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RESEARCH ARTICLE

Effect of Varied Levels of Gibberellin, Anti-Gibberellin and Cytokinin on Growth and Tuberization in Potato

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ABSTRACT

Plant hormones play a significant role in potato tuberization. While Gibberellins inhibit tuberization, anti-gibberellins (PBZ and CCC) and cytokinins promote tuberization in potato. The experiment demonstrated the clear roles of these hormones in facilitating tuberization in potato. Treatment with 25 ppm GA significantly increased plant height (44.56 cm) compared to the control (32.22 cm), while CCC, a Gibberellins (GA) inhibitor, reduced the plant height by inhibiting GA-induced stem elongation. Hormonal treatments affected the number of nodes, leaves, branches, SPAD readings, stem girth, root length and root weight with significantly higher values observed compared to the control. 50 ppm GA showed the highest number of stolons per plant (6.33), but surprisingly resulted in a few tubers per plant (1.7) compared to the control (1.8). However, both 5 ppm and 10 ppm BAP led to significantly more tubers per plant (2.1 and 3.2 respectively), as did 5 ppm and 10 ppm Kinetin (2.8 and 2.6 respectively). Regarding tuber fresh weight, all treatments except CCC and higher concentration of kinetin showed significantly higher fresh weight compared to control. In summary, 50 ppm Gibberellins increased the stolon number, while 10 ppm BAP increased the tuber number and fresh weight in potato.

Keywords: Potato , Plant Hormone , Gibberellins ,Anti-gibberellins , Cytokinin , Tuberization

INTRODUCTION

Potato (*Solanum tuberosum* L.), a tuber crop is the fourth most important food crop after wheat, rice and maize and is being cultivated worldwide in temperate, tropical and sub-tropical zones. It is the major food crop of the world and by far the most important food crop in terms of quantities produced and consumed worldwide ranking fourth position (375 million metric tons/year) after maize, rice and wheat with an estimated production area of 17.78 million hectares at an average yield of 21.09 tons/ha (FAOSTAT, 2022). The production and consumption of potato is growing in the developing world, whereas it is decreasing in the developed world (Keijbets, 2008). Potato is an important food and cash crop in eastern and Central Africa, playing a major role in national food security and nutrition, poverty alleviation, and income generation , providing employment in the production, processing, and marketing sub-sectors (Merga and Dechassa, 2019). Potatoes are grown for their starchy tubers, which are produced underground from the stolons. Besides starch, tubers are also rich in proteins, fats, dietary fibers and an array of essential nutrients and the vital amino acids like leucine, isoleucine and tryptophan. However, the cultivation of this cool-season crop faces geographical constraints. Potatoes thrive best in specific conditions characterized by shorter days, lower temperatures and sandy soils with acidic pH, which promote tuberization. Genetic, environmental and physiological factors influence tuberization to a large extent (Vreugdenhil and Struik, 1989). Available evidence indicate that the photoperiod, temperature, irradiance, and physiological age of the mother tuber affect tuberization either directly or indirectly by mediating changes in hormonal concentrations

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(Ewing,1995). The process of tuberization involves the cessation of growth in the stolon apical meristem and the induction of first longitudinal and later, random cell division and expansion in the sub-apical region (Xu *et al*., 1998). This is accompanied by deposition of storage carbohydrates (Viola et al., 2007) and proteins (Shewry, 2003) in the tubers.

Several environmental factors, such as photoperiods, temperature, plant hormones, and light were found to regulate tuberization. The photoperiods (Short days 8h light/16h dark) and lower temperature (18° C) has been found to favor tuberization (Tengli *et al*., 2022). With respect to plant hormones, cytokinins favour tuberization, while the gibberellic acid inhibits it. Internally-produced gibberellic acid (GA3), a natural plant hormone (Thomas and Sun 2004; Busov *et al.* 2008) is known to be involved in promoting stolon growth (Koda and Okazawa 1983) while inhibiting tuber formation (Vreugdenhil and Sergeeva 1999). The application of GA3 to potato plants dramatically increased the misshaping, including pointy ends and dumbbells of harvested tubers (Pavlista, 2013).

