PLANT SCIENCE TODAY
Vol 12(3): 1-6
https://doi.org/10.14719/pst.8048

eISSN 2348-1900

RESEARCH ARTICLE

In vitro regeneration potential of *Tecomella undulata* using nodal and shoot tip explants

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Received: 03 March 2025; Accepted: 08 July 2025; Available online: Version 1.0: 04 August 2025; Version 2.0: 16 August 2025

Cite this article: Monika J, Virender D, Shikha Y, Anil KP, Preety V, Jyoti P, Mamta K. *In vitro* regeneration potential of *Tecomella* undulata using nodal and shoot tip explants. Plant Science Today. 2025; 12(3): 1-6. https://doi.org/10.14719/pst.8048

Abstract

Tecomella undulata (Sm.) is an important tree species widely used in India. The present study developed *an efficient in vitro mass propagation* protocol for Rohida. The study evaluated *sterilization agents*, media and plant growth regulators for the clonal propagation of *Rohida* using nodal and shoot-tip explants. The most successful sterilization treatment involved $HgCl_2$ (0.1 %) for 3 min, followed by Bavistin (0.2 %) and Streptomycin (0.2 %) for 15 min. This protocol achieved 100 % explant survival. Among different media compositions, nodal explants showed the highest callus formation (73.3 ± 12.48) in EM_7 (MS + BAP 2.0 mg/L + NAA 0.01 mg/L), while the control showed no callus formation. For shoot-tip explants, EM_7 also resulted in the highest callus formation (86.6 ± 8.18 %) and the control produced no callus. EM_{10} (MS + BAP 2.5 mg/L + NAA 0.1 mg/L) led to the highest shoot initiation (86.7 ± 13.34 %) in nodal explants, with an average of 19 days for shoot induction. Similarly, EM_{10} produced the highest shoot initiation (93.3 ± 6.68) in shoot tip explants, with an average induction time of 11 days. The highest rooting percentage (66.6 %) was observed in EM_4 (Half MS+ IBA 2.5 mg/L) with average of 40 days required for root induction. The survival rate of plantlets transferred to soil + vermiculite (1:1) potting mix under greenhouse conditions was 48 %. These findings provide valuable insights into optimizing propagation techniques for *Tecomella undulata*. In the current study, shoot tip explants proved to be superior to nodal explants for regeneration.

Keywords: explant; MS medium; shoot initiation; survival; *Tecomella undulata*

Introduction

Tecomella undulata (Sm.), commonly known as Rohida is primarily valued for its wood, which is used in furniture making, wood carving and the production of farming tools. It also possesses medicinal properties. It is used in the treatment of bacterial and fungal infections, aids in cancer therapy, reduces toxicity, alleviates pain and inflammation, supports weight management and helps regulate the immune system (1). In addition to its practical and medicinal uses, the species plays a vital role in environmental conservation, particularly in arid areas. It helps stabilize shifting sand dunes and provides protection for wildlife. Rohida is especially beneficial for reforestation projects in arid regions due to its resistance to drought and fire (2). However, Rohida is currently classified as a vulnerable species due to extensive logging driven by the high demand for its timber, slower growth rate and limited natural regeneration. Over-exploitation for timber and fuel, coupled with poor natural regeneration and the loss of genetic material, has placed the tree on the brink of extinction (3). Plant tissue culture is a wide term that refers to culture of plant parts such as (cells, tissues or organs) in artificial media, under aseptic conditions with controlled environments. Tissue culture makes it easier to produce and grow plant material that is genetically homogeneous and lacking pest-related diseases. Micropropagation is a usual way of multiplying plant species when conventional propagation techniques are unable to satisfy planting stock needs, frequently because of problems such as low germination rates or poor seed set. Shoot regeneration from explants with pre-existing meristems, such nodal segmental explants have been observed in several cases. The procedure entails starting cultures from mature explants. It is listed as endangered in Rajasthan and on the verge of extinction in Punjab under Section 38 of the Biological Diversity Act of 2002. The World Conservation Monitoring Centre of the United Nations Environment Programme has classified *T. undulata* as Category I - Indeterminate (4). Clonal propagation of hardwood tree species, such as *T. undulata*, is critical for forest enhancement programs, ensuring the availability of high -quality planting material. Traditional seed propagation is limited by poor viability, genetic variability and inadequate conservation practices (5). Additionally, cross-pollination may lead to loss of desirable silvicultural traits. Despite previous efforts to propagate *T. undulata in vitro* using seedlings, there are limitations in this approach. Propagation from mature nodal explants has yielded shoot cultures; however, rooting remains inconsistent and difficult (6). Consequently, there is a need for improvements in shoot multiplication, long-term subculturing and a deeper understanding of root induction in this species (7). The objective of the present study is to develop an efficient *in vitro* propagation protocol for large-scale multiplication of the ethnomedicinal tree *T. undulata*. This study emphasizes callus induction from nodal explants, given their high regenerative potential and evaluates the effect of various plant growth regulators on propagation outcomes.

