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Effect of cytokinins and auxin on shoot proliferation of cotyledonary nodes derived from axenic seedling of tamarind (*Tamarindus indica* L.)

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ABSTRACT

An experiment was conducted in the plant tissue culture laboratory of the Department of Horticulture, University of Agricultural Sciences, Dharwad during 2001-03, to standardize concentrations of cytokinins and auxin on shoot proliferation of cotyledonary nodes derived from axenic seedling of tamarind. From the present investigation it was clear that among the various combinations of growth regulators, BAP 0.5 mg/l + NAA 0.1 mg/l was the best combination for shoot proliferation of cotyledonary nodes derived form axenic seedling of tamarind.

Key words : Tamarind, Cytokinine, Auxin

Tamarind (*Tamarindus indica* L.) is one of the arid fruits crops grown widely in the tropical and sub-tropical regions of the Indian sub-continent particularly in central and south India. Tamarind is popularly known as 'Indian date'. It is multipurpose tree having high medicinal, industrial and nutritional values in addition to its main use as food, fodder and timber.

Micropropagation provides a rapid, reliable system for the production of large number of genetically uniform plantlets. It offers a method to increase valuable genotypes rapidly and expedite the release of improved varieties. In addition, micropropagation ensures mass production of elite clones from hybrid or specific parental line. It makes the propagules which have good health status and possessing desirable characters available through out the year.

Micropropagation of tree species offers a rapid means of producing clones, planting stock for afforestation, woody biomass production and conservation of elite and rare germplasm (Bonga and Durzan, 1982; Bajaj, 1986). But woody taxa are generally difficult to regenerate under *in vitro* conditions. Recently, some success has been achieved in few leguminous tree species (Dhawan, 1989), tamarind being one among them. Regeneration of plantlets from shoot tips and cotyledons obtained from seedlings of tamarind have been reported by Kopp and Nataraja (1990) and Jaiwal and Gulati (1991). *In vitro* induction of multiple shoots from axillary buds of tamarind has also been reported (Balkrishnamurthy and Ganga, 1997). However, till date there is no standard protocol available for *in vitro* clonal propagation of this tree. Considering the above facts the present investigation was carriedout to standardize the *in vitro* propagation methodology of tamarind.

MATERIALS AND METHODS

Tamarind seeds were collected from the mature pods of elite tree of DTS-1 situated in the Golden Jublee Block, Kumbhapur Farm, Department of Horticulture, Main Agricultural Research Station, Dharwad. They were thoroughly washed and then treated with a detergent Tween-20 (0.1%) for 10 minutes. After thorough washing with double distilled water, the seeds were surface sterilized with 0.1 per cent (w/v) aqueous mercuric chloride solution for 15 minutes followed by washing in distilled water and soaking in sterile water for 4-5 hours. The seeds were individually cultured on solidified half strength MS medium containing two per cent sucrose and 0.7 per cent agar. The cultures were incubated in dark at 25±2^{0}C. Within 15 days of culture, germination occurred and seedlings of 5-8 days were used as the source of explant. The cotyledonary nodes of size 1.0 - 1.5 cm were used as explants from axenic seedlings.

In order to standardize the concentration and type of cytokinin on shoot proliferation different cytokinins mainly 6-benzylaminopurine (BAP) (@ 0.5, 1.00, 2.00 mg/l) and kinetin (KIN) (@ 1, 2, 3 mg/l) were added to MS media. To study the effect of cytokinins and auxin combinations on shoot proliferation different auxin concentration *viz.*, 1 napthaleneacetic acid (NAA) (@ 0.1, 0.2, 0.4 mg/l) were added to the MS media containing BAP 0.5 mg/l and kinetin 1 mg/l. All the cultures were incubated in air conditioned room at a temperature of

25±2°C, with photoperiodic regime of 16 hr light and eight hour dark cycles. Observations were recorded on per cent shoot induction, mean number of days taken for shoot induction, mean number of shoots per explant and per cent response to multiple shoot induction after four weeks of incubation. The data generated from the experiments were statistically analysed as described by Panse and Sukhatme (1967).

RESULTS AND DISCUSSION

In plant tissue culture, cytokinins appear to be necessary for cell divisions. Cytokinins are effective in promoting shoot initiation directly or indirectly. Successful treatment with cytokinins induces the growth of several small shoots from each explant over a period of 4-6 weeks. Some times cytokinins are required for embryogenesis and promotion of direct or indirect adventitious shoot formation. The effect of cytokinins on tissue organ cultures can vary according to the type of cytokinins used, the type of culture, the variety of plant and source of the explant. Hence, the efficacy of different cytokinins such as BAP and kinetin for induction of multiple shoots from cotyledonary node was assessed (Table 1). With these cytokinin treatments it was also observed that multiple shoots were induced directly from the cotyledonary nodes along with some amount of callus in the initial stages of growth. BAP was found to be a best source of cytokinin, since the highest per cent of multiple shoots (100%) along with highest number of shoots (2.25 per explant) were induced after 8 days of inoculation in medium containing 0.5 mg/l BAP. However, with higher concentration of BAP, the multiple shoot production was reduced. The treatment involving KIN showed the maximum number of multiple shoot induction (75%) at a concentration of 1.0 mg/l.

