



Somatic Embryogenesis and Plantlet Formation in Turkey Berry (*Solanum torvum* L.) A Medicinally Significant Plant

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Abstract

An effective protocol was developed for inducing somatic embryogenesis and regenerating plantlets from leaf explants of *Solanum torvum* L. (turkey berry), a plant of significant medicinal importance. This study marks a notable advancement in tissue culture methodologies for medicinal plants. Fast-growing, yellowish nodular callus lines containing somatic embryos were successfully established using an initiation medium supplemented with 2.0 mg/L 2,4-D, 3.0 mg/L 2,4,5-T, and 2.0 mg/L NAA. Direct somatic embryogenesis was observed from cotyledon explants cultured on Murashige and Skoog (MS) medium fortified with varying concentrations of 2,4-D and other growth regulators. Optimal embryo growth and maturation were achieved on MS medium containing 2.0 mg/L 2,4-D and 1.5 mg/L TDZ or 3.0 mg/L 2,4-D and 2.0 mg/L BAP. Germination of well-formed somatic embryos into complete plantlets occurred on MS medium supplemented with 2.0 mg/L TDZ and 2.0 mg/L NAA.

The regenerated plantlets were successfully acclimatized in plastic cups and subsequently transferred to pots for maturation. Upon transplantation into soil, the plants exhibited an 80% survival rate and developed into mature, seed-producing individuals. This optimized technique demonstrates high efficiency in regenerating *S. torvum* plants, offering a sustainable approach for conserving this valuable medicinal species and enabling its large-scale propagation for pharmaceutical applications. The findings underscore the potential of tissue culture techniques in promoting the conservation and utilization of medicinally important plants.

Keywords: *Solanum torvum* L., turkey berry, leaf explants, somatic embryogenesis, plantlet regeneration, medicinal plant propagation, callus induction, growth regulators

Introduction

Solanum torvum L. (turkey berry), a member of the Solanaceae family, is a plant of significant medicinal value, traditionally used for treating a wide range of ailments. Its fruits, seeds, and

vegetative parts possess numerous therapeutic properties, making it a versatile remedy in various cultures. The plant is particularly valued for its antimicrobial, antioxidant, antihypertensive, anti-diabetic, diuretic, sedative, and immune-boosting

effects. The fruit extract has demonstrated hypoglycemic, hypolipidemic, and hepatoprotective properties, which make it a promising candidate for treating diabetes and liver disorders. It is also commonly used for alleviating fever, cough, wounds, pain, and reproductive issues.

Somatic embryogenesis is a key tissue culture technique that enables the regeneration of plants from somatic cells. This process is especially crucial for species like *Solanum torvum*, where conventional propagation methods may be slow or inefficient. Somatic embryogenesis in the Solanaceae family has been explored in various species, revealing its potential in large-scale propagation and conservation of medicinal plants.

In a study on *Solanum torvum*, somatic embryogenesis was successfully induced from leaf explants cultured on media containing various growth regulators, including 2,4-D (2,4-dichlorophenoxyacetic acid), BAP (6-Benzylaminopurine), and TDZ (Thidiazuron). Similar studies have been conducted in other members of the Solanaceae family, such as *Solanum lycopersicum* (tomato) and *Solanum melongena* (eggplant), both of which are well-documented for their ability to undergo somatic embryogenesis.

For instance, Jafari et.al., (2011) demonstrated the induction of somatic embryos in *Solanum melongena* using a combination of auxins and cytokinins, emphasizing the importance of media composition and hormonal regulation for successful embryo development. Similarly, Prakash and Rao (2001) found that high concentrations of 2,4-D were most effective in inducing callus formation and somatic embryogenesis in *Solanum torvum*, which aligns with findings in other Solanaceae species. The ability to produce somatic embryos directly from cotyledon and leaf explants in *Solanum torvum* can be attributed to the action of these growth regulators, which promote the dedifferentiation of cells and subsequent re-differentiation into somatic embryos.

