

Rapid Establishment of Multiple shoot buds and Plant regeneration from Leaf explants of the Medicinal plant *Solanum nigrum* L.

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ABSTRACT

Solanum nigrum, a herbaceous plant belonging to the family *Solanaceae*, is one of the well-documented medicinal plants used in traditional folk medicine worldwide. In the present study, a rapid and reproducible protocol was standardized for multiple shoot bud induction and plant regeneration using leaf explant cultures of *S. nigrum* L. Direct organogenesis of adventitious shoots was achieved on Murashige and Skoog's (MS) medium supplemented with various concentrations and combinations of plant growth regulators (PGRs), namely benzylaminopurine (BAP), kinetin (Kn), thidiazuron (TDZ), and indole-3-acetic acid (IAA). A total of 37 media combinations were tested, among which MS medium fortified with TDZ (2.0 mg/L) and IAA (0.5 mg/L) proved most effective, inducing the highest number of shoots per explant (88.0). Elongated shoots were successfully rooted on MS medium supplemented with indole-3-butyric acid (IBA, 2.5 mg/L), an average of 19.0 roots per shoot with an average root length of 6.9 cm. The regenerated plantlets were acclimatized in earthen bags with a survival rate of 80% and subsequently established under field conditions without exhibiting any morphological or growth abnormalities. This efficient in vitro regeneration protocol can serve as a foundation for large-scale propagation, conservation, and sustainable utilization of this elite medicinal herb.

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1. INTRODUCTION

Traditional medicine forms an inseparable component of global healthcare. The use of medicinal plants is gaining popularity worldwide, including in Western countries, primarily due to their minimal side effects. Numerous phytochemical and pharmacological investigations have provided valuable insights into phytopharmaceuticals. Since medicinal plants remain the primary source of raw materials for plant-based medicines, their demand is increasing rapidly. However, indiscriminate extraction, uncontrolled exploitation, and smuggling for pharmaceutical purposes, along with other anthropogenic pressures, have resulted in the fast depletion of several medicinally important species. Hence, there is an urgent need to develop efficient strategies for their conservation. Conventional propagation methods are often time-consuming and unreliable in ensuring uniformity and performance. This has led to the development of alternative approaches, with plant tissue culture emerging as a powerful tool for rapid clonal multiplication, production of disease-free plants and long-term germplasm conservation. *Solanum nigrum* L., an important herb belonging to the family *Solanaceae*, occupies a notable place in folk medicine. It contains a variety of pharmaceutically important secondary metabolites such as solanine, sapogenin, diosgenin, tigogenin, solanidine, and solamargine [1]. Traditionally, the plant has been valued for its emetic, antispasmodic, and diuretic properties and is used to relieve fever, diarrhea, and eye ailments [2,3]. Beyond its medicinal applications, the leaves and berries of *S. nigrum* are commonly consumed as cooked food or vegetables [4]. Although a few studies have reported plant regeneration through direct or indirect organogenesis using leaf explants of *S. nigrum*, these researches remain limited [5-13].

Considering the medicinal and nutritional importance of this species, the present investigation was undertaken to standardize efficient culture media protocols for induction of multiple shoot buds and plant regeneration from leaf explants. This standardized protocol provides a foundation for conservation and large-scale propagation of this valuable medicinal herb through tissue culture techniques.

2. MATERIALS AND METHODS

Healthy plants of *Solanum nigrum* were obtained from the Horticulture Unit, Andhra University, Visakhapatnam, Andhra Pradesh, India, and maintained in pots containing garden soil. Young leaves were excised from these plants and used as explants for the study. The explant washed with tap water for 30-60 min then surface sterilized with 0.1% mercuric chloride (HgCl₂) solution for 3-4 minutes, followed by thorough rinsing with sterile double-distilled water 3 times for five min. All aseptic manipulations were carried out in a laminar airflow cabinet. Murashige and Skoog (MS) basal medium [14], supplemented with 3% (w/v) sucrose, was used for culture initiation. The pH of the medium was adjusted to 5.8 prior to autoclaving at 121°C and 15psi pressure for 15-20 minutes. The medium was solidified with 0.8% agar-agar, and 15 mL of the medium was dispensed into sterile test tubes (25×150 mm), which were sealed with aluminum foil. Cultures were incubated at 25±2°C under a 16-hour photoperiod with cool white fluorescent light providing 3000lux intensity. Different concentrations of cytokinins viz., benzylaminopurine (BAP), kinetin (KIN) and thidiazuron (TDZ) ranging from 0.5 to 3.0mg/l were tested, individually or in combination with 0.5 mg/L indole-3-acetic acid (IAA) for their effect on shoot induction. Well-developed shoots were carefully separated and transferred to full-strength MS medium supplemented with various concentrations (0.5-4.0 mg/L) indole-3-butyric acid (IBA) for rooting. Rooted plantlets were gently removed from the culture vessels, washed with tap water to remove agar residues and transplanted into disposable sterile plastic cups containing a 1:1 mixture of sterile sand and garden soil. The plantlets were covered with sterile polyethylene bags and hardened in a mist chamber for two weeks. Subsequently, hardened plants were transferred to earthen bags containing a 1:1:1 mixture of garden soil, sand, and farmyard manure and maintained under open field conditions.

