IMPACT OF SIDEROPHORE PRODUCING RHIZOBACTERIA ON GROWTH AND IRON CONTENT IN POTATO

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ABSTRACT: Iron deficiency is a prevalent nutritional disorder that has a significant impact on a large population worldwide. Malnutrition can potentially be mitigated through the implementation of biofortification, a method aimed at enhancing the micronutrient content of staple food items. Plant growth promoting rhizobacteria (PGPR) possess the capacity to enhance the iron concentration in consumable plant tissues by enhancing its availability through various mechanisms. A controlled experiment was conducted to evaluate the capacity of five bacterial isolates (Z1, Z2, Z3, Z4, Z5, Z6) to promote plant growth and enhance the bioavailability of iron (Fe) in potato plants. The iron was administered via a solution that consisted of iron sulphate. The study's results demonstrate that the utilization of PGPR led to a notable enhancement in multiple growth parameters of the plants, encompassing plant height, root length, root fresh and dry weights, shoot fresh and dry weights, and iron content, in comparison to plants that did not undergo PGPR inoculation. The application of FeSO4 led to a significant increase in the concentration of Fe, with a respective rise of 100% and 173% observed in the grain and shoot, in comparison to the control group. The application of Plant Growth Promoting Rhizobacteria (PGPR) in combination with iron led to a substantial improvement in the levels of iron in both grain and shoot tissues. Specifically, there was a notable increase of 78.64% in grain iron content and a 63.24% increase in shoot iron content, as compared to the control group. The results of this study suggest that the utilization of plant growth-promoting rhizobacteria (PGPR) can augment the absorption of iron (Fe) by plants in the presence of supplementary Fe in the soil. The results of this study indicate that the implementation of microbial assisted biofortification in potato tuber holds promise for addressing micronutrient deficiency in human populations, particularly in regions with limited access to resources.

Keywords: micronutrient, siderophore, human health.

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INTRODUCTION

Micronutrient malnutrition is a significant problem for human health all over the world, but it is most prevalent in nations with limited access to resources (Bhandari and Banjara, 2015). Around two billion individuals are thought to be suffering from deficiencies in vitamin A, iron, and iodine, according to the estimations provided by the World Health Organization (WHO). Poor households and pre-school children are disproportionately impacted by iron deficiency because of the high demand for iron (Shukla et al., 2014). Iron deficiency is a highly widespread nutritional condition that affects between 2.5 and 5 billion individuals around the world (Darnton-Hill et al., 2005). Iron serves as a co-factor for several enzymes in the human body that are responsible for carrying out fundamental tasks.

According to Ahmed et al. (2013), having an insufficient amount of iron in the diet might lead to mental retardation, anemia, and disability. According to Rana et al. (2012), this condition's malnutrition could be improved by increasing the amount of bio-available iron in the diet using iron supplements and food fortification. These efforts are typically quite costly, and it can be challenging to maintain them on a daily basis, particularly in nations where people are suffering from a lack of nutrition (Zhang et al., 2012).As a result, it would

appear to be in everyone's best interest for crop fortification with iron content to be a technique that would be both cost effective and desired in order to satisfy the latent hunger for iron.