Tuberization in potatoes is a complex process regulated by various plant hormones that influence the molecular mechanisms. Plant hormones such as Gibberellins (GA), Paclobutrazol (PBZ), Chlormequat Chloride (CCC), 6-Benzylaminopurine (BAP) and Kinetin play critical roles in regulating the molecular mechanisms of tuberization in potato (Aksenova *et al*., 2012). Gibberellins are generally known to inhibit tuber formation. High levels of GAs promote stem elongation and vegetative growth, which counteracts the initiation of tuberization (Yamaguchi 2008; Gebrehanna *et al*., 2018). PBZ (Paclobutrazol) and CCC (Chlormequat Chloride) are GA biosynthesis inhibitor and thus promote tuberization (Herrera-Isidron *et al*., 2021). By inhibiting GA biosynthesis, PBZ reduces GA levels in the plant, which removes the inhibitory effect of GAs on tuber formation. PBZ treatment leads to the upregulation of tuber-inducing genes and downregulation of GA-responsive growth-promoting genes. This shift in gene expression favors tuber initiation and development (Hedden and Sponsel, 2015). Hormones modulate the expression of genes like *StBEL5* and *StSP6A*, which are critical for tuber development. *StBEL5* is a transcription factor that promotes tuberization, while *StSP6A* is a mobile RNA acting as a signal for tuber induction (Hannapel *et al*., 2021). In summary, the regulation of tuberization in potato involves a delicate balance of hormonal signals that control the expression of key genes and coordinate the growth and differentiation of stolons into tubers.

MATERIAL AND METHODS

Planting material

The experiments were conducted at the Plant Tissue Culture Laboratory, Department of Crop Physiology, University of Agricultural Sciences, GKVK, Bangalore, India. The virus-free planting material of potato (*Solanum tuberosum L*.) cultivar, Kufri Jyothi (KJ) was procured from the Central Potato Research Institute (CPRI), Shimla, India for the experiments.

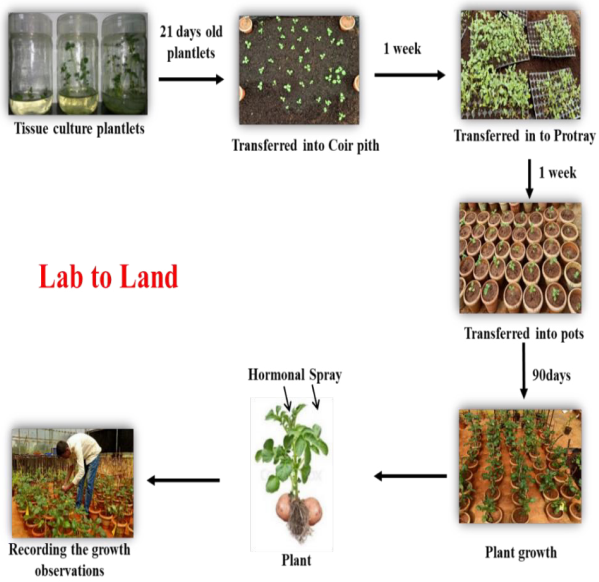
In-vivo studies to examine the role of plant hormones on tuberization in potato

Plant hormones have been shown to regulate tuberization in potato. Studies from our laboratory showed the role of cytokinin inducing mini-tubers in potato (Tengli *et al*., 2022). In this regard, different hormones including anti-GA was tested to examine, whether or not these plant hormones and hormonal combinations influence tuberization in potato. For this experiment, *in vitro* potato plantlets were used. The single node cuttings made from virus free plant material were cultured *in vitro* in MS media and after 21 days, the plantlets developed from single node cuttings were used for further experiments, after their hardening.

Details of the experiment

Potato plantlets grown in tissue culture bottles were hardened by transferring the plants to pot trays and gradually exposing them to increased temperature under a poly house condition. These acclimatized plantlets were then transferred to pots with coir pith to encourage root growth. Once the plantlets displayed a strong root development, they were moved to pots with a soil medium to support overall plant growth. To investigate tuberization in potato, various plant hormones such as Gibberellic acid (GA3) and anti-GA like paclobutrazol (PBZ) and CCC, cytokinin like BAP (Benzyl Amino Purine) and kinetin (cytokinin sources) were applied separately as foliar sprays in different concentrations at stolonization period. The experiment was conducted in three replications following a completely randomized block design to evaluate the influence of these plant hormones on the tuberization process in potatoes.

