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RESEARCH PAPER

# Effect of gibberellic acid on *in vitro* propagation of potato (*Solanum tuberosum* L.)

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**Key Message:** The current study explored that varying concentration of gibberellic acid (GA3) prominently affected the growth factors of potato plant under tissue culture technique. The most effective results were found in treatment T1 with 1 mg/l GA3 for promoting leaf propagation and stem elongation whereas 2 mg/l of GA3 exhibited the lowest result value.

**Abstract**

The impact of various applications of gibberellic acid (GA3) was studied on potato varieties at the rate of 1 mg/l, 1.5 mg/l and 2 mg/l especially for the Lady Rosette cultivar. The objective of this study was to investigate the effect of gibberellic acid (GA3) on different growth parameters of potato plants such as no. of plantlets, total no. of stems and total number of leaves. This research was conducted at the Biotechnology Laboratory, Department of Agriculture Research, Gilgit Baltistan to culture potato seedlings. Thirty beakers were used under various treatments and different growth and developmental parameters were examined i.e., number of plantlets, total number of stems and total number of leaves. The findings showed significant differences ($p > 0.05$) in growth among the different concentrations of gibberellic acid throughout the study period. Based on the results, the treatment with 1 mg/l gibberellic acid was found to be the most effective for leaf propagation in potato. Conversely, the treatment with 2 mg/l gibberellic acid showed the lowest values for the mentioned parameters. Lady Rosetta exhibited the highest number of leaves with good strength, recording 36 leaves, followed by 1 mg/l with 32 leaves, and 1.5 mg/l with 31 leaves. Overall, this research provides valuable insights into the optimization of potato plant propagation, specifically leaf production. The future applications include commercial potato cultivation, seed potato production, potato breeding programs, controlled environment agriculture, and further academic research in the field. 

**Keywords:** Gibberellic acid, *In vitro* propagation, Plant growth regulators, Potato (*Solanum tuberosum* L.), Shoot proliferation

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## Introduction

Potato (*Solanum tuberosum* L.) is a crucial staple crop worldwide, belonging to the Solanaceae family (Zaman et al., 2014; Zaman et al., 2016). Pakistan is involved in potato production, but the country faces challenges in meeting international standards, primarily due to the low quality of its seed (Pakistan Agricultural Research Council, 2020). Currently, Pakistan fulfills only 4% of its seed requirements, with the majority being considered as low quality and infected (Food and Agriculture Organization of the United Nations, 2021). To address this issue, modern techniques such as plant tissue culture are employed to produce disease-free and virus-free seed potatoes (Hussain et al., 2018). Through *in vitro* cultivation under controlled conditions, the growth and health of these plants rely on an optimal combination of growth regulators (Yildirim et al., 2019). Plant tissue culture, also known as aseptic culture, involves the cultivation of cells, tissue, organ and their components in a controlled environment using defined physical and chemical conditions (George et al., 2008). This technique holds significant importance in fundamental and applied research along with various commercial applications (Thorpe, 2007). The concept originated from the pioneering work of German scientist Haberlandt in the early 20$^{th}$ century (Haberlandt, 1902). Initially, the studies focused on root culture, embryo culture and the establishment of the first true callus and tissue culture (Reinert, 2010). Subsequently, amid the 1940 -1960, there was remarkable progress in the development of novel practices and the refinement of existing ones (Murashige, 1974). The discovery of these techniques opened the ways to apply tissue culture in 5 broad areas: activities of cell (Cytology, nutrition, metabolism, morphogenesis embryogenesis and pathology), plant modification and enhancement, production of pathogen free plants and storage of germplasm, mass propagation and the formation of products, primarily secondary metabolites (Reinert, 2003; Mandal et al., 2014). This application began in the mid-1960s (Reinert, 2003) and continued to expand throughout the 1990s, encompassing an increasing no. of plant species (Mandal et al., 2014). Cell cultures have remained a vital tool for studying fundamental aspects of plant biology and biochemistry (Murashige & Skoog,

1962), while also playing a significant role in molecular biology and agricultural biotechnology research (Thrope et al., 2018).