Somatic embryogenesis is a common feature across the Solanaceae family, with many species demonstrating similar pathways and challenges. In *Solanum melongena* and *Solanum lycopersicum*, successful somatic embryogenesis has been achieved through a similar process of callus induction followed by embryo formation under controlled conditions.

Prakash and Rao (2001). "In vitro Regeneration of *Solanum torvum* L. through Somatic Embryogenesis." Plant Cell, Tissue and Organ Culture, 64(3): 257-263.

This study provided insight into the regeneration of *Solanum torvum* using leaf explants, demonstrating the potential for large-scale propagation and conservation of this valuable medicinal species. Jafari, M., et.al., (2011). "Induction of Somatic Embryogenesis in Eggplant (*Solanum melongena* L.) via Different Growth Regulators." Scientia Horticulturae, 129(3): 515-520. The authors discuss the influence of growth regulators on somatic embryogenesis in *Solanum melongena*, which shares similarities with the regenerative pathways observed in *S. torvum*.

Somatic embryogenesis is a powerful method for plant micropropagation, enabling the rapid production of large numbers of genetically uniform plants, including elite and transgenic varieties (Ammirato, 1987; Roberts et.al., 1995). It is preferred over organogenesis for plant regeneration due to the production of plants from single-cell origins, which ensures true-to-type characteristics (Ammirato, 1983a). This technique also facilitates the creation of artificial or synthetic seeds and is widely used for Agrobacterium-mediated genetic transformation (Rama Swamy et.al., 2005b).

The phenomenon of somatic embryogenesis was first reported by Rienert (1958) and Steward et.al., (1958), and it has since been observed in several medicinal plants, including *Atropa belladonna* (Thomas & Street, 1970), *Carum carvi* (Ammirato, 1977), *Papaver somniferum* (Nessler, 1982), *Tylophora indica* (Mhatre et.al., 1984), *Cichorium intybus* (Hierweghet.al., , 1985),

Urginea indica (Jha, 1986), *Cassia fistula* (Bajaj, 1988), *Tribulus terrestris* (Mohan et.al., 2000), *Psoralea corylifolia* (Sahrawat & Chand, 2001), *Solanum melongena* (Yadav & Rajam, 1997, 1998), and *S. surattense* (Rama Swamy et.al., 2005b). Chakrabarty, D., et.al., (2008). "In Vitro Propagation and Somatic Embryogenesis in *Solanum lycopersicum* L. (Tomato)." Journal of Plant Growth Regulation, 27(2): 153-160.

This work highlights the use of somatic embryogenesis in *Solanum lycopersicum*, a closely related species, providing comparative insights into the culture media and protocols used for efficient plant regeneration.

This study aims to develop an efficient and reliable methodology for plant regeneration through somatic embryogenesis using leaf explants from major *S. torvum* genotypes (turkey berry), a plant known for its medicinal properties. The study aims to optimize the tissue culture conditions to induce somatic embryogenesis, thereby enabling large-scale propagation and conservation of this valuable species.

Objectives:

1. **To evaluate the effect of different plant growth regulators** on the induction of callus formation from leaf explants of *Solanum torvum* genotypes.
2. **To optimize the culture medium** for efficient somatic embryogenesis by testing various concentrations of 2,4-D, BAP, TDZ, and NAA.
3. **To induce somatic embryos directly from callus tissues** using the selected growth regulator combinations and media.
4. **To enhance the germination and plantlet formation** of somatic embryos under optimized culture conditions.
5. **To assess the acclimatization and survival rate** of regenerated plantlets when transferred to soil, ensuring the successful establishment of mature plants.
6. **To establish a reproducible protocol** for the *in vitro* regeneration of *Solanum torvum*,

contributing to its sustainable propagation and conservation.

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.

