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NEUTRALIZATION OF CONTAMINATION AND PHENOLICS DURING *IN VITRO* CULTURE ESTABLISHMENT OF *TERMINALIA CHEBULA* RETZ.

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ABSTRACT

An efficient and standardized method for the micropropagation of *Terminalia chebula* Retz. was developed using nodal explants collected from nursery-grown mother plants under controlled laboratory conditions. This study was carried out during 2023–24 at the Department of Forestry, Indira Gandhi Krishi Vishwavidyalaya (IGKV), Raipur (C.G.), with the objective of improving in vitro propagation techniques for this important medicinal tree species. The main focus of the study was to optimize surface sterilization procedures, reduce phenolic exudation, identify the most suitable season for explant collection, and determine appropriate culture media and plant growth regulator combinations for better shoot initiation. Nodal segments measuring 2–4 cm, collected between March and June, showed better survival, lower contamination, and higher regeneration potential, with minimum contamination and maximum bud break. Among the sterilization treatments tested, the highest percentage of aseptic culture establishment was achieved using Bavistin, followed by Streptomycin and mercuric chloride. In contrast, treatments involving lower disinfectant concentrations in combination with sodium hypochlorite resulted in heavy contamination. Phenolic browning, which is a major problem when culturing mature woody tissues, was effectively controlled by pre-treating explants with an antioxidant solution containing ascorbic acid, citric acid, and polyvinyl pyrrolidone, followed by repeated subculturing on Woody Plant Medium (WPM). This approach significantly reduced tissue damage and medium discoloration, leading to improved explant survival and growth. Seasonal variation had a noticeable effect on in vitro performance, with explants collected during active growth periods showing better establishment and regeneration than those collected during monsoon and winter seasons. Shoot initiation also varied with different hormone treatments. The highest bud break percentage was recorded on WPM supplemented with 3.5 mg/L BAP, 0.5 mg/L NAA, and 0.5 mg/L kinetin. Overall, the optimized protocol produced consistent and reliable results from tree-derived explants, despite their moderate regenerative capacity, and can serve as a useful method for the large-scale propagation and conservation of *Terminalia chebula*.

Keywords: *Terminalia chebula*, Micropropagation, Phenolics, Sterilization, Shoot initiation.

Introduction

Terminalia chebula Retz., commonly known as Harad, is an important medicinal and multipurpose tree species that is widely used in traditional systems of medicine and agroforestry (Gowda, 2002; Dhiman, 2003). It is a major component of the classical Ayurvedic formulation *Triphala* and is valued for its antioxidant, digestive, immunomodulatory, and rejuvenating properties. These medicinal attributes have greatly increased its pharmaceutical and

commercial importance (Khan *et al.*, 2015; Phukan, 2019). However, due to high market demand and indiscriminate harvesting from natural forests, the natural populations of this species are gradually declining (Gowda *et al.*, 2011; Nakandalage *et al.*, 2021).

Conventional propagation through seeds is often limited by poor germination, dormancy, low viability, and high genetic variability, which result in inconsistent and low-quality planting material (Rajini,

2014; Prajapati *et al.*, 2020). Similarly, vegetative propagation through traditional methods is restricted by seasonal dependency and low rooting success (Thakur and Rana, 2008). As a result, there is a growing need to develop efficient clonal propagation techniques to ensure sustainable production and conservation of this valuable species.

Plant tissue culture provides a promising alternative for the rapid multiplication of uniform, disease-free, and true-to-type planting material (George *et al.*, 2008). However, micropropagation of woody perennial species such as *T. chebula* is often hindered by several challenges, including high contamination rates, phenolic exudation, tissue browning, and poor regeneration responses, particularly when mature explants are used (Preece and Compton, 1991; Bhatia and Ashwath, 2004). Although some attempts have been made to establish *in vitro* propagation protocols for this species, lack of standardization and inconsistent results continue to be major limitations (Sanjeeva *et al.*, 2013; Nakandalage *et al.*, 2021).

In view of these constraints, the present study was undertaken to develop a standardized and efficient micropropagation protocol for *Terminalia chebula* using nodal explants. The study focused on optimizing surface sterilization methods, controlling phenolic browning, identifying suitable seasons for explant collection, and determining appropriate culture media and plant growth regulator combinations for improved shoot initiation. The ultimate objective was to facilitate large-scale clonal propagation and contribute to the conservation and sustainable utilization of this important medicinal tree species.

