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Auxin-mediated regulation of *in vitro* potato microtuberization: Differential roles of IAA, NAA and 2,4-D in growth and tuber development

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Abstract

This study evaluated the influence of three auxins, namely Indole-3-acetic acid (IAA), Naphthaleneacetic acid (NAA), and 2,4-dichlorophenoxyacetic acid (2,4-D), on *in vitro* microtuberization in potato at two concentrations (1 and 2 ppm). Growth parameters, including plant height, number of nodes, number of leaves, percentage of microtuberization, microtubers per plant, microtuber fresh weight, and microtuber diameter, were assessed and compared with an untreated control. Among the treatments, 1 ppm IAA significantly enhanced vegetative growth and microtuberization, producing the highest plant height, node number, leaf number, microtuberization percentage (48.6%), and microtuber fresh weight (31.8 mg); 2 ppm IAA completely inhibited microtuber formation. In contrast, NAA reduced vegetative growth but markedly promoted microtuber induction. The 2 ppm NAA treatment recorded the highest microtuberization percentage (68.4%), the greatest number of microtubers per plant (0.72), the highest fresh weight (39.4 mg), and the largest microtuber diameter (4.43 mm). Conversely, 2,4-D negatively affected microtuberization, with 2 ppm completely suppressing microtuber formation despite increasing node and leaf numbers. The findings demonstrate that auxin type and concentrations exert distinct effects on potato microtuberization. While low concentrations of IAA favor vegetative growth and moderate microtuber induction, NAA, particularly at 2 ppm, is more effective for enhancing microtuber yield and quality. In contrast, 2,4-D is unsuitable for *in vitro* microtuber production due to its inhibitory effects on tuber initiation and development. These results provide useful insights for optimizing hormone-based protocols for efficient potato microtuber production.

Keywords: Microtuber, Indole-3-acetic acid (IAA), Naphthalene acetic acid (NAA), and 2,4-dichlorophenoxyacetic acid (2,4-D), Temperature

Introduction

Potato (*Solanum tuberosum* L.) is one of the most important food and cash crops worldwide, serving as a major source of carbohydrates, vitamins, minerals, and dietary fiber. In addition to its nutritional value, potato plays a vital role in ensuring global food security owing to their high productivity and adaptability to diverse agro-climatic conditions (Kloosterman *et al.*, 2013) [9]. The availability of healthy, disease-free seed tubers is essential for sustainable potato production. However, conventional seed tuber multiplication is slow and often limited by the accumulation of pathogens, seasonal availability, and the high cost of transportation and storage (Gopal and Chauhan, 2010) [8]. Consequently, *in vitro* microtuberization has emerged as an efficient biotechnology-based approach for the rapid multiplication, conservation, and distribution of high-quality seed material (Seabrook, 2005; Gopal and Chauhan, 2010) [26, 8]. Microtuberization is a complex developmental process regulated by genetic, environmental, and physiological factors. Among these, plant growth regulators play a pivotal role in coordinating tuber induction, initiation, and enlargement (Bharath and Mohan Raju, 2024) [3]. Plant hormones, particularly auxins, are key regulators of plant growth and development, influencing cell division, cell elongation, vascular differentiation, organogenesis, and sink establishment (Sauer *et al.*, 2013) [25]. Their coordinated interaction with other phytohormones governs developmental transitions, including the formation of storage organs such as potato tubers.

Among naturally occurring auxins, indole-3-acetic acid (IAA) is the principal endogenous auxin involved in numerous physiological processes, including cell expansion, apical dominance, root initiation, and tissue differentiation. During tuber development, IAA regulates stolon growth, carbohydrate partitioning, and the early stages of tuber initiation (Roumeliotis *et al.*, 2012) [23]. Low concentrations of exogenous IAA have been reported to stimulate microtuber induction and improve tuber quality, whereas excessive concentrations may disrupt hormonal homeostasis and suppress tuber formation. These concentration-dependent responses suggest that precise regulation of IAA is critical for successful *in vitro* microtuberization (Leyser, 2018) [15].

