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RESEARCH PAPER

Effect of phytohormones on root development of potato (*Solanum tuberosum* L.)

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Key Message: This research explores the effect of indole-3-butyric acid (IBA) and Gibberellic acid (GA_3) on the root development of three potato varieties (Barna, Kuroda, and Bartina) and concludes that addition of 1.5 ml of IBA for Barna and 2 ml of IBA for Kuroda produced the highest number of roots.

Abstract

This research study investigated the effect of phytohormones on root development in three varieties (Barna, Kuroda, and Bartina) of potato (*Solanum tuberosum* L.). The study was conducted at the Tissue Culture Laboratory, Agriculture Research Directorate, Gilgit. The Murashige and Skoog medium was used as the plant growth medium for the experiments. Different concentrations of indole-3-butyric acid (IBA) and Gibberellic acid (GA3) were supplemented to the medium to find out their effects on root development. The root length, root weight, number of roots, and total root weight were measured as parameters for root proliferation. The addition of IBA and GA3 to the medium resulted in increased root length in all potato varieties. The highest shoot lengths were recorded in the 2 ml IBA treatment, with values of 6.50 cm for Barna, 7.03 cm for Kuroda, and 6.80 cm for Bartina. The tuber weight increased with higher concentrations of GA3 (1 ml, 1.5 ml, and 2 ml) for all three varieties. The highest mean tuber weights were recorded in the 2 ml GA3 treatment, with values of 7.46 g for Barna, 6.20 g for Kuroda, and 6.86 g for Bartina. The research findings suggest that the application of phytohormones specifically IBA and GA_3 can significantly enhance root development in these varieties. The concentration of phytohormones identified in this study may pave the way for the development of targeted and efficient protocols in potato micropropagation ultimately contributing to sustainable and efficient agricultural practices in the future. . Conclusively, these results can contribute to the improvement of potato production through tissue culture techniques.

Keywords: Gibberellic acid (GA3), Indole-3-butyric acid (IBA), Micropropagation, Root development, *Solanum tuberosum*

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Introduction

Potato (*Solanum tuberosum*) is an herbaceous vegetable crop belonging to the solanaceae family. It grows well in cooler climates and is classified as a cool-season crop (Pandey et al., 2008). Potatoes require well-drained sandy loam soil that is fertile and loose. They can adapt to a variety of soil types and grow best when the soil pH ranges between 5.5 and 6.8. As the most important vegetable crop worldwide, potatoes rank fourth in terms of cultivation, following wheat, rice, and maize (Zaman et al., 2014). These short, thick tubers are underground stems that store starches and sugars (Chandrasekara & Kumar, 2016). Potatoes are valued for their nutritional content and taste, boasting 75 % water content. A 100 g of potatoes contains approximately 22 g of carbohydrates, 2 g of protein, 13 mg of calcium, 17 mg of vitamin C, 11 mg of riboflavin, 1.2 mg of niacin, 90 kilocalories of energy, and traces of various minerals and fiber. Potatoes can be enjoyed in various forms including baked, boiled and cooked. It can be used in combination with other vegetables or as popular snacks such as finger chips (Hassanpanah et al., 2006; Zaman et al., 2016). Additionally, potatoes have medical importance aiding in digestion, preventing constipation, protecting against colon cancer, reducing cholesterol absorption, and revealing antioxidant, anti-cancer, and cardio-protective properties.

In Pakistan, potatoes are cultivated during three different growing seasons namely spring, summer, and autumn depending on the geographical location. The spring season starting February to March is appropriate for potato cultivation in Pakistan with harvest taking place in April and May. In the northern hilly areas and Azad Jammu and Kashmir, the summer crop is sown from March to May with harvesting occurs in August and September. In southern Punjab, Sindh, and the plains of Baluchistan, the autumn crop is sown from

September to October, and harvesting takes place in January and February. The share of production for the spring, summer, and autumn crops is approximately 7-10 %, 15-20 %, and 70-75 %, respectively. Punjab contributes the highest share of production and cultivated area, specifically during the autumn and spring crops. In KPK, the main potato-producing districts are Nowshera, Dir, and Mansehra, while in Balochistan, it is Pishin, Killa Saifulla, and Kalat (Memon, 2015).