Materials and Methodology

The experiment was carried out in the Tissue Culture Laboratory at C.G. Forest Godhi Nursery, Godhi, Mandir Hasaud, Chhattisgarh, located approximately 14 km from Indira Gandhi Krishi Vishwavidyalaya, Raipur. Healthy nodal explants of *Terminalia chebula* were collected from selected mother plants maintained in the nursery and used for *in vitro* studies.

Sterilization of Explants

The collected explants were first washed thoroughly under running tap water to remove dust and surface impurities. They were then treated with 0.1% Tween-20 solution for 15 minutes with intermittent shaking, followed by three to four rinses with double-distilled water. Further surface sterilization was performed under aseptic conditions inside a laminar airflow cabinet using Bavistin and Streptomycin for

different durations to control fungal and bacterial contamination. This was followed by treatment with freshly prepared mercuric chloride or sodium hypochlorite solution for 3–9 minutes. After sterilization, the explants were rinsed 6–7 times with sterile double-distilled water to remove any residual chemicals. The cut ends were then trimmed aseptically before inoculation.

Removal of Phenolic Exudation

To minimize tissue browning caused by phenolic exudation, the explants were subjected to antioxidant treatment prior to inoculation. The nodal segments were pre-treated with a solution containing ascorbic acid (100 mg/L), citric acid (50 mg/L), and polyvinyl pyrrolidone (25 mg/L) for a specified duration. In addition, these compounds, along with adenine sulphate (50 mg/L), were incorporated into the culture media. Serial subculturing was carried out up to four times at regular intervals to further reduce the accumulation of phenolic compounds and to improve explant survival and growth.

Inoculation and Culture Conditions

For shoot initiation, the sterilized and pre-treated nodal explants were vertically inoculated onto Murashige and Skoog (MS) and Woody Plant Medium (WPM) supplemented with different concentrations and combinations of cytokinins (BAP and Kn) and auxins (NAA, IAA, and IBA). The culture media were fortified with 30 g/L sucrose and solidified with 7 g/L agar. The pH was adjusted to 5.8 prior to autoclaving. The inoculated cultures were incubated at $26 \pm 2^\circ\text{C}$ under a 16-hour light and 8-hour dark photoperiod with 70–80% relative humidity. Observations on shoot initiation, contamination rate, and survival percentage were recorded after two to three weeks of culture.

Results

Effect of Explant Size on Culture Establishment

Leafless nodal segments measuring 2–4 cm in length showed better establishment and early culture initiation compared to longer or thinner explants. These explants exhibited higher survival rates, reduced tissue damage, and faster bud break. This clearly indicates that selecting an optimal explant size plays a crucial role in achieving successful *in vitro* culture establishment.

Effect of Surface Sterilization Treatments on Aseptic Culture Establishment

Surface sterilization treatments (Table 1) showed significant differences at the 5% level of significance, with a coefficient of variation of 12.97%. Among the

ten treatments evaluated, the highest percentage of aseptic culture establishment (77.5%) was recorded under treatment S9, which involved 3% Bavistin for 30 minutes, 0.2% Streptomycin for 20 minutes, and 0.1% mercuric chloride for 8 minutes. The second-highest establishment rate (52.5%) was observed under treatment S8. In contrast, treatments using lower concentrations of disinfectants in combination with sodium hypochlorite resulted in heavy contamination. The maximum contamination rate (92.5%) was recorded under treatment S1, indicating its poor effectiveness in controlling microbial infection. These results highlight the importance of selecting appropriate sterilization agents and exposure durations for obtaining healthy and contamination-free cultures.

