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Kinetin-mediated enhancement of microtuberization in potato: Insights into growth regulation and tuber development

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Abstract
The present study was conducted to evaluate the influence of kinetin on growth characteristics and microtuberization of potato (*Solanum tuberosum* L.) under *in-vitro* conditions. The experiment was carried out using different concentrations of kinetin (0.5, 1.0 and 2.0 ppm) along with a control treatment to determine its effect on vegetative growth and microtuber development. Kinetin application significantly affected plant growth parameters, including plant height, number of nodes and number of leaves, with higher concentrations showing a reduction in vegetative growth compared to the control. However, kinetin exhibited a positive influence on microtuber induction and development. Among the treatments, 1 ppm kinetin recorded the highest percentage of microtuberizing plants (91.67%), maximum number of microtubers per plant (1.77), highest microtuber fresh weight (81.5 mg), and greater microtuber diameter (6.58 mm) compared to the control. The results indicated that an optimum concentration of kinetin enhanced tuber initiation and improved microtuber quality, whereas higher concentrations showed reduced effectiveness. The findings suggest that kinetin, particularly at 1 ppm concentration, plays an important role in promoting microtuberization by stimulating cell division and regulating physiological processes involved in tuber development. Therefore, kinetin supplementation can be effectively utilized in potato tissue culture systems for enhanced production of quality microtubers.

Keywords: Potato, kinetin, microtuberization, cytokinin, tuberization

Introduction
Potato (*Solanum tuberosum* L.) is an economically important crop, and the production of disease-free quality planting material through tissue culture has become an essential component of modern potato improvement programmes. Microtuberization, the process of producing miniature tubers under controlled in-vitro conditions, offers several advantages, including rapid multiplication, easy storage and transportation, and the availability of pathogen-free seed material. The success of potato microtuberization depends on several factors, including genotype, culture medium composition, carbohydrate availability, environmental conditions, and the application of suitable plant growth regulators. Among these factors, cytokinins, particularly kinetin, have been recognized as key regulators involved in the initiation and development of microtubers (Dessoky *et al*., 2016; Bharath and Mohan Raju, 2024) [6, 3].

Kinetin is an important synthetic cytokinin that plays a crucial role in regulating plant growth, development, and morphogenesis. Cytokinins are a class of plant hormones primarily involved in promoting cell division, cell differentiation, chloroplast development, nutrient mobilization, and the regulation of source-sink relationships in plants. Since its discovery, kinetin has been extensively used in plant tissue culture systems due to its ability to stimulate cellular proliferation and influence developmental pathways. In-vitro propagation of plants largely depends on the precise balance of plant growth regulators, and kinetin is one of the most widely applied cytokinins for controlling morphogenic responses and improving regeneration efficiency (Olga *et al*., 2022) [16].

Kinetin influences potato microtuberization by promoting cell division and differentiation in stolon tissues, which are the primary sites for tuber initiation.

The application of kinetin stimulates meristematic activity, enhances cellular proliferation, and facilitates the transition of stolons into tuber-producing structures. Cytokinins are known to promote sink formation by increasing metabolic activity and redirecting assimilates towards developing tubers. Through modulation of hormonal balance, particularly the interaction between cytokinins, auxins, and other growth regulators, kinetin contributes to the regulation of tuber initiation and subsequent tuber enlargement. Kinetin has been reported to regulate tuberization under both tuber-inducing and non-inducing conditions, demonstrating its significant role in the physiological mechanisms associated with potato tuber development (Raspor *et al*., 2012) [18].

Several studies have demonstrated the positive influence of kinetin on potato microtuber formation. Kinetin application to single nodal cuttings has been shown to enhance microtuber induction by improving cell division and developmental processes associated with tuber formation (Dessoky *et al*., 2016) [6]. The incorporation of kinetin into culture media has resulted in improvements in microtuber number, size, fresh weight, and diameter, indicating its potential in optimizing in-vitro tuber production. The concentration of kinetin is a critical factor determining the success of microtuberization, as an optimum level promotes tuber development, whereas excessive concentrations may disturb hormonal balance and negatively affect plant growth.

