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Thidiazuron-induced regulation of growth and microtuber development in Potato

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Abstract
Thidiazuron (TDZ), a cytokinin-like plant growth regulator, plays an important role in promoting microtuberization in potato. The present study evaluated the effect of TDZ at 0.5, 1.0, and 2.0 ppm on the growth and microtuberization of potato under controlled *in vitro* conditions using genetically uniform plants. TDZ application significantly suppressed vegetative growth, with plant height, number of nodes and leaves decreasing progressively as TDZ concentration increased. In contrast, TDZ markedly enhanced the microtuberization process. The highest concentration (2.0 ppm) produced the greatest percentage of microtuberizing plants (77.67%), compared with 43.50% in the control, and significantly increased microtuber fresh weight (40.33 mg) and diameter (4.59 mm). Although the number of microtubers per plant increased slightly with increasing TDZ concentration (1.10 at 2.0 ppm versus 0.42 in the control), differences among TDZ treatments were not statistically significant. The results demonstrate a dose-dependent effect of TDZ, where higher concentrations suppress vegetative growth while promoting microtuber initiation and enlargement. Among the concentrations tested, 2.0 ppm TDZ was the most effective for enhancing microtuber production and quality, suggesting its potential application in efficient in vitro seed potato production systems.

Keywords: Potato, Thidiazuron, microtuber, *In vitro*, concentration

Introduction
Potato (*Solanum tuberosum* L.) is one of the world's most important food crops, serving as a major source of carbohydrates, vitamins, and minerals for millions of people. The production of disease-free and high-quality planting material is essential for sustaining potato productivity. Conventional seed tuber multiplication is often constrained by low multiplication rates, pathogen transmission, and high storage and transportation costs (Bharath and Mohan Raju, 2023) [2]. *In vitro* microtuber production has emerged as an efficient alternative for rapid multiplication of pathogen-free seed material and serves as a valuable tool for germplasm conservation, breeding, and commercial seed potato production (Naik and Buckseth, 2018) [8].

Microtubers are miniature tubers produced under *in vitro* conditions through the manipulation of environmental factors and culture media (Bharath and Mohan Raju, 2023) [2]. Their formation is regulated by several factors, including genotype, sucrose concentration, and photoperiod, temperature, and plant growth regulators. Among these, cytokinins play a crucial role in regulating cell division, shoot differentiation, and tuber induction. Optimizing the type and concentration of plant growth regulators is therefore critical for improving the efficiency of microtuber production (Uyen and Struik, 2021) [10].

Thidiazuron (TDZ), a phenylurea-derived cytokinin-like plant growth regulator, has gained considerable attention because of its strong cytokinin activity and its ability to regulate morphogenesis in a wide range of plant species (Bharath and Mohan Raju, 2024) [1]. Besides stimulating shoot regeneration, TDZ has been reported to promote tuber induction by influencing endogenous cytokinin metabolism and interacting with auxin and gibberellin pathways (Yagiz *et al*., 2020). Previous studies have demonstrated that TDZ enhances microtuber formation in potato by promoting cell division, differentiation, and the expression of genes associated with tuber development (Celikel *et al*., 2021) [3]. However, its effects are highly concentration-dependent, with different TDZ levels exerting varying influences on vegetative growth and microtuber development (Nagy *et al*., 2023) [7].

Although several reports have highlighted the potential of TDZ in promoting potato microtuberization, information regarding the optimum concentration that maximizes microtuber induction while maintaining desirable microtuber characteristics remains limited. Understanding the concentration-dependent effects of TDZ is therefore important for developing efficient *in vitro* propagation protocols suitable for commercial seed potato production.

The present study was undertaken to evaluate the influence of different concentrations of TDZ (0.5, 1.0, and 2.0 ppm) on *in vitro* microtuberization of potato. The effects of TDZ on vegetative growth parameters, including plant height, number of nodes, and number of leaves, as well as microtuberization percentage, number of microtubers, fresh weight, and microtuber diameter, were assessed to identify the most effective concentration for enhanced microtuber production.

