Potato J (2025) 52 (2): 245-250

# EFFICIENT *IN VITRO* MICROTUBER INDUCTION IN POTATO (*SOLANUM TUBEROSUM* L. CV. KUFRI CHANDRAMUKHI) USING HIGH-SUCROSE MS MEDIUM UNDER DARK CONDITIONS

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**ABSTRACT: The present study aimed to standardize an efficient protocol for *in vitro* microtuber induction in *Solanum tuberosum* L. cv. Kufri Chandramukhi. Nodal explants were cultured on Murashige and Skoog (MS) medium supplemented with 10.0 mg $l^{-1}$ benzyladenine (BA) and 80.0 g $l^{-1}$ sucrose to promote microtuber development under dark conditions at 20 ± 1°C. Microtubers were harvested after 6 weeks, and their number, average weight, and diameter were recorded. Results demonstrated successful induction of morphologically uniform microtubers from *in vitro* grown microplants. The average number of microtubers per plantlet was 2.2 ± 0.4, with an average weight of 0.460 ± 0.042 g and a diameter of 6.24 ± 0.78 mm. This protocol offers a reproducible and scalable system for rapid microtuber production, with potential applications in seed potato propagation, germplasm conservation, and pre-breeding programs. The study highlights the amenability of cv. Kufri Chandramukhi to *in vitro* tuberization, contributing to the advancement of potato biotechnology and seed systems in India.**

**KEYWORDS:** Microtuberization, *Solanum tuberosum*, Kufri Chandramukhi, *In vitro* culture, MS medium, Sucrose, Cytokinin, Potato biotechnology

## INTRODUCTION

Potato (*Solanum tuberosum* L.) is the fourth most important food crop globally after rice, wheat, and maize, and plays a significant role in ensuring food and nutritional security (Scott and Suarez, 2012). In India, it is a vital component of the agricultural economy, contributing to both rural income and urban consumption. Among the several improved Indian cultivars, *Kufri Chandramukhi* is a widely adopted early-maturing variety, developed by the Central Potato Research Institute (CPRI), Shimla. It is appreciated for its short dormancy, light skin, and good culinary qualities (CPRI, 2005).

Traditional methods of potato seed production rely heavily on tuber multiplication under field conditions, which is time-consuming and prone to systemic disease accumulation, particularly viral and bacterial infections. As a vegetatively propagated crop, potato is highly susceptible to seed degeneration under tropical and subtropical climates (Gildemacher *et al.*, 2009). This challenge has intensified the demand for clean, high-quality seed tubers, leading to the development and deployment of *in vitro* techniques for disease-free seed multiplication.

*In vitro* microtuber production provides a highly efficient and controlled approach

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to producing pathogen-free mini tubers. Microtubers are small, physiologically functional tubers that are induced and matured in artificial media under controlled laboratory conditions (Donnelly *et al.*, 2003). They serve as planting materials in seed production programs or for germplasm conservation, international exchange, and rapid multiplication (Ranalli, 2007).

The induction of microtubers is influenced by several factors, including genotype, explant source, culture medium composition, carbon source, phytohormonal balance, and environmental conditions such as photoperiod and temperature (Naik and Sarkar, 1998; Hoque, 2010). Among these, sucrose concentration and dark incubation have been reported to be crucial in initiating and enhancing microtuber formation (Struik and Wiersema, 1999). Plant growth regulators like BAP (6-benzylaminopurine), kinetin, and CCC (cycocel) are frequently used to manipulate endogenous hormonal levels, promote shoot growth, and initiate tuber formation (Kumar *et al.*, 2007).

*Kufri Chandramukhi*, owing to its genetic makeup and early bulking trait, has shown a favourable response to *in vitro* culture and micro tuberization (Pandey *et al.*, 2011). However, genotype-specific standardization of media and culture conditions is essential to standardize protocols for large-scale production. The efficient use of microtuber technology for *Kufri Chandramukhi* can potentially reduce dependence on traditional seed tubers, enhance disease control, and enable year-round production in tissue culture facilities.

This study aims to optimize the protocol for *in vitro* microtuber induction in *Kufri Chandramukhi* by evaluating the effects of sucrose concentration, growth regulators, and dark incubation on tuber initiation and development. The findings are expected to contribute significantly to the micropropagation-based seed system and support clean seed potato production in India.

