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IN-VITRO MULTIPLICATION AND MICROTUBERIZATION OF *SOLANUM TUBEROSUM* L. USING DIFFERENT PGPR

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ABSTRACT

The study investigated the effect of inoculation with the plant growth promoting rhizobacteria isolated from rhizosphere on the growth, quality and performance of minitubers of two varieties, Lady Rosetta and Mozika of potato (*Solanum tuberosum* L.). Both varieties were grown in vitro from using shoot tip as explants on MS medium. After 2 to 3 weeks, when rooted plantlets were transferred to liquid MS, and three PGPR bacterial strains were added to the nutrient solution. We evaluated the effect of inoculation on the potato growth and minituber yield. This research focuses on the effective utilization of plant growth-promoting rhizobacteria (PGPR) in the *in vitro* cultivation of potatoes to fulfill the requirements for sustainable improvement of micropropagation results. A total of 24 PGPR bacterial strains were isolated, of which three (*Enterobacter kobei* {LRR4}, *Providencia vermicola* {LRR5}, and *Serratia fonticola* {LRR12}) were selected for *in vitro* experimentation pertaining to potato. The mean shoot length of 10.95 ± 0.04 cm was achieved on Murashige and Skoog (MS) medium supplemented with the LRR5 strain in the Lady Rosetta, whereas the maximum shoot length recorded in the Mozika was 9.17 ± 0.26 cm when subjected to LRR5 treatment. In the Mozika variety, the highest fresh tuber weight recorded was 0.43 ± 0.01 g, which occurred following exposure to the LRR5 strain. Conversely, in the Lady Rosetta variety, the maximum tuber weight of 0.507 ± 0.005 g was documented in plantlets treated with the LRR4 strain. Both varieties showed slightly enhanced SOD and POD activity when treated with the selected strains. Similarly, a maximum chlorophyll content of $1.278 \pm 0.008 \mu\text{mol m}^{-2}$ was found when the plantlets were treated with strain LRR5 in var. Mozika and contrarily, in var. Lady Rosetta, the total chlorophyll content was $1.292 \pm 0.004 \mu\text{mol m}^{-2}$ in leaves that subjected to strain LRR4.

Keywords: Potato, Microtuberization, In vitro multiplication, PGPR.

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INTRODUCTION

Research on plant growth-promoting rhizobacteria (PGPR) in potato development is significant for improving agricultural productivity and sustainability. The potato, a vital food crop, requires considerable phosphorus inputs, but traditional fertilization raises environmental issues (Chea et al., 2021). Historical studies have progressed from identifying PGPR strains to exploring their mechanisms and effects in various conditions (de la Paz-Osorio et al., 2023). The rising need for sustainable practices highlights the importance of incorporating PGPR in potato

micropropagation systems (Tkachenko et al., 2021).

Despite advancements, optimizing PGPR application in in vitro potato growth is still underexplored (Aloo et al., 2021). While many PGPR strains have been studied for their beneficial traits, their growth-promoting effects in vitro are inconsistent and dependent on the potato genotype (Kargapolova et al., 2020; Grigoryan et al., 2022). Additionally, there are debates regarding the effectiveness of various PGPR species, with some studies showing significant benefits and others indicating limited effects (Oswald et al., 2010; Aloo, 2023). This inconsistency

hinders the establishment of standard protocols for PGPR application, potentially affecting seed tuber production and field performance (Soumare et al., 2021).

The objective of this study is to assess the application of PGPR in in vitro potato development, focusing on their growth mechanisms, strain effectiveness, and impact on microplant acclimatization and yield. By consolidating existing research, this study seeks to address the identified knowledge gap and offer guidance for enhancing PGPR utilization in potato tissue culture, thereby promoting sustainable seed production and crop management.

METHODOLOGY

Present study was conducted in Plant Developmental and Regenerative Biology Laboratory (PDRBL), Institute of Botany, University of the Punjab, Lahore. For continuous availability of explants, healthy tubers of both potato varieties, i.e., Mozika and Lady Rosetta were used to establish plants during mid-October, 2021 under natural growing conditions following the standard protocols in the field at the Institute of Microbiology and Molecular Genetics, University of the Punjab, Lahore.

Surface sterilization of explants and multiplication

For in vitro micropropagation of potato, shoot tips were used as explants. After bringing in the laboratory, the shoot tips were thoroughly rinsed under flowing tap water for about 10-15 minutes. The explants were then immersed in 20% solution of household bleach (sodium hypochlorite; NaOCl) containing few drops of Tween-20 keeping on the magnetic stirrer with continuous shaking for about 20 minutes. They were then rinsed four to five with autoclaved distilled water. The explants were then immersed in to 0.1% solution of mercuric chloride (HgCl_2) for 5 minutes with continuous stirring followed by washings with autoclaved distilled water under strict aseptic conditions.

Within the working cabinet, the necrotic ends of the explants were removed and the sterilized tips (approximately 1 cm) were carefully placed on the nutrient medium. The tubes were again enclosed using the polypropylene sheets and rubber bands. At $25 \pm 2^\circ\text{C}$, the culture tubes were then provided with the 16 h photoperiod using cool white florescent light ($40 \mu\text{mol}/\text{m}^2/\text{s}$ photon flux density, Philips, Karachi, Pakistan). After every fourteen days, the growing plantlets were transferred to fresh medium after segmenting them into explants of same size (approximately 1 cm) to ensure the continuous availability of the plantlets.

