in enhancing shoot number from shoot tip explants, while hypocotyl segments remained unresponsive (Table 2). Average shoot length was 1.5 cm or below in most of the treatments. For improving the shoot length different growth regulators were used as noted before. Among them IAA (1 mgL^{-1}) incorporated medium enhanced shoot length by 1cm within 15 days of subculturing.

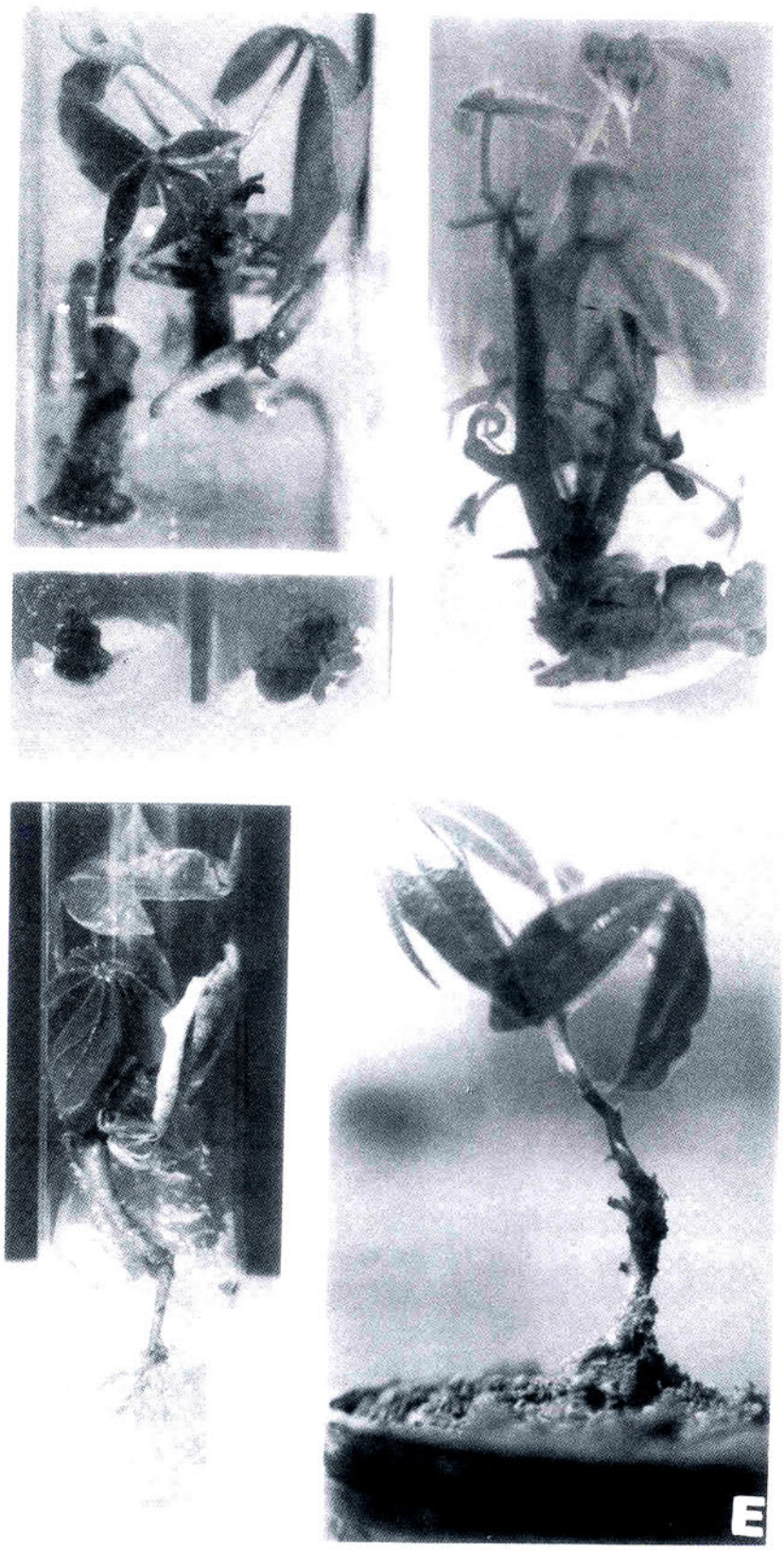

Fig. 2. A & B: Response of shoot tip and hypocotyl explants on BAP (2 mgL^{-1}) + 2% sucrose. C: Response of shoot tip on MS medium fortified with BAP (2 mgL^{-1}) + 3% sucrose. D: Induction of roots from excised shoot placed on IAA (2 mgL^{-1}) supplemented half MS strength medium. E: Potted *in vitro* regenerated plantlet.