Material and Methods

To study the Influence of Thidiazuron (TDZ) on microtuberization in potato

In this study, the effect of Thidiazuron (TDZ) on microtuberization was examined under *in vitro* grown plants growing on MS media supplemented with sucrose were given different concentrations of Thidiazuron (TDZ). These Thidiazuron were supplied to the MS media at the rate of 0, 0.5, 1.0 and 2.0 ppm separately. After the treatments imposition, plants were kept at a temperature of 18 °C under short day conditions. At the end of 45 days, the growth observations such as plant height (cm), number of nodes, number of leaves, per cent microtuberizing plant (%), number of microtubers per plant, fresh weight of microtubers (mg) and microtuber diameter (mm) were made and continued up to 90 days after inoculation. The entire experiment was conducted with an adequate number of replicates to ensure the reliability and repeatability of the results. Control was included in the experiment to validate the results.

Results and Discussion

Study the Influence of TDZ on microtuberization in potato

Thidiazuron, a cytokinin analog is renowned for its involvement in stimulating various physiological processes in plants particularly its impact on microtuberization in potato (Celikel *et al*., 2021) [4]. Extensive research has demonstrated Thidiazuron's capacity to induce microtuberization in potato cultures (Yagiz *et al*., 2020). The induction of microtubers by Thidiazuron appears to be concentration-dependent, with different concentrations of TDZ producing diverse effects on microtuber initiation and development in potato.

The experiment was conducted in a controlled environment using potato plants of identical age and genetic background with the application of thidiazuron to the experimental group at a concentration of 0.5 ppm, 1 ppm and 2 ppm, while the control group received no treatment.

The study explored the impact of Thidiazuron on microtuberization in potato revealing a significant difference in plant height among various treatments. In the control group without Thidiazuron, the plant height was 14 cm, while the 0.5 ppm Thidiazuron treatment exhibited a reduced plant height of 10.16 cm. Furthermore, the 1 and 2 ppm Thidiazuron treatments resulted in lower plant heights of 7.25 cm and 5.63 cm, respectively (Table 1; Plate 1a). These findings suggest that, the presence of Thidiazuron had a suppressive effect on potato plant growth leading to a decrease in plant height. Thidiazuron not only influenced cytokinin pathways but may also affect auxin and gibberellin dynamics leading to observed alterations in plant height (Cosic *et al*., 2022). Thidiazuron might induce a shift in resource allocation towards tuberization at the expense of vegetative growth (Table 1; Plate 1a).

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

Thiadiazuron (TDZ)			
Treatments	**Plant height (cm)**	**Number of nodes**	**Number of leaves**
Control	14[a]	42.06[a]	41.65[a]
TDZ-0.5 ppm	10.16[b]	10.16[b]	11.18[b]
TDZ-1.0 ppm	7.25[c]	6.16[c]	8.43[c]
TDZ-2.0 ppm	5.63[d]	5.38[d]	7.8[c]
CD (P<0.01)	0.475**	0.48**	0.595**
SE(m)	0.16	0.161	0.20
SE(d)	0.226	0.228	0.283
CV (%)	4.224	2.48	2.842

** Significant at 1% level

The results of our study clearly explain that, the plants treated with 2 ppm of Thidiazuron exhibited a significantly lower number of nodes per plant (5.38) compared to the control group (42.06). Notably, concentrations of 0.5, 1.0 and 2 ppm of Thidiazuron resulted in 10.16, 6.16, and 5.38 nodes per plant, respectively. Therefore, an increase in Thidiazuron concentration had a suppressive effect on the number of nodes per plant (Table 1; Plate 1a).

The study focused on assessing microtuberization and monitoring the number of leaves per plant. A substantial difference in the number of leaves per plant was observed between the control group and the Thidiazuron-treated groups. In this study, the number of leaves per plant in the control treatment was 41.65, which was highly significant compared to the Thidiazuron treatments at a concentration of 0.5, 1.0 and 2 ppm. Specifically, the number of leaves per plant for these concentrations was 11.8, 8.43, and 7.8, respectively. Consequently, it was observed that, higher concentrations of Thidiazuron had a suppressive effect on the number of leaves per plant (Table 1; Plate 1a).