Biofortification is a process that can be used to produce staple foods that are higher in micronutrient content using mineral fertilizer, conventional breeding methods, and transgenic techniques; however, the success of these methods has not been constant (Murgia et al., 2012). The creation of siderophores by plant growth promoting rhizobacteria increases soil fertility and crop productivity, as well as the iron content of food crops, as reported by Rana et al. (2012). Siderophores are organic compounds with a low molecular weight that have a high affinity for the element iron. Siderophores can be broken down into one of four primary categories: catecholates, hydroxamates, hydroxypyridonates, or aminocarboxylates. By coordinating with iron atoms on the surface of minerals, siderophores can produce iron complexes that are extremely stable and speed up the process of dissolving iron-containing minerals. Rhizobacteria that encourage plant growth produce siderophores, then release those siderophores into the environment around them. Next, they disperse the iron by creating an iron chelate complex, and last, they transport the iron to the plant through its growing roots (Khalid et al., 2015). According to Boukhalfa and Crumbliss (2002), plants are able to absorb these iron siderophores complexes by using transporter proteins that are situated on the plasma membrane of the root.In addition, the presence of these rhizobacteria promotes plant growth by reducing the plant's susceptibility to disease and increasing its ability to boost nitrogen fixation, phosphate solubilization, synthesis of phytohormones, and formation of organic acid (Ahemad and Kibret, 2014). The use of plant growth promoting microorganisms (PGPR) increased the amount of iron that was found in rice grain. As a result, biofortification of plants using PGPR is regarded as a risk-free method for increasing the amount of iron present in the various edible plant parts and reducing the severity of the condition's malnutrition.

The potato has emerged as a significant staple crop for both agricultural producers and consumers worldwide, including Pakistan. The potato is considered one of the most significant crops due to its high production rate and nutritional value. The potato is of significant importance on a global scale in ensuring food security and addressing issues of hunger (Devaux et al., 2014). In Pakistan, potato is considered one of the significant food crops, following wheat, maize, and rice, as highlighted by Rauf et al. (2007). The current investigation was carried out with the goal of enhancing the iron uptake, general growth, and yield of potato using rhizobacterial inoculation as a means of addressing this problem.

MATERIALS AND METHODS

In this investigation, we selected six (Z1, Z2, Z3, Z4, Z5 and Z6) different plant growth-promoting bacterial strains that had previously been thoroughly characterized and demonstrated the potential to make siderophore.

Preparation of the inoculum for seed coating: Growing the selected strains in a medium consisting of glucose and peptone broth allowed for the preparation of inoculum. At a temperature of 28.1 degrees Celsius, flasks that contained inoculums were kept warm. Measuring the optical density at 535 nm allowed for the upkeep of a uniform cell density (107-108 CFU mL^{-1}) throughout the experiment. Each culture's inoculum was injected into sterile peat at a volume of 100 $mLkg^{-1}$. The mixture was then placed in an incubator for 24 hours at a temperature of 28 minus 1 degree Celsius. In order to inoculate the seeds, they were first dressed in a slurry that was made by combining clay, peat, and a sugar solution that was diluted to 10%. In the case of the control with no inoculation, the seeds were coated with the same bacterial suspension that had been sterilized in an autoclave.

Pot experiment: An experiment in a pot was carried out to investigate the impact of six different bacterial strains (Z1, Z2, Z3, Z4, Z5 and Z6) with and without iron on the growth and iron content of potato. To achieve this goal, pots were loaded with ten kilograms of clay loam soil that had an electrical conductivity of $1.14dSm^{-1}$ and a saturation percentage of 32. Each pot received five potato seeds, which were planted at a depth of five centimeters. All the isolates were put through tests both on their own and in conjunction with an iron sulphate solution applied to the soil at a concentration of 28 ppm per pot. In addition, a control treatment was kept, which involved neither the application of iron nor PGPR.

Another treatment was performed, which consisted solely of the application of iron without any inoculation. All the treatments were organized in accordance with a completely randomized design (CRD), and each replication was performed three times. In each container, the recommended amounts of NPK were administered as urea, diammonium phosphate, and sulphate of potash, respectively. At maturity, the plants were collected, and data regarding the yield contributing parameters were recorded. The root and the shoot of the plants were evaluated for their levels of Fe concentration. The method proposed by Wolf (1982) was used to carry out the digestion of plant materials. In order to accomplish this, 0.1 grammes of oven-dried and crushed plant samples were placed inside of a digestion tube, and then 2 milliliters of concentrated sulfuric acid were added to the tube. The samples were kept at room temperature overnight for observation. After that, one milliliter of 35% extra pure H_2O_2 was poured into the digesting tube