The experiment utilized various hormonal sources including Benzyl Amino Purine (BAP) and kinetin at a concentration of 5 and 10 ppm alongside gibberellins and anti-gibberellin (anti-GA) sources such as paclobutrazol at 5 and 10 ppm and Chlormequat Chloride (CCC) at 5 and 10 ppm. These hormones were applied through foliar sprays at 45 days after sowing, specifically during the initiation of stolonization and tuber development stages. The growth observations were recorded at 90 days after planting on plant height, number of nodes, number of leaves, stem girth, SPAD, root length, root fresh weight, number of stolons per plant, number of tubers per plant and tuber fresh weight was examined (Flowchart 1).



Flowchart 1: Schematic representation of lab to land. Transfer of tissue cultured plants from laboratory conditions to greenhouse conditions and studied for phytohormone response on tuberization.

RESULTS AND DISCUSSION

Effect of plant hormones on tuberization under in-vivo conditions

This study investigated the effect of different plant hormones on the growth parameters of potato plantlets. The tested plant hormones encompassed Gibberellic Acid (GA) at 25 and 50 ppm, Paclobutrazol (PBZ) at 5 and 10 ppm, CCC at 5 and 10 ppm, Benzylaminopurine (BAP) at 5 and 10 ppm, and Kinetin (KIN) at 5 and 10 ppm. The control group received water treatment. The study aimed to assess the various growth parameters such as plant height, leaf number, root length and overall biomass as influenced by the plant hormones. By comparing the results of the hormone-treated groups with the control, the study seeks to elucidate the specific effects of each hormones and their concentrations on potato plantlet growth and development besides tuberization. This research holds significance for understanding how diverse hormonal treatments can be employed to enhance the growth of potato plantlets potentially impacting agricultural practices and future crop yields.

The results indicate that, different plant hormonal treatments showed significantly higher plant height compared to control. Plants treated with GA showed significantly higher plant height compared to those treated with GA inhibitors and cytokinin. Although GA inhibitors such as CCC and Paclobutrazol reduce plant height, the reduction was not as low as that of control plants (Table 1; Plate 1). The results of the experiment also revealed a significant variation in the number of nodes across different hormonal treatments. Except 25 ppm GA, all other treatments had significantly higher number of nodes per plant compared to control. GA inhibitors are shown to exhibit a significantly higher number of nodes (Table 1; Plate 1). In conclusion, the findings suggest that certain concentrations of PBZ (5 and 10ppm) and Kinetin (10ppm) resulted in a notable increase in the number of nodes compared to the control treatment. Likewise, the number of leaves remained unaffected by 25 and 50 ppm GA (20.44 and 20.78, respectively) compared to the control treatment (19.11). However, other hormonal treatments resulted in increased leaf number and the data is highly significant These results offer valuable insights into the impact of various hormonal concentrations on the growth parameters of *in-vivo* cultivated potato plantlets (Table 1; Plate 1).

Gibberellic acid present in leaves, stems and below-ground parts plays a crucial role in the tuberization process of potato (Sattelmacher and Marschner, 1979). Throughout the plant's life cycle, gibberellic acid functions to stimulate the growth of various plant organs by enhancing both cell division and cell elongation (Pal *et al*., 2023). When potato plants were sprayed with gibberellic acid (GA) during the emergence of the 15th leaf, it resulted in an increased leaf area and elevated total gibberellin content. GA also led to an augmentation in chlorophyll content

Table 1. Effect of different concentrations of hormones on various growth parameters in in-vivo grown potato plantlets (Var. Kufri Jyoti)

Treatments		Plant height (cm)	No. of nodes	No. of leaves	No. of branches
Control	Water	32.22^{g}	17.78^{f}	19.11^{e}	1.32^{b}
GA	25 ppm	44.56^{a}	18.56^{ef}	20.44^{e}	1.22^{bc}
	50 ppm	42.22^{b}	20.22^{e}	20.78^{e}	1.33^{b}
PBZ	5 ppm	41.89^{b}	38.22^{a}	38.22^{ab}	1.32^{b}
	10 ppm	35.44^{f}	35.00^{bc}	36.33^{bc}	1.67^{a}
CCC	5 ppm	36.11^{ef}	29.33^{d}	35.33^{c}	1.00^{c}
	10 ppm	37.94^{de}	38.00^{a}	37.67^{abc}	1.11^{bc}
BAP	5 ppm	40.33^{bc}	38.11^{a}	38.11^{ab}	1.22^{bc}
	10 ppm	38.89^{cd}	31.33^{d}	38.89^{a}	1.11^{bc}
Kinetin	5 ppm	36.22^{ef}	34.11^{c}	32.89^{d}	1.67^{a}
	10 ppm	38.56^{cd}	36.33^{ab}	39.00^{a}	1.11^{bc}
CD (P<0.01)		1.952**	2.098**	2.302**	0.219**
SE(m)		0.661	0.711	0.78	0.074
SE(d)		0.935	1.005	1.103	0.105
CV (%)		2.969	4.018	4.165	10.052

** Significant at 1% level

per leaf, but the increase in leaf area was more pronounced causing a reduction in chlorophyll per unit area. Consequently, the treated leaves appeared paler compared to untreated leaves (Verdun, 1959).