The results showed a significant increase in the percentage of microtuberizing plants in the Thidiazuron treatment (77.67 %) compared to the control treatment (43.50%). However, 0.5 ppm and 1 ppm concentrations of Thidiazuron resulted in per cent microtuberizing plants of 58.33 % and 67 %, respectively. The percentage of plants exhibiting microtuber formation was significantly higher in the 2 ppm concentration of Thidiazuron treatment group (67 %) compared to the control group (43.50 %). The application of Thidiazuron demonstrated a pronounced effect on microtuberization in potato plants with a notable 77.67 % occurrence of microtuberization observed in the Thidiazuron treatment (Figure 1a; Plate 1b). This enhancement suggests that, Thidiazuron has a stimulatory effect on the initiation and development of microtubers in potato plants.

Thidiazuron is a cytokinin-like plant growth regulator that stimulates shoot proliferation and enhances tuberization in various plant species. The observed increase in microtuberization can be attributed to Thidiazuron's activity promoting cell division and differentiation leading to microtuber formation. This aligns with previous studies demonstrating cytokinin-induced enhancement of tuberization in potato (Pavlista *et al*., 2003) [9]. In addition, Thidiazuron influences the expression of genes related to tuberization in potato (Nagy *et al*., 2023) [7].

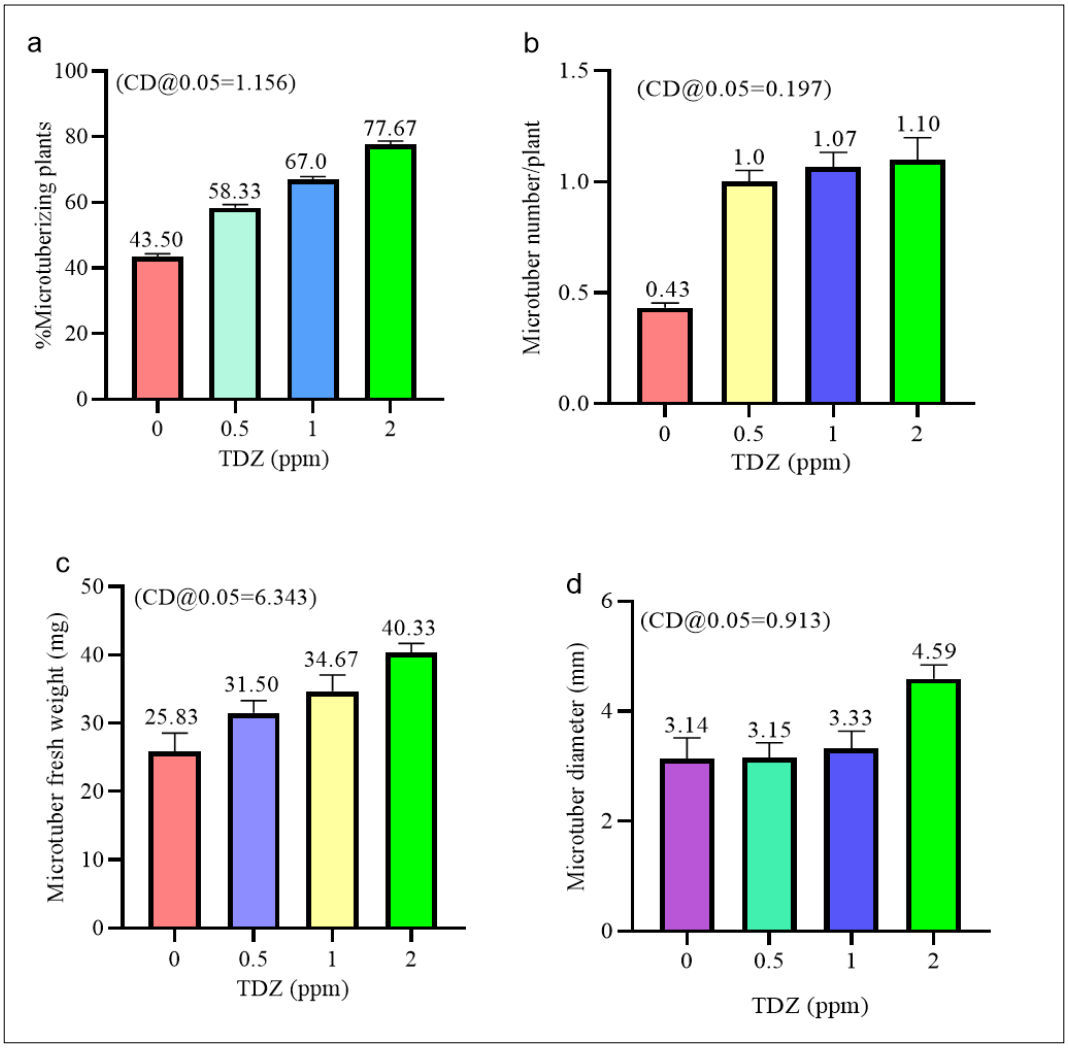

Fig 1: Effect of different concentrations of TDZ on micro-tuberization response in potato. (a) Per cent micro-tuberizing plants (b) Micro-tuber number per plant (c) Micro-tuber fresh weight (mg) (d) Micro-tuber diameter (mm).

A concentration of 0.5 ppm of Thidiazuron resulted in a significantly higher number of microtubers per plant (1.0) compared to the control treatment (0.43). Similarly, 1 ppm concentration of Thidiazuron showed a higher number of microtubers per plant (1.07) compared to the control. Interestingly, higher concentrations of Thidiazuron led to a significantly greater number of tubers per plants (1.10) compared to the control treatment (0.42) (Figure 1b; Plate 1b). Overall, the findings suggest that, higher concentrations of Thidiazuron are associated with an increased number of microtubers per plant and vice versa. However, across different concentrations of thiodiazuron, no significant difference was observed for microtuber number.

