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Micropropagation of *Tylophora indica* (Burm. f.) Merr. by multiple bud formation from mature leaf explants without callus intervention

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Abstract. A rapid *in vitro* propagation system has been established by the formation of multiple adventitious shoot buds without callus intervention from mature leaf explant of a highly valuable medicinal plant - *Tylophora indica*. The explant was cultured on Murashige and Skoog's (1962) medium (MS) supplemented with different concentrations and combinations of 6-Benzylaminopurine (6-BAP) and Adenine sulphate for shoot bud initiation and multiplication. 5 mg/l 6-BAP together with 0.5 mg/l Adenine sulphate produced the greatest average number of shoot buds (304 $\pm$ SE 44) per explant. MS medium supplemented with Indole-3-acetic acid (IAA), α-napthaleneacetic acid (NAA), Indole-3-butyric acid (IBA) in different concentrations were used for rooting of the microshoots. Highest efficiency for rooting has been observed in MS with 3 mg/l IAA. Regenerated plants were successfully transferred to the field (72%).

Key words: Direct regeneration; Medicinal plant; Micropropagation; *Tylophora*.

Introduction

Tylophora indica (Burm. f.) Merr. (English-asthma herb or Indian ipecacuanha), a perennial branching climber of the family Asclepiadaceae, is medicinally important particularly for the treatment of asthma and bronchitis. The presence of two alkaloids, Tylophorine and Tylophorinine, are known since long (Govindachari, 1967). Recently this plant has attracted attention of many serious workers because of the presence of anticancerous principles found within it (Bhutani *et al.*, 1984).

The initiation of callus from stem segments and morphogenetic investigation in callus culture has been reported by Rao and Narayanaswami (1970, 1972). The role of auxins in inducing embryogenic calli and other changes during somatic embryogenesis has also been reported (Bera and Roy, 1989). But no report concerning the direct regeneration from explants has so far been published on this valuable medicinal plant. In this communication an efficient and reproducible method of plant propagation through multiple adventitious shoot bud differentiation from the mature leaf explant of *T. indica* is reported. This direct method would be highly useful for large scale propagation as well as for commercial Tylophorine production.

Materials and Methods

Explant

Fully expanded leaf (2.5 - 3.0 cm in length and 1 - 1.5 cm in width) was used as explant and obtained from plants grown in our experimental garden. Leaves were surface sterilized in 70% (v/v) ethanol for 1 minute, followed by continuous shaking for 10-12 minutes in

