Bulletin of Nexus
Multidisciplinary International Research Journal, Double-Blind Peer Reviewed, Open Access Journal
ISSN : 3065-7865 / Volume-2, Issue-2 / February - 2025

Original Article

In Vitro Induction of Shoot in Red Lentil (*Lens Culinaris)*

Arshiya Ansari[1], Archana Tajane[2], Hasnain Shaikh[3], Tanzeel Nachan[4], Samiksha Cheripelli[5], Poonam Gavhane[6], Anaam Ansari[7], Manushri Patil[8], Sapna Patil[9]

[1,2,4,5,6,7,8,9] Assistant professor, B. N. N. College, Bhiwandi (Thane)

[3]T.Y. B. Sc Biotechnology, B. N. N. College, Bhiwandi (Thane)

Manuscript ID:
BN-2025-020206

ISSN: 3065-7865

Volume 2

Issue 2

February 2025

Pp. 36-39

Submitted: 26 Dec 2024

Revised: 12 Jan 2025

Accepted: 16 Feb 2025

Published: 28 Feb 2025

DOI:
10.5281/zenodo.15234318

DOI Link:
https://doi.org/10.5281/zenodo.15234318

Abstract

*The current study was performed with an intention to minimize the in vitro requirements for the production of shoots from red lentil (*Lens culinaris*) with particular emphasis on the role of kinetin, a cytokinin-type plant growth regulator. Surface-sterilized seeds of *L. culinaris* were sown in Murashige and Skoog (MS) medium enriched with differing concentrations of kinetin—0 mg/L (control), 0.6 mg/L, and 0.8 mg/L. The explants were checked on a daily basis to measure shoot initiation and elongation from day 3 up to day 9 post-inoculation. Results were visible at the maximum level applied with kinetin (0.8 mg/L) such that shoot initiation was as early as day 4 whereas there was intensive elongation by day 8. Conversely, the control set (0 mg/L) and 0.6 mg/L kinetin-treated set did not exhibit shoot formation, indicating that there is a threshold requirement of kinetin to induce the morphogenetic response in this species. The experiment strongly indicated that kinetin plays a crucial role in the shoot organogenesis of *. Culinaris*, and 0.8 mg/L to be optimum for the induction as well as the subsequent elongation of shoots. These results are attributed to the overall information on cytokinin-induced shoot regeneration in leguminous crops and could prove to be a useful reference for subsequent plant tissue culture and genetic transformation experiments in crop improvement and green revolution agriculture.*

Keywords: *Lens culinaris, Red lentil, In vitro shoot induction, Kinetin, MS medium, Cytokinin, Tissue culture, Shoot morphogenesis, Plant regeneration.*

Introduction

Lentils (Lens culinaris Medik.) are a cherished staple and a fantastic source of dietary protein, often regarded as superior to other pulse crops (Singh et al., 1968; Gulati et al., 2002). In Bangladesh, lentils take the crown, leading the way in both consumption and the area they occupy among various pulse crops. The demand for lentils has been steadily increasing, thanks to their remarkable nutritional benefits and broad appeal. Given their significant role in agriculture and the economy, it's crucial to focus on enhancing the quality and yield of lentils.

Lentil cultivation is affected by a range of living and non-living components that have the potential to impact considerably the yield, as well as the quality, of the crop. Diseases due to Fungi, bacteria, and viruses, as well as climatic conditions like drought and excessive salinity are significant Hindrances to lentil farmers. Erskine et al. (1994), Taylor and Ford (2008), and Gurusamy et al. (2012) also conducted research exploring ways of improving lentil cultivars through traditional breeding practices. But depending solely on such conventional procedures hasn't yet achieved the same sort of show-stopping agronomic breakthroughs farmers are looking for. In recent history, there has been research in new biotechnology techniques such as molecular breeding, genetic modification, and genome editing with encouraging findings enhancing lentil yield and resistance to stress factors.

Address for correspondence:

Arshiya Ansari, Assistant professor, B. N. N. College, Bhiwandi (Thane)

How to cite this article:

Ansari, A., Tajane, A., Shaikh, H., Nachan, T., Cheripelli, S., Gavhane, P., Ansari, A., Patil, M., & Patil, S. (2025). In Vitro Induction of Shoot in Red Lentil (Lens Culinaris). Bulletin of Nexus, 2(2), 36–39. https://doi.org/10.5281/zenodo.15234318

Quick Response Code:

Website: https://bnir.us

OPEN ACCESS

Marker-assisted selection (MAS) and quantitative trait loci (QTL) mapping have been advanced to an extent that genes for determining favorable traits can now be identified, thus efficiently bringing about improved varieties of lentil. In addition, biotechnological interventions can contribute to enhancing the crop's adaptability to climate change and disease resistance, making future sustainable lentil production possible.

With the increasing demand and problems of lentil production, coordinated application of conventional breeding, molecular techniques, and agronomic management is required to achieve the maximum yield and quality. Research should continue to explore new strategies to overcome the constraints and enhance sustainable lentil production. This paper tries to shed light on the recent advances in lentil improvement, emphasizing significant challenges and solutions for productivity and resilience enhancement.

Materials and Methods

1. Plant Material:

Red lentil (Lens culinaris) seeds were used as the experimental material. The seeds were procured from a certified seed supplier and stored under optimal conditions before use.

2. Surface Sterilization of Seeds:

To prevent microbial contamination, seeds were surface sterilized according to the protocol:

a. Seeds were well washed in flowing tap water for 10 minutes to clean out dust and soil.
b. They were subsequently treated with 70% ethanol for 30 seconds.
c. Ethanol was decanted, and seeds were treated with 0.1% (w/v) mercuric chloride ($HgCl_2$) solution for 5 minutes with shaking continuously.