The nutrient medium or culture generally defines as the medium used to culture the plant cell. This medium contains all the crucial components essential for plants to effectively go through their vital growth stages (Farhatuallah, 2006). If we explore the historical background of gibberellic acid, it was first discovered by Japanese farmers in rice crops. These crops exhibited an exceptional height, reaching up to 12-15 feet (Sponsel, 1995; Hedden & Kamiya, 1997; Hayashi, 2006; Yamaguchi, 2008). Plants depend on various substances to regulate their essential functions generally known as hormones. These hormones act as growth regulators, growth inhibitors and they play intricate roles in plant growth and development. Among all these hormones, gibberellic acid acts as a critical growth regulator. It has a pivotal role in various parts of plant improvement including stem elongation, the senescence cycle, seed development and vitally the coordination of morphological stages in plants which involves cell enlargement (Bakore, 2016).

Gibberellic acids find various applications in agriculture and industries. They are employed in the stem elongation of grapes, facilitating increased height in sugarcanes, and even utilized in brewing industries. However, one of their most significant applications lies in breaking potato seed dormancy (Bryan, 1989). Gibberellins, a class of plant hormones, are commonly employed to promote stem elongation, flowering and breaking the dormancy phase in seeds, buds, corms and bulbs. Gibberellin is available in more than ninety forms but the most commonly used is GA3. This study aims to produce disease-free potato seedlings and examine the impact of various concentrations of gibberellic acid on the growth and development of Lady Rosetta potato variety in tissue culture.

## Materials and Methods

### Site of research

The research experiment was carried out at the Biotechnology Laboratory in the Department of Agriculture Research, Gilgit, Baltistan. The plant material used in this experiment consisted of the cultivated variety of Lady Rosetta potato in Gilgit Baltistan.

### Nutrient medium preparation

The explants were used in a culture medium that was entirely dependent upon the cultural medium provided by the experiment performer. In this experiment, Murashige and Skoog medium "MS" (Hartmann and Kester, 1968) with different concentrations of GA3 was utilized. Murashige and Skoog medium (1962) served as a plant growth medium. A growth medium can be in solid, liquid, or semi-solid form. When 6-8% agar was dissolved in the liquid nutrient medium, it changed into a solid medium. A particularly solidified medium is referred to as a semi-solid medium. On the other hand, a medium without agar remained in a liquid state, known as a liquid medium. Liquid medium was employed for cell suspension cultures. To prepare the liquid medium, 1000 mL of distilled water was combined with 4.43 g of sucrose and 10 mL of iron.

### Growth regulator preparation apparatus

We utilized various laboratory equipments such as a Petri dish, stirrer, shaker, and weight balance, along with substances like GA3 (growth regulator), NaOH (sodium hydroxide), and distilled water, to prepare a specific stock solution of the growth regulator GA3. We added 0.025 ml of the growth regulator GA3 and to dissolve it effectively, incorporated a few drops of NaOH. To ensure complete dissolution of the solution, we stirred it for 2 to 3 minutes using an orbital shaker. Subsequently, we protected the glass vase containing the stock solution from direct sunlight by wrapping it with a piece of paper and stored it at -20 °C to prevent decay.

### Gibberellic acid treatment

In this experiment, three treatments of gibberellic acid (GA3) were utilized: T1 (1 mg/l), T2 (1.5 mg/l) and T3 (2 mg/l). Each treatment was replicated three times, and all treatments were inoculated into 1000 mL of liquid MS medium.

### Plant material and cultural conditions

The sterile potato plantlet, Lady Rosetta, was carefully chosen from the tissue culture lab collection at the Agriculture Research Directorate, Gilgit. To enhance its growth, the sterile potato variety (Lady Rosetta) underwent modification using MS medium supplemented with the growth regulator GA3. The liquid medium consisted of three distinct concentrations of GA3, namely 1 mg/l, 1.5 mg/l and 2 mg/l. The culture was then incubated at a temperature of 25 °C with 16-hour light cycle, providing illumination using white fluorescent tubes (Philips TL 40w/54) that emitted approximately 2,000 lux.

### Statical analysis

The analysis of variance (ANOVA) technique was used to analyze the data. The differences and mean values among treatments were determined by using the least significant difference (LSD) test at a significant level of 5% (Gomes & Gomes, 1984). To conduct the experiment, a completely randomized design was employed.