The interaction between kinetin and sucrose is another important factor influencing microtuber development. Sucrose serves not only as a carbon and energy source but also acts as a signaling molecule that triggers tuberization pathways. The combined effect of sucrose and kinetin enhances assimilate accumulation, cell expansion, and storage tissue development, thereby improving microtuber size and weight. Studies have reported that the synergistic interaction between kinetin and sucrose significantly contributes to enhanced microtuber production and quality under in-vitro conditions (Ali *et al*., 2018; Bharath and Mohan Raju, 2023) [2, 4].

Although kinetin promotes microtuberization, its effects on vegetative growth parameters such as plant height, node formation, and leaf development may vary depending on concentration and culture conditions. Higher cytokinin levels may redirect plant resources from shoot growth towards tuber initiation and development, resulting in reduced vegetative growth. Therefore, determining the optimal kinetin concentration is essential to achieve a balance between plant growth and efficient microtuber production (Dessoky *et al.,* 2016; Olga *et al*., 2022) [6, 16].

Considering the importance of kinetin in regulating potato microtuberization, the present study was undertaken to evaluate the influence of different kinetin concentrations on growth characteristics and microtuber development in potato under in-vitro conditions. Understanding the role of kinetin in microtuber induction and development will contribute to the improvement of tissue culture protocols for rapid multiplication and production of high-quality potato planting material.

Materials and Methods

To study the Influence of cytokinins on microtuberization in potato

Cytokinins play a crucial positive role in the regulation of potato tuberization. Numerous reports have consistently demonstrated that cytokinins stimulate tuber initiation and enhance sink capacity in potato plants. An increase in cytokinin concentration is also associated with an increased number of tubers per plant. Therefore, the present study was conducted to evaluate the effect of kinetin on microtuberization in potato.

Exploring the Microtuberization Potential of Potato Through Kinetin Application

In-vitro grown potato plants cultured on MS medium supplemented with sucrose were treated with different concentrations of kinetin. Kinetin was incorporated into the MS medium at concentrations of 0, 0.5, 1.0, and 2.0 ppm separately. Following kinetin treatment, the cultures were maintained at 18 °C under short-day conditions. After 45 days of inoculation, growth parameters including plant height (cm), number of nodes, number of leaves, percentage of microtuberizing plants (%), number of microtubers per plant, fresh weight of microtubers (mg), and microtuber diameter (mm) were recorded and further evaluated up to 90 days after inoculation. The experiment was conducted with an adequate number of replications to ensure the reliability and reproducibility of the results. A control treatment without kinetin supplementation was included to compare and validate the effects of kinetin on potato microtuberization.

Statistical Analysis

The data on plant height, number of nodes, number of leaves, per cent micro-tuberizing plants, Micro-tuber number per plant, Micro-tuber fresh weight and Micro-tuber diameter were collected during harvest. Further, all the collected data were analyzed by analysis of variance and the means were compared according to DMRT (Duncan's Multiple Range Test) and the mean separation was done at 5 and 1 per cent level of significance.

Results and Discussion

Study the Influence of Kinetin on microtuberization in potato

Cytokinin such as kinetin is known for its cell division stimulation capability influencing structure development by promoting cell growth in the nodal region and contributing to microtuber formation in potato (Dessoky *et al.,* 2016; Olga *et al*., 2022) [6, 16].

Kinetin-Driven Growth Dynamics in Potato (*Solanum tuberosum* L.) Under *In-Vitro* Culture

This study investigates the impact of kinetin on plant height and microtuberization in potato. The experimental design involves comparing a control treatment with three kinetin treatments at a concentration of 0.5, 1 and 2 ppm to assess significant effects on microtuberization. The control treatment displayed significantly greater plant height (15.2 cm) compared to the 2 ppm kinetin-treated group (5.18 cm). However, the other kinetin-treated concentrations namely, 0.5 ppm and 1 ppm resulted in decreased plant heights of 12.58 cm and 11.15 cm, respectively compared to the control treatment (15.2 cm). The findings suggest that, higher concentrations of kinetin a negative impact on plant height in potato plants indicating a potential restricting effect on plant growth (Table 1; Plate 1).

Previous studies on cytokinins and plant height yielded similar results, Riefler *et al*., (2006) [19] demonstrated in his research on *Arabidopsis thaliana* that, the exogenous

application of kinetin led to altered shoot development and reduced plant height. Similarly, Hussain *et al*., (2021) [12] reported in their study on maize that, cytokinin treatment resulted in shorter plants compared to the control group. In the context of potato microtuberization, the influence of kinetin on plant height could be linked to its impact on tuber formation. However, it is plausible that, the increased focus on tuberization might have diverted resources away from overall plant growth leading to the observed decrease in plant height in the kinetin-treated plants.