The data also indicated that, both the 10 ppm PBZ and 5 ppm KIN treatments displayed a significantly higher number of branches (1.67 each) compared to the control treatment (1.32). Interestingly, treatments with 25 ppm GA, 50 ppm GA, 5 ppm PBZ and 10 ppm CCC did not exhibit any significant differences among themselves demonstrating similar numbers of branches (1.22, 1.33, 1.32 and 1.11, respectively) when compared to the control treatment (1.32). Additionally, treatments with 5 ppm BAP, 10 ppm BAP and 10 ppm Kinetin demonstrated numbers of branches (1.22, 1.11, and 1.11, respectively) that were significantly comparable to the control treatment (1.32). These findings suggest that, specific concentrations of PBZ and Kinetin (5 ppm and 10 ppm) significantly promote branching, while certain concentrations of GA, PBZ and CCC maintain a similar number of branches as observed in the control (Table 1; Plate 1). Regarding the stem girth, GA treated plants showed reduced stem girth compared to the control. However, plants treated with GA inhibitors showed improved stem girth over control and GA suggesting the inhibition of GA induced stem elongation (Table 2; Plate 1). In conclusion, the study highlights the concentration-dependent effects of PBZ, BAP, CCC, KIN, and GA on the growth parameters of potato plantlets providing valuable insights for optimizing hormones applications in potato cultivation.

The study also revealed the diverse impacts of different hormonal concentrations on the leaf nitrogen status as assessed through SPAD measurements. Specifically, 10 ppm concentrations of PBZ, CCC, and BAP resulted in significantly higher SPAD readings, while 5 ppm concentrations of CCC, BAP, and 25 ppm GA also led to significantly higher SPAD readings compared to the control treatment. Conversely, 5 ppm KIN and 50 ppm GA showed significantly lower SPAD readings compared to the control treatment (Table 2; Plate 1). It appears, higher concentration of GA stimulates the plant height making the plants to put on vegetative growth with deficit of nitrogen and hence, the SPAD values are low in plants treated with high concentration of GA.

Hormonal treatment also influenced root length and root fresh weight with significantly higher root length and root weight observed under different hormonal treatments compared to control. This suggest that, different plant hormones influence root growth

Table 2: Effect of different concentrations of hormones on various parameters in *in-vivo* grown potato plantlets (Var. Kufri Jyoti)

Treatments		SPAD Reading	Stem girth	Root length (cm)	Root fresh weight (gm)
Control	Water	42.82^{h}	3.31^{cd}	22.00^{f}	0.15^{f}
GA	25 ppm	43.17^{gh}	3.28^{cd}	23.33^{ef}	0.25^{f}
	50 ppm	35.38^{j}	3.08^{d}	24.89^{de}	0.27^{f}
PBZ	5 ppm	84.53^{a}	3.58^{bc}	32.70^{a}	1.23^{a}
	10 ppm	57.94^{b}	3.53^{bc}	30.00^{b}	0.81^{d}
CCC	5 ppm	51.22^{d}	3.42^{cd}	29.94^{b}	0.83^{cd}
	10 ppm	56.42^{c}	3.84^{ab}	26.33^{cd}	1.24^{a}
BAP	5 ppm	44.44^{fg}	3.47^{c}	31.17^{ab}	0.55^{e}
	10 ppm	48.42^{e}	3.96^{a}	26.67^{cd}	0.95^{bc}
Kinetin	5 ppm	41.44^{i}	3.57^{bc}	32.21^{a}	1.01^{b}
	10 ppm	45.19^{f}	3.22^{cd}	27.67^{c}	1.23^{a}
CD (P<0.01)		1.354**	0.331**	1.828**	0.133**
SE(m)		0.459	0.112	0.619	0.045
SE(d)		0.649	0.159	0.876	0.064
CV (%)		1.586	5.591	3.844	10.084