Naphthalene acetic acid (NAA), a synthetic auxin with greater stability than IAA, has been widely employed in plant tissue culture to enhance organogenesis, root induction, and storage organ development. Previous studies have demonstrated that NAA can improve microtuber induction, increase tuber number, and enhance tuber size by promoting cell division and assimilate accumulation. Nevertheless, its effectiveness varies with genotype, culture conditions, and hormone concentration, emphasizing the importance of optimizing its application for efficient microtuber production (Kondhare *et al.*, 2021a; Pruski *et al.*, 2002) [13, 20].

In contrast, 2,4-dichlorophenoxyacetic acid (2,4-D) is a synthetic auxin primarily used for inducing callus formation and somatic embryogenesis in plant tissue culture (Thorpe, 2007) [28]. Although it exhibits strong auxin-like activity, prolonged exposure or high concentrations frequently inhibit organ differentiation and normal morphogenesis. In potato, the influence of 2,4-D on microtuberization remains less understood, and its role may differ substantially from that of IAA and NAA because of its persistent physiological effects and potential disruption of endogenous hormonal balance (Duan *et al.* 2023) [6]. Despite considerable progress in understanding auxin biology, comparative information regarding the influence of natural and synthetic auxins on potato microtuberization remains limited. Direct comparisons of IAA, NAA, and 2,4-D under identical *in vitro* conditions are particularly scarce, leaving a clear gap in optimizing microtuber production protocols. Elucidating these differential responses will improve our understanding of

hormone-mediated regulation of tuber development and contribute to more efficient propagation systems (Salome Prat, 2010) [24].

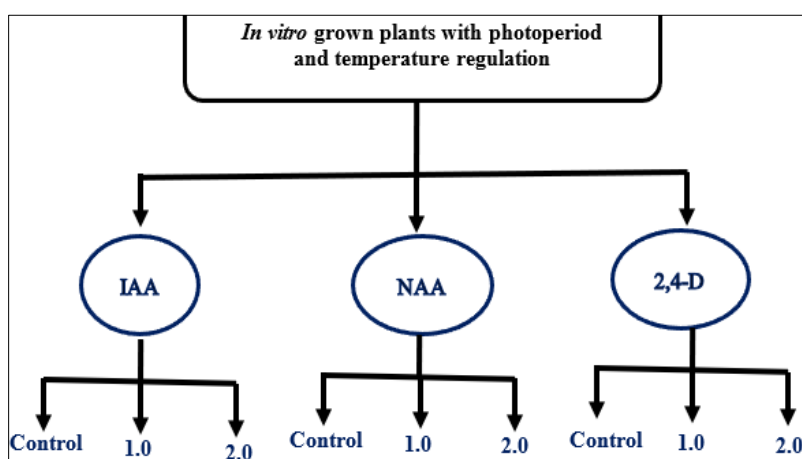
Therefore, the present study was undertaken to evaluate the differential effects of IAA, NAA, and 2,4-D at two concentrations (1 and 2 ppm) on *in vitro* potato microtuberization. The study investigated their influence on vegetative growth traits, including plant height, number of nodes, and number of leaves, as well as microtuberization percentage, microtubers per plant, microtuber fresh weight, and microtuber diameter. The findings provide insights into auxin-mediated regulation of potato microtuber development and identify suitable auxin treatments for enhancing *in vitro* microtuber production.

Materials and Methods

The current study aimed to generate a substantial quantity of high-quality potato planting material through the *in vitro* propagation method. The objective was to optimize various growth factors such as photoperiods, carbon sources, plant hormones, temperature and light. The research was carried out at the Plant Tissue Culture Laboratory, Department of Crop Physiology, University of Agricultural Sciences, Bangalore. This chapter provides details regarding the experimental material used and outlines the methods employed in the investigation.

To study the influence of auxins on microtuberization in potato

Some reports in the past showed the positive effect of auxins on tuberization (Kondhare *et al.*, 2021a and Wang *et al.*, 2018a) [13, 29]. In this context, the effect of different sources of auxin on tuberization was examined in the present study. Auxins such as IAA, NAA and 2,4-D at a concentration of 0, 0.5 and 1ppm were supplemented to the MS media separately and the plants were grown under *in vitro* condition. A temperature of 18 °C was maintained and the plants were kept under short day conditions. After 45 days of incubation, the growth observation such as percent microtuberizing plant (%), number of microtubers per plant, fresh weight of microtuber (mg) and microtuber diameter (mm) were made and continued up to 90 days after inoculation (Flowchart 1).



