Research Article
Crop Life

Direct Adventitious Shoot Induction and Plant Regeneration Using Shoot Tip Explants of Medicinal Herb *Solanum nigrum*

Subba Tata Sape, Nataraj Dade, Sudhakara Rao Pola

[The author informations are in the declarations section. This article is published by ETFLIN in Crop Life, Volume 1, Issue 1, 2025, Page 1-4. DOI 10.58920/etflin000000 (pending update; Crossmark will be active once finalized)]

Received: 20 April 2025
Revised: 31 July 2025
Accepted: 19 August 2025
Published: 13 November 2025

Editor: Siti Subandiyah

Keywords: Adventitious shoot induction, In vitro regeneration, Shoot tip explants, Plant tissue culture, Solanum nigrum, Micropropagation.

Abstract: *Solanum nigrum* L., a medicinally essential species of the family Solanaceae, was regenerated in vitro via high-frequency direct adventitious shoot induction from shoot tip explants. Murashige and Skoog (MS) medium supplemented with benzylaminopurine (BAP) or thidiazuron (TDZ) (0.5–3.0 mg/L), singly or in combination with indole-3-acetic acid (IAA) (0.5 mg/L), was evaluated for adventitious shoot induction. The highest regeneration efficiency, i.e., (98.5 ± 0.38% shoot induction; 142.2 ± 0.33 shoots per explant) was obtained on MS medium containing TDZ (2.0 mg/l) with IAA (0.5 mg/l). Rooting was optimized on MS medium supplemented with indole-3-butyric acid (IBA). The best rhizogenesis of shoots was observed on MS medium supplemented with IBA (2.5 mg/L). The well-developed plantlets with healthy roots were successfully acclimatized and established in the field, achieving a 90% survival rate with no observable phenotypic variations. This efficient and reproducible protocol provides a valuable platform for genetic transformation and other biotechnological applications in the medicinal plant *S. nigrum*.

Introduction

The technique of plant tissue culture has proven to be highly effective, serving as a means of multiplying plants at high rates under controlled phytosanitary conditions (1). Moreover, the tools of plant tissue culture are being applied to a wide range of biotechnological endeavors, particularly to the clonal propagation and genetic improvement of crop and medicinal plants (2-3). *Solanum nigrum*, belonging to the family Solanaceae, contains several medicinally critical secondary metabolites such as solanine, sapogenin, diosgenin, tigogenin, solanidine, solamargine, etc (4). In traditional folk medicine, the berries are widely valued and have been used as an emetic, antispasmodic, and diuretic, as well as a remedy for fever, diarrhea, and different eye ailments (5-6). Apart from its medicinal uses, the leaves and berries of this herb are often eaten as food or cooked and served as a vegetable (7). *In vitro* adventitious shoot development is crucial for the propagation and conservation of medicinally and economically important plants, serving as a key tool in genetic transformation for the production of transgenic plants. Shoot tip culture in particular is extensively applied due to its high regeneration potential, attributed to the dome of totipotent cells that functions as a central hub for diverse developmental processes (8-9). Despite its importance, relatively few studies have focused on plant regeneration using shoot tip explants of *S. nigrum*. To date, reports on *in vitro* direct regeneration through adventitious shoot induction remain limited. However, this approach has been highlighted as a promising method for achieving mass propagation and long-term conservation of the species (10-15). Hence, the present study aims to establish a rapid and efficient protocol for direct adventitious shoot regeneration using shoot tip explants of *S. nigrum* with applications in conservation, sustainable production of elite clones, and genetic transformation studies.

