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SHORT COMMUNICATION

IN VITRO SHOOT MULTIPLICATION OF *TECOMELLA UNDULATA* (SM.) SEEM. – AN ENDANGERED TREE SPECIES

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An efficient protocol for *in vitro* propagation of *Tecomella undulata* through multiple shoot formation from nodal segment explants has been developed. MS basal medium+1.5 mg l^{-1} BAP+0.02 mg l^{-1} IAA was found to be the most effective media for maximum (95%) regeneration of nodal explant at 25±1°C and 16/8 hours cycle of light (2000 lux fluorescent tubes) and dark. Nodal segment showed maximum (9) shoot induction per explant. Twenty-nine shoots per nodal segment explant were observed on MS+0.75 mg l^{-1} BAP+0.01 mg l^{-1} IAA within 3 weeks. The rooting percentage (66%) and average number of days (8) for root induction were recorded by following the two-step procedure. In the first step, a 48 hour treatment of ½ MS medium (liquid) + 2.5 mg l^{-1} IBA was given to isolated shoots. In the second step these shoots were transferred to hormone free ½ MS medium. Seventy-three per cent survibility of plantlets was recorded in potting mixture composed of drained soil+vermiculite (3: 1, v/v).

***Key words*: Hormone, *in vitro* propagation, *Tecomella undulata*.**

There are a large number of reports available for development of micro propagation technique for plants (Chaturvedi *et al.* 1995, Dilta *et al.* 2000, Nagaraju *et al.* 2000, Singh *et al.* 2001). However, the number is limited in case of tree crops because establishment of *in vitro* cultures of woody plants is greatly hampered by browning of the explant. But recently progress has been made in *Tectona grandis* (Khatri *et al.* 2001), *Melia azedarach* (Shahzad and Siddiqui 2001), *Eucalyptus tereticornis* (Sharma and Ramamurthy 2000). *Tecomella undulata*, an evergreen tree, a member of family Bignoniaceae, is commonly known as 'Rohira', 'Marwar Teak', or 'Desert Teak'. The wood is very hard and is used for making toys, fine carving work, furniture and agricultural implements (Jindal *et al.* 1987). This plant is propagated through seeds but seed viability declines with time and is maximum immediately after harvest. It is reported to decline to zero after one year (Chakravarty and Chand 1975). Indiscriminate felling for timber and fuel, coupled with poor regeneration have severely depleted the natural population of this valuable tree, with an associated loss of germplasm. It has, therefore, become imperative that steps be taken to preserve the high quality germplasm (Arya *et al.* 1992). The purpose of present study was to develop and optimize a simple *in vitro* protocol for the micropropagation of *Tecomella undulata*.

Healthy and elite adult trees (about 20 years age) of *Tecomella undulata* growing at the farmers' field at village Bhirani, District Hanumangarh (Rajasthan), were selected for explants. Shoots were harvested from these trees during the month of March-May. Nodal segment explants (1.5-2.5 cm long and 0.5-1.0 cm thick) were washed thoroughly with tap water by adding a few drops of Polysorbate-80. These were surface sterilized with 0.1% (w/v) mercuric chloride for 3 minutes and washed 4-5 times with double distilled water. Shoot tips and internodal segment were also tried as explants. Murashige and Skoog medium (Full and half strength) was used.

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Different auxins (IAA, IBA and NAA) and cytokinin (BAP) at various concentrations and combinations were evaluated for rapid multiple shoot induction from the explants. The cultures were kept at 25±1°C, 2000 lux of light from fluorescent tubes and 60-70% relative humidity. The cultures were exposed to 16/8 hours cycle of light and dark. Experiments were performed with a minimum of 10 replicates and repeated thrice. Observations were recorded after an interval of 3 weeks. The shoots regenerated from the explants were harvested after 3 weeks and sub-cultured on different multiplication media. Individual shoots (2.7 cm length) were excised after 3 weeks of the first sub-culturing and transferred to rooting media. The root induction media tried were half strength MS medium with 0.5-4.0 mg l^{-1} IBA. Root induction was observed by the two-step method. In the first step, 12-36 hours treatment of ½ MS medium (liquid) + IBA was given to isolated shoots on filter paper bridge. In the second step, these shoots were transferred to hormone free ½ MS medium with agar. An initial dark period of one week was given to the cultures. The rooted plants were transferred to pots containing drained soil+vermiculite (3:1, v/v) in climate controlled green house.