Flowchart 1: Schematic representation of different auxin sources viz. Indole acetic acid (IAA), Naphthalene acetic acid (NAA) and 2,4-dichlorophenoxy acetic acid (2,4-D) on microtuberization in potato.

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 Auxins on microtuberization in potato

This research aimed to explore the influence of Indole Acetic Acid (IAA) treatment on microtuberization in potato plants specifically, examining plant height, node count and leaf count. Two different concentrations of IAA, 1 ppm and 2 ppm were administered and their effects were compared to a control treatment.

IAA-Induced Morphogenesis and Growth Regulation in Potato (*Solanum tuberosum* L.)

The findings indicate that, applying 1 ppm concentration of IAA resulted in significantly increased plant height compared to the control treatment. However, plant height under 2 ppm IAA concentration (6.40 cm) was comparable to the control treatment (6.44 cm) with lower height observed under the 1 ppm IAA treatment. In summary, a higher concentration of IAA demonstrated a suppressive effect on plant height (Table 1). The group treated with 1 ppm of IAA displayed a significantly higher number of nodes compared to the control group. On the contrary, the 2 ppm IAA concentration led to a lower number of nodes per plant (6.6) compared to the control treatment (8.02). The results indicated a significant increase in the number of nodes per plant in the 1 ppm IAA treatment suggesting a positive influence on node development in potato plants at this concentration (Table 1). There was a notable difference in the number of leaves per plant between the IAA treated group and the control group. Specifically, potato plants treated with a 1 ppm concentration of IAA exhibited a significantly higher number of leaves (10.56) compared to those in the control treatment (8.04). Conversely, the 2 ppm IAA concentration resulted in a lower number of leaves per plant (6.56) compared to the control treatment (8.04). In conclusion, the 1 ppm concentration of IAA treatment was identified as the optimal concentration for promoting more leaves per plant in relation to microtuberization in potatoes (Table 1).

Table 1: Effect of IAA on plant growth in potato under in-vitro condition.

Treatments	IAA		
	Plant height (cm)	Number of nodes	Number of leaves
Control	6.44 ^b	8.02 ^b	8.04 ^b
IAA-1 ppm	9 ^a	10.6 ^a	10.56 ^a
IAA-2 ppm	6.4 ^b	6.6 ^c	6.56 ^c
CD (<i>p</i> < 0.01)	0.344**	0.183**	0.272**
SE(m)	0.11	0.059	0.087
SE(d)	0.156	0.083	0.123
CV (%)	3.393	1.566	2.324

** Significant at 1% level

Influence of Indole-3-Acetic Acid on Microtuber Induction and Development in Potato Under In-Vitro Conditions

The findings revealed a notable disparity in the percentage of plants undergoing microtuberization between the IAA-treated group and the control group. The group exposed to 1 ppm IAA exhibited a substantially higher percentage of plants (48.6%) developing microtubers compared to the control group (24.4%). Conversely, 2 ppm concentration of IAA completely prevented microtuber formation recording a 0% compared to the control treatment. Consequently, a higher concentration of IAA negatively impacted microtuberization in potato (Figure 1a). The experiment's results on microtubers per plant underscored the significant influence of IAA treatment on microtuberization in potato plants. The group treated with 1 ppm IAA displayed a

significantly greater number of microtubers per plant (0.48) compared to the control group (0.24). Conversely, the 2 ppm IAA treatment inhibited microtuber formation entirely registering 0.0 microtubers compared to the control treatment. Ultimately, higher concentrations of IAA suppressed the microtuber development in potato. Therefore, 1 ppm concentration of IAA is deemed optimal for inducing microtuber formation in potato (Figure 1b). The study also unveiled a substantial improvement in microtuber fresh weight in plants treated with 1 ppm IAA compared to the control group. Plants treated with 1 ppm IAA demonstrated a noticeable increase in microtuber fresh weight (31.8 mg) compared to the control group (27.0 mg). However, the 2 ppm concentration exhibited inhibitory effects on microtuber fresh weight compared to the control treatment. Statistical analysis confirmed a significant difference between the two treatments with the experimental group displaying a higher mean fresh weight of microtubers (Figure 1c). Indole acetic acid (IAA), a well-known auxin, plays a crucial role in regulating various physiological processes including cell expansion and differentiation thereby influencing plant growth and development (Elsabagh *et al.*, 2022; Bharath and Mohan Raju, 2023) [7, 4]. Prior studies have suggested that, auxins such as IAA, plays a key role in initiating tuber formation in potato plants (Sorce and Lorenzi, 1995) [27]. To gain a comprehensive understanding of the positive impact of IAA on microtuberization, further research is imperative to unravel the specific genetic and molecular mechanisms at play. Examining the gene expression patterns and signaling pathways activated by IAA can yield valuable insights into the regulatory networks governing tuber development in potato (Kolachevskaya *et al.*, 2019) [11]. The results revealed a non-significant difference in microtuber diameter between the IAA-treated group and the control group. In the experimental group treated with 1 ppm concentration of IAA, the microtubers displayed a notably larger diameter (3.59 mm) compared to the control group (3.16 mm). Conversely, higher concentration of IAA had no tuber. This suggests that, applying IAA at a concentration of 1 ppm positively influences the development and growth of microtubers in potato (Figure 1d).