Effect of Season of Explant Collection on Contamination and Shoot Initiation

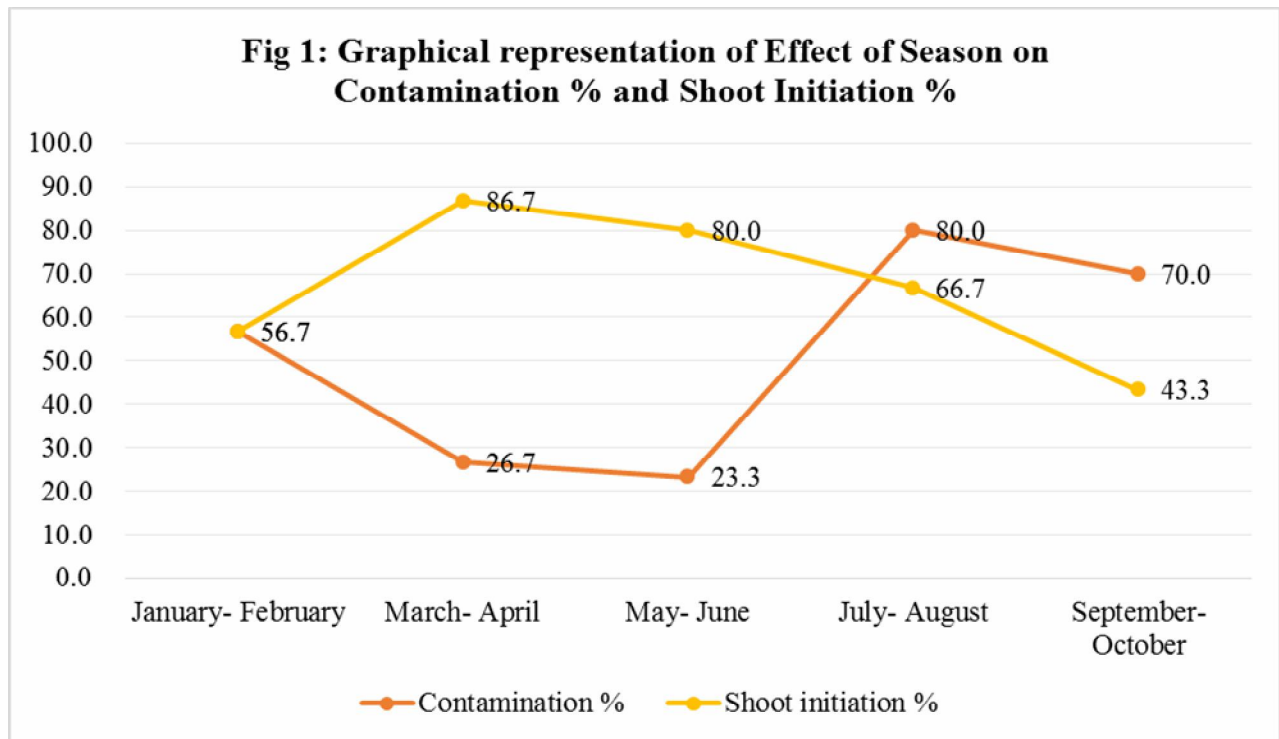
Seasonal variation had a significant effect on contamination levels and shoot initiation in *Terminalia*

chebula. The lowest contamination rate (23.3%) and the highest shoot initiation percentage (86.7%) were recorded during March–April, followed by May–June, as illustrated in Fig. 1. These months correspond to the active growth phase of the plants, which favored better in vitro response.

In contrast, the highest contamination level (80.0%) was observed during July–August, likely due to increased humidity and microbial activity during the monsoon season. Similarly, the lowest shoot initiation rate (26.7%) was recorded during November–December, when plant growth activity was minimal. These findings indicate that the season of explant collection plays a crucial role in determining culture success and should be carefully considered for efficient micropropagation.

Table 1: Sterilization treatment for Culture Initiation

Code	Treatments	Survival %	Contamination %
S1	1% Bavistin (30 minutes) + 0.1% Streptomycin (10 minutes) + 0.1% Sodium Hypochlorite (5 minutes)	7.5	92.5
S2	1% Bavistin (45 minutes) + 0.1% Streptomycin (10 minutes) + 0.1% Sodium Hypochlorite (10 minutes)	27.5	72.5
S3	2% Bavistin (30 minutes) + 0.1% Streptomycin (20 minutes) + 0.2% Sodium Hypochlorite (5 minutes)	10	90
S4	2% Bavistin (45 minutes) + 0.2% Streptomycin (20 minutes) + 0.2% Sodium Hypochlorite (10 minutes)	22.5	77.5
S5	3% Bavistin (30 minutes) + 0.2% Streptomycin (20 minutes) + 0.2% Sodium Hypochlorite (5 minutes)	32.5	67.5
S6	3% Bavistin (30 minutes) + 0.2% Streptomycin (20 minutes) + 0.1% Mercuric Chloride (3 minutes)	37.5	62.5
S7	3% Bavistin (45 minutes) + 0.2% Streptomycin (20 minutes) + 0.1% Mercuric Chloride (5 minutes)	42.5	57.5
S8	3% Bavistin (30 minutes) + 0.2% Streptomycin (30 minutes) + 0.1% Mercuric Chloride (7 minutes)	57.5	42.5
S9	3% Bavistin (30 minutes) + 0.2% Streptomycin (20 minutes) + 0.1% Mercuric Chloride (8 minutes)	77.5	22.5
S10	4% Bavistin (30 minutes) + 0.2% Streptomycin (20 minutes) + 0.1% Mercuric Chloride (9 minutes)	52.5	47.5