Materials and Methods

Media preparation

To prepare for tissue culture, all equipment, glassware and phyta jars were autoclaved at 121 °C and 15 psi for 60 min. The MS media components were mixed with sterile double distilled water and the pH was adjusted to 5.6 using 1N HCl or 1N NaOH. Half- and full-strength MS media containing sucrose (3 % w/v weight/volume) was made with different PGR ratios and 0-0.5 mL/L plant preservative combinations, BAP (6-benzyl amino purine 1-2 mL/L), NAA (naphthalene acetic acid 0-0.4 mL/L) and 0-1 mg/L IBA. The media solidifying agent is 0.8 % agar media. The MS culture (8) medium is sterile at 121 °C and 15 pressure for 15 min. Plant growth regulators were incorporated into sterile medium at temperature of 50-60 °C. The PGRs were filtered using a 22 µm syringe filter before being introduced to the medium. The media were dispensed into phyta jars, UV-treated overnight and autoclaved again at 121 °C for 15 min.

Explant collection

Explants of *T. undulata* were collected from healthy, mature (20 -year-old) plus trees growing at the farmers field at Balasmand, District Hisar (Haryana). Two types of explants (nodal and shoot tip) were used in this study.

Explant sterilisation

Explants were initially washed under running tap water for 10-15 min, followed by immersion in 500 mL sterile distilled water containing 1-2 drops of Tween-20 for 30 min under a laminar airflow hood. Now, the explants with nodes were cut down to 1 -2 cm in size. Mercuric chloride (0.1 %) is used to sterilise the surface for 0-6 min. This is followed by three washings in sterile double-distilled water for 2, 3 and 5 min each. The LAF was used to inoculate the explants into medium with varying PGR ratios. In a parallel experiment, 2 % sodium hypochlorite was used for 5-20 min (after Bavistin treatment) as an alternative to $HgCl_2$. Remaining procedures were identical for both treatments.

Inoculation for callus induction

Explants of *Tecomella undulata* were aseptically excised and placed into phyta jars containing (MS) medium, either at full or half strength, supplemented with various concentrations of plant growth regulators. The cultures were incubated at 28 °C under a 12-16 hr photoperiod with 75 ± 5 % relative humidity for a period of 4-6 weeks. The specific concentrations of growth regulators used to observe and induce callus formation and to assess the explants' responses to different hormonal treatments.

Subculturing and shoot multiplication

Following successful callus induction, the established explants were subcultured onto fresh MS medium to promote shoot multiplication. The initial shoots were carefully excised and transferred to various media formulations containing different combinations of plant hormones. This step aimed to stimulate further shoot development and to evaluate the effects of different hormonal concentrations on shoot proliferation.

Rooting, hardening and acclimatisation

Elongated shoots were aseptically excised and transferred to rooting media consisting of half-strength MS supplemented with various concentrations of auxins (e.g., IBA). Explants were placed in sterilized jam bottles under aseptic conditions using flame-sterilized tools (forceps, scalpels) treated with 96 % ethanol. Cultures were incubated at 25.1 °C under a 16-hr light/8-hr dark cycle with a light intensity of 2000 lux. Rooted plantlets were transferred to pots containing a sterilized soil: vermiculite (1:1) mixture and kept in a greenhouse for hardening. After 4-6 weeks, once the plantlets reached heigh of 12-15 cm, the survival percentage was recorded.

