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Research article

IN VITRO REGENERATION OF *Tecomella undulata* (Sm.) Seem- AN ENDANGERED MEDICINAL PLANT.

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ABSTRACT : A simple efficient method for high frequency of shoot regeneration from internodal explants of endangered medicinal plant, *Tecomella undulata* (Sm.) Seem (Bignoniaceae) was developed. It holds tremendous potential of medicinal values used in traditional remedy for spleen diseases and cancer treatment. The MS medium supplemented with various growth hormones like 6-Benzyladenine (BA), Kinetin (Kn), 2, 4- Dichlorophenoxyacetic Acid (2, 4-D) and α-Naphthalene Acetic Acid (NAA) at different concentrations used for regeneration of *T. undulata*. The present study also describes successful plant regeneration from *in vitro* derived callus of internodal explants. BA and Kn in combination with NAA were used for regeneration of plantlets from callus culture. Shoot multiplication was obtained BA with NAA combinations from the callus culture. The best rooting response was observed on 0.3 mg/l IBA alone. The well rooted plantlets were transferred to polybags containing soil + vermiculite in 1: 1 ratio for hardening. Finally the hardened plantlets were transferred to field conditions and found maximum survivability rates.
Keywords: *Tecomella undulata,* callus, internodal explant and growth regulators.

INTRODUCTION

Tecomella undulata (Sm.) Seem (Bignoniaceae) is a medicinally and economically important plant that originated in India, Arabia [1], appears in the list of endangered plants of Rajasthan[2]. The plant grows under natural conditions in wild, unprotected and is highly exploited. It is commonly known as 'Nakacampanki' in (Tamil) [3]. The drug has been extensively used in ayurvedic system of medicine for treatment of leucorrhoea and leucoderma, enlargement of spleen also used for treatment of urinary discharge due to kapha and pitta. The bark has been used in treatment of syphilis, painful swellings and cancer traditionally. Antibacterial activity has been reported in stem extract as well[4]. The plant has been extensively screened for wide range of pharmacological activities. Khatri *et al.*,[5] demonstrated the hepatoprotective activity of stem bark of *Tecomella undulata* against thioacetamide-induced hepatotoxicity. Ahmad *et al* [6] have evaluated the methanolic extract of plant for its anti inflammatory and analgesic potential by using rat paw edema and tail immersion. Verma *et al.,* [7] isolated an iridoid glucoside undulatin assigned as 4'- O-P-coumaroyl-7, 8-dihydro-8-dehydroxymethylbartsioside structurally by chemical and spectroscopic analysis. Joshi *et al* [8] demonstrated the presence of quinonoid in heartwood and an iridoid glucoside, 6-O-veratryl catalposide from the plant. There is a considerable demand of this plant in India and this demand is met from the natural habitat. It has poor perpetuation, germination rate and limited distribution. This leads to rapid depletion of plant material due to over exploitation of this important plant.

MATERIAL AND METHODS

Plant material and explants source

Tecomella undulata was collected from the Sriuvilliputhur, Virudhunagar District, Tamilnadu. The internodal explants were thoroughly washed under running tap water for 25-30 min and then rinsed in a solution containing the surfactant Tween-20 (2 drops in 100 ml solution). Subsequently, they were surface sterilized with 0.1% (w/v) $HgCl_2$ solution for 1-2 min, followed by 3 to 5 rinses with sterile distilled water in a clean air cabinet. The surface-sterilized explants were aseptically cut into 1-2 cm segments and were carefully inoculated onto the MS culture media [9]. All the chemicals and growth regulators were used are analytical grade and purchased from Hi Media Pvt. Ltd. Mumbai, India.

Culture media and culture conditions

The culture media consisted of MS salts augmented with 3% (w/v) sucrose and gelled with 0.8% (w/v) agar (Hi-Media, India). The MS medium is supplemented with combination of various auxins and cytokinins. All plant growth regulators were added to the medium before autoclaving. The pH of the medium was adjusted to 5.6 to 5.8, followed by autoclaving at 121°C at 15 psi (1.06 kg/cm^2) pressure for 15 to 40 minutes. 15ml of medium was dispensed in sterilized culture tubes. The cultures were incubated at 28 ± 2°C and 60 µmol m^{-2} s^{-2} light intensity under 12 hours photoperiod with cool-white fluorescent tubes (Philips, India) and 55% relative humidity.

Statistical analysis

A minimum of ten culture tubes were developed for all combinations previously explained. Analysis of variance (ANOVA) and mean separations were carried out using Duncan's multiple range tests to assess the statistical significance. $P \leq 0.05$ was considered to be statistically significant, using statistical software SPSS ver. 14 (SPSS Inc., Chicago, USA).