Materials and Methods

Shoot tip segments were used as explants. The explants were initially washed with tap water, followed by the Tween 20 solution. After that, the explants were surface-sterilized by dipping in 70% alcohol for approximately 30 s, followed by treatment with 0.1% $HgCl_2$ for 3 to 4 min, and then washed several times with sterile double-distilled water. The explants were inoculated into the culture medium. The entire above process was performed aseptically under a laminar airflow cabinet. Murashige and Skoog media (16) were used in the study, with a carbohydrate content of 3% sucrose. The pH of the media was adjusted to 5.8 before autoclaving at 15 pounds pressure and temperature at 121 °C for 15-20 min. Gelling of the press was done with 0.8% agar-agar. The cultures were maintained under a cool fluorescent light intensity of approximately 3000lux for 16 h, with a temperature of 25 ± 2 °C. Each treatment consisted of 10 replicates and was repeated twice.

The effect of cytokinins (BAP and TDZ) on the induction of adventitious shoot proliferation was observed singly or in combination with indole-3-acetic acid (IAA). Six different concentrations of BAP and TDZ (0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 mg/L) were tested singly for shoot induction, and combinations with IAA (0.5 mg/L) were also tested for the same purpose. To initiate rooting, the shoots were transferred to MS medium supplemented with IBA (indole-3-butyric acid) (0.5-4.0 mg/L).

The rooted plantlets were transferred to the pots containing a mixture of loam soil and cow dung manure (2:1), watered, and covered with transparent polypropylene bags to maintain high humidity conditions. The pots were kept in daylight for seven days, after which the polypropylene cover was removed and the potted plants were left to preserve in the open environment.

All experiments were conducted in triplicate, with 10 replicates per treatment in each run. Data were recorded for percentage response, the number of shoots per explant, the number of roots per shoot, and the root length per root. The mean and standard error (SE) were calculated for each treatment.

Results and Discussion

The shoot tips of *S. nigrum* were cultured on twenty-five media. The results of the experiment were evaluated for various tissue culture responses at regular intervals. Among the 25 types of media, the maximum shoot induction rate (98.5 ± 0.38%) and the maximum average shoot number per shoot tip (142.2 ± 0.33) were obtained on MS medium supplemented with TDZ (2 mg/L) and IAA (0.5 mg/L) after eight weeks of culture (see **Table 1**). The explants did not exhibit any shoot formation on MS medium without plant growth regulators (PGRs). In contrast, shoot induction was observed in all PGR-supplemented treatments; however, the response was scanty. The supplementation of MS media with different concentrations of TDZ, with or without IAA combination, resulted in effective adventitious shoot induction from shoot tip explants, compared to media fortified with BAP alone or in combination with IAA. The results indicated that the combination of cytokinin with IAA stimulated the rapid induction of adventitious shoots, likely due to their synergistic effect (17), a phenomenon previously reported in the *in vitro* multiplication of several plant species (10, 18). The *in vitro* response has been progressive from 0.5 to 2.0 mg/L cytokinin (BAP/TDZ) singly or in combination with IAA (0.5 mg/L). At the same time, beyond the concentration of (2 mg/L) BAP/TDZ alone or combined with IAA (0.5 mg/L), the percentage shooting and average shoot number per shoot tip were found to decrease in the present study. Kiran *et al*. (19) reported similar findings in *Phyllanthus amarus* regeneration studies. Earlier researchers who worked on *S. nigrum*'s shoot tip explants shoot multiplication reported 2 to 24.8 shoots per shoot tip on MS medium supplemented with BAP/KIN/TDZ alone or combined with IAA (10-15). The present study investigated the *in vitro* response to the medium MS+TDZ (2 mg/L) + IAA (0.5 mg/L), which was optimal for shoot multiplication and exhibited a superior outcome compared to earlier *in vitro* responses on Solanum nigrum shoot tip explants (see **Figure 1**). Handy number roots were developed from the base of the shoot after 2 weeks of culture on MS medium supplemented with different concentrations of IBA (see **Table 2**). The rooting response varied with IBA concentrations. MS medium supplemented with IBA (2.5 mg/L) produced the maximum rhizogenesis, i.e., rooting frequency (100%), root number (19.0 ± 0.18 per shoot), and root length (6.9 ± 0.09 cm per root). These results align with the findings of Kolar *et al*. (20).