Nodal segment explants were found to be the best as compared to shoot tip and internodal segment explants. Murashige and Skoog medium containing 1.5 mg l^{-1} BAP+0.02 mg l^{-1} IAA was found to be the best establishment medium (Table 1). On this medium, highest number of explants (95%) responded positively. An average of 9 shoot buds appeared, within 3-5 days of culture. Cytokinin and auxin are required in appropriate ratio for efficient shoot regeneration. They have a synergistic effect on continuous cell division (Rout *et al.* 1995). Similar results have been reported in rose cultivars.

The shoots regenerated from explants were harvested after 3 weeks and sub-cultured on different multiplication media (Fig.1a). The transfer of regenerated shoots on to simple, hormone-free MS medium did not lead to multiplication. The induction of multiplication in regenerated shoots was achieved on MS medium supplemented with 0.75 mg l^{-1} BAP+0.01 mg l^{-1} IAA (Fig.1b). About 29 shoots per explant were induced

Table 1. Effect of plant growth regulator on multiple shoot induction from nodal segment explant of *Tecomella undulata* on MS medium.

Treatment (PGR mgl^{-1})	% Explants response	Number of shoots/ explant
MS Control	25.90±0.21	1.46±0.18
BAP+IAA		
0.10+0.00	29.39±0.19	2.05±0.12
0.50+0.00	38.63±0.20	2.83±0.12
1.00+0.00	48.03±0.23	4.21±0.23
1.50+0.00	70.45±0.26	6.01±0.11
2.00+0.00	61.81±0.33	5.60±0.11
2.50+0.00	56.36±0.19	5.31±0.21
3.50+0.00	43.63±0.22	3.32±0.25
1.00+0.01	60.90±0.19	5.62±0.18
1.00+0.02	65.90±0.47	6.33±0.24
1.00+0.05	57.27±0.28	6.15±0.29
1.00+0.10	52.27±0.34	6.01±0.22
1.50+0.01	90.00±0.46	7.28±0.15
1.50+0.02	95.00±0.32	9.67±0.12
1.50+0.05	88.18±0.70	8.73±0.18
1.50+0.10	78.18±0.33	8.64±0.10
2.00+0.01	66.36±0.29	7.13±0.29
2.00+0.02	71.36±0.26	8.57±0.26
2.00+0.05	62.72±0.80	8.31±0.20
2.00+0.10	55.90±0.16	8.22±0.21
2.50+0.01	61.81±0.20	7.02±0.10
2.50+0.02	66.81±0.38	8.43±0.19
2.50+0.05	59.54±0.33	7.79±0.10
2.50+0.10	54.09±0.29	7.57±0.27
BAP+NAA		
1.00+0.01	44.54±0.40	4.11±0.25
1.00+0.10	41.81±0.28	3.70±0.11
1.50+0.01	68.63±0.33	5.67±0.18
1.50+0.10	65.45±0.55	5.23±0.11
2.00+0.01	57.72±0.30	5.52±0.15
2.00+0.10	55.00±0.21	5.37±0.14
BAP+IBA		
1.50+0.01	69.57±0.30	5.72±0.28
1.50+0.10	66.36±0.35	5.43±0.15

(± S.E.)