RESULT AND DISCUSSION

Tecomella undulata plants were efficiently regenerated from *in vitro* regeneration. When internodal explants from field-grown mature plants of *Tecomella undulata* were cultured on MS medium supplemented with various concentrations of BA, 2, 4-D and Kn. Callus induction was observed on MS medium supplemented with different concentrations of BA, 2, 4-D and Kn alone and in combination with NAA. Callus was initiated along the cut portions after 15 days of inoculation, initially bulging of internodes were observed. Depending on the concentration and combination of growth hormones used a wide range of variation in frequency of callus formation and nature of callus was observed. The concentration of BA with NAA (2.0+ 0.02 mg/L) developed creamish brown compact callus (Table-1). Browning of calli was perhaps due to the synthesis and exudation of phenolic compounds from the tissues. The transfer of calli to fresh medium or medium supplemented with Charcoal/ Polyvenyl pyrrolidine was found to be effective in reducing the browning of callus [10].

A combined effect of different cytokinins (BA and Kinetin) and auxin (NAA) in various combinations supplemented MS medium were used for shoot regeneration. MS basal medium devoid of growth regulators served as control. After two weeks of subculture, shoot buds were emerged on callus surface. High frequency and maximum number (7.6±0.5) of multiple shoots were induced on 2.0+0.02 mg/l BA with NAA supplemented MS medium (Table-2). The superiority of BA over Kn has been reported for shoot bud initiation [11]. Cytokinins have been known to break dormancy of axillary buds resulting in the formation of microshoots [12]. In a tree species, *Wrightia tomentosa* the percentage of bud breakage was significantly higher on media supplemented with BA (2.22 µM - 8.86 µM/l) [13].

The shoot promoting effect of auxin and cytokinin combinations on organogenic differentiation has been well established in several systems [14]. Maximum shoot multiplications occur only in BA is reported in a number of plants [15]. The degree of callus initiation and shoot multiplication varied considerably with the BA concentrations [16]. Subculturing in BA was resulted much amount of undesirable basal callus with development of the proliferated shoots. This inverse relationship between shoot multiplication rate and shoot length in response to exogenous growth regulator was also observed by Castillon and Cornish [17]. Well developed shoots with a length of (3-5cm) were excised and transferred to MS medium supplemented with different concentrations of auxins such as IBA and IAA (0.1- 0.4 mg/l). In IBA supplemented MS medium the number of roots, root length were high when compared to IAA supplemented medium. High frequency and maximum number of roots were induced on MS medium supplemented with 0.3mg/l IBA (Table-3).

Similar response was also observed in the callus formation and shoot multiplication of *Oroxylum indicum* [18]. Ragavendra singh et al., [19] achieved *in vitro* adventitious shoot regeneration in *T. undulata*. The treatment having IAA (0.1 mg/l) and zeatin (2.5 mg/l) in combination yielded maximum number of shoots from hypocotyle and cotyledonary nodes. Pretreatment of regenerated shoots in a mixture of NAA, IAA and IBA (5.0 mg/l, each) for 36 hours was found to be the best for root induction. Survival of 75% was observed from the plantlets kept for hardening, whereas 62% hardened plants survived in field.

According to the selection of hormone and its further modification especially during proper developmental stage may be necessary to obtain desired multiplication rate and shoot length. Here, we have used a combination of growth regulators for shoots elongation. MS medium fortified with 2.0+0.02 mg/l BA and NAA supported better results as compared to other concentrations of BA in terms of period required for number of shoots regenerated and shoot length. In case of Kn + NAA, maximum numbers of shoots (5.2) per explant was recorded in the medium fortified with 2.0+0.02 mg/1 (Table 1).

Table 1: Effect of cytokinins and auxins supplemented individually and in various combinations on callus culture of *Tecomella undulata.*

Growth regulators	Concentration of growth regulators (mg/L)	% of callus formation	Intensity of callus	Nature of callus
Control	--	--	--	No callus formed
BA	0.5	24	+	Brown loose, fragile
	1.5	43	+	Brown loose, fragile
	2.0	57	++	Cremish brown, fragile
	3.0	54	++	Light brownish white, compact
Kn	0.5	26	+	Brown loose, fragile
	1.5	39	+	Light brownish white, compact
	2.0	44	+	Light brownish white, compact
	3.0	52	++	Cremish brown, fragile
2,4-D	0.5	32	+	Brown loose, fragile
	1.5	35	+	Dark brown, fragile
	2.0	51	++	Light brownish white, compact
	3.0	58	++	Cremish brown, fragile
BA+NAA	0.5+ 0.02	55	++	Light brownish white, compact
	1.5+ 0.02	92	+++	Light brownish white, compact
	2.0+ 0.02	84	+++	Cremish brown, fragile
	3.0+ 0.02	71	+++	Cremish brown, fragile
Kn+NAA	0.5+ 0.02	53	++	Brown loose, fragile
	1.5+ 0.02	67	++	Dark brown, fragile
	2.0+ 0.02	76	++	Light brownish white, compact
	3.0+ 0.02	79	++	Brown loose, fragile

(+) low; (++) moderate; (+++) high.

Our results similar to the micropropagation protocol of *Operculina turpethum*. Rapid shoot bud proliferation (85.33%) along with a maximum of 14 shoots in each bud was observed when cultured in Murashige and Skoog medium supplemented with 1.0 mg l-1 BAP. Proper elongation (5-6 cm) of the primarily induced shoots was achieved by subculturing in 0.5 mg l-1 GA_3 plus 0.1 mg l-1 Kin [20].