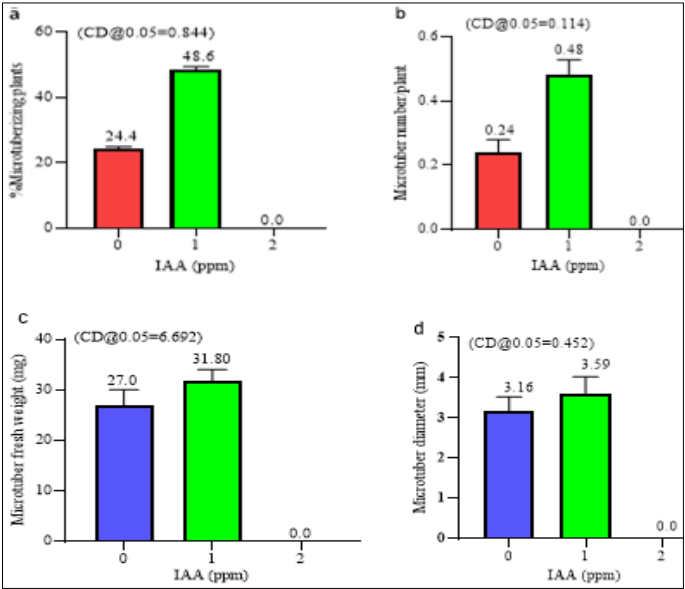


Fig 1: Effect of different concentrations of Auxins source (IAA) on micro-tuberization response in potato. (a) Per cent micro-tuberizing plants (b) Micro-tuber number per plant (c) Micro-tuber fresh weight (mg) (d) Micro-tuber diameter (mm)

Microtuberization is a pivotal process in potato cultivation significantly impacting both yield and tuber quality. Plant hormones, particularly auxins like Indole Acetic Acid (IAA) plays a crucial role in regulating various plant developmental processes. The IAA is renowned for stimulating cell expansion and enhancing nutrient transport within plants (Mondal *et al.*, 2020) [18]. Consequently, promoting cell enlargement in developing tubers may contribute to the larger microtuber size observed in the present study. Additionally, IAA is implicated in the formation of adventitious roots indirectly influencing tuberization by enhancing nutrient uptake and assimilation (Larskaya *et al.*, 2015) [14].

The study by Wang *et al.* (2018a) [29] suggests that, low concentrations of IAA promote tuberization affecting various cellular processes, while higher concentrations exhibit inhibitory effects offering new insights into the molecular mechanisms regulating potato tuberization. Peng *et al.* (2012) [19] indicated that, exogenous auxins enhance the production and quality of potato microtubers benefiting their overall growth. Moreover, Zhang *et al.* (2005) [30] found that, the shoot length, root number and root fresh weight of potato explants increase with higher concentrations of IAA treatment indicating an interactive effect on microtuberization. Borna *et al.* (2019) [5] demonstrated that, MS media supplemented with IAA yields the best response for *in vitro* microtuber induction from stem cuttings, establishing an efficient shoot regeneration system from these *in vitro* raised microtubers.

NAA-Induced Morphogenesis and Growth Regulation in Potato (*Solanum tuberosum* L.)