Statistical analysis

All experiments were conducted using a completely randomized design (CRD) with a minimum of five replicates per treatment and repeated three times for consistency. Data were analysed as mean ± standard error. Analysis of variance (ANOVA) was used to determine significant differences in shoot length and shoot number using OPSTAT software (http://14.139.232.166/opstat/).

Results and Discussion

Explant sterilization

The sterilization of explants is a crucial and challenging step in establishing aseptic cultures. Explant selection and collection were done carefully for the rapid growth of the explants and to reduce the rate of contamination. The selection of infected and damaged explants may lead to a higher risk of contamination in the culture medium. The nodal and shoot tip explants of *T. undulata* was treated with different sterilizing agents and durations placed on MS medium. The survival percentage was observed on the 21st day after inoculation. The most effective sterilization treatment for both nodal and shoot tip explants of *Tecomella undulata* was ST_8. When the explants were treated with 0.1 % $HgCl_2$ for 3 min, followed by 15 min exposure to a mixture of Bavistin (0.2 %) and streptomycin (0.2 %) resulting in 100 % survival rate for the shoot tip explants with 86.6 % survival rate for the nodal explants with lower contamination levels seen in Table 1 and Fig. 1. Similar results were observed in various plant species concerning the impact on contamination rates in explants obtained from mature trees. Nodal explants from different tree species were sterilized with 0.1 % mercuric chloride for 15 min (9, 10). From the previous author findings reported that phenolic leaching was not a significant concern in this species (11).

Table 1. Time durations and concentrations of different sterilizing agents for sterilization of explants of *Tecomella undulata*

Sr. No.	Code	Durations of $HgCl_2$ (0.1 %) in minute	Durations of Bavistin (0.2 %) in minute + Streptomycin (0.2 %) minute	Durations of Bavistin (0.2 %) in minute + Streptomycin (0.4 %) in minute	Nodal explant	Shoot tip explant
					Survival percentage	Survival percentage
1	ST_0	0	-	-	0	0
2	ST_1	1	-	-	0	33.3
3	ST_2	2	-	-	36.6	40
4	ST_3	3	-	-	53.3	60
5	ST_4	4	-	-	23.3	23.3
6	ST_5	5	-	-	10	13.3
7	ST_6	6	-	-	0	10
8	ST_7	3	10	-	43.3	56.6
9	ST_8	3	15	-	86.6	100
10	ST_9	3	20	-	50	60
11	ST_{10}	3	-	10	43.3	53.3
12	ST_{11}	3	-	15	63.3	70
13	ST_{12}	3	-	20	50	60