The results regarding the number of nodes per plant in the control group exhibited a significantly higher average number of nodes per plant (46.73) compared to the kinetin treatment (13.13). Furthermore, kinetin concentrations of 0.5 and 1 ppm resulted in decreased numbers of nodes per plant (44.43 and 35.83, respectively) when compared to the control treatment (46.73). In summary, the application of kinetin significantly impacted the number of nodes per plant in potato with the control treatment showing a higher node count. It implies a suppressive effect of kinetin on microtuberization in potato (Table 1; Plate 1). A pertinent study by Hajare *et al.* (2021) [9] demonstrated that, the application of kinetin led to a reduction in lateral shoot development in tobacco plants.

The study also revealed a significant difference in the number of leaves per plant between the control and the kinetin-treated plants. In the control treatment potato plants exhibited a significantly higher number of leaves (47.63) compared to those subjected to kinetin treatment at 0.5 ppm and 1 ppm, with 45.86 and 36.6 leaves, respectively. Interestingly, a higher concentration of kinetin resulted in a sudden drop in the number of leaves per plant. Therefore, this finding suggest that the application of kinetin negatively influenced leaf development in potato plants (Table 1; Plate 1).

Table 1: Effect of Kinetin on plant growth in potato under i*n-vitro* condition

Kinetin (KIN)			
Treatments	**Plant height (cm)**	**Number of nodes**	**Number of leaves**
Control	15.2[a]	46.733[a]	47.633[a]
KIN-0.5 ppm	12.58[b]	44.43[b]	45.86[b]
KIN-1.0 ppm	11.15[c]	35.83[c]	36.6[c]
KIN-2.0 ppm	5.18[d]	13.13[d]	13.7[d]
CD (*P*<0.01)	0.438**	1.277**	0.731**
SE(m)	0.148	0.43	0.246
SE(d)	0.209	0.608	0.348
CV (%)	3.276	3.006	1.677

** Significant at 1% level

The critical role of sucrose and Kinetin in microtuber formation was highlighted by Ali *et al*. (2018) [2]. They observed that, an increase in sucrose concentration in the medium could enhance microtuber production to a certain extent, while high concentrations (100g) negatively affected the germination rate of plantlets. Emaraa *et al*. (2017) [7] suggested that, the concentrations of plant growth regulators, sucrose and Kinetin in MS medium can collectively improve multiplication, plant growth and micro-tuberization in potato.

Kinetin in Action: Unlocking the Microtuberization Potential of Potato Under *In-Vitro* Conditions: The study also revealed a substantial influence of kinetin on microtuberization in potato plants. Specifically, the percentage of plants exhibiting microtuberization at 1 ppm concentration of kinetin treatment was significantly higher at 91.67%, compared to the control treatment at 42.83%. Additionally, the 0.5 ppm and 2 ppm concentrations also showed significantly higher percentages of microtuberization at 55.17% and 83.33%, respectively compared to the control treatment. This suggests that, the application of kinetin at a concentration of 1 ppm positively influences on the initiation and development of microtubers in potato plants resulting in a 91.67% microtuberizing plant (Figure 1a; Plate 1).

The application of different concentrations of kinetin treatments specifically 0.5 ppm and 2 ppm resulted in significantly higher microtuber numbers per plant with values of 0.67 and 1.57, respectively compared to the control treatment with a value of 0.43 tubers. Additionally, the 1 ppm concentration of kinetin treatment demonstrated a substantial increase in microtuber formation recording 1.77 microtubers per plant compared to the control treatment's. Overall, the kinetin-treated potato plants displayed a remarkable enhancement in microtuberization compared to the control group (Figure 1b; Plate 1).

The present study demonstrated that kinetin significantly enhanced microtuberization in potato under in-vitro conditions. Among the tested concentrations, 1 ppm kinetin showed the highest response, with 91.67% microtuberizing plants compared to 42.83% in the control. The 0.5 and 2 ppm kinetin treatments also improved microtuber induction, confirming the positive role of kinetin in tuber initiation and development.