The control group demonstrated a significantly greater plant height (6.44 cm) compared to the 1 ppm and 2 ppm NAA treatments (3.28 cm and 1.95 cm, respectively). Plants in the control group exhibited robust vertical growth indicating a positive influence on overall plant height without the addition of NAA. This implies that, under the specified experimental conditions, the absence of NAA favored increased plant height. In contrast to the control group, plants treated with 1 ppm and 2 ppm concentrations of NAA displayed reduced plant height. The decline in plant height in the NAA-treated group (3.28 cm and 1.95 cm) signifies a suppressive effect of NAA concentrations on vertical growth. The inhibitory impact on plant height may be linked to the specific concentration of NAA used in the treatment suggesting a concentration-dependent response (Table 2).

Table 2: Effect of NAA on plant growth in potato under *in-vitro* condition.

Treatments	NAA		
	Plant height (cm)	Number of nodes	Number of leaves
Control	6.44 ^a	8.02 ^a	8.04 ^a
NAA-1 ppm	3.28 ^b	5.44 ^b	5.38 ^b
NAA-2 ppm	1.95 ^c	3.94 ^c	3.87 ^c
CD (P<0.01)	0.106**	0.135**	0.268**
SE(m)	0.034	0.043	0.086
SE(d)	0.048	0.061	0.122
CV (%)	1.963	1.666	3.334

** Significant at 1% level

Auxins such as NAA has been recognized as regulators influencing tuber formation in potato by controlling the expression of genes involved in tuber initiation (Liu *et al.*, 2014) [17]. These findings contradict some studies suggesting positive correlations between exogenous auxin application and tuberization (Klopotek *et al.*, 2010) [10]. However, reports on

concentration dependent effects of auxins on potato physiology indicate that, an optimal concentration is crucial for the desired response (Liu *et al.*, 2014) [17]. It is possible that, the concentration used in this study surpassed the threshold for promoting tuberization.

The control treatment exhibited a significantly higher number of nodes per plant (8.02) compared to plants treated with 1 ppm of NAA (5.44). However, the 2 ppm concentration of NAA treatment significantly reduced the number of nodes per plant (3.94) compared to the control treatment (8.02). This result suggests that, the application of NAA at a concentration of 2 ppm had a suppressive effect on the development or proliferation of nodes in potato plants, potentially influencing microtuber formation (Table 2). Alexopoulos *et al.*, (2006) [1] demonstrated that, the exogenous application of auxins including NAA, can impact tuberization in potato. Similarly, Kolachevskaya *et al.*, (2017) [12] discovered that, the application of auxins influences both the number and size of tubers in potato. These studies collectively support the notion that, NAA as an auxin can indeed influence the potato tuberization process.

The experiment unveiled a notable difference in the number of leaves per plant between the control group and the 2 ppm NAA treatment. The control treatment displayed a statistically significant increase in leaf count (8.04) compared to the 1 ppm and 2 ppm NAA-treated plants which showed counts of 5.38 and 3.87, respectively. Potato plants in the control group exhibited a robust vegetative growth pattern characterized by a higher number of leaves per plant with a mean leaf count of 8.04 indicating vigorous foliar development (Table 2).

Conversely, the application of NAA at a concentration of 1 ppm and 2 ppm resulted in a noticeable impact on leaf development. The treated plants showed a statistically significant reduction in the number of leaves per plant compared to the control. The mean leaf count for the 1 ppm and 2 ppm NAA treatments was 5.38 and 3.87 leaves per plant, respectively (Table 2).

NAA-Mediated Regulation of Microtuberization and Tuber Development in Potato

The analysis of results unveiled a statistically significant difference in the percentage of plants undergoing microtuberization between the NAA-treated group and the control group. Specifically, the group treated with 1 ppm NAA demonstrated a significantly higher percentage of plants (60.6%) undergoing microtuberization compared to the control group (24.4%). Interestingly, the group treated with 2 ppm NAA exhibited a significantly higher percentage of plants (68.40%) undergoing microtuberization compared to the control group (24.4%). This discovery suggests that the application of NAA at this concentration positively influences the initiation and development of microtubers in potato plants (Figure 2a).

The average number of microtubers per plant was significantly higher in 1 and 2 ppm NAA-treated group (0.68 and 0.72 tubers, respectively) compared to the control group (0.24). This observation indicates that the application of either 1 or 2 ppm concentration of NAA positively influence the formation of microtubers in potato plants (Figure 2b).