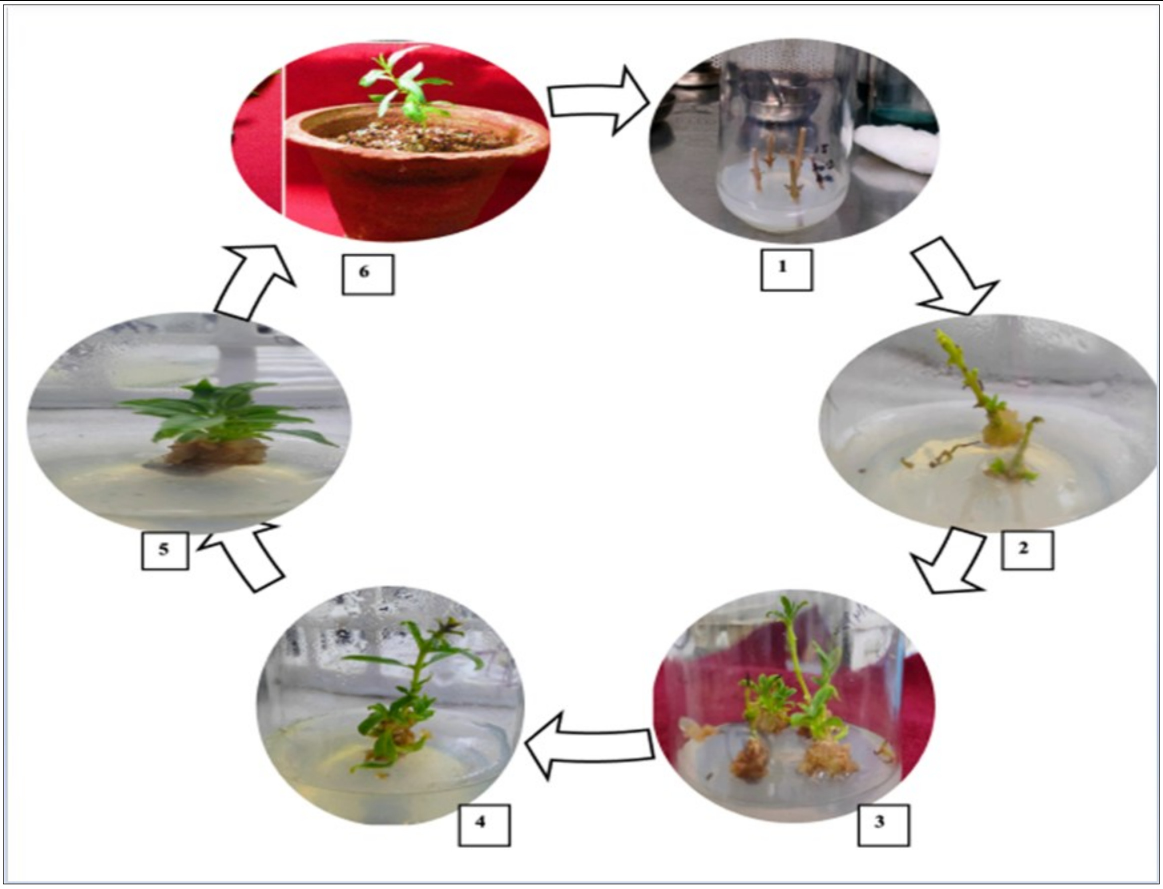

Fig. 1. Steps of *in vitro* propagation of *Tecomella undulata.* (1) Explant inoculation, (2) Shoot initiation, (3) Callus formation, (4) Shoot initiation in shoot tip explant, (5) Nodal tip explant shoot initiation and callus formation, (6) Hardening of explant.

Callus formation

The most effective and highest (86.6 ± 8.18) callus formation percentage was found in shoot tip explant when treated with EM_7 (MS+ BAP 2.0 mg/L + NAA 0.01 mg/L) concentration as compared to nodal explant. The nodal explant treated with same treatment resulted in 73.3 ± 12.48 callus formation (Fig. 3 and Table 2). While, in both explant the control showed zero callus development. It is more effective to use combinations of cytokinin and auxins rather than BAP alone for the establishment of shoot tip and nodal explants. Shoot tip explants were found to be more successful as compared to nodal explants. Similar results were observed when studied

Table 2. Effect of different combinations on *in vitro* callus formation of nodal and shoot tip of explants of *Tecomella undulata* on MS modified media

Sr. No.	Media	% Callus formation	
		Nodal explant	Shoot tip explant
1	EM_0 (control)	0.0 ± 0.00	0.0 ± 0.00
2	EM_1 (MS + BAP 0.5)	33.3 ± 0.00	40.0 ± 6.66
3	EM_2 (MS + BAP 1.0 mg/L)	40.0 ± 6.66	53.3 ± 13.34
4	EM_3 (MS + BAP 1.0 mg/L + IAA 0.5 mg/L)	46.6 ± 8.16	60.0 ± 12.48
5	EM_4 (MS + BAP 1.5 mg/L + IAA 0.5 mg/L)	53.3 ± 13.34	66.6 ± 14.92
6	EM_5 (MS + BAP 1.0 mg/L + NAA 0.01 mg/L)	60.0 ± 12.48	73.3 ± 12.48
7	EM_6 (MS + BAP 1.5 mg/L + NAA 0.10 mg/L)	66.6 ± 14.92	80.0 ± 13.34
8	EM_7 (MS + BAP 2.0 mg/L + NAA 0.01 mg/L)	73.3 ± 12.48	86.6 ± 8.18

