Volume: 5 Issue: 3 Pages: 54-57

Acclimatization and Hardening of *Solanum torvum* (Swartz)-A Medicinal Plant

*1 Dr. Ghan Singh Malothu

*1 Associate Professor, Department of Botany, Kakatiya Government College (A) Hanumakonda, Telangana, India.

Article Info.
E-ISSN: 2583-6528
Impact Factor (QJIF): 8.4
Peer Reviewed Journal
Available online:
www.alladvancejournal.com

Received: 11/Feb/2026
Accepted: 09/March/2026

***Corresponding Author**
Dr. Ghan Singh Malothu
Associate Professor, Department of Botany, Kakatiya Government College (A) Hanumakonda, Telangana, India.

Abstract

Acclimatization (hardening) is a crucial step in the establishment of *in vitro* regenerated plants, ensuring their successful transfer from controlled laboratory conditions to the external environment. *Solanum torvum*, an ethno medicinally important shrub, was selected to standardize the hardening protocol for improved survival and propagation. *In vitro* rooted plantlets derived from leaf, shoot tip, and nodal cultures were carefully washed and transferred to micro pots containing different sterilized substrates: red soil, red soil + sand (1:1), red soil + sand + farmyard manure (1:1:1), and red soil + sand + vermiculite (1:1:1). The plantlets were maintained under high humidity in a walk-in chamber (25–27 °C), and polythene covers were gradually perforated and removed over four weeks. Among the substrates tested, red soil + sand + vermiculite supported the highest survival rate (92% in nodal cultures, 78% in shoot tip cultures, and 72% in leaf cultures), followed by red soil + sand + farmyard manure, while the lowest survival was observed in red soil alone. New leaf initiation occurred within six days in the vermiculite mixture, indicating superior adaptation. Acclimatized plants displayed normal morphology, flowering, and fruiting comparable to donor plants. The study demonstrates that a soil mixture containing red soil, sand, and vermiculite provides an effective medium for acclimatization, ensuring high survival rates and vigorous growth. This protocol can be applied for large-scale propagation and conservation of the medicinally important *S. torvum*.

Keywords: *Solanum torvum*; micropropagation; acclimatization; hardening; *in vitro* regeneration; lab-to-land transfer; plant survival; medicinal plant conservation; potting substrates; vermiculite mixture.

Introduction

Plant tissue culture has emerged as a powerful tool for the mass multiplication, conservation, and genetic improvement of medicinally important and endangered plant species. However, the success of micro propagation largely depends on the survival of *in vitro*–derived plantlets after their transfer from the controlled laboratory environment to ex vitro conditions. The transition phase referred to as acclimatization or hardening, is considered one of the most critical steps in plant tissue culture (Hazarika, 2003).

Plantlets regenerated *in vitro* are continuously exposed to a highly regulated microenvironment characterized by aseptic conditions, low light intensity, high relative humidity, and nutrient-rich media supplemented with sugars that support heterotrophic growth. As a consequence, these plantlets exhibit a culture-induced phenotype with poorly developed cuticle, non-functional stomata, and reduced photosynthetic activity, which makes them highly vulnerable to desiccation, light stress, temperature fluctuations, and pathogen attack when directly exposed to field conditions (Preece and Sutter, 1991). To overcome these limitations, plantlets must be gradually acclimatized to lower humidity, higher irradiance, fluctuating temperature, and biotic stress in order to ensure successful establishment.

Several studies have emphasized that the benefit of any micro propagation protocol can only be realized through the successful lab-to-land transfer of regenerated plantlets (Saxena, 1993; Ahuja, 1993a, b). Acclimatization enhances survival percentage, induces physiological and anatomical changes for autotrophic growth, and ensures the vigorous establishment of plantlets in soil. Soil substrate composition, light intensity, and humidity regulation are critical factors influencing survival during this phase.

Solanum torvum Swartz (Turkey berry), a spiny, perennial shrub belonging to the family Solanaceae, is valued for its ethno medicinal and pharmacological importance. Its fruits and roots are used in traditional medicine as anti-inflammatory, antimicrobial, and hepatic protective agents. The species also holds ecological significance as a rootstock for cultivated eggplants due to its resistance against soil-borne pathogens. Given its medicinal relevance and utility, developing an efficient acclimatization protocol for *in vitro*–derived *S. torvum* is essential for both conservation and large-scale propagation.