In Pakistan, the potato cultivation area experienced growth, expanding from 138 thousand hectares (kha) in 2010 to 175 kha in 2013, only to decline sharply to 159 kha in 2014. Similarly, the potato production in the country reached its peak at 3802 kilo tonnes in 2013 but witnessed a significant drop to 2901 kilo tonnes in 2014 (Majeed & Muhammad, 2018). Potato production provides higher energy and nutritional value per unit area compared to wheat, rice, and maize (Malik, 1995). However, Pakistan's per acre yield is lower than other countries. Micro tubers of potatoes have become a significant focus due to their characteristics such as small size, light weight, unlimited production seasons, ease of storage, rapid growth rate, and high survival rate during cultivation. These micro tubers also serve as valuable germplasm resources for exchange (Peng et al., 2012).

The production of micro tubers is influenced by a myriad of factors such as temperature, plantlet age and health, genotype, mineral nutrition, carbon sources, exogenous auxins, plant growth retardants, and other environmental factors. In a series of experiments conducted with virus-free potato seedlings, the application of plant growth regulators and active charcoal were applied to micro tubers to achieve optimal results (Roodbar Shojaei et al., 2009). Exogenous auxins were found to improve the production and quality of potato tubers. Active charcoal, when added at a concentration of 0.5 %, yielded a shortened growth period and increased number of larger potatoes. Potato diseases represent a significant challenge to yield, with common diseases in Pakistan. These diseases caused by fungal and viral pathogens, lead to economic losses. Selecting suitable potato varieties is crucial as different varieties have distinct characteristics, properties, and uses. In Pakistan, various potato varieties (Kuroda, Roko, Barna, Brtina, Asterix, Diamant, Cardinal, Granola, Patrones, Multa, Provento, Asterix, Espirit, Lady Rosseta, Meridian, Sagitta, and Quincy) are cultivated. These varieties cater to different purposes such as early harvest, export, processing, and new introductions.

Tissue culture techniques are widely employed for asexual propagation of plants including potatoes. These techniques enable the production of virus-free seed potatoes called micro tubers, facilitating rapid propagation rates and improved potato production (Borna et al., 2019). Plant hormones, particularly auxins, play a vital role in influencing the physiological processes of plants at low concentrations. Rooting hormones, a type of auxin hormone, accelerate plant growth and increase the likelihood of successful root development, resulting in higher-quality roots (Shah et al., 2015; Jan et al., 2015). Therefore, this research study aimed to investigate the effect of phytohormones, specifically Indole-3-butyric acid (IBA) and Gibberellic acid (GA_3), on root development in different varieties of potato (*Solanum tuberosum* L.).

Materials and Methods

Study area

The experiment was conducted at the tissue culture laboratory of Agriculture Research Directorate Gilgit, where *in vitro* techniques were employed to enhance potato production through micro-propagation and germplasm conservation.

Media preparation

Murashige and Skoog medium (1962) was utilized as the plant growth medium in this study. A growth medium serves to provide support for the growth of plants and can be in the form of solid, liquid, or semi-solid. When 6-8 % agar was dissolved in the liquid nutrient medium, it solidified into a medium suitable for initiation of plant cultures. On the other hand, a liquid medium remains in its liquid form without the addition of agar. Liquid medium is commonly used for cell suspension cultures. In this experiment, a liquid medium was prepared by adding 4.43 g of sucrose and 10 ml of iron to 1000 ml of distilled water.

Stock solution of hormones

A stock solution of hormones is a versatile and widely applicable solution that can be used in various media and concentrations. To create a 10 mg/L stock solution of IBA, combine 1.0 g of IBA powder with 2.0-5.0 ml of Ethyl alcohol or 1N NaOH in a 100 ml flask. Dissolve the powder by stirring. Sterilize the solution by autoclaving, and store aliquots of the IBA at -20 °C for future use. For Gibberellic acid, prepare a stock solution with a concentration of 1 mg ml^{-1}. Take 100 mg of Gibberellic acid powder and dissolve it in 80 ml of molecular biology grade water in a 250 ml beaker. Stir the solution until the Gibberellic acid is fully dissolved.

Plant material and data collection

Plant material consisting of three potato cultivars, namely Barna, Kuroda, and Bartina, was obtained from the growth room of the tissue culture laboratory. The cultured roots of these potato cultivars were treated with a modified MS medium supplemented with indole-3-butyric acid (IBA) and Gibberellic acid (GA_3). The liquid MS medium contained different concentrations of IBA and GA3, specifically 1 mg/l, 1.5 mg/l, and 2 mg/l. The following parameters were measured to assess root proliferation: root length (cm), root weight (g), number of roots, and total root weight (g). Data was collected for each of these parameters.