Co-culturing of plantlets with selected plant growth promoting rhizobacteria

Twenty days old plantlets of approximately of equal size

with well-developed roots were selected for further experiments. Under aseptic conditions, they were removed carefully from the agar medium and were transferred to MS broth. To the culture tubes the 100 μl of the selected bacterial strains were dispensed when their optical density (OD) was 1.0. The tubes were again placed at $25 \pm 2^\circ\text{C}$ with 16-hour photoperiod.

Morphological and yield parameters

Various growth and yield parameters of the in vitro grown plantlets were measured to assess the effect of the plant growth promoting rhizobacteria. These parameters include the shoot length (cm), root length (cm), shoot fresh weigh (g), root fresh weight (g), number of branches and tubers per plantlet, and the fresh weight of tuber (g).

Determination of activity of superoxide dismutase

For extract preparation, fresh leaf samples, approximately 100 mg were taken and immersed in liquid nitrogen. After adding 0.1 g Polyvinylpyrrolidone (PVP) the leaves were pulverized to fine powder using a pre cooled pestle and mortar. To the pulverized sample was transferred to the centrifuge tube and 2.0 ml of phosphate buffer having pH 7.2 was added to it. After homogenization, the samples were centrifuged at 12,000 rpm at 4°C for 20 minutes. The supernatant was collected to a new tube which was then used for further analyses.

The activity of superoxide dismutase (SOD) was measured through the modified method of Maral et al. (1977). The assay assesses the SOD activity which inhibit the Nitroblue Tetrazolium (NBT) reduction. During photochemical reaction, methionine and riboflavin generate superoxides (O_2^-) which cause NBT reduction to blue coloured formazan. Superoxide dismutases compete for the superoxides which are being converted to hydrogen peroxide (H_2O_2) and molecular oxygen (O_2), thus decreasing the formazan production. The decreased NBT reduction is monitored by spectrophotometry for quantification of SOD activity. Thus, one unit of SOD activity is the amount of enzyme which is responsible for 50 percent inhibition in NBT reduction. For measuring the SOD, 3.0 ml of reaction mixture (0.1 mM EDTA, 13 mM methionine, 50 μM NBT, 50 mM phosphate buffer and 2 μM riboflavin) were added to 20 μl of the supernatant. In control, the reaction mixture lacked the extract. All the samples were then exposed to 40-W fluorescent cool-white light ($40 \mu\text{mol}/\text{m}^2/\text{s}$ photon flux density) for 10-15 minutes. The absorbance was then measured at 560 nm through a spectrophotometer and SOD activity (U/mg of protein) was calculated by applying the formula;

Percent inhibition (%)

$$= \frac{\text{Absorbance of control sample} - \text{Absorbance of experimental sample}}{\text{Absorbance of control sample}} \times 100$$

Measurement of peroxidase activity

The peroxidase activity of the in vitro grown plantlets was measure by the Racusen and Foote (1965). The reaction mixture was prepared by thorough mixing of 10 µl of the enzyme extract, 200 µl of 1% Guaiacol, and 2.5 ml of 100 mM phosphate buffer. The optical density (OD) was then determined after adding 100 µl of 0.3% H₂O₂ keeping the absorbance at 470 nm. The POD was quantified by applying the formula below:

$$\text{Peroxidase activity (mg/g protein/min)} = \frac{A \times df}{EU \times Wt. \times 1000}$$

Where, A represents increased OD/minute, df is the dilution factor, EU is the amount of extract used and Wt. is the weight of sample.

Total chlorophyll content

Chlorophyll content (µmol m⁻²) of the fresh leaf samples was determined by using DMSO (dimethyl sulfoxide)

method. For this 0.1 g fresh leaves were incubated in 10 ml of DMSO in darkness at room temperature for 48 hours. Then absorbance of chlorophyll extract was measured at 645 and 663 nm as described by Arnon (1949). The total chlorophyll content was determined by applying the following formula;

$$\text{Total chlorophyll (µmol m}^{-2}\text{)} = 20.2 \times A(645) + 8.02 \times A(663)$$

Statistical Analysis

All the data obtained during the experiments were subjected to statistical analyses using the statistical tools SPSS 21.00 and least 3 replicates were used to calculated the mean values.

RESULTS

Plantlets of potato varieties Mozika and Lady Rosetta were established on MS (Murashige and Skoog, 1962) basal medium as shown in Figure 1. Then, 30 days old plantlets of uniform size were then sub-cultured to MS broth supplemented with either of the selected bacterial stains, i.e., LRR4, LRR5 and LRR12.