the effect of various concentrations and combinations of (PGRs) on callus formation from shoot explants of *Tecomella undulata* (Sm.). The explants were cultured on MS media with different doses of these regulators. The highest callus formation (91.2 %) was observed on MS medium supplemented with BAP and 2,4-D at 3.0 + 0.5 mg/L, followed by 90.4 % callus formation at 2.5 + 0.5 mg/L or 2,4-D alone at 3.0 mg/L (12). The cytokinin and auxins are the most extensively used plant growth regulators in plant tissue culture and auxins play a valuable role in the callus formation and different types of auxins showed various effects reported (11). Similar findings were observed in callus formation and shoot multiplication of *Oroxylum indicum* (13).

Shoot tip explant and nodal explant for shoot initiation

The effect of cytokinin and auxin combinations on shoot initiation in Rohida explants were shown in Table 3 and Fig. 1 (4, 5). For nodal explants, the highest shoot initiation (86.7 ± 13.34) was observed on medium EM_{10} (MS + BAP 2.5 mg/L + NAA 0.10 mg/L) in 19 days, followed by EM_9 (MS + BAP 2.0 mg/L + NAA 0.01 mg/L) with (80.0 ± 8.18) in 16 days. The control (no growth regulators) showed (26.6 ± 6.66) initiation in 9 days. For shoot tip explants, the highest shoot initiation (93.3 ± 6.68) occurred on EM_{10} in 13 days, followed by EM_9(86.6 ± 8.18) in 11 days. The control showed (33.3 ± 0.00) initiation in 10 days. The MS basal medium with EM_{10} (MS + BAP 2.5 mg/L + NAA 0.10 mg/L) showed the best results for shoot initiation in Rohida as compared to the other growth regulator compositions. Results of several studies were consistent with our findings. The successful *in vitro* adventitious shoot regeneration in *T. undulata* was found in best response of about 11 shoots per explant was recorded in the treatment with IAA (0.1 mg/L) +BAP (2.5 mg/L) (14). The maximum shoot initiation was observed in shoot tip explants on the medium supplemented with BAP (1.0 mg/L) concentration (15).

Shoot multiplication rate in nodal and shoot tip explant

The effect of different growth regulator combinations and concentrations on *in vitro* shoot multiplication rate in Rohida were observed at 7th, 15th and 30th days of culture in Table 4 and 5. The highest average number of shoots per nodal explant was observed on medium EM_6 (MS + BAP 1.00 mg/L + IAA 0.02 mg/L), with (2.0 ± 0.45, 2.6 ± 0.25 and 3.2 ± 0.58) shoots at 7th, 15th and 30th days, respectively. When treated with similar treatment in shoot tip was found more effective with (2.2 ± 0.37, 3.4 ± 0.25 and 4.0 ± 0.00 after 7, 15 and 30 days, respectively). Shoot elongation was higher on media with higher concentrations of BAP and IAA and the number of shoots increased with the concentration of BAP and IAA. Previous workers reported maximum shoot multiplication rate in MS + 0.06 μM IAA + 4.4 μM BA (15). The success of *in vitro* rooting depends on various factors, such as the type and strength of the basal medium and concentration of auxins and the presence of any added substances (16).

Rooting

After successful shoot multiplication, the explants were transferred for rooting stage were presented in Table 6 and Fig. 1(6). For the rooting purpose, high-quality and well-grown, 2-3 cm explants were selected. The maximum root initiation percentage (66.6 %) was observed in medium EM_4 (Half MS +

Table 3. Effect of different combinations on *in vitro* shoot initiation of nodal and shoot tip explants of *Tecomella undulata*