Leaf explants (1 cm) were collected from 3-month-old *Solanum torvum* plants. The explants were washed thoroughly with running tap water for 15 minutes, followed by surface sterilization using 0.1% mercuric chloride (HgCl₂) for 3 minutes. After sterilization, the explants were rinsed with sterile distilled water to remove any residual contaminants. Murashige and Skoog (1962) (MS) medium, supplemented with necessary nutrients, was prepared and autoclaved at 15 psi for 20 minutes. The pH of the medium was adjusted to 5.8 using 1 N NaOH. For shoot bud induction, leaf explants were cultured in the MS medium under controlled conditions with a 16-hour photoperiod at 25±2°C and exposed to white fluorescent light (80 μM·m⁻²·s⁻¹).

To evaluate somatic embryogenesis, leaf explants were cultured in MS medium supplemented with various concentrations of growth regulators, such as 2,4-Dichlorophenoxyacetic acid (2,4-D), 2,4,5-Trichlorophenoxyacetic acid (2,4,5-T), and Naphthaleneacetic acid (NAA). A 3% (w/v) concentration of sucrose was included in all media. The medium was autoclaved at a pressure of 1.06 kg/cm² for 20 minutes, and the pH was adjusted to 5.8, with the agar concentration set to 0.8%. All cultures were maintained at 25±2°C, with a 16-hour light/dark cycle provided by cool white, fluorescent light (30 μmol·m⁻²·s⁻¹). After 6

weeks, the number of explants showing signs of somatic embryogenesis, including globular and advanced embryonic stages, was recorded. The callus was then dissected, and the resulting somatic embryos (globular stage) were transferred to MS basal medium supplemented with BAP (0.5-3.0 mg/L) and TDZ (0.5-3.0 mg/L) for continued growth and maturation.

For germination, the globular or cotyledonary embryos were transferred to MS medium containing TDZ (0.5-3.0 mg/L) and NAA (3.0 mg/L). The embryos were incubated in a $25\pm 2^\circ\text{C}$ environment with constant illumination ($35\ \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) from fluorescent lights for 15 days. Afterward, the regenerated plantlets were carefully removed from the test tubes, and the agar was washed off with tap water. The plantlets were then transplanted into plastic cups filled with vermiculite and kept in a greenhouse until they were ready for field transplantation.

Statistical analysis was conducted using a standard error (SE) test to assess the number of embryos, their development, and germination success. Duncan's New Multiple Range Test was applied for statistical comparison. Each treatment included 30 replicates, and each experiment was repeated three times to ensure the consistency and reliability of the results.

This approach is expected to provide valuable insights into the plant regeneration of *Solanum torvum* through somatic embryogenesis, further enhancing our understanding of its potential for large-scale propagation.

The successful induction of somatic embryogenesis in *Solanum torvum* not only serves as an effective method for propagating this medicinal plant but also plays a significant role in the conservation of its genetic resources. This tissue culture technique offers a means for the mass production of plants with desirable traits, which is essential for ensuring a continuous supply of raw materials for pharmaceutical and medicinal applications. Like other species in the *Solanaceae* family, somatic embryogenesis in *S.torvum* requires the precise optimization of

growth regulator combinations, underscoring the value of this method in enhancing both the sustainability and medicinal potential of the plant.

Results and Discussion

1. Somatic Embryogenesis Induction

Leaf explants of the studied plant species were cultured on MS medium supplemented with varying concentrations of auxins: 2,4-D (0.5–4.0 mg/L), 2,4,5-T (0.5–4.0 mg/L), and NAA (0.5–4.0 mg/L). Somatic embryos developed directly from the surface of explants within 6–8 days of culture initiation. The embryos progressed through distinct developmental stages, including globular, heart, torpedo, and cotyledonary stages, as observed in Figure 1 (Fig. 1a–f).

Callus formation initiated at the cut edges of the explants, and pre-embryos appeared surrounding the explants, which evolved into fully formed embryos. These embryos further differentiated into plantlets with roots and shoots (Fig. 1). No embryogenic response was observed in the MS basal medium without auxins, confirming the critical role of auxin in somatic embryogenesis.