along the sides, and the tube was turned while it was doing so. After putting the tube in a digesting block for twenty minutes, it was then heated to a temperature of 350 degrees Celsius. After that, the tube was removed, allowed to cool, 1 mL of H_2O_2 was added to it, and it was heated once more for 20 minutes. The same method was carried out up until the point where the substance lost its color. To determining the amount of iron present, a volume of colorless extract of fifty milliliters was produced using distilled water and then preserved. By using an atomic absorption spectrophotometer, we examined a variety of plant part samples for the presence of iron buildup. These included grain, shoots, and roots.

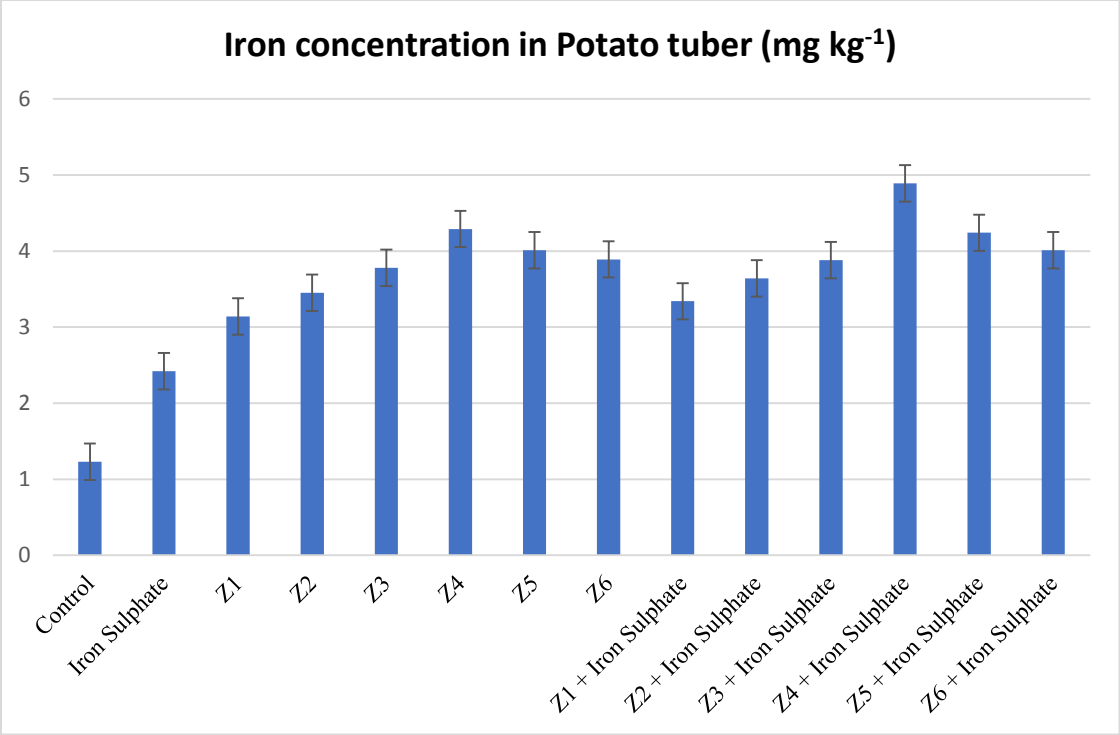
Soil iron concentrations were determined using the ammonium bicarbonate-DTPA (AB-DTPA) extractable technique (Soltanpour et al., 1976). Statistical techniques were used to analyze the data (Steel et al., 1997). Duncan's Multiple Range Test (DMRT) (Duncan, 1955) was used to compare the means.