The results demonstrate a statistically significant difference in microtuber fresh weight between the 1 ppm and 2 ppm NAA treatment groups. Microtubers from the 2 ppm NAA-treated plants exhibited a significantly higher fresh weight (39.4 mg) compared to those from the control group (27 mg). Interestingly, the 2 ppm concentration of NAA showed a significantly higher microtuber fresh weight (39.40 mg) compared to the control treatment (27 mg). The observed increase in microtuber fresh

weight in the 2 ppm NAA treatment group suggests a positive influence of 2 ppm NAA on tuber development in potatoes (Figure 2c).

The experiment's results revealed a statistically significant difference in microtuber diameter between the NAA-treated group and the control group. Microtubers from the 2 ppm NAA-treated group exhibited a higher average diameter (4.43 mm) compared to those from the control group (3.16 mm). However, between control 1 ppm NAA, non-significant differences observed. This finding suggests that, Naphthalene Acetic Acid at a concentration of 2 ppm positively influence microtuberization in potato (Figure 2d).

The findings from the current experiment align with previous reviews particularly the work of Kondhare *et al.* (2021a) [13] who emphasized the crucial role of auxin biology in the formation of potato tubers. The observed auxin content pattern in stolons during different stages of potato tuber formation corresponds to its levels during various developmental phases of storage roots. Roumeliotis *et al.* (2013) [22] identified the involvement of the PIN family of proteins in potato and discussed the potential role of StPIN proteins in redistributing auxin in the early stages of tuber development. In a separate study, Liljana *et al.* (2012) [16] highlighted the genotypedependent *in vitro* tuberization capacity of potato noting that, the most effective approach for micropropagation and *in vitro* microtuberization involves the use of MS composition with auxin. The overall study underscores the significance of auxins and their concentration in influencing various facets of potato tuber development and *in vitro* microtuberization.

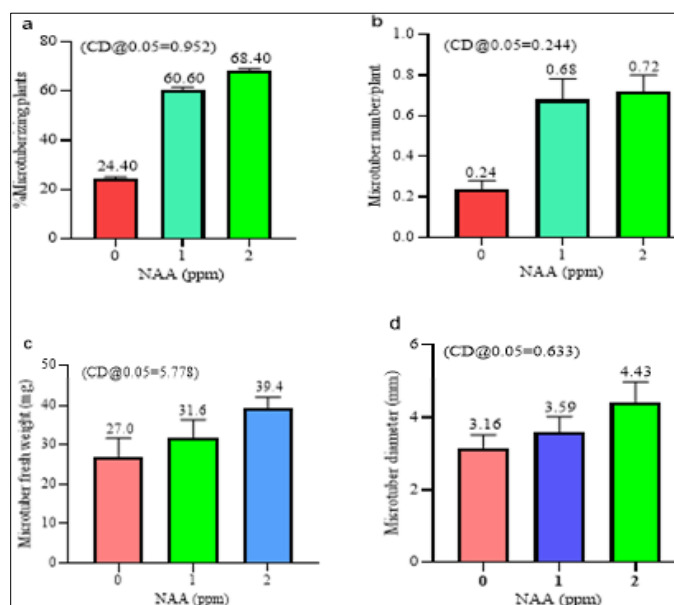


Fig 2: Effect of different concentrations of Auxins source (NAA) on micro-tuberization response in potato. (a) Per cent micro-tuberizing plants (b) Micro-tuber number per plant (c) Micro-tuber fresh weight (mg) (d) Micro-tuber diameter (mm).

2,4-D-Induced Morphogenesis and Growth Regulation in Potato (*Solanum tuberosum* L.)

The control treatment demonstrated a significantly greater plant height (6.44 cm) compared to the 1 ppm and 2 ppm concentrations of 2,4-D which measured 4.32 cm and 5.30 cm, respectively. This suggests that, the absence of 2,4-D positively influenced the vertical growth of potato plants. Potato plants treated with 2 ppm concentration of 2,4-D displayed a significantly reduced plant height (5.30 cm) compared to the control treatment (6.44 cm) and

an increased plant height compared to the 1 ppm concentration of 2,4-D (4.32 cm). Microtuber formation in the control treatment was also significantly higher than in the 1 ppm and 2 ppm concentrations of 2,4-D. The results indicate that, natural growth conditions without 2,4-D application favored microtuberization in potato (Table 3).