Statistical analysis

The statistical data was analyzed using the analysis of variance (ANOVA) technique. The differences among treatment means were compared using the least significant difference (LSD) test at a 5 % probability level.

Results

Effect of IBA on shoot length (cm) of different varieties of potato

Table 1 presents the effect of different concentrations of indole-3-butyric acid (IBA) on three varieties of potatoes: Barna, Kuroda, and Bartina. The control group shows the potatoes without any IBA treatment. The data includes measurement of shoot length (cm) for each treatment and variety. The results show that increasing concentrations of IBA had a positive effect on shoot length for all three potato varieties. The highest shoot lengths were recorded in the highest IBA concentration treatment (2 ml) for all three varieties. For the Barna variety, the control group had a mean shoot length of 4.30 cm, while the IBA treatments of 1 ml, 1.5 ml, and 2 ml resulted in shoot lengths of 5.33 cm, 6.10 cm, and 6.50 cm, respectively. For the Kuroda variety, the control group showed a mean shoot length of 4.63 cm, while 1 ml, 1.5 ml, and 2 ml IBA treatments of resulted in shoot lengths of 5.866 cm, 6.53 cm, and 7.03 cm, respectively. For the Bartina variety, the control group had a mean shoot length of 5.20 cm, while the IBA treatments of 1 ml, 1.5 ml, and 2 ml yielded the shoot lengths of 6.03 cm, 6.40 cm, and 6.80 cm, respectively. The coefficient of variation (C.V) was calculated to assess the variability within each variety. The C.V values ranged from 12.88 % to 17.24 %, indicating moderate to high variability in shoot length among the different treatments within each variety. The standard error, mean, and standard deviation were also calculated to provide additional information about the data. Based on the LSD test ($P \leq 0.05$), means in each row followed by the different letter are significantly different, indicating that the IBA treatments had a significant effect on shoot length compared to the control group for all three potato varieties.

Table 1 Effect of different concentrations of IBA on shoot length (cm) of different varieties of potato

Treatment	Barna	Kuroda	Bartina
Control	4.30^{c}	4.63^{c}	5.20^{b}
IBA (1 ml)	5.33^{b}	5.866^{b}	6.03^{ab}
IBA (1.5 ml)	6.10^{ab}	6.53^{ab}	6.40^{a}
IBA (2 ml)	6.50^{a}	7.03^{a}	6.80^{a}
CVC	0.89	0.69	1.06
St. Error	0.36	0.28	0.43
Mean	5.55	6.01	6.108
C.V	17.24	16.69	12.88
Std. Dev.	0.95	1.0	0.78

Means in each row followed by the same letter are not significantly different at LSD test ($P \leq 0.05$).

Effect of GA3 on tuber weight (g) of different varieties of potato

Table 2 presents the results of an experiment investigating the effect of different concentrations of gibberellic acid (GA3) on three varieties of potatoes: Barna, Kuroda, and Bartina. Table includes the average tuber weight (g) for each variety under different treatments, as well as additional statistical measures such as the coefficient of variation (C.V), standard error, mean, and standard deviation. The results indicate that for Barna, the average tuber weight ranged from 5 g (in the control) to 7.46 g (with 2 ml of GA3), with significant differences observed between the latter treatment and all others in the same row. The coefficient of variation for Barna was 2.15, and the mean tuber weight across all treatments was 5.89 g, with a standard deviation of 1.62 g. Similarly, for Kuroda, the average tuber weight varied from 4.16 g (in the control) to 6.20 g (with 2 ml of GA3), with significant differences noticed between the latter treatment and all others in the same row. The coefficient of variation for Kuroda was 1.94, and the mean tuber weight across all treatments was 4.85 g, with a standard deviation of 1.40 g. For Bartina, the average tuber weight ranged from 4.50 g (in the control) to 6.86 g (with 2 ml of GA3), with significant differences found between each treatment and all others in the same row. The coefficient of variation for Bartina was 2.38, and the mean tuber weight across all treatments was 5.7 g, with a standard deviation of 1.58 g.