RESULTS

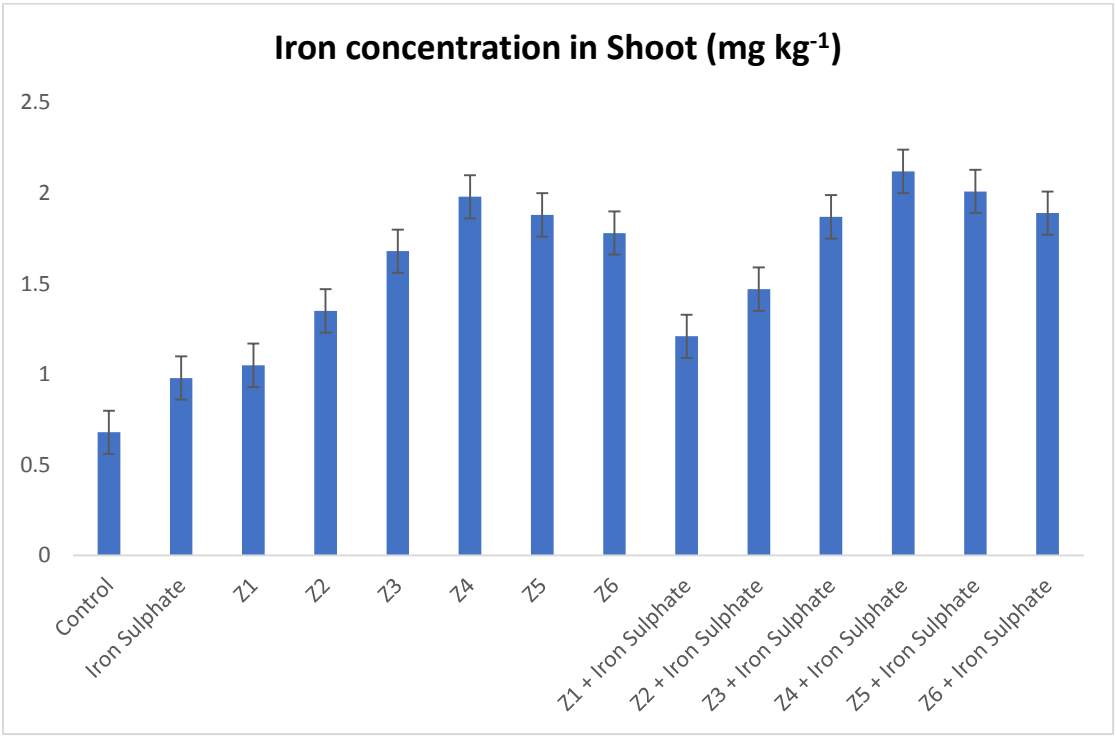
When compared to the uninoculated control plant, the performance of selected bacterial isolates was greatly enhanced in the presence of iron; however, the results of plant growth and yield are presented in Table 1. These findings demonstrated that certain isolates of bacteria considerably boosted plant stature. Plant height was shown to be increased with both isolate Z1 and Z4, however the maximum plant height was observed by using a combined application of isolate Z4 and Fe. This resulted in a 84.35% increase in plant height compared to control plants that were neither treated nor infected. A considerable increase in number of tubers per plant was also found with PGPR inoculation compared to control and iron-treated plants; however, the largest number of tubers per plant was recorded by the combined application of Fe and Z4; this resulted in a 74.89% increase in number of tubers per plant. In a similar vein, the straw yield was greatly improved by each PGPR isolate. The administration of both Fe and Z2 together resulted in a much greater rise in straw yield (68.57%) compared to the uninoculated control group, indicating a statistically significant difference from other isolates. According to the findings concerning potato root length, it was discovered that root length had been greatly improved by all of the isolates. When compared to the other isolates, the findings produced by Z4 and Z3 exhibited the greatest improvement in root length (67.89%), and these results were much better than those produced by any of the other isolates. In addition, the use of isolate Z2 in conjunction with iron resulted in the greatest increase in root dry weight, while usage of isolate Z3 alone resulted in the greatest increase in root fresh weight. In a similar manner, the maximum root dry weight was seen with simultaneous inoculation of isolate Z1 and iron. This result was 84% higher.