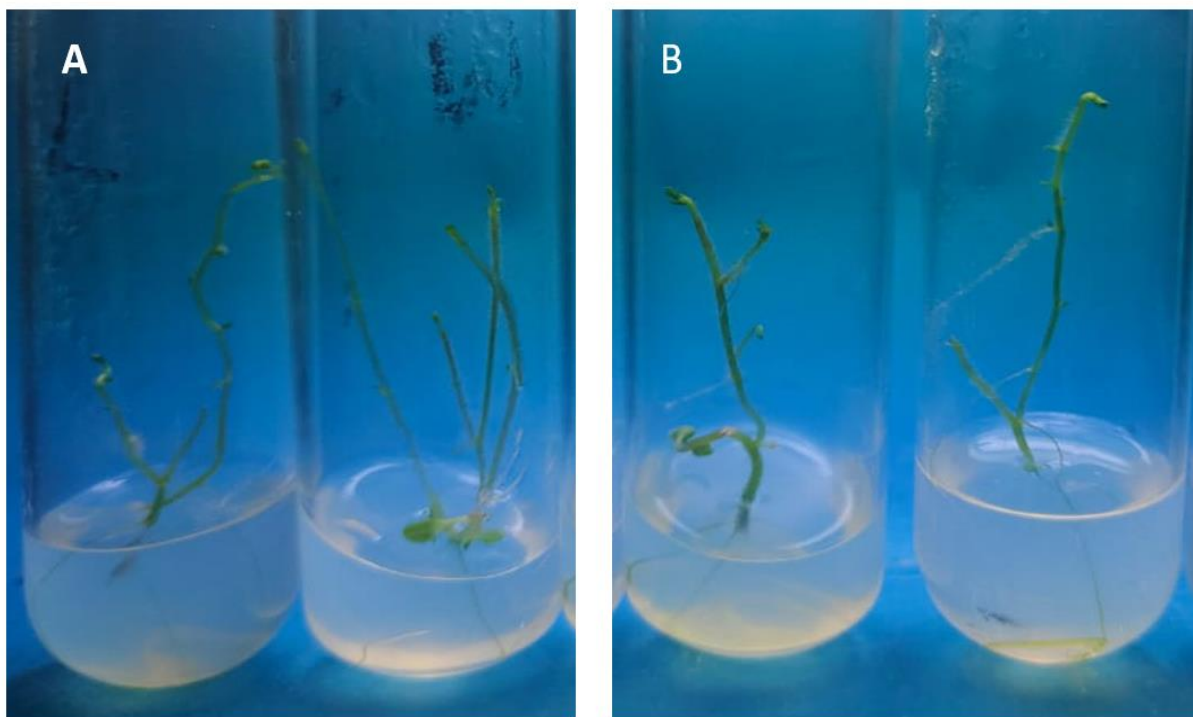


Figure 1: Potato plantlets obtained on MS (Murashige and Skoog, 1962) basal medium. (A) var. Mozika and (B) var. Lady Rosetta.

Effect of selected PGPR strains on morphological parameters

The selected bacterial strains, LRR4, LRR5, and LRR12, caused a significant increase in the shoot length of both potato varieties, i.e., Mozika and Lady Rosetta, under in vitro

conditions on MS broth (Figure 2). However, the difference was more pronounced in var. Lady Rosetta as compared to var. Mozika. Nonetheless, in both varieties showed maximum shoot length in the presence of strain LRR5. Under control conditions, increase in shoot length of var. Mozika was $5.925 \pm$

0.47 cm, which was 9.17 ± 0.26 cm when the plantlets were exposed to strain LRR5. The applications of strains LRR4 and

LRR12 also showed a significant increase in the shoot lengths up to 7.25 ± 0.64 cm and 7.63 ± 0.06 cm, respectively.

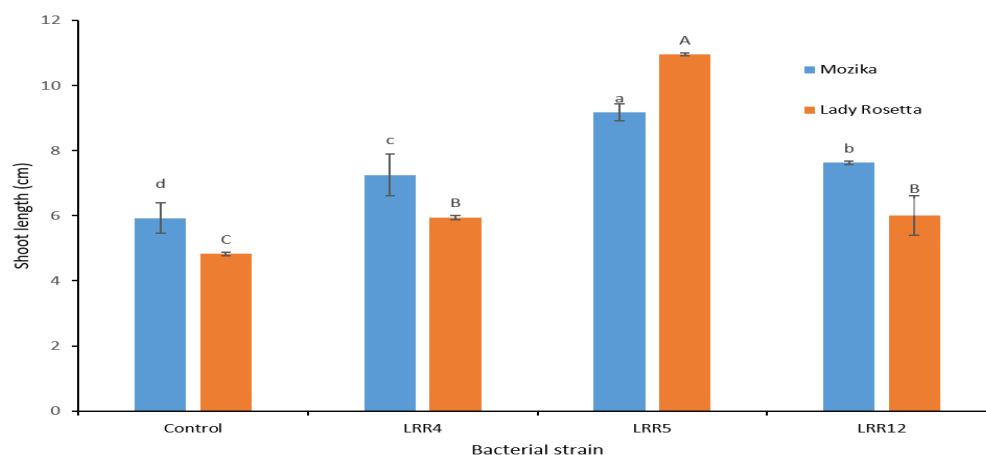


Figure 2: Increase in shoot length (cm) of potato varieties Mozika and Lady Rosetta under in vitro conditions. Mean of 4 replicates $\pm$ standard error; different alphabets represent significant difference using Duncan's Multiple Range Test (DMRT) at $p=0.05$.

In case of the var. Lady Rosetta, the increase in shoot lengths was 4.83 ± 0.06 to 10.95 ± 0.04 cm when the medium was supplemented with bacterial isolate LRR5 and the control, respectively. The other two selected strains, i.e., LRR4 and LRR12, also caused a significant increase in the shoot length up to 5.95 ± 0.06 and 6.0 ± 0.61 cm, respectively.

