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# Comparative Effects of Indole-3-Acetic Acid and Gibberellic Acid on In Vitro Growth of Potato Plantlets for Seed Production

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## ABSTRACT

This study investigates the effect of Indole – acetic acid (IAA) and Gibberellic acid (GA3) applied individually and in combination, on the in vitro growth of Solanum tubersosum L. microshoots. The results showed that optimized concentrations of these growth regulators significantly enhanced shoot elongation, nodal proliferation and root initiation. Combined treatments showed a stronger synergistic effect than individual applications. The study observed to enhanced dormancy breaking and sprout development. These results suggest the combined use of IAA and GA3 is an efficient approach for large scale production of healthy potato plantlets.

***Keywords:*** *Solanum Tuberosum L.; In Vitro Propagation; Indole-3-Acetic Acid (IAA); Gibberellic Acid (GA3); Microshoots; Shoot Elongation; Seed Tuber Production.*

## INTRODUCTION

In vitro propagation has emerged as a reliable technique for the rapid multiplication of high-quality, disease-free potato seed material (Mohapatra & Batra, 2017). The effectiveness of this approach largely depends on the use of plant growth regulators, particularly gibberellins and auxins, which regulate key physiological processes such as node formation, shoot elongation, and root development (Kumari, 2023). Gibberellic acid ($GA_3$) and auxins such as indole-3-acetic acid (IAA) and indole-3-butyric acid (IBA) are widely recognized for their role in enhancing morphogenetic responses in potato tissue culture systems (Kumari, 2023).

Optimization of growth regulator concentrations in Murashige and Skoog (MS) medium is essential for improving culture establishment, survival rates, and biomass accumulation across different potato cultivars (Makau et al., 2022). Previous studies have demonstrated that the combined application of auxins with cytokinins such as benzylaminopurine (BAP) significantly enhances multiple shoot induction during micropropagation. Furthermore, the interaction between gibberellic acid and synthetic auxins has been shown to improve shoot proliferation uniformity and promote efficient rhizogenesis in potato (Kumari, 2023).

In addition to hormonal regulation, recent studies have explored sustainable approaches such as the incorporation of bio-organic amendments, including mycorrhiza and vermicompost, to enhance nutrient uptake and tuberization efficiency (Boubaker et al., 2023). Moreover, the use of tissue culture-derived plantlets in hydroponic systems offers an effective alternative to conventional soil-based propagation by reducing land dependency and production costs.

Despite these advancements, the precise balance of growth regulators, particularly $GA_3$ and IAA, remains a critical factor influencing the success of in vitro propagation. Standardization of these hormonal concentrations, along with sucrose levels, is essential for scaling up the production of resilient potato varieties (Makau et al., 2022).

Therefore, the present study aims to evaluate the physiological responses of a widely cultivated potato variety to varying concentrations of gibberellic acid and indole-3-acetic acid under controlled in vitro conditions. The objective is to determine the optimal hormonal combination for enhancing shoot development and root biomass (Stupko & Lugovtsova, 2023; Abdella et al., 2023).

## METHODOLOGY

### Plant Collection and Explant Source

In the present study, plant material of potato (*Solanum tuberosum* L.) was collected and utilized for the establishment of in vitro cultures under controlled tissue culture conditions. Healthy and disease-free seed tubers were initially selected as the source material to ensure the production of pathogen-free plantlets.

### Preparation of Tubers for Inoculation

Healthy potato tubers (*Solanum tuberosum* L.) were selected and thoroughly cleaned before inoculation. The tubers were washed three times with sterile distilled water to remove surface debris. Subsequently, surface sterilization was carried out by immersing the tubers in 70% (v/v) ethanol for 3– minutes, followed by three rinses with sterile distilled water to eliminate residual alcohol.

Further sterilization was performed using 0.1% (w/v) mercuric chloride ($HgCl_2$) solution for 2 minutes with continuous agitation. After treatment, the tubers were rinsed multiple times, 3 washes with sterile distilled water to completely remove traces of the sterilizing agent.

The surface-sterilized tubers were then aseptically transferred to culture vessels containing suitable plant tissue culture medium.

### Preparation of Tuber Germination Medium

To facilitate in vitro germination and development of potato (*Solanum tuberosum* L.) explants, a suitable plant tissue culture medium was prepared. As tissue culture-derived plantlets are known to be healthy and disease-free, tubers were used as explants for the induction of shoots and roots under controlled conditions.

The basal medium was supplemented with plant growth regulators, specifically indole-3-acetic acid (IAA) and gibberellic acid ($GA_3$), to evaluate their effect on shoot and root induction from tuber explants.

The pH of the medium was adjusted to 5.8 using 1 N sodium hydroxide (NaOH) before the addition of the gelling agent. Phytagel was added at a concentration of 0.2% (w/v) as a solidifying agent. The medium was then heated in a microwave oven.

Approximately 15 mL of the prepared medium was dispensed into culture tubes, which were subsequently sterilized by autoclaving at 121°C and 15 psi for 15 minutes. After autoclaving, the medium was allowed

to cool and solidify under sterile conditions. The prepared culture media were then used for aseptic inoculation of surface-sterilized tuber explants.

**Transplantation and Hardening of In Vitro Plantlets**

Following successful in vitro germination, well-developed plantlets of potato (*Solanum tuberosum* L.) were carefully removed from the half-strength Murashige and Skoog (MS) medium, ensuring minimal damage to the root system. Residual culture medium adhering to the roots was gently removed under sterile conditions.