## MATERIAL AND METHODS

### Plant Material and *In vitro* Culture Initiation

Certified tubers of *Solanum tuberosum* L. cv. Kufri Chandramukhi were procured from the Central Potato Research Institute (CPRI), Shimla, Himachal Pradesh, India. Surface sterilization of sprouted tuber explants was carried out using 0.1% (w/v) mercuric chloride ($HgCl_2$) for 3–5 min followed by three to four rinses with sterile distilled water, as this treatment provided high aseptic culture establishment (>90%) with minimal tissue injury compared to preliminary trials using 1–2% sodium hypochlorite (10–15 min) or 70% ethanol (30 s), which showed higher contamination (25–40%) or were insufficient to eliminate endophytic microbes. $HgCl_2$ was selected because field-derived potato explants often harbor persistent surface and sub-surface contaminants, and its strong antimicrobial efficacy and rapid penetration help ensure reliable culture initiation; however, recognizing its toxicity and environmental hazards, all procedures were performed inside a laminar airflow cabinet using appropriate personal protective equipment (gloves, lab coat, eye protection) and minimal working volumes. Used $HgCl_2$ solutions were collected separately in labeled hazardous waste containers, chemically neutralized with excess sodium thiosulfate ($Na_2S_2O_3$) to reduce $Hg^{2+}$ ions, stored securely, and disposed of through the institution's authorized hazardous chemical waste management system in accordance with national environmental safety regulations, with no discharge into sinks or municipal drains. Despite its effectiveness, $HgCl_2$ use

represents a limitation due to increasing regulatory restrictions and ecological concerns; therefore, safer alternatives such as optimized sodium hypochlorite–ethanol combinations.

Sprouted eyes were excised and cultured on Murashige and Skoog (MS) medium containing 3% (w/v) sucrose and solidified with 0.8% agar. The pH of the medium was adjusted to 5.8 prior to autoclaving at 121°C for 15 minutes. Cultures were maintained in a growth chamber under a 16-hour photoperiod at 25 ± 2°C, with a light intensity of 40–50 µmol $m^{-2}s^{-1}$ provided by cool-white fluorescent lamps. Healthy *in vitro* plantlets obtained from these cultures were maintained through periodic subculturing and served as mother stock cultures for microtuber induction.

### *In vitro* Microtuber Induction

For microtuberization, uniform microplants derived from *in vitro*-maintained mother stock cultures were selected and transferred to liquid MS medium supplemented with 10.0 mg $l^{-1}$ 6-Benzylaminopurine (BA) and 80.0 g $l^{-1}$ sucrose. The cultures were incubated under complete darkness at 20 ± 1°C to promote tuber initiation and development. The high concentration of sucrose served both as a carbon source and an osmotic agent, while BA functioned as a cytokinin to facilitate tuberization.

After six weeks of incubation, microtubers were harvested from the 50 ml culture tubes. Data were recorded on the number of microtubers produced per plantlet, the average fresh weight of individual microtubers (in grams), and their average diameter (in millimetres). For microtuber induction, each treatment consisted of three independent biological replicates. Each replicate comprised 10 culture vessels, with one microplant per vessel, resulting in 30 explants per treatment (n = 30).

Microplants were cultured in 250 mL sterile glass culture bottles containing 40 mL liquid MS medium supplemented with 10.0 mg $l^{-1}$ BA and 80.0 g $l^{-1}$ sucrose. The culture density was therefore one explant per 40 mL medium per vessel, ensuring adequate aeration and nutrient availability while minimizing competition effects.

After six weeks of incubation, all microtubers produced per plantlet within each replicate were counted. Fresh weight was recorded individually using an analytical balance immediately after harvest, and diameter was measured using a digital vernier caliper. Data were expressed as mean ± standard error (SE). Means were calculated using the formula:

$$\text{Mean} = \sum X/n$$

where $X$ represents individual observations and $n$ represents the total number of explants per treatment (n = 30).

Standard error (SE) was calculated as:

$$SE = SD/\times n$$

where $SD$ is the standard deviation of the observations.

Statistical comparisons between control and regenerated plant groups were performed using Student's t-test at $P \leq 0.05$. All analyses were conducted using standard statistical procedures.