The most effective auxin for somatic embryogenesis was NAA, which showed a maximum response at 2.0 mg/L. This concentration induced the highest number of embryos (16.0 per explant) and achieved 100% embryogenesis frequency. Similar results were observed with 2.0 mg/L 2,4-D, producing a comparable frequency of embryogenesis. 2,4,5-T also facilitated embryogenesis, with the highest embryo frequency (12.3 ± 0.32 per explant) observed at 2.0 mg/L. However, increasing auxin concentrations beyond 3.0 mg/L reduced both the frequency and number of embryos, likely due to auxin-induced inhibition of embryo development. This observation aligns with earlier studies highlighting the negative effects of high auxin concentrations on embryogenesis [Gaj, 2004; Roberts *et al.*, 2009].

2. Direct vs. Callus-Mediated Embryogenesis

The embryos in this study were induced directly from leaf explants, minimizing the risk of somaclonal variation commonly associated with callus-mediated regeneration. Such direct embryogenesis is crucial for producing genetically stable plantlets, as observed in other species like groundnut (*Arachis hypogaea*) and chickpea (*Cicer arietinum*) [Premanand *et al.*, 2020; Samson *et al.*, 2018].

3. Embryo Germination

Globular and cotyledonary stage embryos were transferred to MS medium supplemented with 2.0 mg/L BAP and 2.0 mg/L 2,4-D for germination. Under these conditions, germination was achieved within 15–20 days, characterized by elongation of embryos, unfolding of cotyledons, and the emergence of the shoot apex. The highest frequency of germination (80.2%) was observed with a combination of 3.0 mg/L NAA and 2.0 mg/L TDZ, which induced robust shoot elongation and cotyledon development (Fig. 1d–f). TDZ, known for promoting somatic embryo germination, was especially effective in this process [George *et al.*, 2021; Gaj *et al.*, 2004].

4. Plantlet Development and Acclimatization

Fully formed plantlets were transferred to MS medium supplemented with 3.0 mg/L NAA and 2.0 mg/L TDZ for rooting and shoot elongation. Complete plantlets developed within two weeks

(Fig. 1h). These plantlets were hardened in a hormone-free MS liquid medium for two weeks and acclimatized in vermiculite-filled poly cups. Out of 400 regenerants, 200 survived initial acclimatization, and 150 plants were successfully transplanted to individual containers with an 80% survival rate.

5. Comparative Analysis with Previous Studies

The findings of this study align with previous reports on somatic embryogenesis in legumes and solanaceous crops, where specific auxin and cytokinin combinations significantly influenced embryo development and germination. Notably, the observed effectiveness of TDZ in germination parallels results in chickpeas and groundnuts, where cytokinin-enriched media promoted efficient germination (Patel *et al.*, 1994; Premanand *et al.*, 2020).

This study demonstrated the successful induction of somatic embryogenesis directly from leaf explants, circumventing issues of somaclonal variation associated with callus-mediated regeneration. The optimized use of NAA and TDZ facilitated high-frequency embryo formation, germination, and subsequent plantlet development, offering a robust protocol for clonal propagation and genetic conservation.