The present study was undertaken to standardize a hardening protocol for *in vitro* regenerated *S. torvum* plantlets, focusing on the effect of different potting substrates on survival, growth, and field establishment.

Objectives

The present investigation was carried out with the following objectives:

1. To develop an efficient protocol for hardening/acclimatization of *in vitro* regenerated *Solanum torvum* plantlets.
2. To evaluate the effect of different potting substrates (red soil, red soil + sand, red soil + sand + farmyard manure, red soil + sand + vermiculite) on survival and growth of plantlets.
3. To assess the survival rate and morphological performance of plantlets derived from different explants (leaf, shoot tip, and nodal cultures) during acclimatization.
4. To establish a reliable lab-to-land transfer method for large-scale propagation and conservation of the medicinally important shrub *S. torvum*.

Materials and Methods

In this study, plant material was collected from the Department of Botany at Kakatiya University, located in Hanumakonda, Telangana, India (18.0° N, 79.58° E). Unlike Solanum torvum, which typically produces black seeds, white seeds were discovered in the Turkey berry (Solanum torvum). The plant specimen was preserved as a herbarium collection in the Department of Botany at Kakatiya University, with taxonomic authentication conducted by Prof. V. S. Raju, a renowned expert from the Plant Systematics Laboratory at Kakatiya University.

Plant Material and Selection of Plantlets

In vitro regenerated plantlets of *Solanum torvum* Swartz were obtained from leaf, shoot tip, and nodal explants cultured on standardized regeneration and rooting media. Plantlets with 3–5 nodes and at least 10 healthy roots measuring 3–5 cm in length were selected for acclimatization studies.

Preparation of Plantlets for Acclimatization

The rooted plantlets were gently removed from culture vessels, and traces of agar medium were carefully washed off using sterile distilled water to minimize contamination and facilitate root-soil contact.

Potting Substrates

Hardened plantlets were transferred to sterile plastic micro pots containing the following potting mixtures:

1. Red soil
2. Red soil + sieved sand (1:1)
3. Red soil + sieved sand + farmyard manure (1:1:1)
4. Red soil + sieved sand + vermiculite (1:1:1)

All substrates were sterilized prior to use to prevent microbial contamination.

Acclimatization Procedure

Plantlets in micro pots were covered with transparent polythene bags to maintain 80–85% relative humidity (RH) and placed in a walk-in growth chamber at 25–27 °C under controlled conditions. To gradually adapt the plantlets to ambient conditions, polythene covers were perforated after two weeks and completely removed after four weeks.

Following initial acclimatization, the hardened plantlets were transferred to earthenware pots containing garden soil and maintained under shady field conditions for one month. Subsequently, the plants were shifted to open field conditions for further growth and establishment.

Assessment of Survival and Growth

Plant survival percentage was recorded across different substrate treatments. Morphological responses, including new leaf initiation, overall vigor, and similarity to donor plants, were also documented. Comparative survival of plantlets derived from different explants (leaf, shoot tip, and nodal cultures) was analyzed.

Results and Discussion

Effect of Substrates on Acclimatization

In vitro rooted plantlets of *Solanum torvum* obtained from leaf, shoot tip, and nodal cultures were successfully acclimatized on four different potting mixtures. Survival percentage and leaf initiation varied significantly with substrate composition (Table 1).

Among the tested substrates, the mixture of red soil + sieved sand + vermiculite (1:1:1) supported the highest survival rates, with 72% survival in leaf culture plantlets, 78% in shoot tip culture plantlets, and 92% in nodal culture plantlets. This substrate also promoted the earliest initiation of new leaves (8 days), indicating superior adaptation and growth. The second-best performance was recorded in red soil + sieved sand + farmyard manure (1:1:1), with survival rates of 58%, 65%, and 77% for plantlets from leaf, shoot tip, and nodal cultures, respectively.

By contrast, plantlets transferred to red soil alone showed poor survival (10–18%) and delayed new leaf formation (18 days). The red soil + sand mixture (1:1) performed moderately, with survival rates ranging between 24–48% and new leaf emergence in 14 days. Thus, the choice of substrate was found to be critical for the successful acclimatization of *S. torvum* plantlets.

