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Research Article

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PGPR Mediated Fe-Biofortification of *T. aestivum* L.

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ABSTRACT

Introduction: Due to the high prevalence of Fe deficiency in human populations, especially in developing nations, increasing Fe concentration in cereal crops is a major global challenge. Biofortification of cereals such as wheat with Fe is an attractive approach that can significantly compensate for Fe malnutritionand human health issues. Under nutrient imbalance circumstances, bio-fertilizers have been reported to play a vital role in plant development.

Aims/Objectives: The current study was designed to evaluate the potential of PGPR to be used as bio-fertilizers and appraised their capability to enhance plant growth, quality and nutrient content, in combination with Fe treatment to combat malnutrition.

Methodology: PGPR were screened for auxin producing ability and biochemically characterized for various growth promoting attributes. Fe in various combinations with PGPR was applied in soil and as foliar spray to the plants. Various growth and biochemical parameters were recorded. Wheat leaves were subjected to atomic absorption spectrometric (AAS) analysis.

Results: Five bacteria with maximum auxin production potential were selected as bio-fertilizers. 36% improvement in fresh weight was recorded in *Pseudomonas* sp.(FS2) inoculations in the presence of 100µg/ml Fe under laboratory conditions, whereas, in wire house setup, plants supplemented with *Jeotgalicoccus aerolatus* (Tr3)exhibitedremarkable elevation in sugar content 298% with 100µg/ml foliar application of Fe. Data obtained from AAS showed that plants treated with *Burkholderia cepacia* (FS1) and *Proteus mirabilis* (Pc3) exhibited 2.17 and 2.28 ppm Fe content as a result of soil and foliar spray of 200µg/ml Fe respectively.

Conclusion: Foliar application of Fe along with bio-fertilizers has proven to be a practical approach to improve grain quality and Fe content. Hence, biofortification of cereal crops with micronutrients and bio-fertilizers can assure a sustainable, eco-friendly and cost-effective solution to cure malnutrition and food security in developing countries.

Key Words: Biofortification, Fe malnutrition, Bio-fertilizers, Wheat, Agricultural-Biotechnology, Green solution

INTRODUCTION

Iron is fourth most abundant element on earth and an essential nutrient for all organisms. Despite of its abundance, it is not available to plants in large amounts. In aerobic soil, it is present mostly in the form of Fe^{+3} and generally form insoluble hydroxides and oxyhydroxides, making it usually unavailable toboth plants and microorganisms. Fe involves in basic processes i.e., photosynthesis, respiration and chlorophyll biosynthesis in plants. To maintain iron homeostasis within the plant cell, iron ions are stored in vacuoles and sequestered within ferritins. Plants grown in Fe-deficient soils have low Fe content in their edible parts, resulting in Fe deficiency in humans.[1]

The human body needs Fe to produce oxygen transport proteins i.e., haemoglobin and haem enzymes along with other enzymes containing iron which are essential to produce energy, thyroid function, and immune defense. Insufficient iron absorption will primarily cause the mobilization of storage iron, then inadequate Fe transport to the bone marrow and decrease haemoglobin levels causing anemia. Children's mental and psychomotor development can be slowed by iron deficiency anemia resulting in enhanced morbidity and mortality of child and mother at childbirth.[2]

Plant growth promoting rhizobacteria (PGPR) play vital role in plant growth and development and can be used as bio-fertilizers. PGPR are directly engaged in enhanced uptake of nitrogen, solubilization of minerals such as zinc, phosphorous, synthesis of phytohormones i.e., auxin and production of siderophores for iron chelation to make it accessible to the plant roots and hence improve the nutrient content and crop

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quality. Biofortification of cereals using bio-fertilizers can be considered as a potential beneficial measure, which along with breeding assortments, can prompt expanded micronutrient concentrations in crops, other than improving yield and soil fertility. Cereal-based foods, such as wheat, are a major dietary component in developing countries, but they have low Fe concentrations and bioavailability.[3] Biofortification of food crops with Fe through agricultural approaches is one of the most widely used methods for reducing the prevalence of Fe deficiency in human populations. The aim of this study was to evaluate plant growth enhancement potential of bio-fertilizers along with Fe micronutrient in improving quality, yield and Fe content of wheat crop to combat malnutrition and improve plant and human health.

MATERIALS AND METHODS

Screening and Growth Improvement Attributes of Bio-fertilizers (PGPR)

Bacterial isolates were screened for their IAA producing potential following Ahmed and Hussnain.[4] Out of eighteen inoculants, only five isolates i.e., *Proteus mirabilis* (Pc3), *Jeotgalicoccus aerolatus* (Tr3), *Burkholderia cepacia* (FS1), *Pseudomonas aeruginosa* (DS4) and *Pseudomonas* sp.(FS2) were found to be positive for IAA production and further evaluated for their growth promoting attributes such as Zinc, phosphate solubilization and ammonification following Bunt and Rovira[5], Paul and Sinha[6] and Cappuccino and Sherman.[7]

Soil analysis

Before plant growth experiment, soil was analyzed to determine different properties of soilfollowing Motsara and Roy.[8]

Plant Growth Experiment

Plant growth experiments were conducted using selected isolates(Pc3, Tr3, FS1, DS4 and FS2)with and without 100 and 200µg/ml Fe ($FeSO_4.7H_2O$) treatment. Certified seeds of *Triticum aestivumL*. (var. Fd-08) were procured from Punjab Seed Corporation, Pakistan. 0.1% $HgCl_2$ was used for surface sterilization of seeds. Optical densities of bacterial cultures were adjusted at 600 nm to 10^6–10^7 CFU/mL and then seeds were supplemented with these cultures for 1 hour. 175g sterilized sieved soil was taken per pot for laboratory experiment and eight seeds were sown per pot with four replicates whereas, for wire house set up, seven seeds were sown per pot containing 2.7kg sieved soil in triplicates. Soil (before seed sowing) and foliar (to seedlings after four weeks of sowing) application of micronutrient was done. After 27- 30 days of growth period, laboratory grown seedlings were harvested while for wire house, plants were harvested at maturity.Various growth parameters i.e.,shoot and root length, leaf length, width and area, number of leaves, number of grains per plant,spike length, total yield, 100 grains weightwere recorded for treated and non-treated plants. Similarly, biochemical constituents of plants were also noted i.e.,pigment content [Wellburn[9]], protein content [Lowry et al.[10]], sugar content [Dubois et al. [11]].