## Results

### Length of plantlets

In this research, the length of plantlets was examined by the application of phytohormone GA3. The results showed that the application of GA3 had a significant effect on the length of plantlets. Particularly for the treatment T1 where GA3 was applied at the concentration of 1 mg/l and plantlets exhibited maximum length (Fig. 1). Data showed increase in the length for the Lady Rosette plantlets were 13.5 cm, 13 cm and 11 cm, respectively. These measurements were taken after the plantlets had undergone

GA3 treatment. The findings revealed that the application of GA3 at a concentration of 1 mg/l proved to be beneficial to promote the growth and elongation of the plantlets. It is important to note that researchers considered the use of a standard dose of $GA_3$ and this concentration of GA3 proved to be highly effective for the development of shoots.

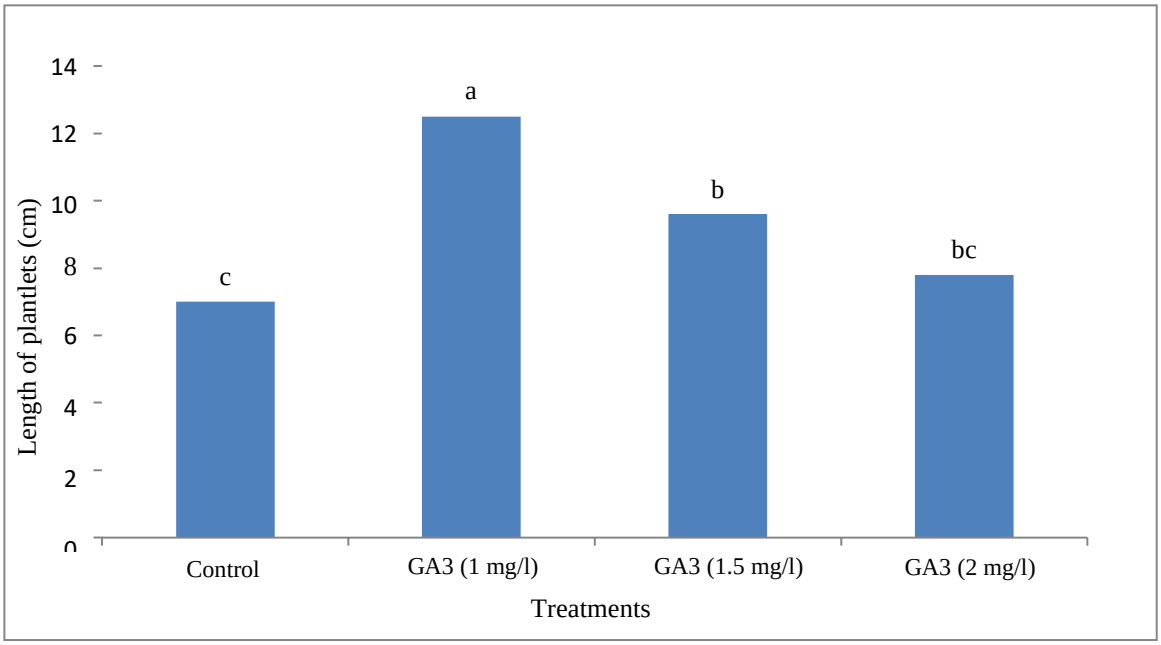


**Fig. 1** Effect of various concentrations of GA3 on the length of potato plantlets

**Total number of stems**

The data presented in Fig. 2 illustrates the total number of stems in the variety Lady Rosatte under the influence of different concentration of gibberellic acid. The statical analysis of the study was conducted with the significant level of > 0.05%. The findings show that there is a significant difference between all the treatments. The variety Lady Rosseta exhibited varying no. of stems in response to different concentrations of gibberellic acid. It is evident that the application of GA3 had a noticeable impact on stem growth. Among all the treatments, the most significant results were found with the concentration of 1 mg/l GA3 which resulted in the growth of three stems followed by 2 mg/l with the production of same results. At a concentration of 1.5 mg/l of GA3, the lowest numbers of stems (2.0) were obtained. Conclusively, gibberellic acid (GA3) with a concentration of 1 mg/l proved to be the most effective in promoting stem growth and development.