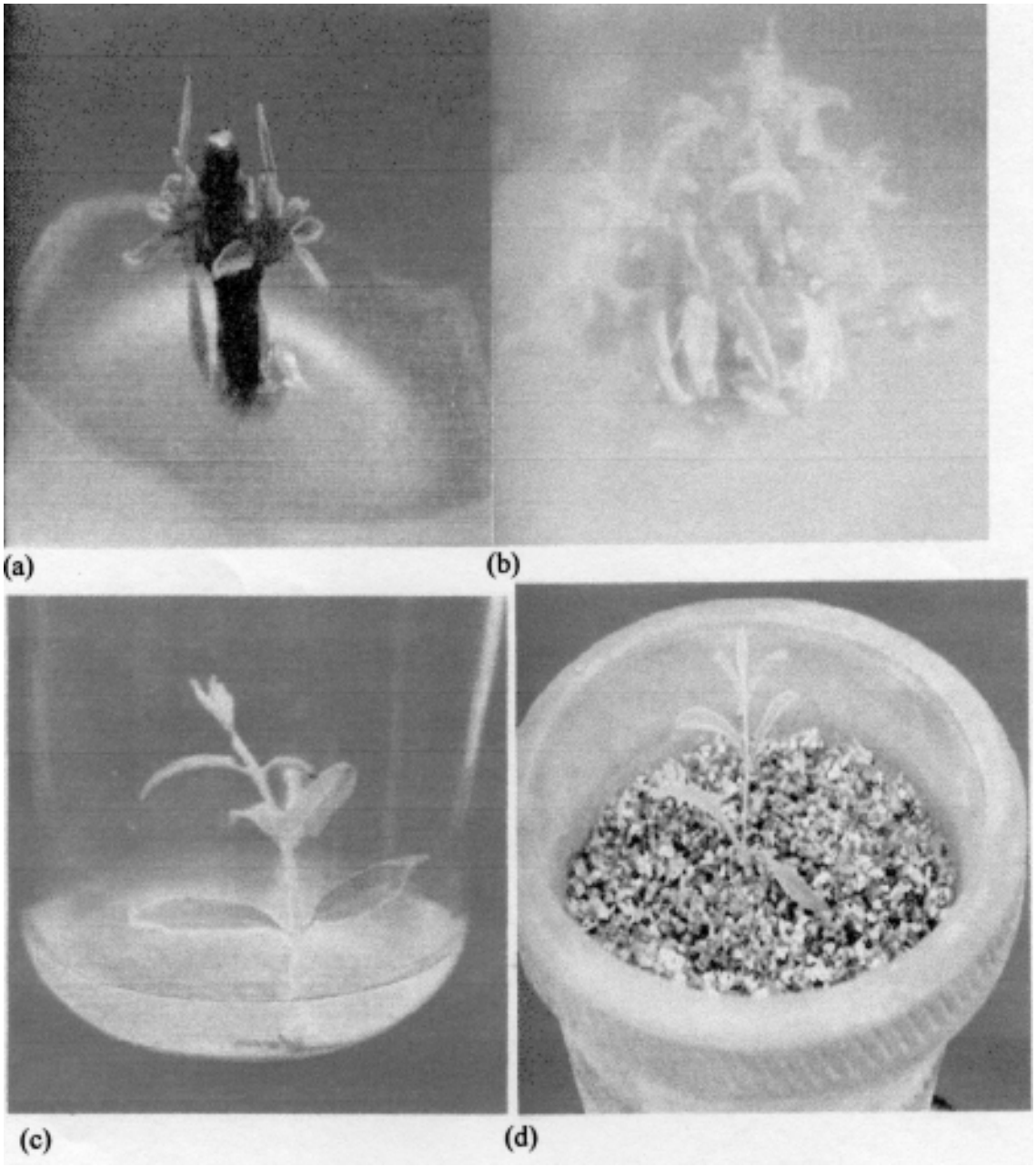

Fig. 1. (a) Shoots on nodal explant, (b) Multiplication of shoots, (c) Root induction on shoot and (d) Plantlet in the pot of *Techomella undulata* (SM) seem

within 3 weeks (Table 2). Better shoot multiplication was obtained by decreasing the concentration of both BAP and IAA. In order to avoid callus induction from the cut end, it was necessary to reduce concentration of IAA from 0.02 mg l^{-1} to 0.01 mg l^{-1}. The sub-cultured shoots could be multiplied for one year; it was essential to sub-culture the shoots on fresh multiplication medium after an interval of 3 weeks. Similar findings have been reported in *Eucalyptus tereticornis* (Sharma and Ramamurthy 2000).

Individual shoots of about 2.7 cm length were excised after 3 weeks of the first sub-culturing and transferred onto rooting media. Out of nine media (with agar and

Table 2. Effect of plant growth regulator on formation of multiple shoots/explant from sub-cultured shoots of *Tecomella undulata* on MS medium.

Treatment (PGR mgl^{-1})	% Explants response	Number of shoots/ explant
MS Control	0.00+0.00	0.0+0.0
BAP+IAA		
0.50+0.01	21.00+0.16	2.4+0.04
0.50+0.02	18.33+0.11	2.2+0.07
0.50+0.05	15.00+0.18	1.9+0.05
0.75+0.01	29.33+0.10	2.7+0.06
0.75+0.02	27.66+0.12	2.5+0.08
0.75+0.05	20.00+0.15	2.0+0.05
1.00+0.01	30.66+0.14	2.3+0.04
1.00+0.02	21.66+0.12	2.1+0.09
1.00+0.05	16.33+0.21	1.8+0.14

(± S.E.)

without agar) tried, half strength MS medium supplemented with IBA was found to be the most effective for root induction from isolated shoots. The rooting percentage (66%) and average number of days for root induction were recorded by following the two step procedure (Khatri *et al.*, 2001). In the first step, a 48 hours treatment of ½ MS medium (liquid) + 2.5 mg l^{-1} IBA was given to isolated shoots on filter paper bridge. In the second step these shoots were transferred to hormone free (1/2 MS medium with agar. An initial dark period of one week favoured root induction. From a single shoot, 4-6 roots were produced (Fig. 1c). Similar method of rooting of micropropagated shoots has been applied in *Streculia urens* (Purohit and Dave 1996). The rooted plantlets were removed gently from the bottles after 3 weeks of root induction and washed 4-5 times with distilled water to remove adhered medium to prevent contamination. The plantlets were transferred to pots in climate controlled green house.