Table 1. Effect of various cytokinins on shoot proliferation from excised shoot tip and hypocotyl fragments of *Sterculia foetida.*

Hormone concentration in mgL^{-1}		Average length of shoots (cm) Mean* ± SD		Average number of shoots formed Mean* ± SD	
		Shoot tip	Hypocotyl	Shoot tip	Hypocotyl
Control		1.14 ± 0.16	-	1.00 ± 0.00	-
BAP	0.1	1.55 ± 0.27	-	1.10 ± 0.30	-
	1.0	1.80 ± 0.26	-	1.40 ± 0.58	-
	2.0	1.95 ± 0.51	1.06 ± 0.10	3.10 ± 0.58	2.45 ± 0.66
	3.0	2.22 ± 0.27	0.92 ± 0.13	2.05 ± 0.70	1.65 ± 0.47
	5.0	1.56 ± 0.39	-	1.50 ± 0.50	-
KN	0.1	1.22 ± 0.32	-	1.00 ± 0.00	-
	1.0	1.40 ± 0.38	-	1.30 ± 0.45	-
	2.0	1.52 ± 0.21	-	1.60 ± 0.58	-
	3.0	1.39 ± 0.33	-	1.55 ± 0.58	-
TDZ	0.05	1.49 ± 0.39	-	1.40 ± 0.66	-
	0.5	1.30 ± 0.37	-	1.00 ± 0.00	-

*: 20 replications
SD - standard deviation
-: indicates no response

Table 2. Effect of cytokinin and auxin combinations on shoot formation from shoot tip fragments of *Sterculia foetida.*

Hormone concentration in mgL^{-1}	Average number of shoots formed Mean* ± SD	Average length of shoots (cm) Mean* ± SD
Control	1.14 ± 0.16	1.00 ± 0.00
BAP+ KN		
1.0 1.0	1.41 ± 0.34	1.45 ± 0.58
2.0 1.0	1.62 ± 0.29	1.80 ± 0.81
1.0 2.0	1.31 ± 0.28	1.35 ± 0.65
BAP + NAA		
2.0 0.1	1.26 ± 0.37	1.40 ± 0.66
2.0 1.0	1.48 ± 0.42	1.30 ± 0.55
BAP + IAA		
2.0 0.1	1.46 ± 0.37	1.05 ± 0.21
2.0 1.0	1.55 ± 0.30	1.20 ± 0.50
BAP+IBA		
2.0 0.1	1.08 ± 0.19	1.25 ± 0.53
2.0 1.0	1.21 ± 0.31	1.15 ± 0.35

*: 20 replications
SD: standard deviation

Regenerated shoots were isolated and placed on root inducing medium containing half strength MS medium fortified with IAA, IBA and NAA and various auxins (Table 3). Roots were formed in all auxin incorporated media, except with IBA (0.1 mgL^{-1}). Single root or 2 roots were formed in most of the treatments. IAA at 2 mgL^{-1} induced 3-4 roots from cut end

Table 3. Rooting response of regenerated shoots obtained from shoot tip explants of *Sterculia foetida* using various auxin concentrations on half strength MS medium.

Auxin conc. in mgL^{-1}	Number of days required for rooting	Average length of roots (cm) Mean* ± SD	Average number of roots formed Mean* ± SD
NAA			
0.1	43	2.00 ± 0.56	1.70 ± 0.47
1.0	50	2.20 ± 0.37	1.39 ± 0.39[b]
2.0	35	2.37 ± 0.32	1.10 ± 0.31[b]
IAA			
0.1	68	2.75 ± 0.33	1.65 ± 0.49
1.0	60	3.17 ± 0.75	1.80 ± 0.50[b]
2.0	60	3.51 ± 0.48	2.70 ± 0.47[b]
IBA			
0	0	0	0
1.0	70	2.02 ± 0.12	1.35 ± 0.58
2.0	70	2.18 ± 0.31	1.50 ± 0.50[b]
NAA + IAA			
1 each	50	2.14 ± 0.32	1.25 ± 0.43[b]
NAA + IBA			
1 each	50	1.77 ± 0.23	1.40 ± 0.48[b]
IAA + IBA			
1 each	45	1.60 ± 0.39	1.20 ± 0.40[b]

*: 20 replications
'b' indicates basal callus formation

of the micro shoots implanted, whereas at 0.1-1 mgL^{-1}IAA induced mostly single root (Fig. 2D). Slight callus formation was observed from basal end of micro shoots in some tested rooting concentrations. IAA was reported for rooting earlier in some woody taxa (Anuradha and Pullaiah, 1999). Rooted plantlets were placed in liquid quarter strength MS basal medium as stated before, transferred to pots containing sterilized sand, soil and manure mixture (1:1:1) and covered with polythene bags. Liquid half strength MS basal medium was watered to these plants. Polythene bags were perforated and constantly removed. Plants were acclimatized to room temperature, later shifted to the green house after 14 days (Fig. 2E) and kept under shade for 30 days, then planted in the field with 40% recorded plant survival. Trials are underway for further improving the survival rate.

In the present study an efficient protocol was developed for *in vitro* propagation of *Sterculia foetida* using shoot tip and hypocotyl explants. It may also be a source of propagules of selected superior trees for use in reforestation programmes and for germplasm conservation.

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梧桐幼苗之幼莖及頂芽培植體的莖再生

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摘　　要

從梧桐幼苗獲得幼莖及頂芽的培植體，培養於含有不同濃度的細胞分裂素及生長素的 MS 培養基中。所用的細胞分裂素有 BAP、KCN 及 TDZ，生長素則有 INN、NAA 及 IBA。培養基中有些同時含有細胞分裂素及生長素，惟有些培養基中僅含有細胞分裂素或生長素。再生的莖則培養於長根的培養基中，其中含有不同種類及濃度的生長素，如 IAA、IBA、NAA (0.1 ~ 2 mg/L)及 IAA (2 mg/L)。等莖長出 3 ~ 4 根後，則培植於盆中，盆中的混合土含有砂、土壤及腐植質(比例為 1:1:1)。然後把盆栽小植物放置於室外，使小植物能夠適應室外的生長環境，惟其存活率只有 40%。

關鍵詞：活體外繁殖、幼莖(胚軸)、頂芽培植體、梧桐。

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