Table 2 Effect of different concentrations of GA_3 on tuber weight (g) of different varieties of potato

Treatment	Barna	Kuroda	Bartina
Control	5.00^{b}	4.16^{b}	4.50^{a}
GA_3 (1 ml)	5.10^{b}	4.30^{ab}	5.06^{a}
GA_3 (1.5 ml)	6.00^{ab}	4.73^{ab}	6.36^{a}

GA_3 (2 ml)	7.46[a]	6.20[a]	6.86[a]
CVC	2.15	1.94	2.380
St. Error	0.88	0.7957	0.973
Mean	5.89	4.85	5.7000
C.V	27.64	28.913	27.74
Std. Dev.	1.62	1.40	1.58

Means in each row followed by the same letter are not significantly different at LSD test ($P \leq 0.05$).

Discussion

Potatoes rank as the fourth most important food crop after maize, wheat, and rice, given their high production volume, yield, and profitability for farmers. Potatoes possess significant nutritional value, including protein, iron, thiamine, niacin, fiber, and various other nutrients. Pakistan achieves self-sufficiency in potatoes for domestic consumption by depending on locally produced seed potatoes for over 99 % of its requirements. In a research study conducted by Kumlay & Ercisli (2015), various concentrations and combinations of plant growth regulators were employed to stimulate callus formation, shoot proliferation, and root regeneration in three potato (*Solanum tuberosum* L.) cultivars. It was observed that nodal explants under long-day conditions outperformed leaf segments in terms of callus induction. Despite the effectiveness of this approach, the conventional seed production system for potatoes, which relies on seed tubers, has inherent drawbacks, including issues related to poor seed health and low multiplication rates (Beukema & Zaag, 1990). Tissue culture techniques play a vital role in the rapid production of seeds on a large scale. Auxins, particularly indole-3-butyric acid (IBA) play a vital role in root development and differentiation. They enhance cell division in the root zone and intensify adventitious root initiation through the production of endogenous enzymes (Husen & Pal, 2007; Frick & Strader, 2018). Cultivation of potato in Pakistan has seen significant growth due to its nutritional value, high production volume, and economic benefits for farmers. The application of auxins especially IBA has been found to promote root development and enhance potato yield. Tissue culture techniques particularly meristem culture have proven effective in obtaining virus-free potato plants and rapid seed production. Factors such as hormones, light conditions, and growth regulators play crucial roles in micro-propagation and development of potato plants.

Indole-3-butyric acid (IBA) is considered the most important auxin, as it is highly stable and exhibits low toxicity (Ludwig-Müller et al., 2000; Hartma6nn et al., 2007). Frick and Strader (2018) reported that auxins play a crucial role in root development by enhancing cell division in the root zone. Root initiation is influenced by various factors including both external and internal elements, such as the type and concentration of endogenous plant growth regulators. Husen and Pal (2007) discovered that the application of IBA affects protein synthesis and RNA production leading to increased root formation through accelerated cell division. Auxins stimulate adventitious root initiation by promoting the production of endogenous enzymes that facilitate cell division, differentiation, and multiplication. In an experiment conducted by Carswell & Locy (1984) on sweet potato (*Ipomoea batatas* L.) cv. Jewel, callus growth was found to be optimal in media containing 1.0 mg/L NAA and 10 mg/L IBA. Root growth, on the other hand, was best observed in media with 1.0 mg/L NAA and 0.1 mg/L IBA. Kumlay (2014) highlighted that the micropropagation of potatoes is influenced by various factors, including cultivar characteristics, culture medium, temperature, season, photoperiod, and plant growth regulators (PGRs).

Similar to our findings, Memon (2015) highlighted the significance of potato as the primary crop vegetable in Pakistan, ranking fourth among food crops and fifth in production. It serves as a crucial food source for both humans and animals, and is also utilized in the production of alcohol and starch. Due to its nutritional value and high production capacity, farmers are increasingly drawn to potato cultivation. The cultivation area has significantly expanded from 3,000 hectares at the time of independence to 179,300 hectares in Pakistan. This substantial increase in production volume reflects the farmers' keen interest in potato cultivation. Freshly harvested potato tubers consist of approximately 79 % moisture and 21 % dry matter, predominantly starch in the form of amylase and amyl pectin. Potatoes are highly nutritious, containing vitamins (B1, B2, and B3), minerals (potassium, phosphorus, and magnesium), folate, pantothenic acid, and riboflavin.