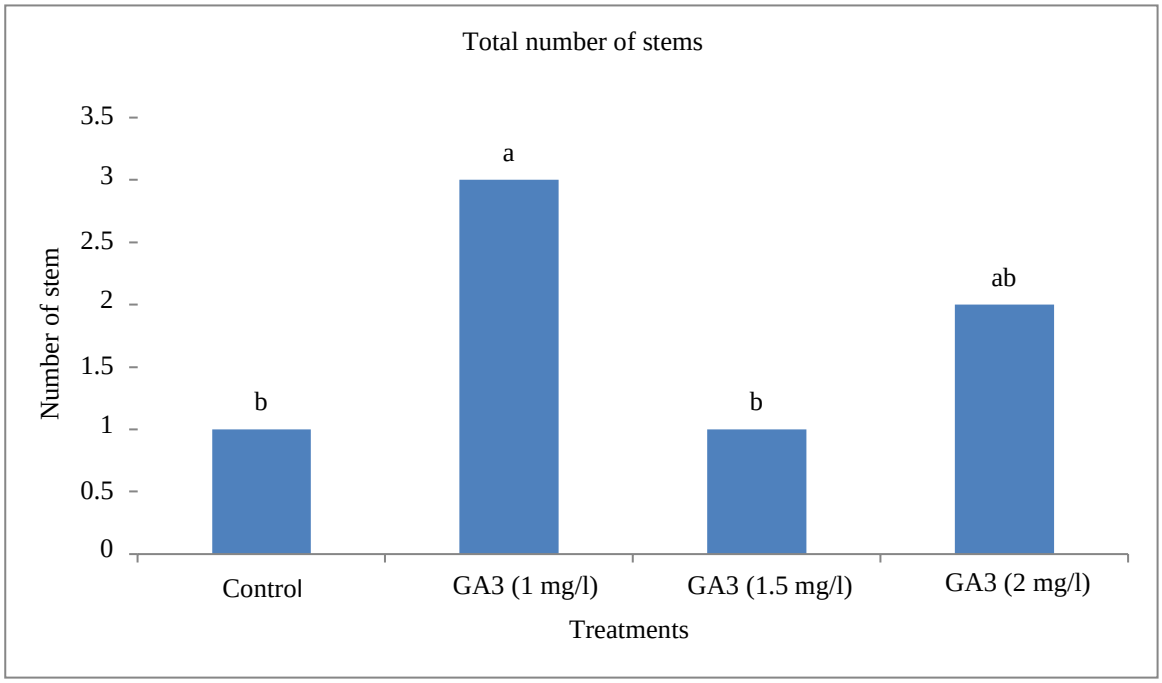


**Fig. 2** Effect of various concentrations of GA3 on number of stems in Lady Rosseta

**Total number of leaves**

The present research focused on investigating the impact of gibberellic acid (GA3) on the production of total number of leaves in potato. Especially, this study aims to determine the effect of GA3 on variety Lady Rossate. Notably, the variety Lady Rosseta showed maximum growth in the number of leaves of 35, 37 and 35 (Fig. 3). Under treatment T1, the first plantlet showed a higher number of leaves as compared to other plantlets.