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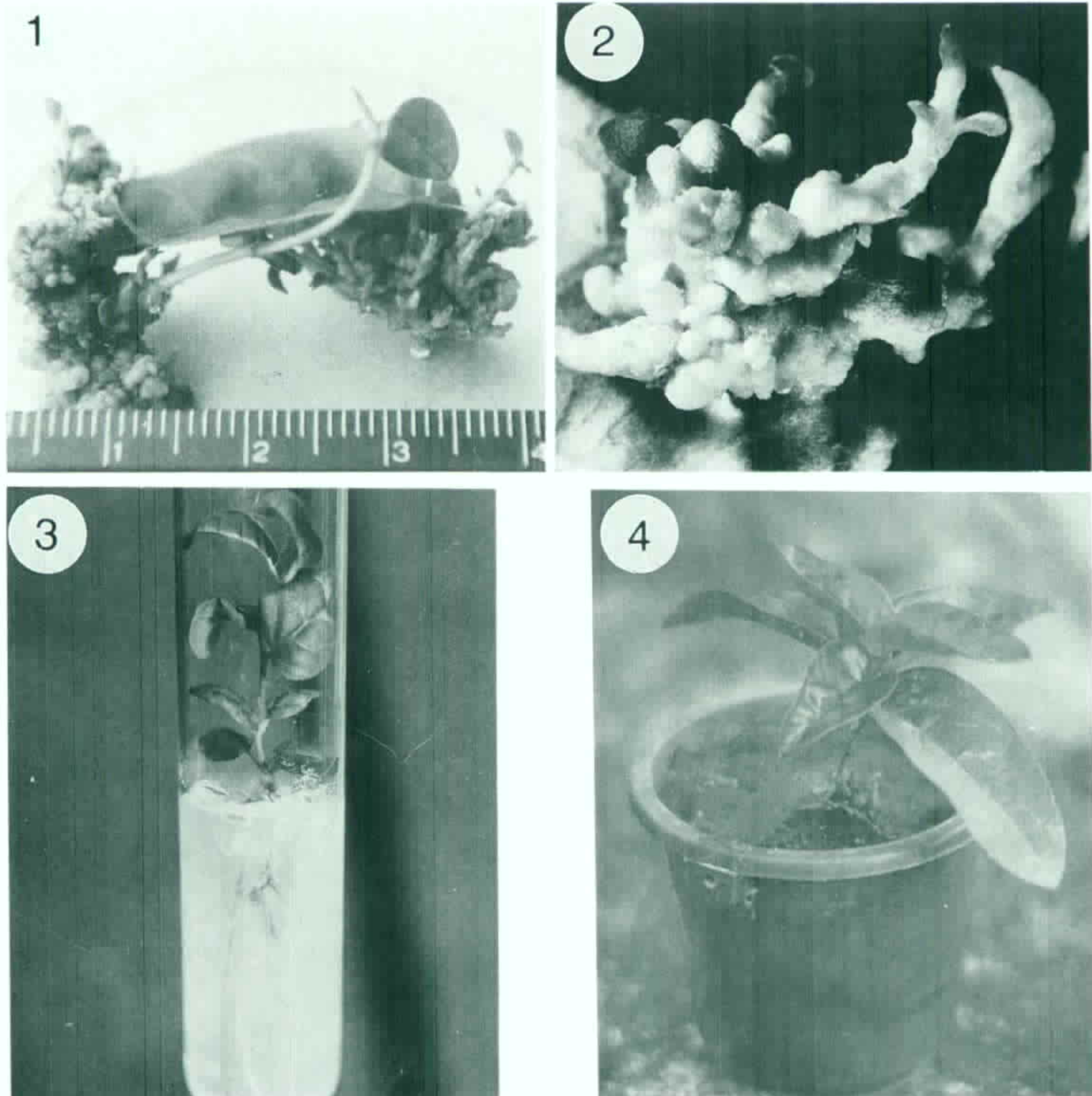
0.1% (w/v) $HgCl_2$ with 5% Teepol. After repeated rinses with sterilized water, the leaf as a whole or part of it were cultured.

Culture Media and Culture Condition

Full strength or half strength Murashige and Skoog's (1962) medium with 3% (w/v) sucrose and 0.6% (w/v) agar (Hi-media bacteriological) was used as the basal medium. It was supplemented with various growth substances such as 6-BAP, Adenine sulphate, IAA, NAA and IBA in different concentrations (Tables 1 and 2). All the media were adjusted to pH 5.8 before autoclaving at 121°C, 1.05 kg/cm² for 15 minutes. The explants were cultured onto 20 ml agarified culture medium in 15 × 2.2 cm culture tubes. The cultures were maintained at 25 ± 1°C temperature under 16 hours photoperiod of 35.7 μE/cm²/sec from cool white fluorescent light at 55-60% constant relative humidity.

Shootbud Initiation and Multiplication

The leaf explants were cultured on MS media supplemented with only 6-BAP (0.5, 1, 2, 5 and 10 mg/l) or 6-BAP (0.5, 1, 2, 5 and 10 mg/l), together with Adenine sulphate (0.5 mg/l) in each case.



Figs. 1-4. 1, Shoots developed from apical and basal part of a whole leaf explant. (× 1.8); 2, Shoots developed from cut end of sectioned leaf explant. (× 5); 3, Rooted shoot. Note the formation of multiple roots. (× 1.2); 4, Regenerated plant, two months after transplanting in soil. (× 0.5).

Rooting of the Regenerated Shootlets

Microshoots of 0.75-1.0 cm length (4-6 week culture) were excised and transferred to full strength MS medium (agarified as well as liquid), supplemented with IAA, NAA and IBA, each in 1, 2, 3 and 5 mg/l, for rooting.

Transfer to the Field

All the rooted microshoots were successfully transferred to the pot through successive acclimatization process and ultimately transplanted and grown in the field.

Results and Discussion

Shoot buds differentiated from the apical as well as basal part of the whole leaf (Fig. 1). When leaf segments were used, shoot buds were originated from the cut surfaces of the lamina. Shoot buds were mostly formed from the cut edges of the midrib and of the side veins of both adaxial as well as abaxial surfaces but more profusely from the surface which was in direct contact with the medium. Shoot bud began to appear after 15-20 days in culture. The highest average number of shoot bud per explant (304 ± SE 44) was found in media containing 5 mg/l 6-BAP and 0.5 mg/l Adenine sulphate after 30-40 days of culture (Table 1).