Explant Response during Hardening

Among different explant sources, nodal culture–derived plantlets consistently showed the highest survival percentages across all substrates, followed by shoot tip and leaf culture plantlets. This suggests that nodal cultures possess greater physiological stability and adaptability during the transition from *in vitro* to ex vitro conditions.

Physiological Basis of Acclimatization

The higher survival rates in substrates containing vermiculite or farmyard manure may be attributed to improved aeration, moisture retention, and nutrient availability, which facilitate the gradual transition from heterotrophic to autotrophic growth. *In vitro* plantlets generally possess poorly developed cuticle and stomatal function, making them prone to desiccation and stress when directly exposed to fluctuating field conditions (Hazarika, 2003). The gradual reduction of relative humidity and controlled exposure to light in the walk-in chamber enabled stomatal regulation, improved photosynthetic activity, and promoted leaf initiation.

Previous reports also indicate that acclimatization induces physiological adjustments, such as the accumulation of carbohydrate reserves (Capelladse *et al*., 1991), increased antioxidant enzyme activity (Van Huylenbroeck & Debergh, 1996), and improved photosynthetic competence, which collectively enhance plantlet survival. The early initiation of new leaves in vermiculite-based substrate observed in the present study supports the role of optimized soil aeration and water balance in reducing transplant shock.

Table 1: Effect of various substrates on hardening of *in vitro* rooted plantlets of *S.torvum*

Substrates	No. of Plantlets transferred	Survival rate (%) after 35 days			Days required for new leaf formation
		Leaf culture*	Shoot tip culture*	Nodal culture*	
Red soil	50	15	10	18	18
Red soil + sieved sand (1:1)	50	24	28	48	14
Red soil + sieved sand + farm yard manure (1:1:1)	50	58	65	77	10
Red soil + sieved sand + vermiculite (1:1:1)	50	72	78	92	8

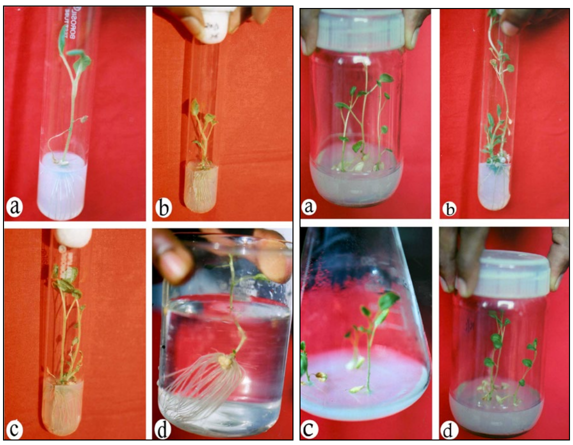
* Regenerated plantlets from the respective culture

Lab-to-Land Transfer

After four weeks of acclimatization, hardened plantlets were successfully transferred to garden soil in earthen pots and later established in field conditions. The acclimatized plants exhibited normal growth, flowering, and fruiting comparable to donor plants, demonstrating that the developed hardening protocol was effective in ensuring long-term survival and field establishment.

Implications for Conservation and Propagation

The present study highlights the importance of substrate optimization for the acclimatization of *S. torvum*. With survival rates as high as 92% in nodal culture plantlets, the standardized protocol provides a reliable approach for large-scale propagation and conservation of this ethnomedicinally important shrub. These findings also support the broader application of acclimatization techniques in plant tissue culture programs aimed at medicinal plant conservation and commercial cultivation.