The results of our study indicated that, Thidiazuron concentration of 0.5 ppm led to a non-significantly higher microtuber fresh weight (31.50 mg) compared to the control group (25.83 mg). However, the microtuber fresh weight at 1 ppm and 2 ppm concentrations of Thidiazuron was significantly elevated, measuring 34.67 mg and 40.33 mg, respectively, compared to the control group (25.83 mg) (Figure 1c; Plate 1b). These findings suggest a positive impact of Thidiazuron on the development and growth of microtubers in potato. The observed increase in microtuber fresh weight in the Thidiazuron-treated groups implies that, Thidiazuron may play a crucial role in promoting tuberization and enhancing the overall yield of microtubers in potato.

The results demonstrated a non-significant increase in microtuber diameter in the group treated with 0.5 ppm and 1 ppm concentration of TDZ compared to the control treatment. However, a higher concentration of Thidiazuron resulted in a significant increase in microtuber diameter in potato (4.59 mm) compared to the control. The trend, however, suggests that, increasing the concentration of Thidiazuron gradually enhances the microtuber diameter in potato. This enhanced microtuber diameter was consistently observed across various concentrations of TDZ indicating a dose-dependent effect on media concentration. The microtubers in the Thidiazuron treatment group displayed a significantly larger size compared to those in the control group, indicating that, Thidiazuron has a promotional effect on the development and enlargement of microtubers during the tuberization phase of potato growth (Figure 1d; Plate 1b).

It was shown that liquid MS medium, incorporating Thidiazuron and utilizing whole plants as explants proved to be effective for efficient microtuber production in potato Yagiz *et al*., (2020), Furthermore, Isam *et al*. (2022) [6] demonstrated that, shoot induction was successfully achieved from callus treated with an MS medium containing Thidiazuron. Subsequent cultivation of plantlets on MS medium enriched with Thidiazuron and either 80 or 90 g/l sucrose for two months resulted in a substantial formation of microtubers per explant.