These results clearly reveal that BAP is the best source of cytokinin for induction of multiple shoots from cotyledonary nodes in case of tamarind. The results are in confirmity with the findings of previous workers in various species. The optimal level of BAP for shoot bud development from seedling explants of mangosteen (*Garcinia mangostana*) was 5.0 mg/l. However higher concentrations were also effective, but shoot buds were clustered and stunted (Goh *et al.*, 1995). Among the other cytokinins tested, BAP was more effective than KIN with maximum shoot proliferation from nodal explants in jack fruit when used at 0.5 mg/l (Rahman and Blake, 1988). Umer and Jagadishchandra *et al.* (1993) obtained the highest number of shoots from seedling explants of *Chloroxylon swietenia* (Satinwood) on MS medium containing 0.5 mg/l BAP.

After having assessed the type of cytokinins and its concentration for multiple shoot induction, the results were further utilized for other treatment combinations with auxin to know the synergistic effect (Table 2).

The highest number of multiple shoots (2.50) and per cent (100%) shoot multiplication in early days (8.00) were recorded in the medium containing BAP 0.5 mg/l + NAA 0.1 mg/l. This may be due to the reason that auxins at lower concentrations show synergistic effect of

Table 1 : Effect of cytokinins on shoot proliferation of cotyledonary nodes derived from axenic seedlings of tamarind

Tr. No.	*BM + Treatments	Per cent shoot induction	Mean number of days taken for shoot induction	Mean number of shoots per explant	Per cent response to multiple shoot induction
T_1	BAP 0.5 mg/l	85.00	8.50	2.25	100.00
		(67.20)			(90.00)
T_2	BAP 1.0 mg/l	72.25	9.50	2.15	50.00
		(58.20)			(45.00)
T_3	BAP 2.0 mg/l	62.30	11.00	1.65	75.00
		(52.14)			(60.00)
T_4	BAP 3.0 mg/l	55.22	13.25	1.40	50.00
		(47.96)			(45.00)
T_5	KIN 1.0 mg/l	65.20	7.25	1.90	75.00
		(53.85)			(60.00)
T_6	KIN 2.0 mg/l	47.80	9.50	1.75	50.00
		(43.74)			(45.00)
T_7	KIN 3.0 mg/l	43.50	11.50	1.75	50.00
		(41.16)			(45.00)
	S.E.±	0.667	0.126	0.029	1.49
	C.D. (P=0.01)	2.790	0.528	0.121	5.91

* BM – Murashige and Skoog medium
Figures in parenthesis indicate arcsin values

Table 2 : Effect of cytokinins and auxin on shoot proliferation from cotyledonary node derived from axenic seedlings of tamarind

Tr. No.	*BM + Treatments	Per cent shoot induction	Mean number of days taken for shoot induction	Mean number of shoots per explant	Per cent response to multiple shoot induction
T_1	BAP 0.5 mg/l	85.00	9.00	2.25	100.00
		(67.21)			(90.00)
T_2	BAP 0.5 mg/l + NAA.1 mg/l	86.66	8.00	2.50	100.00
		(68.53)			(90.00)
T_3	BAP 0.5 mg/l + NAA.2 mg/l	79.50	13.00	2.00	75.00
		(63.08)			(60.00)
T_4	BAP 0.5 mg/l + NAA.4 mg/l	0.00	0.00	0.00	0.00
		(0.00)			(0.00)
T_5	KIN 1.0 mg/l	65.00	7.50	2.00	75.00
		(53.73)			(60.00)
T_6	KIN 1.0 mg/l + NAA 0.1 mg/l	68.66	7.25	2.00	90.00
		(55.93)			(71.56)
T_7	KIN 1.0 mg/l + NAA 0.2 mg/l	57.50	9.57	0.00	0.00
		(49.32)			(0.00)
T_8	KIN 1.0 mg/l + NAA 0.4 mg/l	0.00	0.00	0.00	0.00
		(0.00)			(0.00)
	S.E.±	0.92	0.08	0.021	1.129
	C.D. (P=0.01)	3.78	0.33	0.086	4.64

* BM – Murashige and Skoog medium
Figures in parenthesis indicate arcsin values

cytokinin. These findings are in close agreement with those of Splittstoesser and Mohamed Yasseen (1991) who also reported multiple shoot induction in cotyledonary nodes explant in medium containing BA 0.2 mg/l + NAA 0.1 mg/l in case of axenic seedlings of tamarind. However, the other treatments comprising NAA at higher concentration (>0.2 mg/l) resulted in only considerable callus formation. Similar results were obtained by Pierik (1987). From the present investigations it was clear that among the various combinations of growth regulators, BAP 0.5 mg/l + NAA 0.1 mg/l was the best combination for shoot proliferation of cotyledonary nodes derived from axenic seedlings of tamarind.

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