Under in vitro conditions, bacterial isolates showed a positive effect on the root length of both potato varieties, i.e., Mozika and Lady Rosetta (Figure 3). Similar to the

shoot length, the longest roots of both varieties were observed in the plantlets co-cultured with strain LRR5, however, the effect was more pronounced in var. Lady Rosetta in comparison to var. Mozika. In var. Mozika, a significant increase in root length from 4.9 ± 0.41 to 6.1 ± 0.7 cm was observed when the plantlets were treated with strain LRR5. The var. Lady Rosetta showed similar behaviour of increasing root length, which increased from 3.05 ± 0.4 to 6.8 ± 0.41 cm in the presence of strain LRR5.

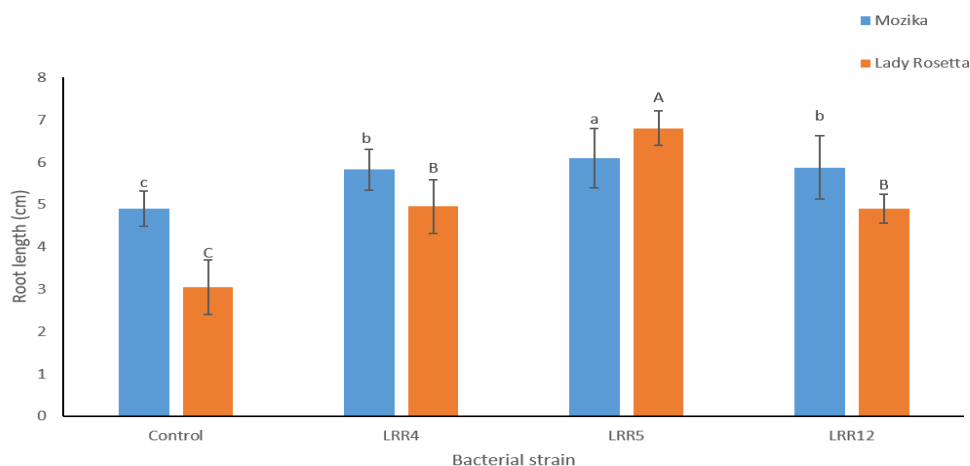


Figure 3: Effect of rhizobacteria on root length (cm) of potato varieties Mozika and Lady Rosetta under in vitro conditions. Mean of 4 replicates $\pm$ standard error; different alphabets represent significant difference using Duncan's Multiple Range Test (DMRT) at $p=0.05$.

Figure 4 shows fresh weights of shoots of the plantlets of both potato varieties when co-cultured with the selected bacterial stains under in vitro conditions. In case of var. Mozika observed a significant increase in the fresh weight of shoots from 0.22 ± 0.004 to 0.245 ± 0.02 g in plantlets treated with strain LRR4 compared to the control plantlets. On the other hand, the strains LRR5 and LRR12 caused reduction in the fresh weights of shoots. In case of var. Lady Rosetta, shoots

with minimum fresh weights were observed in untreated plantlets where the mean fresh weight was 0.19 ± 0.004 g. The application of all the selected bacterial strains significantly increased the fresh weight, and the maximum weight of shoots was observed in the plantlets treated with strain LRR5, which was 0.495 ± 0.006 g. The fresh weights of shoots were 0.318 ± 0.02 and 0.327 ± 0.008 g, when the plantlets were treated with strains LRR4 and LRR12, respectively.

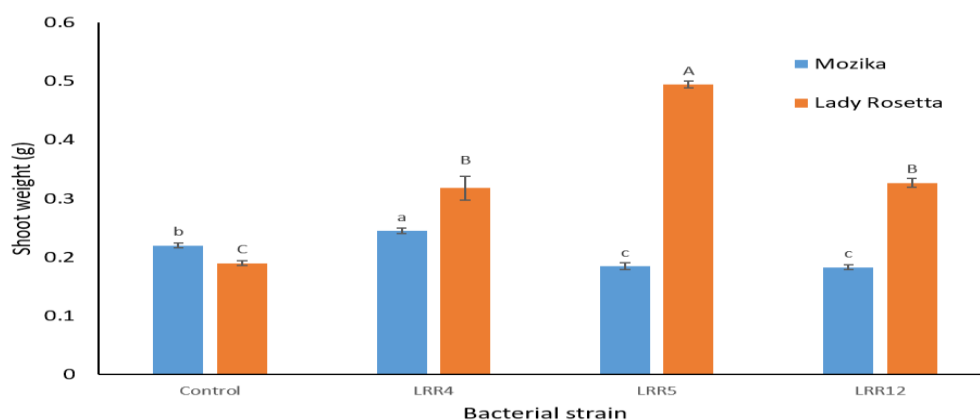


Figure 4: Effect of rhizobacteria on fresh weight of shoot (g) of potato varieties Mozika and Lady Rosetta under in vitro conditions. Mean of 4 replicates $\pm$ standard error; different alphabets represent significant difference using Duncan's Multiple Range Test (DMRT) at $p=0.05$

Under in vitro conditions, the fresh weights of the roots of potato plantlets of both varieties treated with the selected bacterial strains have been shown in Figure 5. All the selected three strains caused a significant increase in the fresh weights of the plantlet roots of both varieties. In both varieties, maximum fresh weights of roots were observed when the plants were exposed to the strain LRR5; however, the effect was more noticeable in the var. Lady Rosetta. In var. Mozika, the fresh weight of the root was 0.113 ± 0.004

g under control conditions. However, it increased significantly up to 0.185 ± 0.006 and 0.182 ± 0.004 g due to the treatments provided with LRR5 and LRR12, respectively. In case of var. Lady Rosetta, the fresh weight of roots showed an increase from 0.095 ± 0.003 to 0.255 ± 0.005 g in the presence of strain LRR5. However, the fresh weights of roots of the plantlets treated with the strains LRR4 and LRR12 were 0.182 ± 0.007 and 0.183 ± 0.004 g, respectively.