** Significant at 1% level

(Table 2; Plate 1). The GA concentration of 25 ppm (4.72) demonstrated a significantly higher number of stolons per plant compared to the control treatment (1.61). The GA concentration of 50 ppm exhibited an even higher number of stolons per plant (6.33) surpassing not only the control (1.61) but also all other treatments. Both the 5 and 10 ppm concentrations of PBZ resulted in a significantly higher number of stolons per plant (4.11 and 4.33 respectively) compared to the control (1.61) treatment. The 5 ppm and 10 ppm concentrations of CCC led to a significantly higher number of stolons per plant (4.44 and 3.66 respectively) compared to the control (1.61) treatment. Nevertheless, the stolon count was lower than that observed with GA treatments. Both the 5 ppm and 10 ppm concentrations of BAP resulted in a significantly higher number of stolons per plant (3.55 and 2.56, respectively) compared to the control treatment (1.61). However, the stolon count was lower when compared to GA treatments, suggesting a differential effect on stolon development. The 5 ppm and 10 ppm concentrations of Kinetin led to a significantly higher number of stolons per plant (4.44 and 2.33 respectively) compared to the control treatment (1.61) (Figure 1; Plate 1).

Stolon initiation in potato significantly coincides with the period of tuber initiation (Claassens and

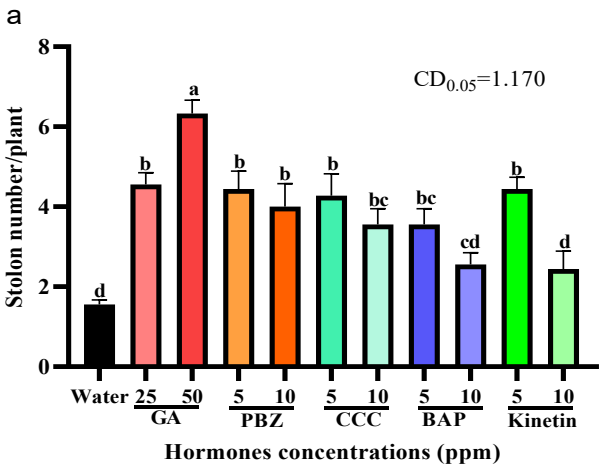


Fig. 1. Influence of different phytohormones on tuberization in potato: (a) Stolon number per plant.

Vreugdenhil, 2000). Evidently, the hormonal status is not uniform for each stolon and variations may even exist between individual tips of a branched stolon. This lack of synchronization in various developmental steps is frequently overlooked in descriptions of tuber formation. Additionally, gibberellins are known to prevent, inhibit or delay tuber initiation (Vreugdenhil and Struik, 1989; Pharis, 1991). Consequently, the reduction of gibberellin levels under short-day

(SD) conditions has a dual effect as it decreases stolon elongation and facilitates tuber initiation. However, this does not imply that, gibberellins are the sole determinants of tuber initiation. They exert a negative influence, while other factors play a positive regulatory role and under short-day (SD) conditions, the application of gibberellin does not prevent tuber formation (Hannapel, 2007) suggesting GA alone may not inhibit tuberiztion in potato rather controlled by many factors.

The results illustrate the varied impacts of different concentrations of plant hormones on the number of stolons per potato plant. Gibberellic Acid, particularly at 50 ppm emerged as the most effective hormone in promoting stolon development. However, other hormones such as PBZ, CCC, BAP and Kinetin also exhibited a significant effects indicating their potential roles in regulating specific aspects of potato plantlet growth (Figure 1; Plate 1).

The 25 ppm GA concentration (2.4) significantly enhanced the number of tubers per plant compared to the control treatment (1.8). Surprisingly, the 50 ppm GA concentration (1.7) resulted in a significantly lower number of tubers per plant compared to both the control (1.8) and other hormone-treated samples. Al-Doori (2023) demonstrated that, applying gibberellic acid (GA3) through foliar spray at three-day intervals during the tuberization stage led to a decrease in tuber yield. This finding aligns with Yopp's (2018) observation, where daily spraying of GA3 on the shoots from plant emergence up to three weeks at 10 ppm resulted in a reduction in total tuber number. In essence, the application of GA3 is associated with a decrease in tuber number and overall tuber yield in potato.