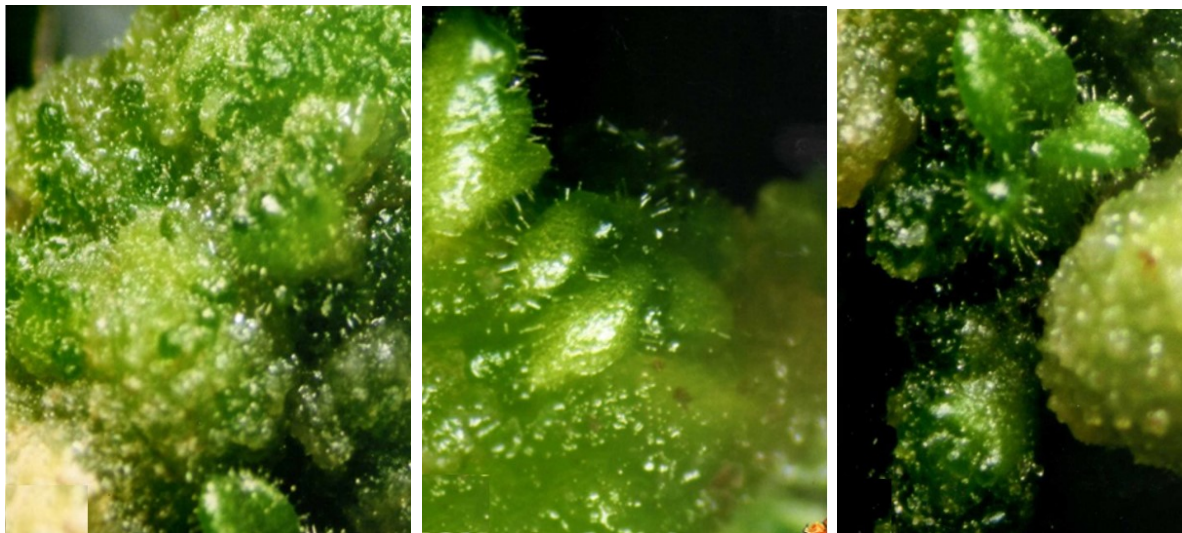
Table 1: Effect of Different Concentrations of 2, 4-D, 2,4,5-T, and NAA on Somatic Embryogenesis Induction from Leaf Explants of Turkey Berry (*Solanum torvum* L.)

Hormones	Concentration (mg/L)	% of Cultures Responding	Number of Embryos/Explant (Mean ± SE)	Callusing Response
2,4-D	0.5	90	3.5 ± 0.47	++
	1	100	6.0 ± 0.43	+++
	2	100	14.2 ± 0.34	+++
	3	80	10.3 ± 0.32	++
	4	70	9.2 ± 0.42	+
2,4,5-T	0.5	70	4.0 ± 0.32	+
	1	80	7.3 ± 0.43	++
	2	100	12.3 ± 0.32	++
	3	100	11.2 ± 0.33	+
	4	90	7.0 ± 0.82	+
NAA	0.5	70	2.0 ± 0.62	++
	1	80	8.3 ± 0.43	+++
	2	100	16.0 ± 0.33	+++
	3	95	11.2 ± 0.32	+++
	4	60	6.0 ± 0.65	++

Callusing **Response Scale**: +++: High callus formation, ++: Moderate callus formation, +: Low callus formation, --: No callus formation

The highest number of embryos per explant was observed at 2.0 mg/L NAA (16.0 ± 0.33) with 100% culture response and high callus formation (++++)

Among the tested auxins, NAA demonstrated the most effective induction of somatic embryogenesis compared to 2,4-D and 2,4,5-T.



a

b

c



Fig. 1. Somatic embryogenesis and plant regeneration in Leaf explant cultures of *S. torvum*. a, b, c) Embryogenic callus induction on MS +NAA (2.0mg/L) globular, heart, torpedo, d) Maturation of Somatic embryos on MS+2.0 mg/L 2, 4-D+1.5mg/L TDZ e) matured shoots after six weeks germination of somatic embryos, f) matured shoots after twelve weeks of germination of somatic embryos

Relation to Solanaceae Plants

The results align well with findings from studies on plants in the Solanaceae family, such as *Solanum tuberosum* (potato), *Capsicum annuum* (bell pepper), and *Solanum melongena* (eggplant), where auxins and cytokinins have similarly been shown to play vital roles in somatic embryogenesis and plant regeneration.

For example:

In *Solanum tuberosum*, low concentrations of 2,4-D were found to be essential for initiating callus, with cytokinin supplementation (e.g., BAP or kinetin) enhancing the development of somatic embryos and shoot regeneration.