Effect of Antioxidant Treatment on Phenolic Exudation and Tissue Browning

Phenolic exudation and tissue browning were prominent during the initial stages of culture establishment, with severe browning and necrosis observed at the cut surfaces soon after inoculation. This adversely affected explant survival and growth. However, pre-treatment of explants with an antioxidant

solution containing ascorbic acid (100 mg/L), citric acid (50 mg/L), and polyvinyl pyrrolidone (PVP) (25 mg/L) for 20 minutes, followed by repeated subculturing on Woody Plant Medium (WPM), proved to be highly effective in controlling browning. This treatment helped maintain tissue viability, reduced necrosis, and significantly improved explant survival and overall culture performance.

Table 2: Effect of Different treatment for removal of Phenolics of explants.

Treatment code	Media	Media + Additives*	Dip in Antioxidant mixture** duration	Transfer to another bottle			
				I	II	III	IV
B1	MS	-	-	++++	+++	+++	++
B2	MS	yes	-	+++	++	++	+
B3	MS	-	10 minutes	+++	++	+	+
B4	MS	-	20 minutes	+++	++	+	-
B5	MS	-	30 minutes	+++	++	+	+
B6	MS	-	40 minutes	+++	++	+	+
B7	WPM	-	-	+++	++	+	+
B8	WPM	-	10 minutes	+++	++	+	+
B9	WPM	-	20 minutes	++	+	-	-
B10	WPM	-	30 minutes	++	+	+	-
B11	WPM	-	40 minutes	+++	+	+	+

* Ascorbic acid (100mg/l) + Citric acid (50mg/l) + Adenine sulphate (50mg/l) + PVP (25mg/l)

** Ascorbic acid (100mg/l) + Citric acid (50mg/l) + PVP (25mg/l)



Fig. 2: Treatment response for removal of Phenolics in different media and after serial transfers.

Effect of Culture Media and Growth Regulators on Shoot Initiation

Shoot initiation response varied significantly among the different hormonal treatments, with a coefficient of variation of 15.50%. The highest bud break percentage (63.3%) was recorded on Woody Plant Medium (WPM) supplemented with 3.5 mg/L BAP, 0.5 mg/L NAA, and 0.5 mg/L kinetin, indicating the effectiveness of this hormone combination for

shoot induction. No shoot initiation was observed in the control treatment, highlighting the essential role of plant growth regulators in *in vitro* culture. Moderate shoot initiation responses were obtained on Murashige and Skoog (MS) medium supplemented with various combinations of BAP and NAA. These results suggest that both the type of basal medium and the concentration of growth regulators play a crucial role in influencing shoot initiation in *Terminalia chebula*.