In contrast, Duan *et al.* (2023) [6] demonstrated a dose-dependent inhibition of tuber formation in response to 2,4-D in their research on potato cultivation. The inhibitory effect on microtuberization is attributed to 2,4-D's role as an auxin-like herbicide. Auxins regulate various plant growth processes including tuberization and excessive concentrations of synthetic auxins like 2,4-D may disrupt the hormonal balance necessary for proper tuber initiation and development (Rashid, 2018;) [21].

Table 3: Effect of 2,4 D on plant growth in potato under *in-vitro* condition

Treatments	2,4-D		
	Plant height (cm)	Number of nodes	Number of leaves
Control	6.44 ^a	8.02 ^b	8.04 ^b
2,4 D-1 ppm	4.32 ^c	7.84 ^c	7.88 ^c
2,4 D-2 ppm	5.3 ^b	9.24 ^a	9.22 ^a
CD (P<0.01)	0.127**	0.252**	0.324**
SE(m)	0.041	0.081	0.104
SE(d)	0.058	0.114	0.147
CV (%)	1.705	2.16	2.773

** Significant at 1% level

The control group exhibited a mean of 8.02 nodes per plant slightly lower than the 2 ppm 2,4-D treated group with a mean of 9.24 nodes per plant. The observed difference between the groups was found to be statistically significant indicating a clear impact of the 2 ppm 2,4-D treatment on node development. These findings suggest a stimulatory effect of the 2 ppm 2,4-D herbicide on node development in potato plants. The increased number of nodes has the potential to influence overall plant growth and productivity. However, it is important to consider the complex nature of plant responses to plant hormones particularly in potato tuberization (Table 3).

The experiment's findings highlight a substantial difference in the number of leaves per plant between the 1 ppm 2,4-D treatment (7.88) and the control group (8.04). Specifically, potato plants treated with 2 ppm of 2,4-D exhibited a notably higher number of leaves (9.22) compared to the control group (8.04). The average number of leaves per plant in the 2 ppm 2,4-D treatment group was 9.22, while the control group exhibited an average of 8.04 leaves per plant. Statistical analysis confirmed the statistical significance of this difference indicating a positive influence of the 2 ppm 2,4-D concentration on leaf development in potato plants (Table 3).

2,4-D-Mediated Regulation of Microtuberization and Tuber Development in Potato

The results revealed a significant difference between the control group and the groups exposed to 1 ppm and 2 ppm concentrations of 2,4-D emphasizing the impact of 2,4-D on microtuber development in potato plants. The control treatment demonstrated a significantly higher percentage of microtuberizing plants (24.4%) compared to the experimental group exposed to 2 ppm of 2,4-D (0.0%). In contrast, the groups treated with 1 ppm and 2 ppm concentrations of 2,4-D exhibited lower percentages of microtuberizing plants (12.80%) and (0.0%), respectively compared to the control treatment. Robust microtuber formation was observed in the control group suggesting that, the absence of

2,4-D positively influenced the tuberization process in potato plants (Figure 3a).

The experiment results indicated a significant difference in microtuber formation between the control treatment and the 2 ppm concentration of 2,4-D in potato plants. The control treatment exhibited a significantly higher number of microtubers per plant (0.24) compared to the plants exposed to the 1 ppm concentration of 2,4-D (0.12). The control group, which did not receive any 2,4-D treatment demonstrated robust microtuber development indicating a favorable environment for tuberization. This observation suggests that, the absence of 2,4-D or exposure to lower concentrations may enhance the natural tuber-forming processes in potato. In contrast, potato plants subjected to 2 ppm concentration of 2,4-D exhibited a lower microtuber count per plant (0.0) compared to the control treatment. This outcome implies that, the presence of 2,4-D at this concentration may adversely affect or inhibit the tuberization process in potato (Figure 3b).

The control treatment displayed higher microtuber weight (27 mg) compared to 1 ppm concentration of 2,4-D (22 mg). However, the 2 ppm concentration of 2,4-D inhibited microtuber weight (0 mg) in contrast to the control treatment (27 mg). This observation suggests that, the presence of 2,4-D at the specified concentration negatively impacted microtuber formation in potato plants. The reduction in microtuber weight indicates an inhibitory effect on the tuberization process (Figure 3c).