Sterilant was decanted off and seeds washed 4–5 times with sterile distilled water to remove residues of $HgCl_2$.HgCl

3. Culture Media Preparation:

Murashige and Skoog (MS) medium was used as the basal medium for shoot induction. The medium was supplemented with various concentrations of plant growth regulators (PGRs), including:

- 6-Benzylaminopurine (BAP)
- Kinetin (KIN)
- Indole-3-acetic acid (IAA)

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.

4. Inoculation and Culture Conditions :

a. Seeds were sterilized and inoculated onto the afore-prepared MS medium under aseptic condition in a laminar airflow cabinet.
b. Cultures were incubated at 25 ± 2°C under a photoperiod of 16 hours (supplied by cool fluorescent light) of intensity 2000–2500 lux.
c. The humidity was held at 60–70%.

Shoot Induction and Growth Assessment

- Shoot emergence was monitored at frequent intervals.
- Shoot induction frequency, shoot length, and number of shoots per explant were noted after 4 weeks of culture.

Results and Discussion

The present study aimed to standardize the medium and explant conditions for *in vitro* shoot induction in Lens culinaris. The experiment involved the inoculation of red lentil seeds onto Murashige and Skoog (MS) medium supplemented with different concentrations of kinetin, a cytokinin known to promote shoot formation.

Table 1: Induction of shoot in the MS medium supplemented with Kinetin

Name Of Cytokinin	Concentration (mg/ lit)	Observervation	
		4 Days	8 Days
MS+ Kinetin	0	--	--
	0.6	--	--
	0.8	Initiation of shoot	Elongation of shoot

Effect of Kinetin on Shoot Induction

Seeds were initially prepared under aseptic conditions before being inoculated onto MS media with varying kinetin concentrations (0 mg/L, 0.6 mg/L, and 0.8 mg/L). Observations were recorded between 3 to 9 days post-inoculation to assess shoot initiation and elongation.

Table 1 shows the effect of varying kinetin concentrations on shoot induction. It was found that:

- The control sample (MS medium alone without any addition of kinetin) failed to induce shoots.
- Seeds in 0.6 mg/L kinetin failed to show shoot initiation.
- The highest level (0.8 mg/L kinetin) showed apparent shoot initiation after 4 days, followed by significant shoot elongation by day 8.

This indicates that kinetin plays an important part in shoot morphogenesis and 0.8 mg/L is the optimal concentration for maximum shoot induction.

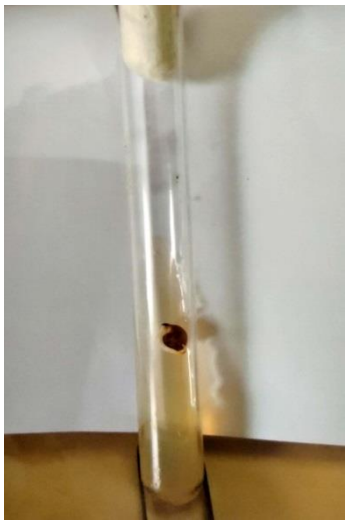

Figure 01: Shoot formation in MS medium + Kinetin at (0.8mg/lit) after 4 days

Figure 02: Shoot elongation in MS medium + Kinetin at (0.8 mg/lit) after 8 days

Discussion

These findings are consistent with earlier research on the cytokinin activity in stimulating shoot differentiation of legumes. The inability to induce shoots at lower kinetin levels indicates that there exists a threshold kinetin concentration to achieve organogenesis.

The elongation of shoots in the 0.8 mg/L treatment confirms the theory that kinetin not only initiates but also maintains growth of the shoots. This discovery further emphasizes the role of Optimizing cytokinin content in order to bring about maximum regenerability in lentils.

According to previous studies, it is inferred from the current investigation that MS medium containing 0.8 mg/L kinetin is optimum when it comes to inducing and sustaining shoots in *Lens culinaris*. Future studies may explore interactions with other plant growth regulators, such as auxins, to enhance regeneration efficiency further.

Acknowledgment

I am Arshiya Ansari thankful to Ms. Archana Tajane, Head of the Department of Microbiology, B. N. N College, Bhiwandi, for their support and for granting permission to carry out the work.

Financial support and sponsorship

Nil.

Conflicts of interest

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

References:

1. Murashige, T., & Skoog, F. (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologia Plantarum, 15*(3), 473-497. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x
2. Sarker, R. H., & Biswas, A. (2002). In vitro plantlet regeneration and Agrobacterium-mediated genetic transformation of lentil (*Lens culinaris* Medik.). *Plant Tissue Culture, 12*(2), 155-165.
3. Ochatt, S. J., Moessner, A., & Kumar, A. (2013). Lentil. In *Agrobacterium protocols* (pp. 165-180). Humana Press.
4. Sharma, S. K., & Agrawal, V. (2018). Influence of plant growth regulators on callus induction and plant regeneration in lentil (*Lens culinaris* Medik.). *Journal of Plant Biotechnology, 45*(1), 75-82.
5. Roy, P., & Mandal, N. (2017). Effect of cytokinins on *in vitro* shoot induction in lentil (*Lens culinaris* Medik.). *International Journal of Botany Studies, 2*(3), 40-45.
6. Gamborg, O. L., Miller, R. A., & Ojima, K. (1968). Nutrient requirements of suspension cultures of soybean root cells. *Experimental Cell Research, 50*(1), 151-158. https://doi.org/10.1016/0014-4827(68)90403-5