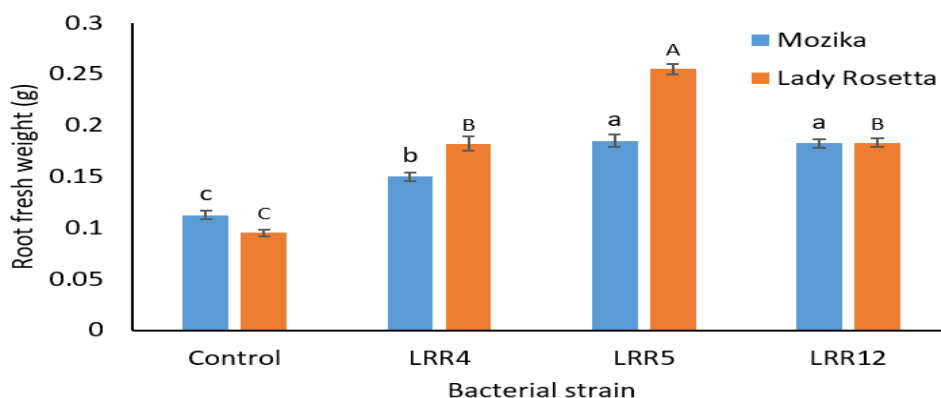


Figure 5: Effect of rhizobacteria on fresh weight of root (g) of potato varieties Mozika and Lady Rosetta under in vitro conditions. Mean of 4 replicates $\pm$ standard error; different alphabets represent significant difference using Duncan's Multiple Range Test (DMRT) at $p=0.05$.

The number of branches per plantlet of both potato varieties, i.e., Mozika and Lady Rosetta was determined when the plantlets were treated with the selected bacterial strains under in vitro conditions (Figure 6). In both potato varieties, the treatments of plantlets with strain LRR5 showed maximum number of branches per plantlet. In var. Mozika, there were 9.0 ± 0.41 branches per plantlet and this number reached to 14.0 ± 0.41 when the plantlets were co-cultured with LRR5. However, there were 10.5 ± 0.28

and 8.0 ± 0.41 branches per plantlet when they were treated with strains LRR4 and LRR12, respectively. A similar pattern was observed in the var. Lady Rosetta where the number of branches per plantlet reached to 11.25 ± 0.47 when treated with strain LRR5 when compared with the control where it was 7 ± 0.41 . On the other hand, there were 8.0 ± 0.41 and 7.0 ± 0.41 branches per plantlet in respect to the treatments provided with strains LRR4 and LRR12, respectively.

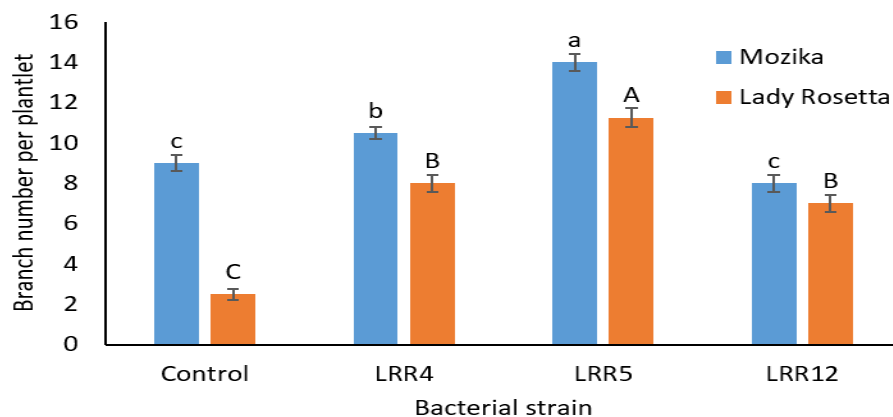


Figure 6: Effect of rhizobacteria on the number of branches of potato varieties Mozika and Lady Rosetta under in vitro conditions. Mean of 4 replicates $\pm$ standard error; different alphabets represent significant difference using Duncan's Multiple Range Test (DMRT) at $p=0.05$

Effect of selected PGPR strains on yield parameters

Tuber number per plantlet and tuber weight are important parameters in determining the yield (Figure 7). Figure 4.8 shows the number of tubers per plantlet. Both varieties showed a considerable increase in the number of potatoes per plantlet.

In var. Mozika, the maximum number of tubers, i.e., 3.5 ± 0.28 , was observed, which was significantly higher than in the control, where this number was only 1.0 ± 0.41 . The treatments using strains LRR4 and LRR12 produced 2.5 ± 0.28 and 1.25 ± 0.25 tubers per plantlet, respectively.



Figure 7: Tubers produced on potato plantlets under the effect of PGPR. (A) var. Mozika and (B) var. Lady Rosetta.

Contrarily, in the var. Lady Rosetta, the highest number of tubers per plantlet was achieved, provided with the treatment using strain LRR12 (Figure 8). There were 7.0 ± 0.28 tubers per plantlet were observed when plantlets were treated with strain LRR12 while in control this number was only 1.5 ± 0.28 . While there were 4.0 ± 0.41 tubers per plant produced when the plantlets were co-cultured with strain LRR4. In var. Lady Rosetta, the strain LRR 5 did not affect the number of tubers per plantlet.