Potting mixture containing drained soil and vermiculite (3:1, v/v) was found most suitable. Seventy-three per cent survivability of plantlets was recorded in this potting mixture (Table 4, Fig. 1d). Survivability of plantlets transferred in potting mixture depends on the manipulation of external environmental factors like temperature, humidity and soil moisture rather than the composition of culture media from which the plantlets were multiplied.

Table 3. Effect of IBA on rooting of micropagated shoots of *Tecomella undulata*.

Treatment (mg l^{-1})	No. of shoots cultured	No. of shoots exhibiting roots	Per cent rooting of shooting	Days to rooting
MS control	10	-	-	-
IBA				
0.50	10	-	-	-
1.00	10	-	-	-
1.50	10	-	-	-
2.00	10	-	-	-
2.50	10	6.60	66.00±0.16	8.0±0.27
3.00	10	-	-	-
3.50	10	-	-	-
4.00	10	-	-	-

(± S.E.)

Table 4. Effect of different potting mixture on survival of *in vitro* raised plantlets of *Tecomella undulata*.

Potting mixture	Number of pots	Per cent survival of plantlets
Vermiculite	15	40.00
Soil + Vermiculite (1:1)	15	53.33
Drained soil + Vermiculite (3:1)	15	73.33
Sand + Soil + FYM (1:1:1)	15	46.66

REFERENCES

Arya, S., Toky, O.P., Harris, S.M. and Harris, P.J.C. (1992). *Tecomella undulata* (rohira): A valuable tree of the *Thar* desert. *International Tree Crop Journal* **7**: 141-147.

Chakravarty, A.K. and Chand, C. (1975). Phenotypic variation in desert teak (*Tecomella undulata*). *Ann. Arid Zone* **14**: 21-24.

Chaturvedi, H.C., Prasad, R.N., Mishra, P., Sharma, M., Bhattacharya, A. (1995). Micropropagation of ornamental plants. In: K.L. Chadha and S.K. Bhattacharjee (eds.), Advances in Horticulture, Vol.12 Ornamental plants, pp. 563-565. Malhotra Publishing House, New Delhi.

Dilta, B.S., Sehgal, O.P., Pathania, N.S. and Subodh Chander. (2000). *In vitro* effect of NAA and BA on culture establishment and bulblet formation in Lily. *J. Ornamental Hort., New Series* **3**: 65-70.

Jindal, S.K., Kackar, M.L. and Solanki, R.R. (1987). Germplasm collection and genetic variability in Rohira (*Tecomella undulata* Sm.) Seem. in western Rajasthan. *Indian J. Forestry* **10**: 52-55.

Khatri, J.H., Kukadia, M.U. and Singh, R.R. (2001). Microproapgation of teak (*Tectona grandis* Linn. F.) *Indian J. Forestry* **24**: 368-371.

Nagaraju, V., Bhowmik, G. and Parthasarathy, V.A. (2000). Component analysis of *in vitro* response of BAP in Gladiolus. *J. Ornamental Hort, (New Series)* **3**: 18-21.

Purohit, S.D. and Dave, A. (1996). Micropropagation of *Sterculia urens* – An endangered tree species. *Plant Cell Reports* **15**: 704-706.

Rout, G.R., Samantaray, S. and Das, P. (1995). Somatic embryogenesis and plant regeneration from callus culture of *Acacia catechu* – A multipurpose leguminous tree. *Plant Cell, Tissue and Organ Culture* **42**: 283-285.

Shahzad, A. and Siddiqui, S.A. (2001). Micropropagation of *Melia azedarach* L. *Phytomorphology* **51**: 151-154.

Sharma, S.K. and Ramamurthy, V. (2000). Micropropagation of 4-years old elite *Eucalyptus tereticornis* trees. *Plant Cell Reports* **19**: 511-518.

Singh, S.K., Kumar, S., Pandey, R.K. and Raghava, S.P.S. (2001). *In vitro* propagation in Dracaena. *J. Ornamental Hort. (New Series)* **4**: 22-24.