Sr. No.	Media	% Shoot initiation (nodal tip explant)	Average no. of days required for shoot induction	% Shoot Initiation (Shoot tip explant)	Average no. of days required for shoot induction
1	EM_0 (control)	26.6 ± 6.66	9	33.3 ± 0.00	10
2	EM_1 (MS + BAP 0.5 mg/L)	33.3 ± 0.00	11	40.0 ± 6.66	9
3	EM_2 (MS + BAP 1.0 mg/L)	40.0 ± 6.66	13	46.6 ± 8.16	7
4	EM_3 (MS + BAP 1.0 mg/L + IAA 0.01 mg/L)	46.6 ± 8.16	10	53.3 ± 13.34	9
5	EM_4 (MS + BAP 1.5 mg/L + IAA 0.02 mg/L)	53.3 ± 13.34	15	66.6 ± 10.55	13
6	EM_5 (MS + BAP 2.0 mg/L + IAA 0.05 mg/L)	60.0 ± 12.48	12	73.3 ± 12.48	8
7	EM_6 (MS + BAP 2.5 mg/L + IAA 0.10 mg/L)	73.3 ± 12.48	18	80.0 ± 13.34	12
8	EM_7 (MS + BAP 1.0 mg/L + NAA 0.01 mg/L)	60.0 ± 12.48	16	66.6 ± 14.92	15
9	EM_8 (MS + BAP 1.5 mg/L + NAA 0.10 mg/L)	66.6 ± 0.00	20	73.3 ± 12.48	10
10	EM_9 (MS + BAP 2.0 mg/L + NAA 0.01 mg/L)	80.0 ± 8.18	16	86.6 ± 8.18	13
11	EM_{10} (MS + BAP 2.5 mg/L + NAA 0.10 mg/L)	86.7 ± 13.34	19	93.3 ± 6.68	11

Table 4. Effect of different combinations on *in vitro* shoot multiplication rate at different time intervals in *Tecomella undulata* of nodal explant

Sr. No.	Media	Average no. of shoots/explant		
		(7th day)	(15th day)	(30th days)
1	EM_0 (control)	1.0 ± 0.00	1.2 ± 0.20	1.4 ± 0.25
2	EM_1 (MS + BAP 0.50 mg/L + IAA 0.01 mg/L)	1.0 ± 0.00	2.2 ± 0.20	2.4 ± 0.25
3	EM_2 (MS + BAP 0.50 mg/L + IAA 0.02 mg/L)	1.2 ± 0.20	1.6 ± 0.25	1.8 ± 0.49
4	EM_3 (MS + BAP 0.75 mg/L + IAA 0.01 mg/L)	1.4 ± 0.25	1.8 ± 0.37	2.8 ± 0.37
5	EM_4 (MS + BAP 0.75 mg/L + IAA 0.03 mg/L)	1.6 ± 0.40	2.2 ± 0.37	2.6 ± 0.51
6	EM_5 (MS + BAP 1.00 mg/L + IAA 0.01 mg/L)	1.8 ± 0.37	2.4 ± 0.25	3.0 ± 0.55
7	EM_6 (MS + BAP 1.00 mg/L + IAA 0.02 mg/L)	2.0 ± 0.45	2.6 ± 0.25	3.2 ± 0.58

Table 5. Effect of different hormones on *in vitro* shoot multiplication rate at different time intervals in *Tecomella undulata* on shoot tip of explant

Sr. No.	Media	Average no. of shoots/explant		
		(7th day)	(15th day)	(30th days)
1	EM_0 (control)	1.2 ± 0.20	1.4 ± 0.25	2.0 ± 0.55
2	EM_1 (MS + BAP 0.50 mg/L + IAA 0.01 mg/L)	1.4 ± 0.25	2.4 ± 0.25	2.6 ± 0.25
3	EM_2 (MS + BAP 0.50 mg/L + IAA 0.02 mg/L)	1.4 ± 0.25	2.6 ± 0.25	2.8 ± 0.58
4	EM_3 (MS + BAP 0.75 mg/L + IAA 0.01 mg/L)	1.6 ± 0.25	2.8 ± 0.20	3.4 ± 0.68
5	EM_4 (MS + BAP 0.75 mg/L + IAA 0.03 mg/L)	1.8 ± 0.37	3.0 ± 0.32	3.6 ± 0.25
6	EM_5 (MS + BAP 1.00 mg/L + IAA 0.01 mg/L)	2.0 ± 0.32	3.2 ± 0.20	3.8 ± 0.20
7	EM_6 (MS + BAP 1.00 mg/L + IAA 0.02 mg/L)	2.2 ± 0.37	3.4 ± 0.25	4.0 ± 0.00