Figure 9 represents the fresh weight of tubers in both varieties when treated with selected bacterial strains under in vitro conditions, which is an important yield parameter. In both potato varieties, the applications of bacterial strains significantly increased the tuber weight,

although the increase was more pronounced in the var. Lady Rosetta. In var. Mozika, the maximum fresh weight of the tuber, i.e., 0.43 ± 0.01 g, was observed after the treatment with strain LRR5, while in the control, the fresh weight per tuber was 0.105 ± 0.003 g. The fresh weights of tubers obtained from the plantlets received the applications of either strain LRR4 or LRR112 were 0.175 g. Contrarily, in var. Lady Rosetta, maximum tuber weight, i.e., 0.507 ± 0.005 g, was observed in the plantlets treated with the stain LRR4, which was significantly higher in comparison with control where its weight was 0.15 ± 0.004 g. The tubers obtained after the treatments with LRR5 and LRR12 were of 0.235 ± 0.006 and 0.265 ± 0.003 g, respectively.

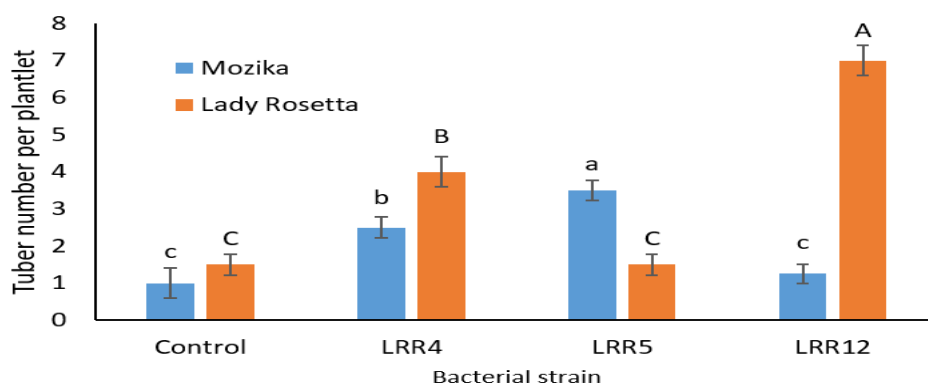


Figure 8: Effect of rhizobacteria on the number of tubers per plantlet of potato varieties Mozika and Lady Rosetta under in vitro conditions. Mean of 4 replicates $\pm$ standard error; different alphabets represent significant difference using Duncan's Multiple Range Test (DMRT) at $p=0.05$

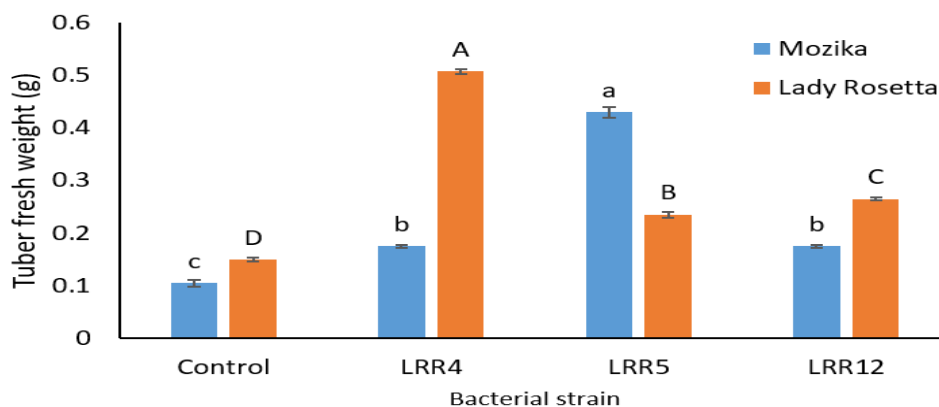


Figure 9: Effect of rhizobacteria on the fresh weight (g) of tubers of potato varieties Mozika and Lady Rosetta under in vitro conditions. Mean of 4 replicates $\pm$ standard error; different alphabets represent significant difference using Duncan's Multiple Range Test (DMRT) at $p=0.05$

Effect of selected PGPR strains on biochemical parameters

Figure 10 shows the superoxide dismutase (SOD) activity of

the leaf tissues of both potato varieties when treated with selected bacterial strains. Both varieties showed slightly enhanced SOD activity when treated with the selected

strains. The varieties Mozika and Lady Rosetta showed Maximum SOD activities when treated with LRR5 and LRR4, respectively. In var. Mozika the SOD activity increased from 4.28 ± 0.04 to 5.11 ± 0.04 U/g of tissue when the plantlets were treated with strain LRR5 in comparison with the control. However, the plantlets treated with strains LRR4 and LRR12 exhibited SOD activities 4.9

± 0.03 and 4.79 ± 0.01 U/g of tissue, respectively. In var. Lady Rosetta, a significant increase in SOD activity from 4.45 ± 0.12 to 5.18 ± 0.02 U/g of tissue was observed when the plantlets were exposed to LRR4 in comparison to the control. The plantlets treated with other two strains, i.e., LRR5 and LRR12 exhibited SOD activities 5.07 ± 0.12 and 4.52 ± 0.04 U/g fresh weight, respectively.