Subsequently, these plantlets were removed from the

Table 1. Direct induction of adventitious shoots from shoot tip explants of *Solanum nigrum* L. using different PGRs after 8 weeks of culture.

No.	MS media with PGR (mg/L)	Shooting (%)	Shoot no./ shoot tip
1.	MS	00.0 ± 0.00	00.0 ± 0.00
2.	BAP (0.5)	42.0 ± 0.06	08.8 ± 0.11
3.	BAP (1.0)	55.0 ± 0.14	10.2 ± 0.06
4.	BAP (1.5)	62.5 ± 0.11	13.8 ± 0.16
5.	BAP (2.0)	82.0 ± 0.24	26.3 ± 0.14
6.	BAP (2.5)	75.0 ± 0.19	22.4 ± 0.13
7.	BAP (3.0)	63.0 ± 0.12	15.0 ± 0.02
8.	TDZ (0.5)	45.2 ± 0.23	15.2 ± 0.17
9	TDZ (1.0)	60.0 ± 0.19	24.5 ± 0.20
10.	TDZ (1.5)	62.3 ± 0.22	31.3 ± 0.18
11.	TDZ (2.0)	75.5 ± 0.32	53.0 ± 0.33
12.	TDZ (2.5)	68.2 ± 0.30	44.5 ± 0.15
13.	TDZ (3.0)	66.0 ± 0.22	39.2 ± 0.24
14.	BAP (0.5) ± IAA (0.5)	68.7 ± 0.19	34.6 ± 0.18
15.	BAP (1.0) ± IAA (0.5)	74.0 ± 0.30	29.7 ± 0.38
16.	BAP (1.5) ± IAA (0.5)	78.7 ± 0.19	30.5 ± 0.69
17.	BAP (2.0) ± IAA (0.5)	82.1 ± 0.34	47.2 ± 0.69
18.	BAP (2.5) ± IAA (0.5)	66.6 ± 0.18	24.2 ± 0.76
19.	BAP (3.0) ± IAA (0.5)	58.7 ± 0.38	17.6 ± 0.52
20.	TDZ (0.5) ± IAA (0.5)	80.4 ± 0.19	60.2 ± 0.29
21.	TDZ (1.0) ± IAA (0.5)	85.2 ± 0.10	85.5 ± 0.20
22.	TDZ (1.5) ± IAA (0.5)	89.2 ± 0.16	110.6 ± 0.19
23.	TDZ (2.0) ± IAA (0.5)	98.5 ± 0.38	142.2 ± 0.33
24.	TDZ (2.5) ± IAA (0.5)	92.4 ± 0.22	121.3 ± 0.18
25.	TDZ (3.0) ± IAA (0.5)	80.3 ± 0.26	96.9 ± 0.22

Table 2. *In vitro* rhizogenesis of regenerated *S. nigrum* shoots using the PGR (IBA) after 2 weeks of culture.

No.	MS media with PGR (mg/L)	Rhizogenesis		
		Root induction (%)	Root no./ shoot	Root length (cm)
1.	MS	00.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
2.	IBA (0.5)	55.0 ± 0.21	5.0 ± 0.19	4.0 ± 0.11
3.	IBA (1.0)	60.0 ± 0.11	5.2 ± 0.22	4.3 ± 0.15
4.	IBA (1.5)	68.0 ± 0.14	8.6 ± 0.11	5.2 ± 0.20
5.	IBA (2.0)	75.0 ± 0.16	10.3 ± 0.20	5.8 ± 0.10
6.	IBA (2.5)	100 ± 0.00	19.0 ± 0.18	6.9 ± 0.09
7.	IBA (3.0)	85.0 ± 0.12	14.5 ± 0.24	6.5 ± 0.14
8.	IBA (3.5)	80.0 ± 0.17	8.1 ± 0.15	5.0 ± 0.23
9.	IBA (4.0)	55.0 ± 0.26	5.0 ± 0.13	4.6 ± 0.26