All the treatments were arranged in a Completely Randomized Design (CRD), with all treatments were being replicated three times with 10 replicates per treatment. Data on the percentage response, the average number of shoots per explant, the number of roots per shoot, and root length per root were collected. The collected data were statistically analyzed using Analysis of Variance (ANOVA) using SPSS (Version 10) software package to test for significant differences in the number of shoots, root number, and root length.

3. RESULTS AND DISCUSSION

Successful *In vitro* shoot bud induction and plant regeneration largely depend on the choice of explant and the appropriate combination of plant growth regulators (PGRs). In the present study, multiple shoot induction from leaf explants was evaluated on MS medium supplemented with different concentrations and combinations of PGRs, and the responses are summarized in **Table 1**. The explants did not exhibit any shoot formation on MS medium without plant growth regulators, whereas all PGR supplemented media supported direct shoot induction. MS medium supplemented with cytokinins (BAP/KIN/TDZ) at concentrations ranging from 0.5-3.0 mg/L resulted in moderate shoot induction, producing 2.8-24.6 shoots per explant. The addition of IAA (0.5 mg/L) to BAP/KIN/TDZ significantly enhanced shoot bud proliferation, yielding 6.5-88.0 shoots per explant after eight weeks of culture (**Figure 1**). Notably, shoot bud numbers increased as cytokinin concentrations rose from 0.5 to 2.0 mg/L (alone or in combination with IAA), but higher concentrations led to a decline in shoot induction efficiency [15,16]. Among the tested combinations, MS medium supplemented with TDZ (2.0 mg/L) and IAA (0.5 mg/L) proved to be the most effective, producing an average of 88.0 shoots per explant. Similar synergistic effects of cytokinins and auxins in shoot organogenesis have been reported earlier in *Phyllanthus amarus* [17] and in several Solanaceous members, where a higher cytokinin to auxin ratio is often required for efficient shoot initiation [11,18]. The synergistic action of cytokinins with IAA in stimulating rapid shoot bud induction has also been confirmed in other plant species [19-21]. Compared with previous researches on *S. nigrum*, which achieved 3.2-42.73 shoots per explant using BAP/KIN/TDZ (alone or with IAA) [5-13], the present study demonstrates greater response with up to 88 shoots per explant. For rooting, regenerated shoots were transferred to MS medium supplemented with different concentrations of IBA (**Table 2**). Among the tested concentrations, IBA (2.5 mg/L) was most effective, inducing 100% root formation with an average of 19.0 roots per shoot and a mean root length of 6.9 cm. These results are consistent with the findings of Kolar et al. [6]. Rooted plantlets were thoroughly washed to remove agar residues, acclimatized in inorganic salt solution for 15 days, and then transferred to earthen bags containing a 1:1:1 mixture of garden soil, sand, and farmyard manure. The survival rate of hardened plants was 80%, and the regenerated plants exhibited normal flowering and fruiting without morphological abnormalities.



Figure 1: *In vitro* morphogenesis and Plant regeneration using leaf explants of *S. nigrum*: a) shoot initials, b) multiple shoots, c) shoot cluster, d) root induction, e) rooted plantlet, f) acclimatized plant

Table 1. Effect of Plant growth regulators on multiple shoot induction from *Solanum nigrum* leaf explants

