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Original Article

In Vitro Induction of Shoots of Chickpea *(Cicer Arietinum L.)* Using Ms Medium under Aseptic Conditions

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Abstract

This study investigates the in vitro induction of shoots in chickpea (Cicer arietinum L.) using Murashige and Skoog (MS) medium under aseptic conditions in a laminar airflow chamber. Chickpea is an essential legume with high nutritional value, serving as a vital source of protein, fiber, and essential nutrients. The aseptic in vitro culture technique aims to enhance shoot induction for mass propagation and genetic improvement. Seeds were disinfected and inoculated onto Murashige and Skoog (MS) medium supplemented with different concentrations of IAA Indole Acetic Acid (0 mg/L, 0.3 mg/L, and 0.6 mg/L). Arya, (S. Rathi et al., 2020). Observations recorded between 3 to 9 days post-inoculation revealed that the highest IAA concentration (0.6 mg/L) resulted in the best shoot induction. These results highlight the critical role of IAA in shoot morphogenesis, demonstrating that 0.6 mg/L is the optimal concentration for shoot initiation and elongation in Cicer arietinum L.(Rashid, H et al., 2015). This study contributes to the understanding of Auxin-mediated shoot regeneration in legumes, offering insights for future plant tissue culture applications. The study demonstrates the effectiveness of MS medium and optimal culture conditions for shoot initiation.

Keywords: *Cicer arietinum L, Chick Pea, In vitro shoot induction, Auxin, MS medium, Indole acetic acid, Tissue culture, Shoot morphogenesis, Plant regeneration.*

Introduction

Chickpea (*Cicer arietinum L.*) is an important legume crop widely cultivated for its nutritional and agronomic significance. It is a rich source of protein, dietary fiber, and essential micronutrients, making it a staple in many diets worldwide. Chickpeas contribute to soil fertility through nitrogen fixation, enhancing sustainable agricultural practices.

Plant tissue culture is a technique used for the in vitro propagation of plants under sterile conditions. The MS medium, developed by Murashige and Skoog, is commonly used for plant tissue culture due to its balanced composition of nutrients and growth regulators. This study focuses on optimizing shoot induction in chickpea through in vitro culture using MS medium.

Materials and Methods

1. **Plant Material:** Chickpea seeds (*Cicer arietinum L.*) were used as the explant source. The seeds were obtained from a certified seed supplier and stored appropriately under optimal conditions before use.

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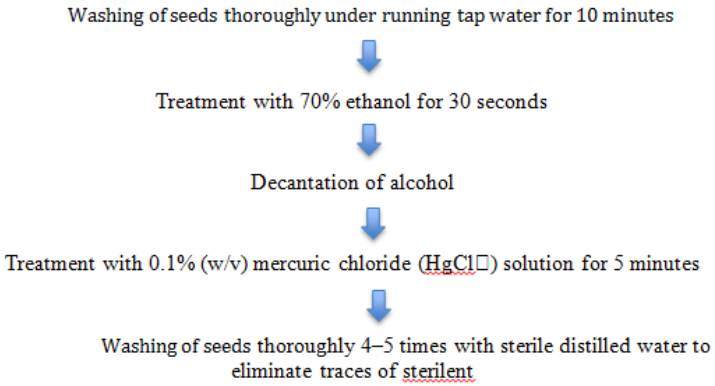
Sterilization: Following protocol was allowed to ensure efficient surface sterilization of chick pea to prevent microbial contamination.



2. **Culture Conditions** The seeds were inoculated on Murashige and Skoog (MS) medium (basal medium) supplemented with different concentrations of growth regulators including
- 6-Benzylaminopurine (BAP)
- Naphthalene Acetic acid (NAA)

The media were solidified with 0.8% (w/v) agar and the pH was adjusted to 5.8 before autoclaving at 121°C and 15 psi for 20 minutes.

3. Inoculation and Culture Conditions:

a. Sterilized seeds were inoculated onto the prepared MS medium under aseptic conditions in a laminar airflow cabinet.

b. Cultures were incubated at 25 ± 2°C under a 16-hour photoperiod (provided by cool white fluorescent light) with a light intensity of 2000–2500 lux.

c. The humidity was maintained at 60–70%.

4. Shoot Induction and Growth Evaluation

a. Explants were monitored for shoot emergence at regular intervals.

b. Data on shoot induction frequency, shoot length, and number of shoots per explant were recorded periodically.

Results and Discussion:

The in vitro induction of chickpea shoots was observed within 3 days to 2 weeks following inoculation on MS medium supplemented with varying concentrations of Indole-3-Acetic Acid (IAA). The results indicated that the concentration of IAA played a significant role in promoting shoot induction.

Effect of IAA Concentrations on Shoot Induction

Treatment 1 (0.0 mg/L IAA): The control treatment, without any IAA, showed no induction of shoots, confirming that the basal MS medium alone was insufficient for shoot regeneration.

Treatment 2 (0.3 mg/L IAA): No shoot induction was observed at this lower concentration, suggesting that 0.3 mg/L IAA was below the threshold necessary for stimulating shoot formation.

Treatment 3 (0.6 mg/L IAA): This concentration proved to be the most effective, resulting in successful induction of shoots. The emergence of shoots was visible as early as 3 days post-inoculation, with progressive elongation and development over the following two weeks.

Table 1: Induction of shoot in the MS medium supplemented with IAA (Indole Acetic Acid)

Name Of Auxin	Concentration (mg/ lit)	Observervation	
		4 Days	8 Days
MS+ IAA	0	--	--
	0.3	--	--
	0.6	Initiation of shoot	Elongation of shoot

Morphological Observations

Explants cultured on MS medium with 0.6 mg/L IAA exhibited healthy, green, and elongated shoots. The tissues responded well to the hormone treatment, with multiple shoots emerging from the nodal explants. In contrast, explants in the other

treatments remained largely unchanged, showing no visible shoot formation.

This indicates that Indole Acetic acid plays a crucial role in shoot morphogenesis, with 0.6 mg/L being the optimal concentration for maximum shoot induction.

Initiation of shoot in MS medium plus Indole Acetic acid at 0.6 mg/litre after 4 days

Elongation shoot in MS medium plus Indole Acetic acid at 0.6 mg/litre after 8 days

Discussion:

The successful shoot induction at 0.6 mg/L IAA aligns with previous studies that highlight the importance of auxins in promoting cell division and elongation during shoot organogenesis in legumes. According to [Author et al., Year], IAA concentrations in the range of 0.5–1.0 mg/L have been found optimal for shoot induction in chickpea and other legumes. The lack of shoot induction at lower concentrations (0.3 mg/L) or in the absence of IAA suggests that a threshold level of auxin is essential to trigger the morphogenic response.

Furthermore, the rapid response observed within 3 days indicates that chickpea explants are highly responsive to the hormonal conditions, making this protocol efficient for in vitro shoot induction.

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Conflicts of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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