Table 2 shows that PGPR inoculation considerably enhanced potato tuber iron content compared to uninoculated iron treated plant for total iron concentration. In contrast to the results obtained with the other isolates, the largest increase in Fe content of potato tubers (78.98%) was found with the combined application of Fe and isolate Z4.

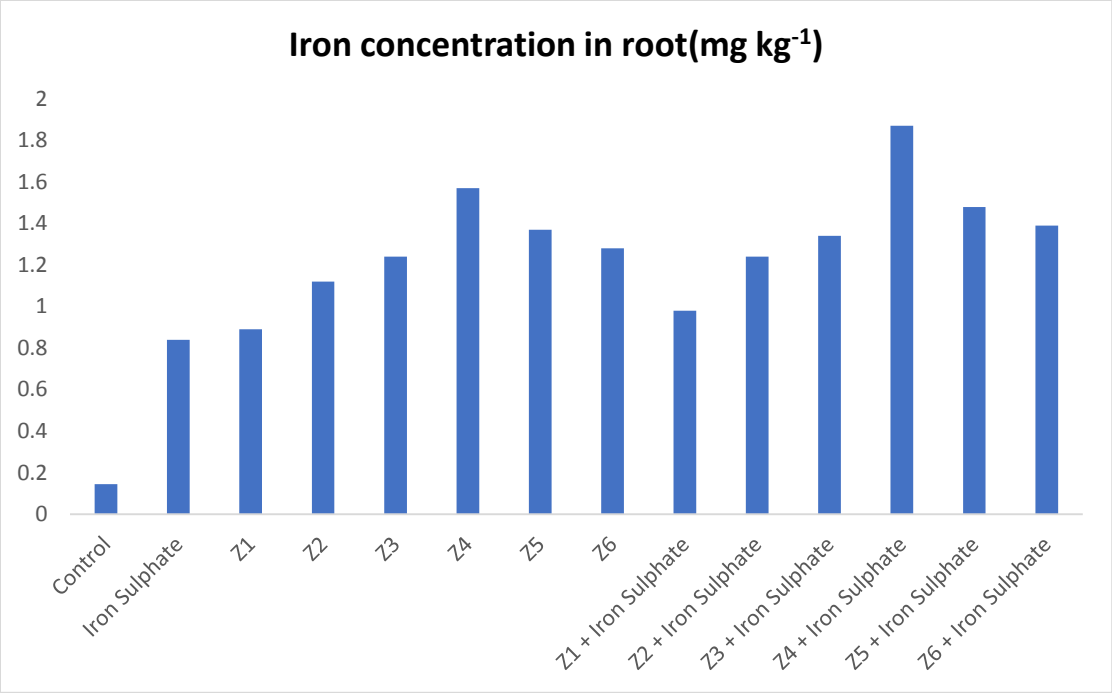
Treatments	Plant height (cm)	Number of tuber/Plant	Straw yield (g)/Pot	Root fresh weight (g)	Root dry weight (g)
Control	27.34	3.99	18.45	11.24	4.496
Iron Sulphate	29.89	4.21	21.24	12.24	4.896
Z1	31.24	4.34	25.45	12.47	4.988
Z2	33.45	4.78	26.74	13.65	5.46
Z3	32.45	5.12	27.88	14.58	5.832
Z4	35.48	5.58	29.65	15.65	6.26
Z5	34.89	4.89	24.78	14.27	5.708
Z6	32.89	5.12	23.68	13.98	5.592
Z1 + Iron Sulphate	33.45	5.21	27.89	14.54	5.816
Z2 + Iron Sulphate	35.47	5.24	29.58	15.67	6.268
Z3 + Iron Sulphate	36.56	5.64	30.14	16.89	6.756
Z4 + Iron Sulphate	40.89	6.45	34.57	17.54	7.016
Z5 + Iron Sulphate	36.58	5.88	26.67	15.65	6.26
Z6 + Iron Sulphate	34.88	5.97	25.87	14.98	5.992



Inoculation also improved the Fe concentration in the shoots. It was found that when Fe and isolate Z3 were applied together, the shoot Fe content increased by as much as 74.98% compared to uninoculated iron-treated plants.