Table 6. Effect of different combinations on *in vitro* rooting % of *Tecomella undulata* on MS modified media

Sr. No.	Media	% Root initiation	Average no. of days required for root induction
1	EM_0 (Control)	-	-
2	EM_1 (IBA 0.5 mg/L)	-	-
3	EM_2 (Half MS + IBA 1.0 mg/L)	33.3	31
4	EM_3 (Half MS + IBA 2.0 mg/L)	-	-
5	EM_4 (Half MS + IBA 2.5 mg/L)	66.6	40
6	EM_5 (Half MS + IBA 3.0 mg/L)	-	-

IBA 2.5 mg/L), with an average of 40 days required for root induction. Whereas no rooting was recorded in control. Similar findings were examined that the impact of various concentrations of (IBA) on *in vitro* rooting and acclimatization of teak (*Tectona grandis*) (17). Present results were similar with previous findings by workers to carried out rooting in *Tecomella undulata* in a liquid medium supplemented with IBA and IAA, were results in low percentage of the plant survival after transplantation (18).

Hardening and transplantation

The plants were carefully established in the culture bottles were taken out of the greenhouse. Once the plantlets were separated and washed under running water to remove any waste media, they were put into plastic bags with different types of potting mix. The hardened plants were then moved to the field in accordance with suggested agricultural techniques. According to the information in Table 7, the best potting mix for plantlets grown *in vitro* was a mixture of soil and vermiculite (1:1), which was followed by soil and FYM (1:1:1), both of which had 42.67 % survival rate. The two potting combinations utilised, *Tecomella undulata* plantlets cultivated *in vitro* in a soil + vermiculite (1:1) mix in the greenhouse had the best survival rate, 48.33 %. The survival rate observed in plantlets grown in a mixture of soil and farmyard manure (FYM) (1:1:1) aligns with previous findings reported that (1:1) mixture of sand and vermiculite also promoted plant survival during transplantation (18). This suggests that nutrient-rich potting media containing organic matter and support the early growth

Table 7. Effect of different potting mixture on survival percentage of *in vitro* raised plantlets of *Tecomella undulata*

Sr. No.	Potting mixture	Percent survival
1	Soil + Vermiculite (1:1)	48.33
2	Sand + Soil + FYM (1:1:1)	42.67

and adaptation of plantlets to field conditions. Similar results were recorded in regenerated plantlets of *Tecomella undulata* were acclimatized and transferred to soil for growth under field conditions and 60 % survived (19). *In vitro* regeneration of *Tecomella undulata* (Sm.) Seem - an endangered medicinal plant (20).

Conclusion

Among the various sterilizing agent treatment, the maximum survival was recorded when shoot tip explants were treated with $HgCl_2$ (0.1 %) for 3 min along with Bavistin (0.2 %) and Streptomycin (0.2 %) for 15 min. MS medium treated with EM_{10} (MS+BAP 2.5 mg/L + NAA 0.10 mg/L) showed the maximum shoot induction using nodal and shoot tip explants, respectively. EM_6 (MS+BAP 1.00 mg/L+ IAA 0.02 mg/L) was found to be the best most effective with 4.0 ± 0.00 in average number of shoots per explant on 30th day of culturing in shoot tip explant. Root initiation percentage was observed maximum when treated with IBA (EM_4 Half MS + IBA 2.5 mg/L) at 40 days.

Acknowledgements

I would be thankful to Dr. Shikha Yashveer and Dr. Anil Kumar Poonia for their technical help and support in this work.

Authors' contributions

MJ participated in analysing results of experiments carried out the field experiment and original draft writing. The conceptualization and supervision of the research was carried out by VD and SY. AK. P contributes to finalization data and teaches me how to use software. JP and PV helps in data analysis. MJ and MK collected plants and subsequently established the culture followed by shoot multiplication, root induction and acclimatization. Mk helps in writing and review editing. All the authors studied and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: The authors declare no conflict of interest.

Ethical issues: None

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Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

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