The promotive effect of kinetin may be attributed to its cytokinin activity, which stimulates cell division, differentiation, and sink establishment in stolon tissues. Cytokinins regulate tuber initiation by enhancing cellular activity and maintaining hormonal balance during tuber development. Raspor *et al*. (2012) [18] reported the involvement of cytokinins in potato tuberization, while Dessoky *et al*. (2016) [6] observed enhanced microtuber formation with kinetin supplementation.

Kinetin application increased the number of microtubers per plant, with the highest number recorded at 1 ppm (1.77 microtubers per plant) compared to 0.43 in the control. This improvement may be due to enhanced cell proliferation, assimilate translocation, and increased sink strength in developing tubers. Similar results were reported by Fufa and Diro (2014) [8] and Rahman *et al*. (2015) [17], who found that cytokinin application improved microtuber induction and development.

The superior response at 1 ppm kinetin indicates the importance of an optimum cytokinin concentration for efficient microtuber formation. Higher concentrations may reduce the response due to hormonal imbalance. Borna *et al*. (2019) [5] and Meenakshi (2020) [15] also reported the beneficial effects of kinetin on microtuber development. Furthermore, Ali *et al*. (2018) [2] highlighted the synergistic role of kinetin and sucrose in enhancing microtuber growth and quality. Therefore, 1 ppm kinetin can be considered an effective concentration for improving potato microtuberization under in-vitro conditions.

Kinetin-Mediated Enhancement of Potato Microtuber Fresh Weight: Similarly, the microtuber fresh weight in the 1 ppm kinetin treatment indicated a positive correlation

between kinetin application and improved tuber development in potato. However, in other kinetin treatments, specifically 0.5 ppm, the microtuber fresh weight was slightly higher (59.17 mg) compared to the control (25.83mg). Conversely, the 2 ppm kinetin treatment exhibited a significantly lower microtuber fresh weight (16.7 mg) compared to the control treatment (25.83 mg) (Figure 1c; Plate 1).

The results demonstrate a statistically significant increase in the fresh weight of microtubers in the group treated with 1 ppm kinetin in comparison to the control group. The mean fresh weight in the 1 ppm kinetin-treated group was 81.5 mg, while the control group exhibited a mean fresh weight of 25.83 mg. The difference between the two groups was found to be statistically significant underscoring the positive impact of kinetin on microtuber development (Figure 1c; Plate 1).

Cytokinin such as kinetin play a crucial role in cell division and differentiation processes. The exogenous application of cytokinin as reported by Hussein *et al*. (2017) has been shown to enhance tuberization in potato. The mechanism by which kinetin promotes microtuberization involves stimulating cell division in the subapical region of stolons ultimately leading to the formation of microtubers. Additionally, kinetin may influence hormonal balance particularly auxins, which are known to regulate tuber initiation and development (Zierer *et al*., 2021; Bharath and Mohan Raju, 2024) [20, 3]. The concentration of 1 ppm was chosen based on previous studies that indicated its effectiveness in promoting tuberization in various plant species (Abbasian *et al*., 2018; Dutta *et al.,* 2024) [1]. However, further studies may be necessary to optimize the concentration for different potato cultivars and under varying environmental conditions.