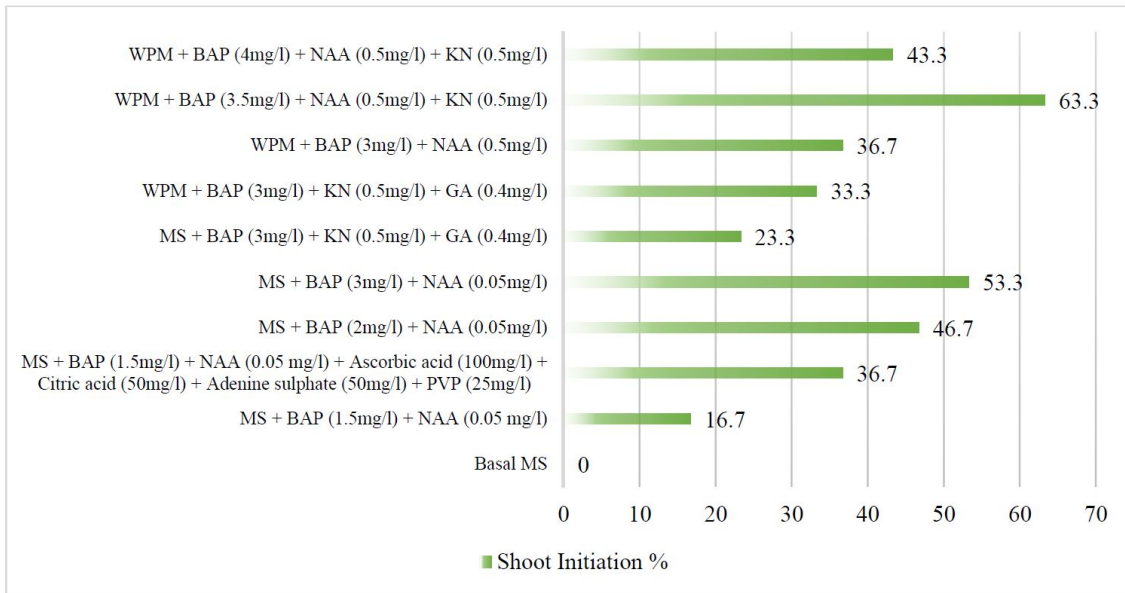


Fig. 3: Shooting response with different concentrations of hormones in MS and WPM media.

Discussion

The results of the present study clearly demonstrate that explant characteristics, sterilization methods, season of collection, control of phenolic exudation, and hormonal balance play crucial roles in the successful micropropagation of *Terminalia chebula*. Proper optimization of these factors is essential for achieving consistent and efficient *in vitro* regeneration.

The superior performance of nodal segments measuring 2–4 cm in length may be attributed to the

presence of actively dividing meristematic tissues and sufficient nutrient reserves, which support early bud break and regeneration. Smaller or excessively thin explants are more prone to desiccation and mechanical damage, whereas longer segments often show reduced responsiveness due to increased tissue maturity. Similar observations were reported by Deshmukh (1997), Bhat (2011), and Phulwaria *et al.* (2012), emphasizing the importance of selecting physiologically active explants for efficient micropropagation.

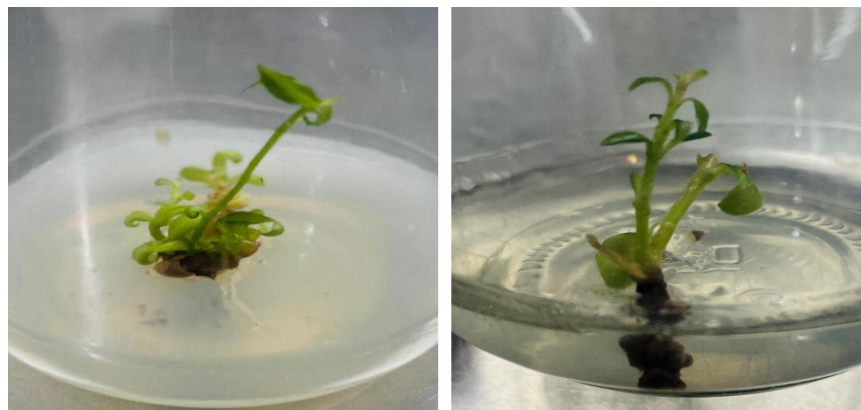


Fig. 4: Shoot Initiation and Shooting response of explants in treatment I3 and I9.

The significantly higher aseptic establishment achieved under treatment S9 highlights the importance of adopting a strong and sequential sterilization protocol for nursery-derived explants. Nursery-grown plants are continuously exposed to soil-borne and airborne microorganisms, resulting in higher endogenous contamination. The combined application of Bavistin, Streptomycin, and mercuric chloride ensured effective elimination of fungal and bacterial contaminants. Mercuric chloride, due to its strong penetrating ability and broad-spectrum antimicrobial activity, proved particularly effective. Similar findings were reported by Deshmukh (1997), Bhat (2011), Choudhary *et al.* (2015), Bhargava *et al.* (2018), and Varma (2021), who also recommended multi-step sterilization procedures for woody plant species.