In *Capsicum annuum*, NAA combined with low cytokinin concentrations promoted embryogenic callus formation, a trend that mirrors the observations in *S.torvum*.

Studies on *Solanum melongena* highlighted the importance of balancing auxins and cytokinins for direct somatic embryogenesis, supporting the findings in this research that auxin concentrations above an optimal threshold negatively impact embryogenesis and slow development.

The ability of *S.torvum* and Solanaceae plants to produce somatic embryos directly without intermediate callus stages minimizes somaclonal variations, ensuring genetic fidelity. Additionally, the generation of synthetic seeds in *S.torvum* parallels advancements in Solanaceae plants, where synthetic seeds are used to preserve germplasm and enable large-scale propagation.

Table 2. Effect of Different Concentrations of BAP and TDZ in Combination with 2.0 mg/L 2,4-D on Somatic Embryo Maturation in Turkey Berry (*Solanum torvum* L.) on MS Medium

Hormone Combination (mg/L)	% of Cultures Responding	Callusing Response (Mean ± SE)
2,4-D + BAP		
2.0 + 0.5	53	64.0 ± 0.30
2.0 + 1.0	60	70.0 ± 0.22
2.0 + 1.5	75	77.0 ± 0.33
2.0 + 2.0	80	89.0 ± 0.23
2.0 + 2.5	70	81.0 ± 0.43
2.0 + 3.0	58	75.0 ± 0.33
2,4-D + TDZ		
2.0 + 0.5	58	69.0 ± 0.34
2.0 + 1.0	60	72.0 ± 0.35
2.0 + 1.5	68	90.0 ± 0.24
2.0 + 2.0	70	86.0 ± 0.26
2.0 + 2.5	45	73.0 ± 0.26
2.0 + 3.0	43	60.0 ± 0.33

Significance and Future Directions

This study lays the groundwork for applying direct somatic embryogenesis and synthetic seed production in crop improvement programs, including transgenic plant development and conservation. The findings hold relevance for

species in *S.torvum* and Solanaceae, demonstrating the universality of the auxin-cytokinin interplay in somatic embryogenesis. Future research could explore the molecular mechanisms underpinning these processes and the role of specific genes and signaling pathways in improving plant regeneration efficiency.

Table 3 Effect of Different Concentrations of NAA and TDZ on Somatic Embryo Germination in Turkey Berry (*Solanum torvum* L.)

Hormone Concentration (mg/L)	Number of Embryos Germinated	% of Embryo Germination ± (SE)
NAA + TDZ (3.0 + 0.5)	18	25.0 ± 0.24
NAA + TDZ (3.0 + 1.0)	25	35.0 ± 0.25
NAA + TDZ (3.0 + 1.5)	35	54.0 ± 0.23
NAA + TDZ (3.0 + 2.0)	50	80.0 ± 0.44
NAA + TDZ (3.0 + 2.5)	40	74.0 ± 0.33
NAA + TDZ (3.0 + 3.0)	32	40.0 ± 0.35

Conclusion

The current study successfully established an efficient protocol for somatic embryogenesis in Turkey Berry (*Solanum torvum* L.), demonstrating that optimized hormone concentrations significantly influence callus induction, somatic embryo production, and plant regeneration. The findings highlight that lower auxin concentrations are critical for initiating callus, maintaining its fresh weight, and supporting somatic embryogenesis. Additionally, cytokinin played a pivotal role in the differentiation of callus and the subsequent regeneration of complete plants.

The regenerated plants exhibited normal growth, flowering, and seed set, with no observable morphological differences compared to plants grown from seed. This consistency underscores the genetic stability and viability of tissue-cultured plants. The methodology also holds potential for large-scale applications, such as the production of transgenic Turkey Berry *S.torvum* L. plants through *Agrobacterium*-mediated transformation or gene gun technology. The successful integration of direct somatic embryogenesis and synthetic seed production further expands its utility in biotechnology and agriculture.

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