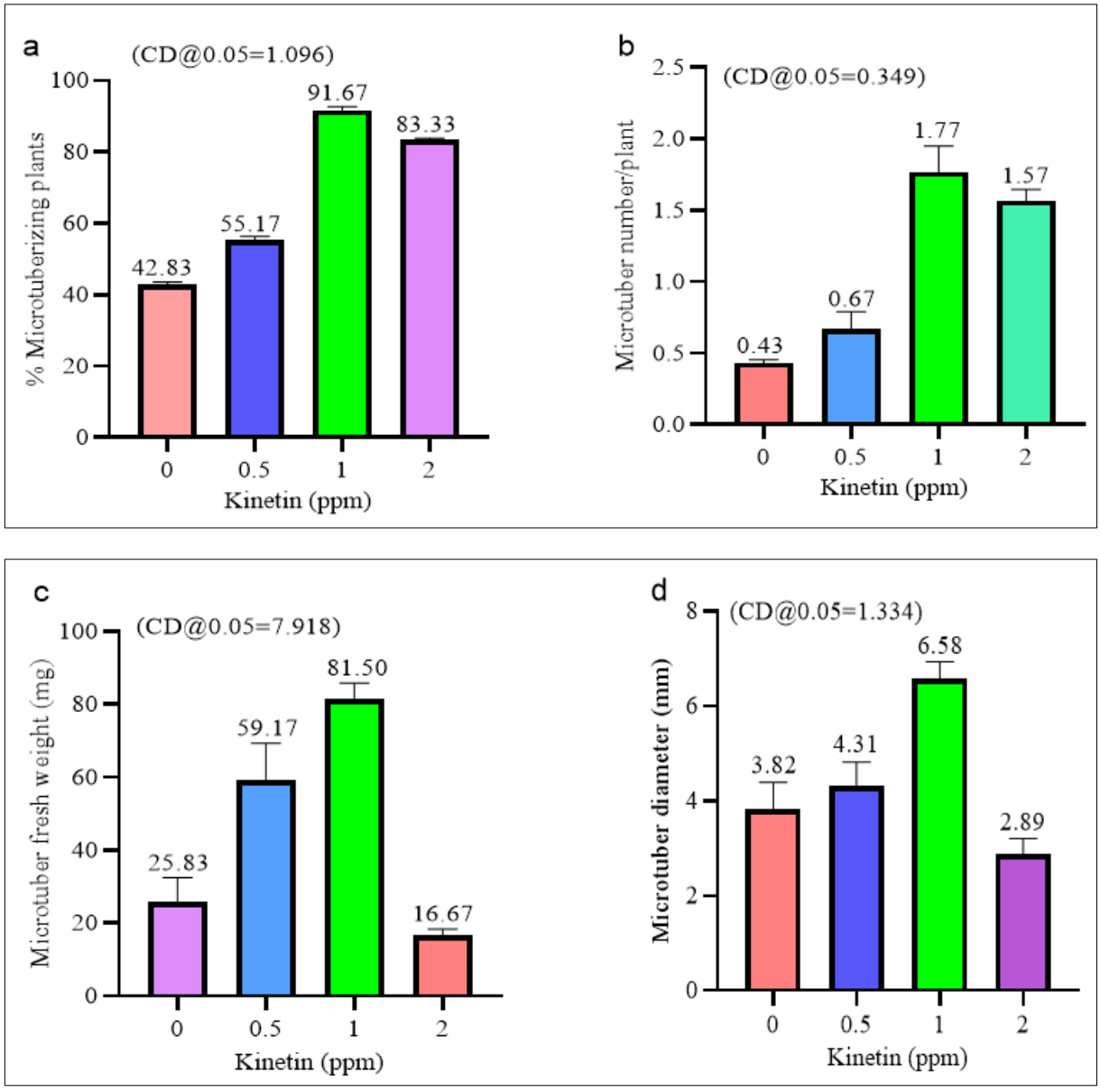

Fig 1: Effect of different concentrations of Kinetin on micro-tuberization response in potato. (a) Per cent micro-tuberizing plants (b) Micro-tuber number per plant (c) Micro-tuber fresh weight (mg) (d) Micro-tuber diameter (mm)