Figure 1. Direct adventitious shoot induction and Plant regeneration using shoot tip explants of Solanum nigrum L. (A) shoot tip showing shoot projection initials, (B) adventitious shoots, (C) multiple shoot cluster, (D) rhizogenesis of plantlet, (E) plantlet showing roots, and (F) acclimatized plants.

cultures, washed free of agar and transferred to pots containing a mixture of loam soil and cow dung manure (2:1). The plants were kept covered with a polythene bag for 10 days to check excessive transpiration. The plants were then successfully established in field conditions. The acclimatized plantlets exhibited a 90% survival rate. Thus, the protocol standardized through this study demonstrates the possibility of developing an efficient *in vitro* propagation system for the successful mass propagation of *S. nigrum* L.

Conclusion

The present experiment successfully standardized a highly efficient and reproducible protocol for the *in vitro* regeneration of the medicinal herb *Solanum nigrum*. Among the various PGR combinations tested, Murashige and Skoog (MS) medium supplemented with 2 mg/L TDZ and 0.5 mg/L IAA was identified as the most effective for inducing profuse adventitious shoot proliferation from shoot tip explants, yielding an average of 142.2 ± 0.33 shoots per explant — a remarkable improvement over previously reported methods. Furthermore, rhizogenesis was optimally achieved on MS medium supplemented with 2.5 mg/L indole-3-butyric acid (IBA), resulting in robust root formation and healthy plantlet development.

The established regeneration system not only offers a rapid and reliable approach for large-scale propagation of *S. nigrum* but also provides a valuable platform for future applications in genetic improvement, secondary metabolite production, and conservation of elite germplasm. In addition, this standardized protocol may facilitate *in vitro* mutagenesis and transformation studies aimed at enhancing the pharmacological properties of this important medicinal plant. Further optimization of acclimatization conditions and metabolite profiling of regenerated plants could strengthen the potential use of this system in commercial and pharmaceutical contexts.

Declarations

Author Informations

Subba Tata Sape ✉
Corresponding Author
Affiliation: Department of Biotechnology, Andhra University, Visakhapatnam 530 003, India.
Contribution: Investigation.

Nataraj Dade
Affiliation: Department of Biotechnology, Andhra University, Visakhapatnam 530 003, India.
Contribution: Investigation.

Sudhakara Rao Pola
Affiliation: Department of Biotechnology, Andhra University, Visakhapatnam 530 003, India.
Contribution: Investigation.

Acknowledgment
The author expresses sincere gratitude to the Head of the Department of Biotechnology for providing the necessary facilities to carry out this investigation, and to Dr. O. Aniel Kumar, former Professor, Department of Botany, Andhra University, for his valuable suggestions during the compilation of this manuscript.

Conflict of Interest
The authors declare no conflicting interest.

Data Availability
The unpublished data is available upon request to the corresponding author.

Ethics Statement
Not applicable.

Funding Information
The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

References

1. Santana MA, Romay G, Matehus J, Vicente Villardon J, Demey JR. A simple and low cost strategy for micropropagation of cassava (Manihot esculenta Crantz). African Journal of Biotechnology, 2009;8(16):3789-3897.

2. Jagannathan V. Present status of plant tissue culture in India. Proceedings of the National Seminar on Plant Tissue Culture, ICAR Publication, New Delhi. 1988;1-10.

3. Rao IU. Understanding in vitro flowering of bamboos by using plant growth regulators. Proceedings of Golden Jubilee Conference on Challenging and Emerging Strategies for Improving Plant Productivity (ISPP). IARI, New Delhi, India. 2008;52-53.

4. Saleem TSM, Chetty CM, Ramkanth S, Alagusundaram M, Gnanaprakash K, Rajan VST, Angalaparameswari S. Solanum nigrum Linn. - A Review. Pharmacognosy Reviews. 2009;3(6):342-345.

5. Kritikar KR, Basu BS. Indian Medicinal Plants. 1987;3:1784-1781.

6. Younes LS, Shibli RA, Tahtamouni RW, Al-Qudah TS, Al Hawmdeh F. Micropropagation of Black Nightshade (Solanum nigrum L.): A promising medicinal plant in Libya. The Libyan Journal of Agriculture. 2020;24(2).