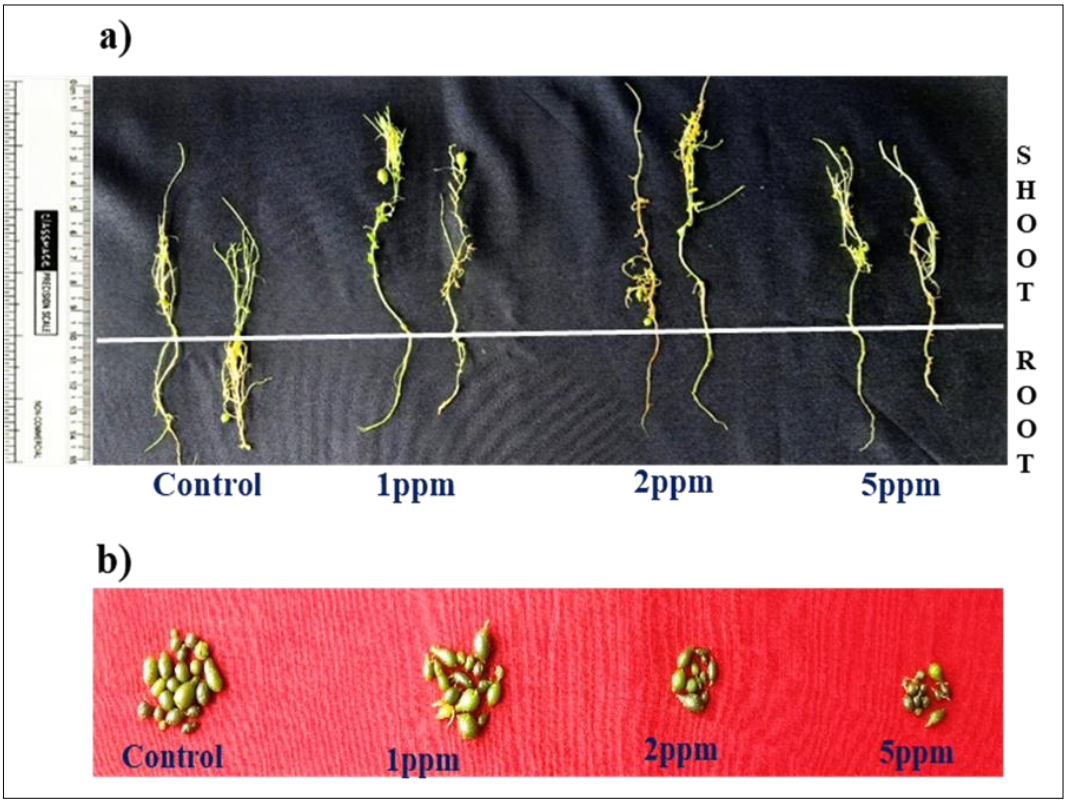

Plate 1: Effect of different concentrations of CCC on microtuberization (a) plant growth and (b) microtubers

Conclusion

The present study demonstrated that thidiazuron (TDZ) significantly influences in vitro microtuberization in potato by suppressing vegetative growth while enhancing microtuber induction and development. Increasing TDZ concentration from 0.5 to 2.0 ppm progressively reduced plant height, number of nodes, and number of leaves, indicating a shift in plant growth from vegetative development toward tuber formation. Conversely, TDZ significantly improved the percentage of microtuberizing plants, microtuber fresh weight, and microtuber diameter. Although the number of microtubers per plant increased with increasing TDZ concentration, the differences among TDZ treatments were not statistically significant. Among the concentrations evaluated, 2.0 ppm TDZ proved to be the most effective, producing the highest microtuberization percentage along with superior microtuber size and fresh weight. These findings suggest that TDZ is an effective plant growth regulator for enhancing *in vitro* potato microtuber production and can be utilized to improve microtuber quality for seed potato production. Further studies on genotype-specific responses and the interaction of TDZ with other growth regulators and culture conditions would help optimize protocols for large-scale commercial microtuber production.

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Conflict of Interest

The authors disclose no conflict of interest

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