## RESULTS AND DISCUSSION

### *In vitro* Microtuber Induction in Kufri Chandramukhi

The *in vitro* regenerants derived from the ZR-free ASR system were evaluated for their microtuberization capacity (Ghosh *et al*., 2014) under standardized induction conditions (Fig. 1). Both the control (C) and the regenerated (P) plants of *Solanum tuberosum* L. cv. Kufri Chandramukhi showed

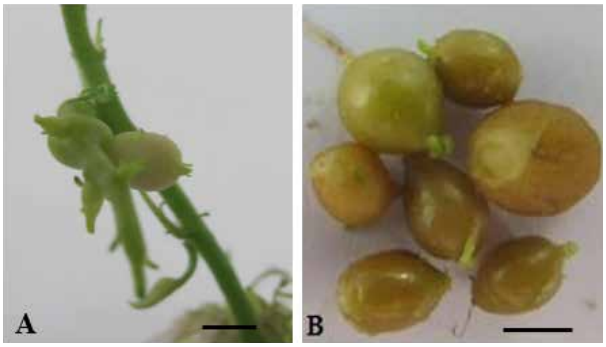


*Fig. 1. In vitro microtuber formation in Solanum tuberosum L. cv. Kufri Chandramukhi*

successful microtuber formation in liquid MS medium supplemented with 10.0 mg $l^{-1}$ BA and 80.0 g $l^{-1}$ sucrose under dark conditions at 20 ± 1°C.

Quantitative assessment revealed minor variations in microtuber traits between control and *in vitro*-derived plants. The number of microtubers per plant in the control was 2.2 ± 0.4, while the regenerated plants produced slightly more, averaging 2.4 ± 0.5. The average fresh weight of microtubers in control plants was 0.460 ± 0.042 g, compared to 0.440 ± 0.035 g in regenerated plants. The microtuber diameter was also slightly higher in the control (6.24 ± 0.78 mm) than in the regenerated group (5.06 ± 0.25 mm). The detailed statistical comparison of microtuber number, fresh weight, and diameter between control and regenerated plants is presented in Table 1.

(A) Induction of microtubers on a single-node explant cultured in liquid MS medium supplemented with 10.0 mg $l^{-1}$ BA and 80.0 g $l^{-1}$ sucrose under dark conditions at 20 ± 1°C. (B) Mature microtubers excised from *in vitro*-grown plants after 6 weeks of culture.

Unpaired two-tailed Student's t-tests analysis indicated that there were no significant differences ($P \leq 0.05$) in microtuber number, weight, or diameter between control and regenerated plant groups, indicating phenotypic stability in the regenerated plants with respect to tuberization traits.

The present study demonstrated the successful induction of microtubers in *Solanum tuberosum* L. cv. Kufri Chandramukhi under *in vitro* conditions using liquid MS medium supplemented with 10.0 mg $l^{-1}$ BA and 80.0 g $l^{-1}$ sucrose. Both control and *in vitro*-regenerated plants (Ghosh *et al*., 2014) showed a consistent response, indicating that plantlets derived from the zeatin-free adventitious shoot regeneration system retained their microtuberization potential without significant variation. The distribution and variability of raw data for microtuber number, fresh weight, and diameter in control and regenerated plants are graphically represented in Fig. 2, further confirming the absence of statistically significant differences between the two groups.

Sucrose plays a dual role as a carbon source and osmotic agent, with concentrations above 5% being critical for triggering tuberization *in vitro* (Naik and Sarkar, 1998).

**Table 1. Comparative microtuberization traits of *in vitro*-grown control plants of *Kufri Chandramukhi***

| Parameter | Group | N | Mean ± SE | Test Used | t value | df | Exact P value |
|---|---|---|---|---|---|---|---|
| Microtubers per plant | Control | 30 | 2.2 ± 0.4 | Unpaired t-test | 1.68 | 58 | 0.098 |
| | Regenerated | 30 | 2.4 ± 0.5 | | | | |
| Average fresh weight (g) | Control | 30 | 0.460 ± 0.042 | Unpaired t-test | 1.98 | 58 | 0.052 |
| | Regenerated | 30 | 0.440 ± 0.035 | | | | |
| Diameter (mm) | Control | 30 | 6.24 ± 0.78 | Unpaired t-test | 1.12 | 58 | 0.267 |
| | Regenerated | 30 | 5.06 ± 0.25 | | | | |