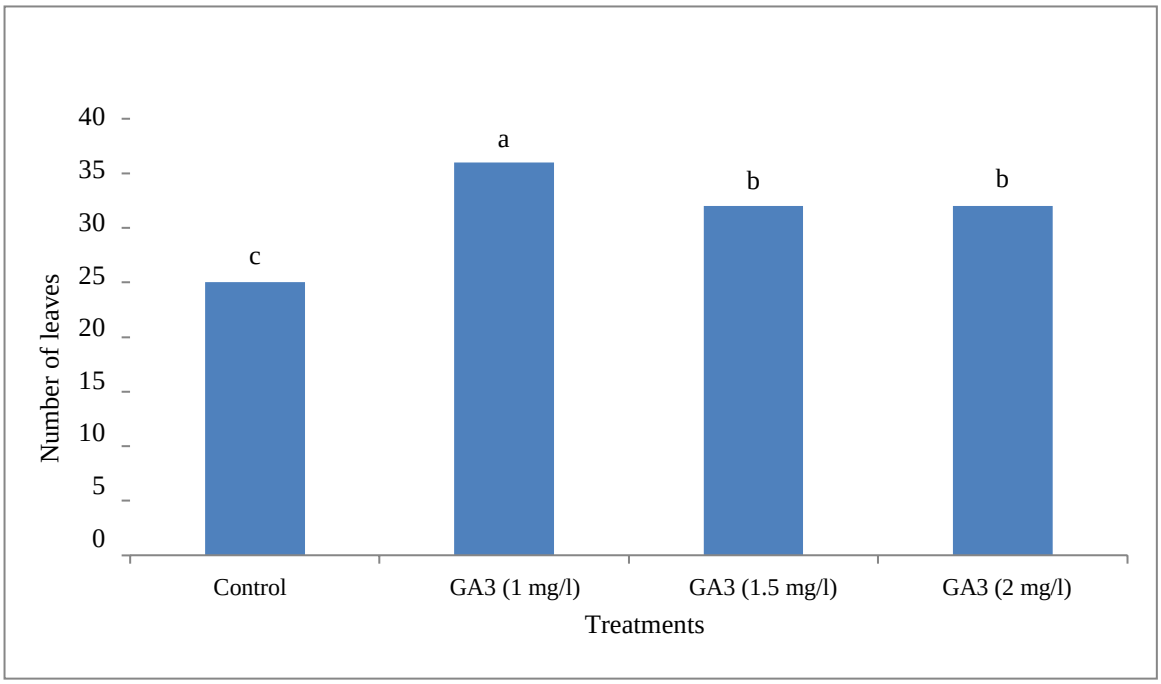


**Fig. 3** Effect of different concentration of GA3 on number of leaves in Lady Rosetta

## Discussion

Potato (*Solanum tuberosum* L.) is among the most significant vegetable crops grown worldwide. After corn, wheat and rice, it is regarded as the world's 4th food crop (Alva et al., 2011). Likewise other crops, potatoes can be reproduced by two means, sexually and asexually i.e., true potato seed and by means of tubers, respectively (Beukema & Van der Zaag, 1990). In 1960, stem cutting was introduced for the first time as a mode of propagation (Dahshan et al., 2018) and tuber propagation as a mean to eliminate the bacterial and fungal pathogens. Furthermore, disease-free planting material is produced by using different propagation techniques such as stem cutting, nodal cutting and leaf bud cutting. Stem cutting technique is considered the most useful amongst them. About 30% potato multiplication in North American and 25% in European rapid multiplication programs used stem cutting technique (Jones, 1988). GA3 is an important plant growth regulator (PGR) for dormancy breakage and shoot differentiation because of the breakdown of apical dominance and cell multiplication (Casimiro et al., 2001). Development of shoots may hasten the growth of leaves. The plants received GA3 at lower concentration (0.1 mg/l) represented more numbers of leaves as compared to others (Abd El-Kafie et al., 2018).

The outcomes of the current research revealed that the application of phytohormone GA3 played a significant role in increasing the length of the plantlets in Lady Rosetta variety. In comparison of control group, the plantlets which were treated with 1 mg/l GA3 showed increased length. It was noticed that after GA3 treatment, the length of plantlets increased 13.5 cm, 13 cm, and 11 cm, respectively. The results proposed that GA3 at a concentration of 1 mg/l proved to be beneficial in promoting the growth and elongation of plantlets. Across the three plantlets, the maximum number of leaves (35-37) was achieved in the plant variety Lady Rosetta. Under treatment T1, the first plantlet exhibited the higher leaf number which was the result of application of GA3 at the rate of 1 mg/l. These findings declared that application of GA3 (1 mg/l) under treatment T1 produced standard and promising results in term of plant performance. Also, the length of plantlet and subsequently the number of leaves improved under treatment T1 by the application of gibberellic acid. This indicates that GA3 had a positive impact on the growth and development of Lady Rosetta plants, resulted in increasing leaf production.