Both 5 and 10 ppm of PBZ and CCC led to a significantly higher number of tubers per plant (2.6 and 2.8 respectively) and (1.9 and 2.2 respectively) compared to the control treatment (1.8). Paclobutrazol, a triazolic fungicide, regulates plant growth by disrupting ent-kaurene oxidase activity in the ent-kaurene oxidation pathway, a critical step in gibberellic acid biosynthesis resulting in decreased gibberellic acid levels (Hanson and Willis, 1992). It inhibits three oxidative steps from ent-kaurene to ent-kaurenoic acid blocking the synthesis of gibberellins in the early phase of the biosynthetic pathway (Rademacher, 2020). This inhibitory effect is supported by lower gibberellic acid levels in treated plants, and some paclobutrazol effects can be reversed by gibberellic acid application (Tsegaw, 2007). Tekalign and Hammes (2005) demonstrated that, thirty days after planting (early stolon initiation), foliar application of PBZ to leaves significantly affected fresh tuber yield in potato plants.

Both 5 and 10 ppm of BAP led to a significantly higher number of tubers per plant (2.1 and 3.2 respectively) compared to the control treatment (1.8). Notably, the 10 ppm BAP resulted in a higher number of tubers per plant (3.2) compared to other concentrations. Similarly, both 5 and 10 ppm Kinetin resulted in a significantly higher number of tubers per plant (2.8 and 2.6 respectively) compared to the control treatment (1.8) (Figure 2; Plate 1). Cytokinins (CKs), classical plant hormones are recognized for promoting potato tuber formation particularly in conventional in vitro systems (Lomin *et al*., 2020). CKs play a role in tuber development in potato (Eviatar-Ribak *et al*., 2013) and the formation of tuber-shaped rounded tumors on agrobacteria-infected plants. Besides stimulating tuber formation, CKs contribute to tuber sprouting (Lomin *et al*., 2020). The morphogenic impact of CKs is rooted in their ability to induce cell divisions (Thu *et al.,* 2017) although their influence extends beyond this aspect. Collectively, the literature emphasizes the crucial role of the CK regulatory system in enhancing tuber yield in valuable tuber-producing crops like potato.

With respect to fresh weight of tubers, except BAP, PBZ and lower concentration of kinetin treatment, in

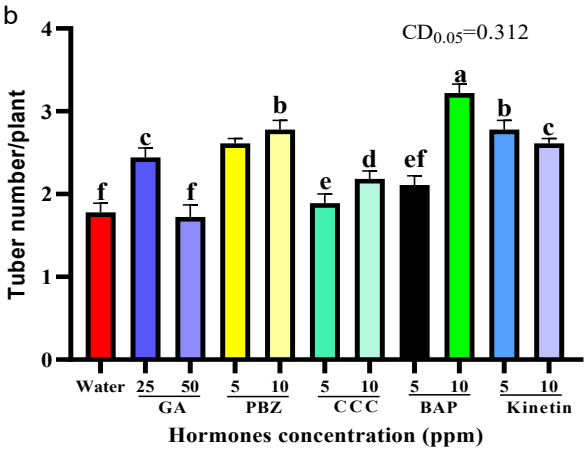


Figure 2: Influence of different phytohormones on tuberization in potato: (b)Tuber number per plant.

all other treatments, the fresh weight did not differ significantly with control treatment (Figure 3; Plate 1). The study reveals varied effects of different plant hormonal concentrations on tuber fresh weight in in-vivo grown potato plantlets. PBZ, BAP, and 5ppm Kinetin exhibit positive influence, while GA, CCC, and 10 ppm Kinetin have negative impacts. These findings provide valuable insights for optimizing growth conditions in potato cultivation by manipulating plant hormone concentrations. Caldiz (1996) demonstrated that, cytokinins when applied as foliar-spray on potato plants, improved tuber yield from 7.2 to 12.7 t/h. Similarly, Chugh and Kumar (2022) found that, 50 ppm of the cytokinin source, 6-benzyl adenine purine (BAP) applied 34 days after planting, significantly influenced early tuberization and increased tuber yield. In another study, El-Areiny *et al*. (2019) showed that, CPPU, a cytokinin source applied as a foliar application at 0.12 M concentration resulted in more tubers per plant and higher tuber yield. Similarly, Malek *et al*. (2021) demonstrated that, foliar application of cytokinin increased the number of tubers per plant, tuber fresh