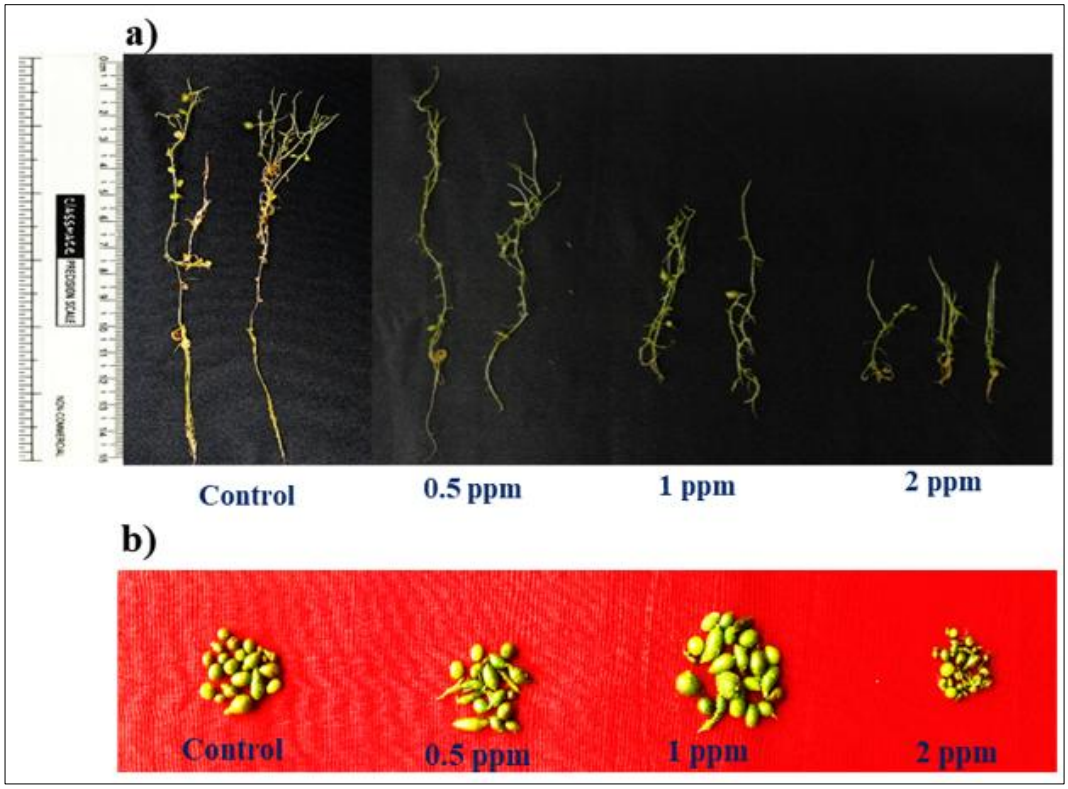

Plate 1: Effect of different concentrations of Kinetin on microtuberization (a) plant growth (b) microtuber.

Kinetin-Mediated Growth and Diameter of Potato Microtubers *In-Vitro*

The results indicated a significant impact of kinetin on microtuber development in potato plants with one notable finding being the microtuber diameter. At a concentration of 0.5 ppm kinetin resulted in a microtuber diameter of 4.31 mm slightly higher compared to the control treatment which had a microtuber diameter of 3.82 mm. However, at a higher concentration of kinetin (2 ppm), the treatment showed a significantly lower microtuber diameter compared to the control. In the 1 ppm concentration of kinetin-treated group, the mean microtuber diameter was 6.58 mm, whereas the control group exhibited a mean diameter of 3.82 mm. The increased microtuber diameter in the kinetin-treated group suggests that, the application of a 1 ppm concentration of kinetin has a positive influence on potato tuber development (Figure 1d; Plate 1).

The present experimental findings are consistent with the prior research on the influence of cytokinins on potato tuber development. For instance, Lal *et al*. (2022) [13] demonstrated that, applying cytokinins promotes tuberization leading to increased tuber yield in potato plants. Likewise, Martinez-Garcia *et al*. (2019) and Bharath and Mohan Raju, (2023) [4] found that, kinetin treatment resulted in larger tuber sizes in another Solanaceae species. It is crucial to note that, while our study showed a significant impact on microtuber diameter at a concentration of 1 ppm of kinetin, higher concentrations may not necessarily yield the same positive effects.

The results obtained in the study corroborate with other workers. In a study by Hoque (2010) [10], *in vitro* tuberization was examined using potato shoot apex and nodal cutting explants on MS medium supplemented with varying concentrations of Kinetin for micro-tuber production. Kinetin identified as one of the plant hormones influencing micro-tuberization *in vitro* was explored in single nodal cuttings by Dessoky *et al*. (2016) [6].

Conclusion

The present study established that kinetin plays a significant role in regulating growth and enhancing microtuberization of potato under in-vitro conditions. Although kinetin application reduced vegetative growth attributes such as plant height, number of nodes, and leaves compared to the control, it significantly promoted microtuber initiation and development. Among the tested concentrations, 1 ppm kinetin exhibited the best response by achieving the highest percentage of microtuberizing plants (91.67%), maximum number of microtubers per plant, increased microtuber fresh weight (81.5 mg), and larger microtuber diameter (6.58 mm). Higher kinetin concentration (2 ppm) showed reduced effectiveness, indicating that an optimum hormonal balance is essential for efficient tuber development. Overall, the findings suggest that 1 ppm kinetin is an effective concentration for improving potato microtuber production and can be utilized in in-vitro propagation systems for quality planting material production. Further studies involving different potato cultivars and optimized combinations of growth regulators may help enhance the efficiency and commercial application of microtuberization techniques.

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Conflict of interest

The authors disclose no conflict of interest

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