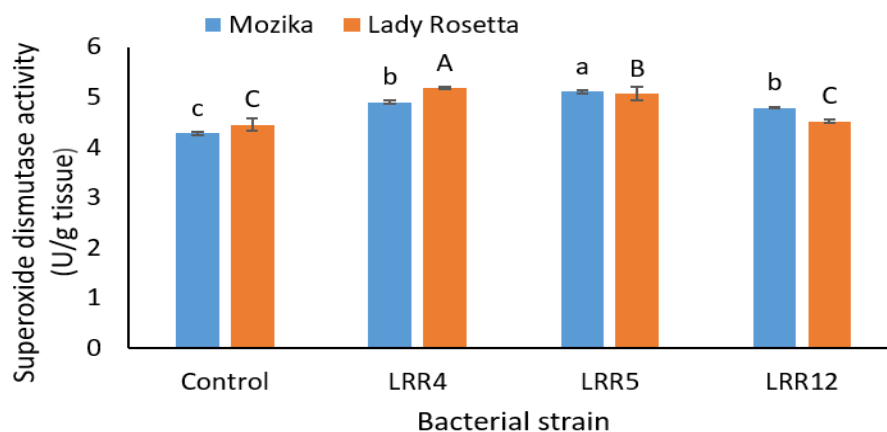


Figure 10: Effect of rhizobacteria on the activity of superoxide dismutase (SOD) in potato varieties Mozika and Lady Rosetta under in vitro conditions. Mean of 4 replicates $\pm$ standard error; different alphabets represent significant difference using Duncan's Multiple Range Test (DMRT) at $p=0.05$

Peroxidase activity (POD) of the plantlets of both potato varieties treated with the selected bacterial strains is shown in Figure 11. Increased POD activities were observed in the plantlets of both varieties treated with either of the selected bacterial strains. In var. Mozika, the maximum POD activity, i.e., 0.828 ± 0.011 U/g protein/min, was observed when the plantlets were treated with the strain LRR5 which was significantly higher than the control, where the plantlets exhibited 0.558 ± 0.006 U/g protein/min POD activity.

However, plants treated with strains LRR4 and LRR12 exhibited 0.602 ± 0.008 and 0.573 ± 0.005 U/g protein/min of POD activities, respectively. In var. Lady Rosetta, the POD activity increased from 0.54 ± 0.03 to 0.67 ± 0.004 U/g protein/min when the plantlets were treated with strain LRR4 in comparison with the control. However, the plantlets treated with strains LRR5 and LRR12 exhibited POD activities 0.615 ± 0.003 and 0.56 ± 0.013 U/g protein/min, respectively.

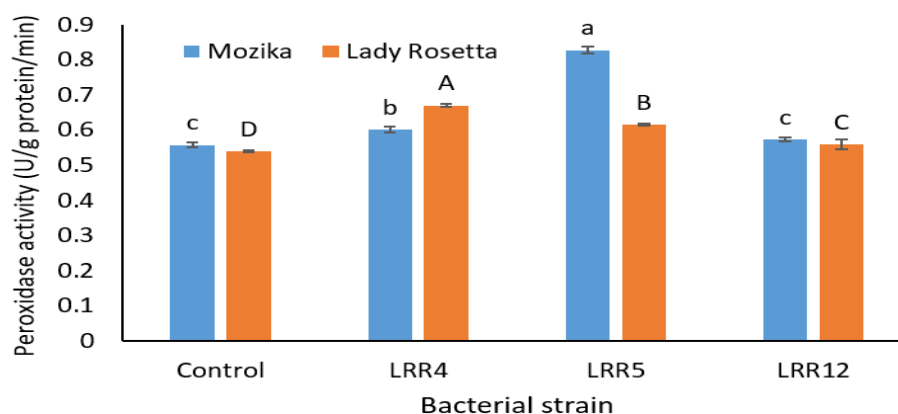


Figure 11: Effect of rhizobacteria on the activity of peroxidase (POD) in potato varieties Mozika and Lady Rosetta under in vitro conditions. Mean of 4 replicates $\pm$ standard error; different alphabets represent significant difference using Duncan's Multiple Range Test (DMRT) at $p=0.05$

Figure 12 shows the total chlorophyll of the leaves obtained from the plantlets of both varieties when treated with the selected bacterial strains. Both varieties showed significantly higher values for total chlorophyll content when treated with either of the selected bacterial strains. In var. Mozika, the total chlorophyll content was increased from 0.985 ± 0.006 to a maximum of $1.278 \pm 0.008 \mu\text{mol m}^{-2}$ when the plantlets were treated with

strain LRR5 in comparison with the control. Contrarily, in var. Lady Rosetta, the total chlorophyll content was increased from $1.05 \pm 0.02 \mu\text{mol m}^{-2}$ in the leaves of the control to $1.292 \pm 0.004 \mu\text{mol m}^{-2}$ in leaves that received strain LRR4 treatments. The leaves of the plantlets treated with LRR5 and LRR12 exhibited 1.222 ± 0.008 and $1.255 \pm 0.025 \mu\text{mol m}^{-2}$ of the total chlorophyll content, respectively.