Asalfew et al. (2016) reported that the application of gibberellic acid (GA3) to potato tubers, both before sowing and after harvesting, effectively breaks dormancy and promotes early sprouting. They found that treatments with GA3 at concentrations of 750 ppm and 1000 ppm not only break dormancy but also lead to earlier sprouting, enhanced yield, and improved quality of potato haulm. The number of sprouting and the sprout length of the tuber are increased. Most likely the dipping treatment of 40 and 50 ppm results in a reduction in the dormancy period by 18-20 days and also demonstrates more effective results in control trial as compared to the application with low concentration of GA3. It was noticed that GA3 treatment at the rate of 750 ppm and 1000 ppm on haulm reduced the dormancy period by 11 days whereas dipping of potato tubers in 40 ppm and 50 ppm solutions decreased the days to emergence by 6 and 8 days, respectively. Hence, the optimum results are found with the application of GA3 at the rate of 750 ppm and dipping of potato tubers in 40 ppm solution. In a previous research study, GA3 was highly effective on morphogenesis and proved efficacious for the adaptation of International Potato Center (CIP) potato saplings in the field (Abd El-Kafie et al., 2018). Akey et al. (2017) evaluated the impact of GA3 on coconut embryo, and its

plantlets grown by *in vitro* propagation wherein the examination was held to see the effect of GA3 on the propagation of zygotic embryo and its conversion into plantlets. Four treatments were applied obtaining five-week culture in two different media; solid medium and liquid medium and after that the embryo was transferred into GA3 free liquid medium culture for the remaining 224 days. The shoot or the saplings plantlets were classified into 4 groups. The first group comprised shoots (10 cm) with less weight, while other three groups were contained plantlets, heights with 10 cm-20 cm > 20 cm-30 cm and >30 cm-40 cm. Later, when the development of plantlets acclimation was done and plantlets were shifted to greenhouse and transferred into black polyethene bags having a mixture of peat moss with highly fertile composites (1: 1, w/w). After 32 weeks, the saplings were moved to a greenhouse and planted in black polythene bags containing a mixture of peat moss and soil (1: 1, w/w). The bags were then covered with transparent polythene and the surrounding area was bound with silt (151.5 cm) to improve atmospheric exchange. Significant results were obtained after 36 weeks, with a high germination percentage recorded in the liquid medium and the tallest plants reaching a height of 20-30 cm.

Azad et al. (2020) reported that the purpose of research work was to develop virus free potatoes through tissue culture techniques such as meristem culture. Two different genotypes i.e., Cardinal and SH-5 were propagated by meristem culture technique. Gibberellic acid (GA3), naphthalene acetic acid (NAA) and mixture of both plant growth regulators (PGRs) were analyzed with different concentrations (200 µl, 300 µl and 400 µl). The purpose of this evaluation was to check the development in vegetative and reproductive phases such as growth of roots, shoots, no. of leaves and nodes in potato varieties, cardinal and SH-5. The significant difference between varieties, hormones and their interaction mean value declared that for two varieties, the most suitable results were found in case of Cardinal variety with root length (0.70 cm), shoot length (2.50 cm), no. of leaves (6.96 cm) and number of nodes (8) as compared to SH-5 that produced root length (0.54 cm), shoot length (1.38 cm), no. of leaves (4.29) and no. of nodes (5.3). An application of gibberellic acid at 300 µl charted by 200 µl concentration on the variety cardinal showed the best results. The maximum root length (1.40 cm) shoot length (3.43cm), no. of leaves (10) and no. of nodes (11) were obtained by applying this mixture with concentration of 300 µl. The usage of 200 µl gibberellic acid affected in the same way on root and shoot length as 300 µl but differed in case of number of leaves and nodes.

## Conclusion

The findings of this research revealed that the concentration of gibberellic acid had a significant influence on various growth parameters of potato (*Solanum tuberosum*) including the number of plantlets, total no. of leaves, and total number of stems. The treatment with 1 mg/l gibberellic acid was found to be the most effective in promoting leaf propagation in potato, while the treatment with 2 mg/l of gibberellic acid showed the lowest values for these parameters. Lady Rosetta demonstrated the highest number of leaves with good strength, followed by 1 mg/l of treatment and 1.5 mg/l of treatment. These findings contribute to the optimization of potato plant propagation techniques, particularly in terms of leaf production. Overall, the study highlights the significance of gibberellic acid as a valuable tool in *in vitro* propagation of potato and offers important insights for improving potato cultivation practices.

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