In conclusion, we described a simple *in vitro* regeneration method for *Tecomella undulata* that would be helpful for mass propagation of this endangered species. Further this study showed that the high frequency organogenesis occurs by using BA and NAA is possible. It could be used as a tool to protect the biodiversity/ natural vegetation of *T. undulata*

Table 2: Effect of cytokinins and auxins supplemented individually and in various combinations on nodal segments of *Tecomella undulata.*

Growth regulators	Concentration of growth regulators (mg/L)	Number of days required for shoots	Number of shoots (Mean±SE)	Shoot length (cm), (Mean±SE)
Control	--	--	--	--
BAP	0.5	10	3.5 ± 0.4	2.1 ± 0.4
	1.5	08	7.4 ± 0.3	3.4± 0.2
	2.0	09	7.9 ± 0.7	3.8 ± 0.7
	3.0	15	4.4 ± 0.2	2.2 ± 0.6
Kn	0.5	18	2.8±0.5	2.0± 0.05
	1.5	12	5.3±0.02	2.4±0.2
	2.0	10	6.7±0.06	2.8±0.4
	3.0	19	3.7±0.2	2.0±0.3
BAP+NAA	0.5+ 0.02	19	5.4±1.5	3.4± 0.5
	1.5+ 0.02	13	6.8±0.05	6.4±0.25
	2.0+ 0.02	11	7.6±0.5	6.8±0.65
	3.0+ 0.02	17	4.8±0.7	3.8±0.82
Kn+NAA	0.5+ 0.02	16	3.8±1.2	3.2±0.45
	1.5+ 0.02	15	4.8±0.8	3.4±0.05
	2.0+ 0.02	12	5.2±0.4	3.6±0.08
	3.0+ 0.02	18	3.8±0.3	2.8±0.92

Table 3: Effect of IBA and IAA on root induction from *in vitro* regenerated shoots of *Tecomella undulata.*

Growth regulators (mg/L)	Frequency of root initiation (%)	Mean No of roots ± SE
IBA		
0.1	69	3.20±0.08
0.2	72	5.70±0.15
0.3	85	11.20±0.02
0.4	82	9.80±0.34
IAA		
0.1	78	7.43±0.42
0.2	83	10.40±0.52
0.3	81	10.20±0.37
0.4	76	9.76±0.16

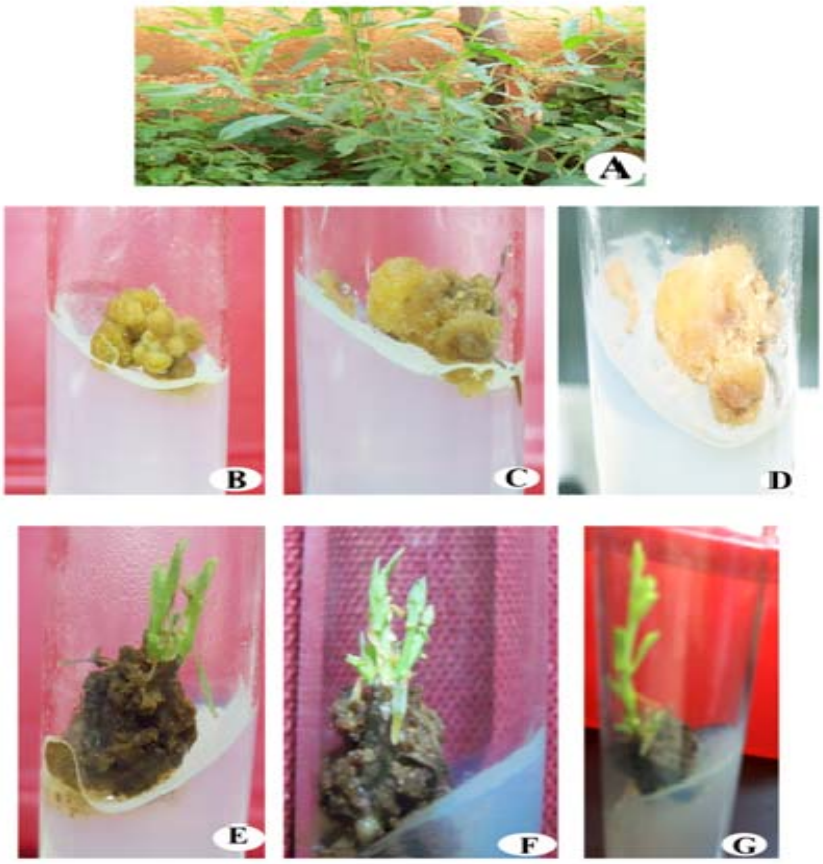

Figure 1 (A-G): Multiple shoot induction and plant regeneration from internodal explants of *Tecomella undulata* A: Habit of *Tecomella undulata* B-D: Callus developed in MS medium containing various growth regulators. E&F: Initiation of multiple shoots from callus, G: Elongation of multiple shoot.

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