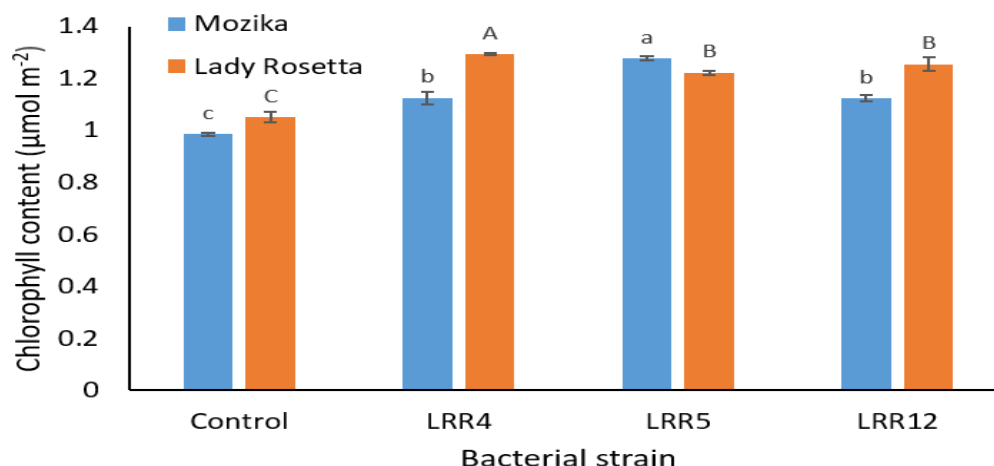


Figure 12: Effect of rhizobacteria on the total chlorophyll content in potato varieties Mozika and Lady Rosetta leaves under *in vitro* conditions. Mean of 4 replicates $\pm$ standard error; different alphabets represent significant difference using Duncan's Multiple Range Test (DMRT) at $p=0.05$.

DISCUSSION

The present experiment showed that the potato plantlets of two varieties, Mozika and Lady Rosetta, inoculated with selected strains of plant growth-promoting rhizobacteria (LRR4, LRR5, and LRR12), exhibited significant effects on morphological, yield, and biochemical parameters under *in vitro* conditions. The findings clearly demonstrated that bacterial strains positively altered plant development and physiological processes, although the degree of these effects varied among bacterial isolates and between the two varieties (Ahmad et al., 2022). Among the strains tested, potassium-solubilizing strain LRR5 had the most pronounced impact on the growth of both potato varieties, with remarkable improvements in shoot and root length, indicating its high growth-promoting potential (Kumar et al., 2020).

This enhancement may be attributed to the ability of LRR5 to synthesize phytohormones such as indole-3-acetic acid (IAA), gibberellins, and cytokinins, which stimulate cell elongation, division, and differentiation (Hayat et al., 2010). PGPR are also known to solubilize phosphate and facilitate the uptake of essential nutrients, thereby improving plant

vigor and biomass accumulation (Bhattacharyya & Jha, 2012). Of particular importance is their positive influence on root development, as a well-developed root system increases water and nutrient absorption (Vessey, 2003). These findings align with previous studies reporting that PGPR-mediated increases in conjugated linoleic acid (CLA) and other phytonutrients enhance root and shoot architecture in potato and other crops (Timmusk et al., 2017).

The observed differences in response between Mozika and Lady Rosetta may be due to genotypic variations in root exudation profiles and bacterial colonization efficiency, which influence plant-microbe interaction dynamics (Compant et al., 2019). Inoculation with PGPR also improved the fresh weights of shoots and roots, with LRR5 again showing the most pronounced effect, particularly in Lady Rosetta. This increase in biomass likely reflects enhanced photosynthetic efficiency and nutrient assimilation promoted by bacterial inoculation (Verma et al., 2021). The higher number of branches per plantlet in LRR5-treated samples may be attributed to improved hormonal balance, especially increased cytokinin levels known to promote lateral shoot initiation (Spaepen et al., 2007).

Yield-related parameters such as the number and weight of tubers were significantly affected by PGPR inoculation. In *Mozika*, strain LRR5 produced the highest tuber number and weight, while in Lady Rosetta, LRR12 and LRR4 were more effective. This variation indicates that plant–bacteria compatibility plays a crucial role in determining the extent of yield enhancement (Sarma et al., 2015). PGPR promote tuber formation by enhancing carbohydrate accumulation in stolons and facilitating translocation of photosynthates to developing tubers (Ahmad & Kibret, 2014). Furthermore, these bacterial inoculants improve nitrogen fixation and phosphorus solubilization, increasing nutrient availability essential for tuber initiation and enlargement (Bhattacharyya & Jha, 2012). These findings are consistent with previous reports showing that inoculation with *Pseudomonas*, *Azospirillum*, and *Bacillus* species significantly enhances tuber number and size in potato (Egamberdieva et al., 2017). Biochemical analyses revealed enhanced antioxidant enzyme activities, including superoxide dismutase (SOD) and peroxidase (POD), as well as increased total chlorophyll content in both varieties treated with PGPR. Elevated SOD and POD activity suggests that bacterial inoculation strengthened the plant's defense mechanisms and oxidative stress control (Kloepper et al., 2004). PGPR can induce systemic resistance (ISR) through activation of antioxidant pathways that scavenge reactive oxygen species (ROS) under stress conditions (Pieterse et al., 2014). The higher chlorophyll levels in inoculated plantlets likely reflect improved photosynthetic capacity and overall plant vitality due to increased uptake of nitrogen, magnesium, and iron—key elements for chlorophyll biosynthesis (Verma et al., 2021). Enhanced chlorophyll content contributes to increased carbon fixation and biomass production, ultimately leading to higher tuber yield (Ahmad et al., 2022).

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