Isolate Z1 alone raised root iron concentration by up to 62.37% compared to uninoculated Fe-treated plants; however, when employed in conjunction with Fe, the rise was much more dramatic (97%).



DISCUSSION

Worldwide, millions of people suffer from iron deficiency. Biofortification is a method for growing food that is high in essential micronutrients. Rhizobia, which aid plant growth through several different methods, may increase iron concentration in edible plant sections. Five bacterial isolates were investigated in this investigation to see if they might boost iron concentration in potato's edible sections when used alone or in conjunction with iron. Our research showed that plant growth, yield, and Fe uptake were all enhanced after being inoculated with PGPR isolates (Dhaliwal et al., 2022).

However, when Fe and PGPR were applied together, the effects were amplified. The growth-promoting effects of PGPR have been linked to boosted nitrogen fixation, P solubilization, phytohormone production, and organic acid synthesis (Aparo et al., 2023). Nitrogen fixation, P solubilization, and the ability to manufacture specific chemicals have all been linked to PGPR inoculation boosting root and shoot growth (Ingle et al., 2023). By increasing P-solubilization and indole acetic acid synthesis, PGPR promoted potato plant development ().

In addition, rhizobacteria's siderophore synthesis aids plant growth by ensuring the plant receives adequate Fe nutrition. Shahid et al. (2023) observed that bacterial isolates of Bacillus cereus UW 85 and *Azotobacter vinelandii* MAC 259 both produced a siderophore that stimulated plant development. There was an increase in plant growth and output after inoculating soybean and potato seeds with siderophore-producing fluorescent Pseudomonas. Similarly, bean strains that produced siderophores increased growth parameters including shoot and root dry weight (Singh et al., 2023). Bacillus megaterium, which was isolated from the tea rhizosphere, was shown to produce siderophores, which stimulated growth and inhibited pathogenicity (Chakraborty et al., 2006).

E. coli with the potential to make siderophore was isolated by Gangwar and Kaur (2009) from rye grass and sugarcane, and this led to increased plant growth and output. In addition, siderophore synthesis by PGPR has been shown to boost soybean growth in non-sterilized soil (Khan et al., 2023).

Our research on Fe concentration in the grain, shoot, and root all point to an increase in Fe levels after PGPR treatment. It was discovered that combining Fe and PGPR inoculation produced the best results. Possible superiority over the control and Fe application treatments in this synergy can be attributed to the siderophore generated by PGPR. Many bacteria produce siderophores, which are ferric iron-specific ligands, in response to low iron levels (Hafeez et al., 2013). According to Kaur et al. (2020), using PGPR increased siderophore production, which in turn improved iron transport from root to grain. Similarly, inoculating wheat seed with three cyanobacterial strains (CW1, CW2, and CW3) and one bacterial strain (PW5) increased the grain's protein content and micronutrients (Fe, Zn, and Cu) concentration (Rana et al., 2012). The increase in iron levels may also be a result of increased ferritin gene expression. Overexpression of the genes for iron

absorption (ferritin) in plants has been found to enhance plant iron content (Dhuldhaj and Pandya, 2017).

Conclusion: The application of siderophore rhizobateria in conjunction with iron (Fe) supplementation resulted in enhanced iron absorption, as well as improved overall growth and yield of potato plants. Hence, it can be inferred that the utilization of iron (Fe) in conjunction with rhizobacteria presents a highly favorable and economically efficient approach for enhancing the iron levels in potato. Further research is required to elucidate the precise mechanism underlying the biofortification of micronutrients by rhizobacteria.

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