Seasonal variation had a significant influence on explant response, with March to June proving to be the most favourable period for culture establishment and shoot initiation. During this phase, plants remain in active growth with higher metabolic activity and lower microbial load, resulting in improved tissue responsiveness. In contrast, the monsoon season (July–August) favoured microbial proliferation due to high humidity and rainfall, leading to increased contamination. Reduced shoot initiation during November–December may be associated with dormancy, lower enzymatic activity, and increased phenolic oxidation. These findings are in agreement with those of Bhat (2011) and Kaur (2018), who also reported seasonal dependency in *T. chebula* micropropagation.

Phenolic exudation and tissue browning posed major challenges during culture establishment, particularly in mature explants. The release of oxidized phenolic compounds leads to tissue necrosis and inhibits growth by creating a toxic microenvironment. The combined use of ascorbic acid, citric acid, and polyvinyl pyrrolidone (PVP) effectively minimized oxidative stress by scavenging free radicals and adsorbing phenolic compounds. Frequent subculturing further reduced toxin accumulation and improved nutrient availability. Similar strategies have been recommended by Deshmukh (1997), Sidhu (2010), Bhat (2011), Yusuf (2005), Dangi *et al.* (2014), and Varma (2021) for controlling browning in woody plant cultures.

The significant variation observed in shoot initiation among hormonal treatments reflects the importance of optimizing growth regulator combinations. The superior performance of WPM medium supplemented with BAP, NAA, and kinetin suggests that lower salt concentration and balanced

hormonal composition are more suitable for nursery-derived explants. Reduced salt levels in WPM help minimize osmotic stress and phenolic toxicity, thereby enhancing tissue viability. The requirement for relatively higher cytokinin and auxin concentrations may indicate lower endogenous hormone levels in mature nursery plants. The synergistic action of BAP, NAA, and kinetin promoted cell division, bud differentiation, and meristematic activity, resulting in improved shoot initiation. Similar responses favouring WPM and BAP-based media were reported by Deshmukh (1997), Ramesh *et al.* (2005), Ravi (2015), Kaur (2018), and Rathore *et al.* (2008) in related species.

Overall, the present study establishes that successful micropropagation of *Terminalia chebula* using nursery explants requires careful optimization of explant size, sterilization protocol, seasonal timing, antioxidant treatment, and hormonal composition. The standardized protocol developed in this investigation provides a reliable foundation for large-scale clonal propagation, conservation, and sustainable utilization of this valuable medicinal tree species.

Conclusions

The present study successfully developed an efficient and standardized *in vitro* culture protocol for *Terminalia chebula* using nodal explants collected from mother plants maintained at the Godhi nursery. This was achieved by carefully optimizing explant size, season of collection, sterilization methods, control of phenolic exudation, and growth regulator combinations. Nodal segments measuring 2–4 cm, collected during March to June, showed better survival, lower contamination, and improved regenerative response. The sequential use of 3% Bavistin, 0.2% Streptomycin, and 0.1% mercuric chloride for 8 minutes proved to be the most effective sterilization treatment for achieving maximum aseptic culture establishment. In addition, antioxidant pre-treatment with ascorbic acid, citric acid, and polyvinyl pyrrolidone (PVP), followed by repeated subculturing on Woody Plant Medium (WPM), successfully minimized phenolic browning and tissue damage, thereby enhancing explant viability. Shoot initiation was significantly improved on WPM supplemented with BAP, NAA, and kinetin, highlighting the importance of a balanced interaction between cytokinins and auxins for successful bud break and shoot development. Although the explants showed moderate regenerative potential, the optimized protocol produced consistent and reliable results. Overall, the standardized procedure developed in this study provides a strong and practical foundation for large-

scale clonal propagation, conservation, and the supply of high-quality planting material of *Terminalia chebula* for agroforestry and medicinal plantation programmes.