7. Wang Y, Xiang L, Yi X, He X. Potential anti-Inflammatory steroidal saponins from the berries of Solanum nigrum L. (European black nightshade). Journal of Agricultural and Food Chemistry. 2017;65(21):4262–4272

8. Gould J, Devey M, Hasegawa, Ulian EC, Peterson G, Smith RH. Transformation of Zea mays L. using Agrobacterium tumefaciens and the shoot apex. Plant Physiology. 1991;95:426-434.

9. Siva PS and Krishna GV. Advancements in horticultural crops using shoot tip culture. The Pharma Innovation Journal. 2022;11(12):1035-1041.

10. Sridhar TM, Naidu CV. High frequency plant regeneration, in vitro flowering of Solanum nigrum (L.) – an important antiulcer medicinal plant. Journal of Phytology. 2011;3(2): 85-93.

11. Kavitha MS, Wesely EG, Mehalingam P. Direct Multiple Shoot Regeneration from Shoot Tip and Nodal Explants of Solanum nigrum L. A Medicinal Herb. Journal of Ornamental and Horticultural Plants. 2012;2(2):65-72.

12. Ugandhar T, Sammaiah D, Ayodhya Ramulu Ch, Anitha Devi U, Balaraju P. Direct multiple shoots proliferation of black night shade (Solanum nigrum L.) from shoot tip explants induced by thidiazuron. Indian Journal of Pharmaceutical and Biological Research. 2015;3(1):71-76.

13. Parvathi D, Venkateshwarlu M, Anitha Devi U, Ugandhar T. Invitro micropropagation from apical bud explants culture of Night shade (Solanum nigrum L.) A medicinally important plant. International Journal of Emerging Technology & Research. 2016; 3(6):1-7.

14. Venkateshwarlu M. Plantlet regeneration from shoot tip explants of Solanum nigrum (L) - A medicinal important plant. International Journal of Research Publication and Reviews. 2021;2(12):1083-1085.

15. Aldabbagh FMK, AlZaidi IHM, Alshamari MAK. Micropropagation and assessment of genetic fidelity of regenerate by RAPD markers of Solanum nigrum. Iraqi Journal of Agricultural Sciences. 2024;55(1):432-439.

16. Murashige T, Skoog F. A Revised Medium for Rapid Growth and Bio Assays with Tobacco Tissue Cultures. Plant Physiology. 1962;15:473-497.

17. Bishopp A, Schaller GE, Kieber JJ. The Yin-Yang of hormones: cytokinin and auxin interactions in plant development. The Plant Cell. 2015; 27(1):44–63.

18. George EF. Plant Propagation by Tissue Culture. Part I, The Technology, Exegetics Ltd, Edington. 1993.

19. Kiran SG , Govindarajulu B, Venugopal RB, Ramgopal Rao S, Kaviraj CP, Jabeen FTZ, Aravind B, Srinatha Rao. High frequency shoot regeneration from Phyllanthus amarus. Indian Journal of Biotechnology. 2004;3:103-107.

20. Kolar AB, Vivekanandan L, Ghouse Basha M. In vitro Regeneration and Flower Induction on Solanum nigrum L. from Pachamalai hills of Eastern Ghats. Plant Tissue Culture and Biotechnology. 2008;18(1):43-48.

Additional Information

How to Cite
Subba Tata Sape, Nataraj Dade, Sudhakara Rao Pola. Direct Adventitious Shoot Induction and Plant Regeneration Using Shoot Tip Explants of Medicinal Herb *Solanum nigrum*. *Crop Life*. 2025;1(1):1-4

Publisher's Note
All claims expressed in this article are solely those of the authors and do not necessarily reflect the views of the publisher, the editors, or the reviewers. Any product that may be evaluated in this article, or claim made by its manufacturer, is not guaranteed or endorsed by the publisher. The publisher remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.