Shoot buds thus formed after 30 days were sectioned into small groups (15-25 shoot buds in each

Table 1. *Shoot bud formation in MS media with different concentrations and combinations of BAP and Adenine sulphate*

Treatment conc. mg/l		Number of shoot bud*	Range of shoot length (cm)
BAP	Adenine-sulphate		
0.5	0	0	0
1	0	0	0
2	0	24±4.4	0.1-0.5
5	0	99±14.5	0.1-1.75
10	0	70±5.9	0.1-1.9
0.5	0.5	0	0
1	0.5	20.5±2.5	0.1-0.6
2	0.5	151±13.5	0.1-2.78
5	0.5	304±44	0.1-4.5
10	0.5	163.5±21.5	0.1-1.6

*Data presented as the mean value ± standard error of shoot bud formed from a leaf after 30 days of culture.

Table 2. *Response of shoots in different rooting media*

Treatment conc. (mg/l)		Number of shoots with roots*	Nature of root	Maximum root length (cm)
MS +	1	52.5	Normal	5.5
IAA	2	73	Normal	6.7
	3	85	Normal	7.8
	5	40	Few abnormal	2.5
MS +	1	41.5	Hypertrophic	0.75
NAA	2	51	Hypertrophic	1.3
	3	59	Hypertrophic	2.5
	5	38.5	Hypertrophic	0.62
MS +	1	46.5	Slender, Brittle	1.5
IBA	2	56	Slender, Brittle	3.7
	3	66.5	Slender, Brittle	5.8
	5	42	Slender, Brittle	1.2
1/2 MS	1	52	Normal	4.8
+	2	70.5	Normal	6.8
IAA	3	75.5	Normal	7.5
	5	35	Few abnormal	1.6

*Data presented as the % of shoots that formed roots after 30 days of culture.

group) and subcultured on the fresh primary inductive medium for further multiplication and growth. Repeated subculture of groups of differentiating shoot clusters in the same medium accelerated the formation of shoots in large numbers. Moreover, since the cycle of shoot multiplication is very short (4-6 weeks), each cycle resulted in an exponential increase in the number of shoots and thus could be continued throughout the year, irrespective of the seasonal variation. The shoot multiplication could be repeated upto 10-12 multiplication cycles with normal morphogenesis and without returning to the original explant source.

IAA showed normal rooting, whereas NAA and IBA produced deformed roots, hypertrophied with NAA and slender and brittle with IBA (Table 2). Half strength MS supplemented with IAA (1, 2, 3 and 5 mg/l) was also tested. Roots were produced within two weeks of culture and the best results were observed in full strength MS supplemented with 3 mg/l IAA (Fig. 3).

All the rooted microshoots were successfully transferred to the pot through successive acclimatization process and ultimately transplanted and grown in the field (60-72%). Most of the plants showed normal and healthy growth after transferring to the field. Morphological variations have not yet been observed among the regenerated clones.

Plants regenerated through callus, cell suspension or protoplast culture are usually associated with a higher degree of somaclonal variants, in contrast to the direct method of plant regeneration (Karp, 1989; Larkin and Scowcroft, 1981). In this investigation, the leaf explant produced large numbers of clones which were morphologically uniform. This observation, along with the prolific rate of shoot multiplication, efficient rooting and field survival, may have some scope in the possible exploitation of the technique for large scale micropropagation of Tylophorine producing *T. indica*.

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Tylophora indica (Burm. f.) Merr. 以成熟葉片培養直接形成多芽之微體繁殖

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利用成熟葉片培養，此不經過癒傷組織階段形成多數不定芽的方式建立了具有高度藥用價值之植物－*Tylophora indica*的快速繁殖系統。培植體培養在添加不同濃度及組合之6-Benzylaminopurine (6-BAP)及Adenine sulphate的Murashige and Skoog's (1962)培養基上供芽體誘導及繁殖。同時含 5 mg/l 6-BAP 及 0.5 mg/l Adenine sulphate 時獲得由一個培植體形成最大平均值(304±SE44)的芽體。添加不同濃度的 Indole-3-acetic acid (IAA)，α-napthaleneacetic acid (NAA)及 Indole-3-butyric acid (IBA)的 MS 培養基供芽體發根，以含 3 mg/l IAA 的 MS 培養基獲得最高的發根率。再生植株已成功地轉植於田間(72%)。