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References

- Bhargava, P., Ravindra, N. and Singh, G. (2018). A modified and improved protocol development for in vitro clonal propagation of *Santalum album* L. from internodal explants. *Tropical Plant Research*, **5**(2), 193–199.
- Bhat, S. K. (2011). *Studies on micropropagation of Terminalia chebula Retz.* (Ph.D. thesis). University of Jammu.
- Bhatia, P. and Ashwath, N. (2004). Improving the quality of in vitro cultures of woody plants. *Plant Cell, Tissue and Organ Culture*, **78**, 121–129.
- Choudhary, M., Jaiswal, S., Singh, R., Arya, I. D. and Arya, S. (2015). A micropropagation protocol for mass multiplication of *Terminalia arjuna*—a valuable medicinal tree. *Advanced Forestry Science*, **2**(1), 1–6.
- Dangi, B., Kaul, V. K., Kothari, S. L. and Kachhwaha, S. (2014). Micropropagation of *Terminalia bellerica* from nodal explants of mature tree and assessment of genetic fidelity using ISSR and RAPD markers. *Physiology and Molecular Biology of Plants*, **20**(4), 509–516.
- Deshmukh, A. J. (1997). *Micropropagation studies in Terminalia chebula Retz.* (M.Sc. thesis). Dr. Y. S. Parmar University of Horticulture and Forestry, Solan, India.
- Dhiman, R. C. (2003). *Studies on genetic variability and improvement of Terminalia chebula* (Ph.D. thesis). Dr. Y. S. Parmar University of Horticulture and Forestry, Solan, India.
- George, E. F., Hall, M. A. and De Klerk, G. J. (2008). *Plant propagation by tissue culture* (3rd ed.). Springer.
- Gowda, M. (2002). Provenance variation in *Terminalia chebula*. *Indian Forester*, **128**(9), 1011–1017.
- Gowda, M., Chandrashekar, K. R. and Rao, G. R. (2011). Conservation and genetic improvement of *Terminalia chebula*. *Journal of Non-Timber Forest Products*, **18**(3), 221–226.
- Kaur, J. (2018). *Propagation of Terminalia chebula Retz. and evaluation of biochemical properties of fruits collected from different sources* (M.Sc. thesis). Punjab Agricultural University, Ludhiana.
- Khan, M. A., Khan, T. and Ahmad, M. (2015). Morphological and medicinal characterization of *Terminalia chebula*. *Journal of Medicinal Plants Research*, **9**(4), 112–118.
- Nakandalage, N., Wijesundara, D. and Perera, A. (2021). Morphological characterization and propagation of *Terminalia chebula*. *Tropical Agricultural Research*, **32**(2), 145–156.
- Phukan, A. (2019). Studies on variability of *Terminalia chebula* in Northeast India. *Indian Journal of Forestry*, **42**(1), 57–62.
- Phulwaria, M., Rai, M. K., Harish, Gupta, A. K., Ram, K. and Shekhawat, N. S. (2012). Improved micropropagation of *Terminalia bellerica* from nodal explants of mature tree. *Acta Physiologiae Plantarum*, **34**, 299–305.
- Preece, J. E. and Compton, M. E. (1991). Problems with explant exudation in woody plant tissue culture. *HortScience*, **26**(10), 1209–1212.
- Rajini, K. (2014). Evaluation of elite genotypes of *Terminalia chebula*. *Journal of Tropical Forest Science*, **26**(3), 356–364.
- Pavan, U., Rao, K. V. and Sadanandam, A. (2005). Micropropagation of *Terminalia bellirica* Roxb.—a sericulture and medicinal plant. *In Vitro Cellular & Developmental Biology – Plant*, **41**(3), 320–323.
- Rathore, P., Suthar, R. and Purohit, S. D. (2008). Micropropagation of *Terminalia bellirica* Roxb. from juvenile explants. *Indian Journal of Biotechnology*, **7**, 246–249.
- Ravi, M. (2015). *Studies on tissue culture and biochemical aspects during morphogenesis in Terminalia arjuna Roxb.* (Ph.D. thesis). Sri Krishnadevaraya University, Anantapuram.
- Sanjeeva, W., Perera, P. and Gunaratne, L. (2013). Regional variation in *Terminalia chebula* in Sri Lanka. *Journal of Forestry Research*, **24**(2), 215–222.
- Sidhu, Y. (2010). In vitro micropropagation of medicinal plants by tissue culture. *Plymouth Student Scientist*, **4**(1), 432–449.
- Thakur, S. and Rana, R. S. (2008). Vegetative propagation of *Terminalia chebula*. *Indian Journal of Forestry*, **31**(2), 287–292.
- Varma, H. S. (2021). *In vitro clonal propagation of teak (Tectona grandis L.) from nodal explants* (M.Sc. thesis). Anand Agricultural University, Anand.
- Yusuf, A. (2005). Clonal propagation of *Anogeissus sericea* var. *nummularia*—a rare tree of arid forestry. *Journal of Sustainable Forestry*, **20**(1), 67–78.