The plantlets were subsequently transplanted into pots containing sterile, moist cocopeat as a growth substrate. To maintain high humidity and prevent desiccation as well as microbial contamination, each pot was covered with a transparent polyethylene bag, creating a controlled environment.

The potted plantlets were then transferred to a polyhouse for hardening under controlled environmental conditions. After approximately 10 days of hardening, the plantlets were gradually exposed to conditions by removing the polyethylene covers.

Thereafter, the plantlets were gently washed with sterile water to remove adhering cocopeat from the roots and were transferred to net pots of an aeroponic system. These plantlets were further evaluated for survival and establishment under aeroponic conditions.

**RESULTS AND DISCUSSION**

**Collection of Potato Tubers and Surface Sterilization**

In this study, disease-free potato plantlets were established using half-strength Murashige and Skoog (MS) medium supplemented with plant growth regulators, specifically indole-3-acetic acid (IAA) and gibberellic acid ($GA_3$). Healthy potato tubers were initially selected as the source material.

The tubers were surface-sterilized using a sequential treatment consisting of 70% ethanol for 4 minutes, followed by 0.1% mercuric chloride ($HgCl_2$) for 2 minutes (Fig. 1). This sterilization protocol effectively eliminated microbial contaminants, ensuring aseptic conditions for subsequent culture initiation. Nodal explants derived from the sterilized tubers were then inoculated onto MS medium under sterile conditions.

The use of these sterilizing agents facilitated successful establishment of axenic cultures, consistent with previously reported protocols employing 0.1% $HgCl_2$ for effective surface sterilization of explants (Omar, 2017; Borna et al., 2019).

**Shoot and Root Induction on Half-Strength MS Medium**

Potato tuber explants cultured on half-strength Murashige and Skoog (MS) medium supplemented with sucrose, indole-3-acetic acid (IAA), and gibberellic acid ($GA_3$) exhibited effective shoot and root induction. The cultures were maintained under controlled conditions with a photoperiod of 16 hours light and 8 hours dark at 26 ± 1°C, which proved optimal for growth and development.

Initial shoot induction was observed within 7–10 days, followed by root formation between 14 days under in vitro conditions. Well-developed plantlets were obtained by 25 days and subsequently transplanted into

cocopeat between 30 days for acclimatization. After successful hardening, plantlets with well-established root systems were transferred to the aeroponic system, where environmental parameters such as temperature and nutrient supply were precisely regulated (Borna et al., 2019; Miriam et al., 2018).

The combined application of IAA and $GA_3$ significantly enhanced vegetative growth and promoted stolon development, a critical factor for improving minituber production in aeroponic systems (Bharath & B, 2024). Sucrose, applied at a concentration of 30 g/L, served as the primary carbon source, supporting cellular metabolism and vigorous plantlet development (Buckseth et al., 2020).

Overall, the integration of controlled micropropagation techniques plays a vital role in the large-scale production of high-quality planting material for efficient aeroponic cultivation systems (Tang et al., 2024).

**Hardening of Potato Plantlets**

The in vitro–raised potato plantlets were successfully transferred to cocopeat for acclimatization. Proper covering (bagging) ensured a controlled microenvironment, allowing the plantlets to harden without any signs of stress or acclimatization issues. By the 12th day of hardening, the plantlets were well established and ready for transfer to the aeroponic system for disease-free seed potato production.

This acclimatization approach ensured a high survival rate during the transition from in vitro to ex vitro conditions, as adequate moisture retention and nutrient management are critical for successful establishment (Kulus & Tymoszuk, 2024; Kafle et al., 2023). Additionally, the use of an aeroponic system provided an optimal environment for root development and tuber initiation, contributing to increased production of virus-free seed tubers (Buckseth et al., 2020).

Overall, the use of properly hardened plantlets established a strong foundation for uniform minituber production while minimizing the risk of pathogen transmission during the transition to a soil-free cultivation system (Toma, 2022).

**Growth Performance of Tissue-Cultured Plantlets for Potato Seed Production**

Hardened tissue-cultured potato plantlets were successfully established in the aeroponic system, where they exhibited healthy growth and produced seed tubers within 90 days. The aeroponic setup facilitated enhanced tuberization compared to conventional soil-based methods, primarily due to precise nutrient delivery and controlled root zone conditions (Abuhena et al., 2022).

The use of tissue-cultured plantlets ensured the availability of uniform, disease-free planting material, supporting efficient and high-density seed production (Sadawarti et al., 2020). Additionally, aeroponic cultivation improved root aeration, minimized pathogen exposure, and enabled year-round production independent of seasonal limitations (Buckseth et al., 2022; Sugiyono et al., 2021).

Overall, the integration of plant tissue culture with aeroponic systems represents a sustainable and efficient approach for large-scale production of high-quality potato minitubers, particularly in resource-limited or non-arable environments (Rajendran et al., 2024).

## CONCLUSION

This study demonstrates that disease-free potato plantlets can be efficiently produced through tissue culture using optimized sterilization and growth conditions. The regenerated plantlets were successfully acclimatized and established in an aeroponic system, resulting in effective minituber production within 90 days.

Overall, the integration of tissue culture and aeroponics provides a reliable, efficient, and scalable approach for the production of high-quality, disease-free potato seed.

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