Štefančič et al. (2005) conducted a research study to investigate the impact of two applied auxins (IAA and IBA) on the development of roots and shoots in leafy cuttings, employing the 'GiSeIA 5' dwarfing cherry rootstock. The results indicated that IBA (indole-3-butyric acid) was more successful than IAA in promoting earlier root development. Moreover, IBA influenced stronger shoot growth and higher root yield compared to IAA. While both auxin treatments increased the percentage of root cuttings without callus, they had no effect on the final percentage of root cuttings or the survival of cuttings. Although the applied auxins did not significantly affect root formation, their application resulted in a higher percentage of qualitative 'GiSeIA 5' leafy cuttings. Overall, IBA played a crucial role in root formation and differentiation, proving to be a more efficient treatment than IAA.

Zhang et al. (2017) emphasized the significance of potatoes as a crucial food crop globally, ranking it just after wheat, rice, and maize. The study investigated the effects of different hormones on micro-tubers of the potato plant and their developmental growth. The researchers successfully examined shoot initiation, multiplication, elongation, and rooting under *in vitro* conditions using nodal explants from the virus-free seedling of the "Diamants" potato variety. The highest percentages of survived nodal segments were observed when surface sterilized with a 0.2 % $HgCl_2$ solution for 5 minutes. The results demonstrated that the best medium for shoot initiation and multiplication, as well as node number and rooting, was PS3 (MS medium containing 3 mg/l GA_3 and 0.1 mg/l Kin), which produced 14 shoots/magenta from a node. Conversely, MS medium without growth regulators yielded the lowest percentages. Benzyl amino purine (BAP) at a concentration of 5mg/1 resulted in the highest values of tuberization percentage, weight, and number of micro-tubers per shoot. The study identified PM3 as the most efficient medium for micro-tuber induction, followed by PM2, while PM1 was deemed unsuitable. Additionally, the researchers successfully transferred plantlets with roots of 4 to 5 cm in length to pots containing sterile greenhouse clay and sand in equal ratios, with a survival rate of approximately 90 %.

Batool et al. (2014) conducted research on obtaining virus-free potatoes using meristem culture. Two potato varieties, namely Cardinal and SH-5, were grown using meristem culture, and growth hormones Gibberellic acid (GA3) and Naphthalene acetic acid (NAA) were applied to promote faster and uniform growth of roots, shoots, leaves, and nodes in the potato plants. The study revealed significant differences between varieties, hormones, and their interactions. Cardinal produced better root length, shoot length, number of leaves, and number of nodes compared to SH-5. The combination of Cardinal variety and Gibberellic acid at 300 µL showed the most suitable results, with maximum root length, shoot length, number of leaves, and number of nodes obtained at a concentration of 300 µ L. A concentration of 200 µL resulted in similar root and shoot lengths but produced fewer leaves and nodes.

López et al. (2009) investigated the effect of indole-3-acetic acid (IAA) and kinetin (K) under blue light (B) and red light (R) on *in vitro* cultured stem cuttings of potato plants (*Solanum tuberosum* L., cv. Miranda). The study found that blue light resulted in thick, short plants with large, well-developed leaves and significant micro-tuber production, while red light produced long, thin plants with small leaves and fewer micro-tubers. The addition of IAA in blue light had no significant effect on plant development, but it slightly lengthened the stem and enhanced tuber formation. In red light, IAA had a pronounced effect on stem reduction and tuber formation. Kinetin increased tuber formation in red light, while in blue light; it strongly promoted tuber formation and increased the root/shoot ratio.

Conclusion

The findings of this research study demonstrated that the application of IBA and GA3 significantly enhanced root development in all potato varieties tested. The highest mean values for root length were observed when using 1.5 ml of IBA for the Barna variety and 2 ml of IBA for the Kuroda variety. Similarly, the highest root length values were recorded when using 1.5 ml of GA3 for the Barna variety. These findings suggest that phytohormones, particularly IBA and GA3, can effectively promote root proliferation in different potato varieties. The findings of this study have practical implications for potato growers and researchers involved in potato breeding and cultivation. The application of IBA and GA3 can be implemented as a strategy to enhance root development in different potato varieties, potentially leading to increased yields and improved quality of potatoes.

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