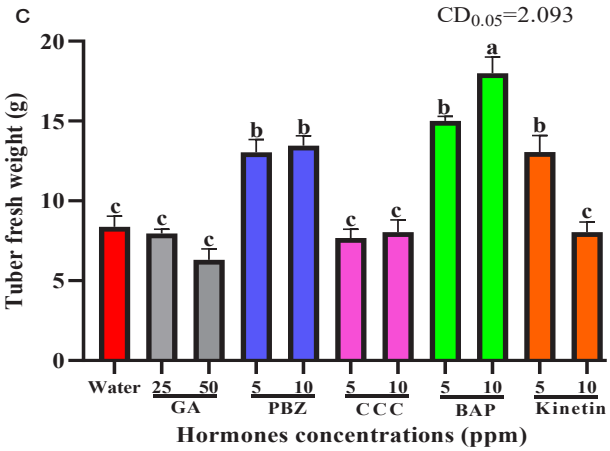


Figure 3: Influence of different phytohormones on tuberization in potato: (c) Tuber fresh weight.

weight, yield/plant and total yield. Hence, the available information unequivocally proves that, cytokinin promotes tuberization, while GA inhibits tuberization in potato. However, the effects of GA can be mitigated with anti-GA applications.

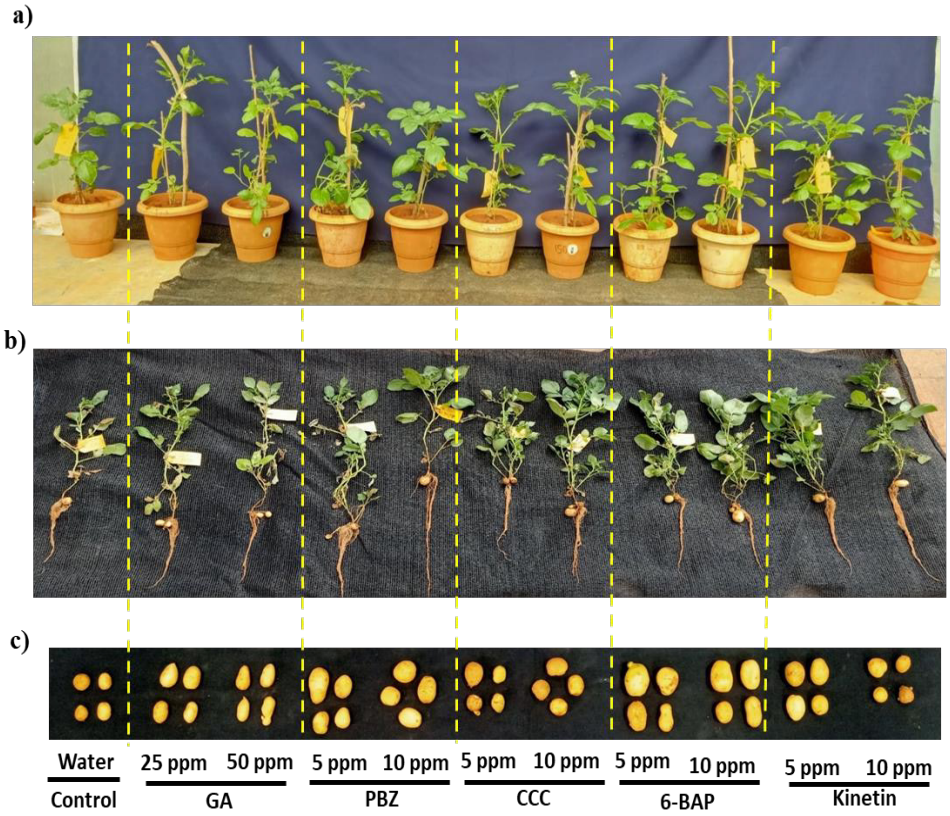


Plate 1: Effect of phytohormones on plant growth and tuberization in potato *under in vivo* condition.

a) Plants in the greenhouse before harvest. b) After uprooting from pots. c) Potato tubers were collected and taken observations.

CONCLUSION

Plant hormones play a significant role in potato tuberization. Gibberellins, anti-gibberellins (PBZ and CCC), and cytokinins promote the processes of stolonization and tuberization in potato. 50 ppm of Gibberellins increased the number of stolons, while 10 ppm of BAP increased the number and fresh weight of tubers in potato.

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Conflict of interest

The authors disclose no conflict of interest

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