S.no.	MS media with PGR(mg/L)	Shooting (%)	Shoot no./ explant
1.	MS	00	00.0±0.0
2.	BAP(0.5)	60	04.8±0.1
3.	BAP(1.0)	68	06.0±0.6
4.	BAP(1.5)	72	10.8±0.4
5.	BAP(2.0)	82	14.0±0.8
6.	BAP(2.5)	75	11.2±0.3
7.	BAP(3.0)	63	07.3±0.2
8.	KIN(0.5)	50	02.8±0.7
9.	KIN(1.0)	62	05.3±0.8
10.	KIN(1.5)	68	08.8±0.6
11.	KIN(2.0)	82	11.4±0.8
12.	KIN(2.5)	66	05.5±0.4
13.	KIN(3.0)	59	03.8±0.6
14.	TDZ(0.5)	66	08.5±0.9
15.	TDZ(1.0)	78	10.6±0.8
16.	TDZ(1.5)	82	14.8±0.4
17.	TDZ(2.0)	95	24.6±0.7
18.	TDZ(2.5)	80	16.0±0.9
19.	TDZ(3.0)	69	10.2±0.1
20.	BAP(0.5)+IAA(0.5)	68	10.0±0.3
21.	BAP(1.0)+IAA(0.5)	75	19.0±0.9
22.	BAP(1.5)+IAA(0.5)	81	26.5±0.5
23.	BAP(2.0)+IAA(0.5)	88	30.0±0.6
24.	BAP(2.5)+IAA(0.5)	78	18.2±0.2
25.	BAP(3.0)+IAA(0.5)	64	09.8±0.8
26.	KIN(0.5)+IAA(0.5)	65	06.5±0.2
27.	KIN(1.0)+IAA(0.5)	60	12.0±0.6
28.	KIN(1.5)+IAA(0.5)	72	15.6±0.8
29.	KIN(2.0)+IAA(0.5)	80	19.8±0.3
30.	KIN(2.5)+IAA(0.5)	70	11.5±0.7
31.	KIN(3.0)+IAA(0.5)	62	07.6±0.4
32.	TDZ(0.5)+IAA(0.5)	79	22.0±0.2
33.	TDZ(1.0)+IAA(0.5)	88	33.6±0.8
34.	TDZ(1.5)+IAA(0.5)	90	56.0±0.6
35.	TDZ(2.0)+IAA(0.5)	100	88.0±0.4
36.	TDZ(2.5)+IAA(0.5)	74	63.4±0.1
37.	TDZ(3.0)+IAA(0.5)	66	29.0±0.2
Analysis of variance			$F= 17.496$ $d.f.= 9,818$ $P<0.001$

Values are Mean±SE. For shoot number per explant for each con. of PGR significant at $P < 0.001$.

Table 2. Effect of Indole-3-butyric acid on rhizogenesis of *S. nigrum* regenerated shoots

S.no.	MS media with PGR(mg/L)	Rhizogenesis		
		Root induction (%)	Root no. /shoot	Root length (cm)
1.	MS	00	00.0±0.0	0.0±0.0
2.	IBA(0.5)	55	05.0±0.9	4.0±0.1
3.	IBA(1.0)	60	05.6±0.2	4.3±0.5
4.	IBA(1.5)	68	08.2±0.1	5.2±0.2
5.	IBA(2.0)	75	10.3±0.7	5.8±0.6
6.	IBA(2.5)	100	19.0±0.8	6.9±0.7
7.	IBA(3.0)	85	14.5±0.6	6.5±0.4
8.	IBA(3.5)	80	08.1±0.5	5.0±0.7
9.	IBA(4.0)	55	05.0±0.3	4.6±0.5
Analysis of variance			$F = 9.843$ $d.f. = 3,445$ $P < 0.001$	$F = 2.347$ $d.f. = 2,374$ $P < 0.001$

Values are Mean±SE. For root number per shoot and root length for each con. of IBA significant at $P < 0.001$

4. CONCLUSION

The present investigation demonstrated that the addition of IAA (0.5 mg/L) to MS medium supplemented with different cytokinins (BAP/KIN/TDZ) was essential for profuse induction of multiple shoot buds from leaf explants of *Solanum nigrum*, compared to media containing cytokinins alone. Among the tested combinations, MS medium fortified with TDZ (2.0 mg/L) and IAA (0.5 mg/L) proved to be the most effective, yielding a superior response of 88.0 shoots per explant. For rhizogenesis, MS medium containing IBA (2.5 mg/L) was optimal, inducing 100% rooting with healthy root development. Thus, the standardized protocol established in this study provides an efficient and reproducible method for multiple shoot induction and complete plant regeneration from leaf explants. This protocol can be effectively employed for large-scale propagation, conservation, and sustainable utilization of *Solanum nigrum*.

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