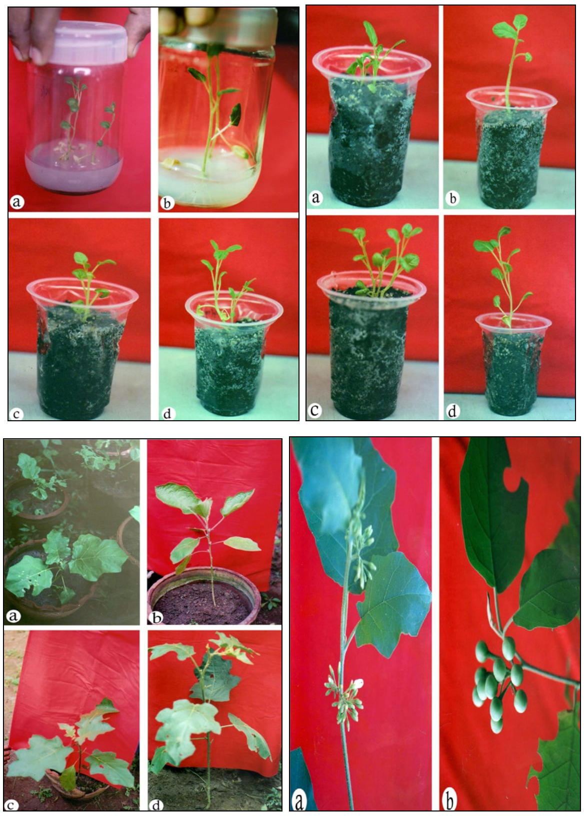


Fig 1: Different stages of formation roots and shifted from lab to land also survived in normal conditions with flowers and fruits.

Conclusion

The present study successfully standardized a hardening protocol for *in vitro* regenerated plantlets of *Solanum torvum*. Among the substrates tested, the mixture of red soil + sieved sand + vermiculite (1:1:1) proved to be the most effective, supporting the highest survival rate (up to 92% in nodal cultures) and promoting early new leaf initiation. Nodal culture–derived plantlets exhibited greater adaptability compared to shoot tip and leaf culture plantlets.

The gradual acclimatization process, involving controlled humidity and stepwise exposure to external conditions, was critical for minimizing transplant shock and ensuring vigorous growth. Acclimatized plants displayed normal morphology, flowering, and fruiting similar to donor plants, confirming their successful establishment in field conditions.

This protocol provides a reliable and efficient approach for the lab-to-land transfer, large-scale propagation, and conservation of the medicinally important shrub *S. torvum*. The findings also emphasize the broader role of acclimatization in improving survival and field performance of micropropagated plants.

References

1. Ahuja MR. Micropropagation of woody plants: Problems, achievements and future prospects. *International Journal of Plant Sciences*. 1993a; 154(4):526–534.
2. Ahuja MR. Clonal propagation of woody species: Concepts and biotechnological approaches. Euphytica. 1993b; 70(1-2):21–34.
3. Capellades M, Lemeur R, Debergh PC. Effects of sucrose on starch accumulation and rate of photosynthesis in Rosa cultured *in vitro*. Plant Cell, Tissue and Organ Culture. 1991; 25(1):21-26.
4. Hazarika BN. Acclimatization of tissue-cultured plants. Current Science. 2003; 85(12):1704-1712.

5. Maene LJ, Debergh PC. Liquid medium additions to established tissue cultures to improve elongation and rooting *in vitro*. Plant Cell, Tissue and Organ Culture. 1985; 5(1):23-33.
6. Preece JE, Sutter EG. Acclimatization of micropropagated plants to the greenhouse and field. In P. C. Debergh & R. H. Zimmerman (Eds.), *Micropropagation: Technology and application*. Dordrecht: Springer, 1991, 71–93.
7. Saxena PK. Micropropagation of woody plants: Uses and limitations. *International Journal of Plant Sciences.* 1993; 154(4):555–562.
8. Van Huylenbroeck JM, Debergh PC. Physiological aspects in acclimatization of micropropagated plantlets. Plant Tissue Culture and Biotechnology. 1996; 2(3):136-141.
9. Van Huylenbroeck JM, Piqueras A, Debergh PC. Photosynthesis and carbon metabolism in acclimatization of micropropagated plants. Acta Horticulturae. 1995; 393, 191-198.
10. Arthan D, Svasti J, Kittakoop P, Pittayakhachonwut D, Tanticharoen M, Thebtaranonth Y. Antiviral isoflavonoid sulfate and steroidal glycosides from *Solanum torvum*. *Phytochemistry.* 2002; 59(4):459–463.
11. Suresh K, Venkataraman R. Ethnomedicinal, phytochemical and pharmacological properties of Solanum torvum Swartz: A review. *International Journal of Pharmaceutical Sciences and Research.* 2012; 3(11):4117-4125.
12. Anbazhakan S, Balu V, Sathish S. Pharmacognostic studies on *Solanum torvum* Swartz. *Journal of Biosciences Research.* 2009; 1(2):89-93.