This study investigated the impact of various concentrations of 2,4-D (2,4-dichlorophenoxyacetic acid) on microtuber formation in potato with a focus on microtuber diameter. A control treatment was compared to the 1 ppm and 2 ppm concentrations of 2,4-D. The results revealed a significant difference in microtuber development particularly in terms of diameter. The control treatment displayed a significantly higher microtuber diameter (3.16 mm) compared to the 1 ppm concentration of 2,4-D (2.88 mm). However, the 2 ppm concentration of 2,4-D inhibited microtuber growth in potato resulting in a diameter of 0 mm. This discrepancy suggests that, the presence of 2,4-D at 2 ppm negatively impacted microtuber growth in potato (Figure 3d).

Although 2,4-D stimulated certain vegetative traits such as node and leaf development at higher concentration, it significantly inhibited microtuber initiation and development. The 2 ppm 2,4-D treatment completely prevented microtuber formation, confirming its unsuitability for potato microtuber production. The study demonstrates that the response of potato to auxins is highly concentration dependent, with naturally occurring and synthetic auxins exhibiting contrasting effects on vegetative growth and tuberization. Among the auxins evaluated, 2 ppm NAA was the most effective treatment for maximizing microtuber production and quality, whereas 1 ppm IAA was most suitable for improving vegetative growth while maintaining satisfactory microtuberization. These findings provide a practical basis for optimizing plant growth regulator combinations in *in vitro* potato microtuber production and offer useful information for large-scale seed tuber production and future studies on the molecular regulation of auxin-mediated tuberization.

Conclusion

Auxin type and concentration significantly influenced *in vitro* growth and microtuberization of potato, demonstrating distinct physiological responses to IAA, NAA, and 2,4-D. Application of 1 ppm IAA promoted vegetative growth by increasing plant height, number of nodes, and number of leaves, while also enhancing microtuberization percentage, microtubers per plant, and microtuber fresh weight compared with the control. Increasing the IAA concentration to 2 ppm exerted an inhibitory effect, completely suppressing microtuber formation, indicating that excessive IAA is detrimental to tuber induction. NAA reduced vegetative growth in a concentration-dependent manner but markedly enhanced microtuberization. The 2 ppm NAA treatment produced the highest percentage of microtuberizing plants, greatest number of microtubers per plant, maximum fresh weight, and largest microtuber diameter among all treatments.

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Conflict of Interest

The authors disclose no conflict of interest.

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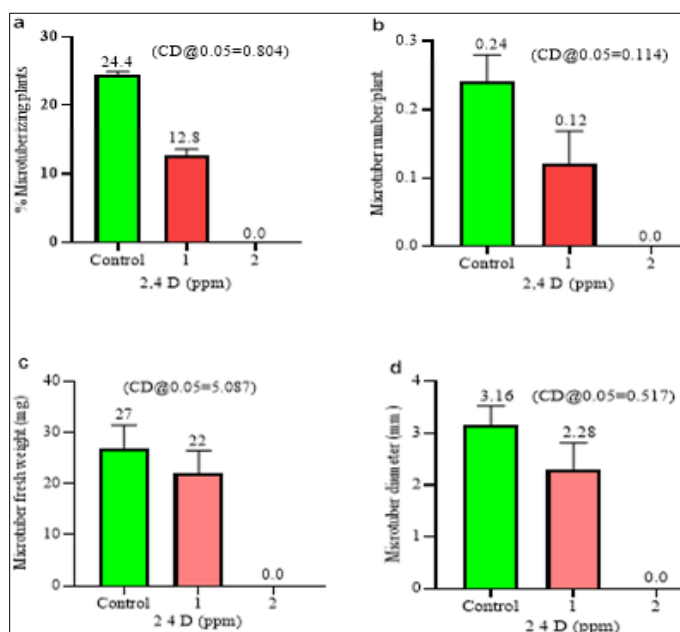


Fig 3: Effect of different concentrations of Auxins source (2, 4-D) on micro-tuberization response in potato. (a) Per cent micro-tuberizing plants (b) Micro-tuber number per plant (c) Micro-tuber fresh weight (mg) (d) Micro-tuber diameter (mm).

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