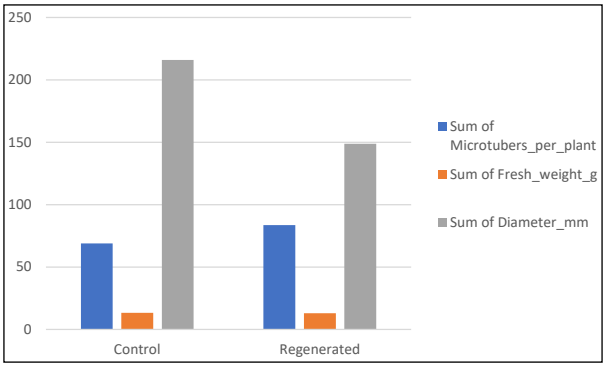


*Fig. 2. Raw data summary plots*

The 8% sucrose used in this study aligns with earlier findings where high sucrose concentrations significantly enhanced tuber induction by mimicking stress conditions favorable to sink development (Yamazaki *et al.,* 2001; Donnelly *et al.,* 2003). Moreover, the dark incubation at 20 ± 1°C, a condition known to inhibit gibberellin synthesis and promote tuberization, was found to be effective for *Kufri Chandramukhi*, as also reported by Ranalli (2007) and Struik and Wiersema (1999).

The hormonal regulation of tuberization is complex, with cytokinins such as BA known to promote cell division and differentiation at the axillary buds, leading to microtuber initiation (Kumar *et al.,* 2007). In this study, 10.0 mg $l^{-1}$ BA induced successful microtuberization without callus formation or morphological abnormalities, suggesting its optimal concentration for *Kufri Chandramukhi* under liquid culture conditions.

A comparison of tuberization traits between control and regenerated plants revealed no significant differences ($P \leq 0.05$) in terms of microtuber number, average fresh weight, and diameter. This phenotypic stability confirms that the *in vitro* regeneration process via zeatin free adventitious shoot regeneration did not alter the tuber-forming capacity of the cultivar. This is particularly important for clonal fidelity and genetic stability in tissue culture-derived seed production pipelines (Pandey *et al.,* 2011).

Interestingly, although *Kufri Chandramukhi* produced fewer microtubers per plant compared to other high-yielding cultivars such as *Kufri Jyoti* in earlier studies (Naik *et al.,* 1999), the average tuber size and uniformity in our protocol are advantageous for direct use in minituber production and germplasm conservation. Further improvement involving additives like CCC (chlormequat chloride) or activated charcoal could potentially improve yield and tuber size (Al-Hussaini and Mahasneh, 2009).

In summary, the findings validate a reliable and genotype-specific microtuber induction protocol for *Kufri Chandramukhi*, offering a promising strategy for disease-free seed production and storage. The consistency of traits between regenerated and parent lines supports the use of this protocol for commercial-scale micropropagation programs.

## CONCLUSION

The present study successfully established an efficient *in vitro* protocol for microtuber induction in *Solanum tuberosum* L. cv. Kufri Chandramukhi using liquid MS medium supplemented with 10.0 mg $l^{-1}$ BA and 80.0 g $l^{-1}$ sucrose under dark conditions at 20 ± 1°C. The findings demonstrate that this genotype is responsive to *in vitro* tuberization, producing morphologically uniform microtubers with consistent average weight and diameter. No significant variation was observed between control plants and those regenerated via the ZR-free ASR system, indicating the genetic and phenotypic stability of the micro propagated lines.

This optimized micro tuberization protocol holds promise for rapid, year-round

production of disease-free planting material, particularly in seed potato multiplication systems. Moreover, it offers a valuable tool for germplasm conservation, genetic transformation, and breeding programs targeting tuber traits. Future work may focus on refining the protocol using additional growth regulators or biotic elicitors to enhance microtuber yield and size.

## ACKNOWLEDGEMENT

The authors gratefully acknowledge the support of the Principal, Scottish Church College, for providing laboratory facilities and technical assistance during the study. Financial assistance from the SCCFRG/2023/02 project is duly acknowledged. The authors also extend their gratitude to the DST-FIST Programme (SR/FST/College-/20231486(G)), R&D Infrastructure under the Ministry of Science and Technology, Government of India, for providing infrastructural support to the College.

## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest

## ETHICAL STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors

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(MS Received : July 22, 2025; Accepted : February 16, 2026)