Atomic absorption spectrometric analysis

To measure Fe content, leaves of treated and non-treated wheat plants, were subjected to atomic absorption spectrometric analysis following Russell et al.[12]

Statistical analysis

The data obtained were analyzed using SPSS (version; 16.0) statistical package (Chicago, USA).

RESULTS

Screening and Growth Improvement Attributes of Bio-fertilizers (PGPR)

Bacterial inoculant FS2 exhibited maximum auxin production upto 318µg/ml. while, FS1 has shown least auxin content i.e., 99.3 µg/ml. Maximum zinc and phosphate solubilizing efficiencyi.e., 265% and 79% was recorded in Tr3 and Pc3 inoculant. Furthermore, appearance of yellowish to brownish color was an indication of ammonia production potential. All inoculants were positive for ammonia production (Fig. 1).

Soil Analysis

Results of soil analysis demonstrated that soil was loamy in texture with (24±3°C) T, (0.78 m/S) EC, (8.4) pH, (0.76%) SOM, (251 mg/kg) N, (2.6 mg kg^{-1}) P and (152 mg kg^{-1}) K. Due to low SOM and high pH, soil could possibly be classified as micronutrients deficient soil.

Plant Growth Assay

A remarkable enhancement in shoot and root length, number of leaves and fresh weight up to 64, 23, 48 and 38% was noted in Tr3, Pc3and FS2 inoculated treatments with 100µg/ml Fe respectively, under laboratory conditions (Fig. 2). In wire house setup, DS4 and FS1 inoculations resulted in significant enhancement in shoot length and number of leaves up to 13 and 23% with 100µg/ml Fe foliar spray respectively, over control (Fig. 3). Plants treated with bacterial inoculant FS1 caused significant increase in leaf length up to 5 and 17% with soil and foliar application of 100µg/ml Fe respectively, over control. Similarly, 16 and 27% enhancement in leaf width was noted in the plants treated with Pc3 and FS2 as a result of 100 and 200µg/ml soil and foliar application of Fe respectively. Maximum increase in leaf area, number of grains per plant, total yield, spike length and 100 grains weight up to 48, 12, 13, 5 and 57% was recorded in treatments

inoculated with FS2, DS4, Pc3and Tr3 with soil and foliar application of 200µg/ml Fe respectively, with respect to control (Fig. 3).

Fe treatments supplemented with bio-fertilizers influenced the biochemical parameters of treated plants as well. In laboratory experiment, bacterial strains Pc3and FS1caused increase in soluble sugar and chlorophyll 'b' content up to 134 and 565% with 100µg/ml Fe while, with 200µg/ml Fe, remarkable improvement in protein, chlorophyll 'a' and total chlorophyll content up to 300, 489 and 604% was recorded in Tr3 inoculations respectively, as compared to non-treated plants (Fig. 2). In wire house set-up, Tr3 inoculations caused significant increment in soluble sugar content up to 134% and 298% with 200 and 100µg/ml soil and foliar application of Fe respectively (Fig. 4). Similarly, Pc3 inoculated plants showed 191 and 119% enhancement in protein content of leaves and *Pseudomonas aeruginosa* (DS4) inoculations exhibited 56 and 45% increase in chlorophyll 'b' content with 100µg/ml Fe soil application and 200µg/ml Fe foliar spray respectively. Increase in protein content of grains was recorded up to 72 and 253% in the plants inoculated with DS4 and Pc3with soil and foliar application of 200µg/ml Fe while FS2 and Pc3inoculations resulted in 244 and 359% and DS4 and Tr3 inoculated plants exhibited 89 and 109% increment in chlorophyll 'a' and total chlorophyll content with soil and foliar applied 100µg/ml Fe respectively.

Atomic absorption spectrometric analysis

According to atomic absorption spectrometric analysis, plants treated with bacterial inoculant Pc3and Tr3 exhibited maximum iron content i.e., 2.20 and 2.24ppm with soil and foliar spray of 200µg/ml Fe treatment, respectively over control (Table 1).

DISCUSSION

Micronutrient malnutrition, specifically Fe deficiency causes hidden hunger leading to severe health issues. Current study was designed to enrich wheat with Fe using bio-fertilizers in a cost effective manner. Results of present study revealed that various growth and bio-chemical constituents of treated plants were significantly increased due to Fe andbio-fertilizer treatment. Under laboratory conditions, plants supplemented with FS2, Tr3, Pc3 showed significant increment in shoot length, root length, number of leaves, fresh weight, soluble sugar, protein and chlorophyll content while, in wire house conditions, various growth parameters i.e., shoot length, leaf length, leaf width, number of leaves increased remarkably in DS4 and FS1 inoculated plants while leaf area, spike length, total yield and 100 grains weight enhanced with FS2, Tr3 and Pc3 inoculations under foliar application of 100 and 200µg/ml Fe respectively.

PGPR possess intrinsic capabilities to produce auxin, which loosens plant cell walls and aids bacteria in absorbing various substances secreted by plant roots, which could explain the increase in shoot length.[13] The application of Fe resulted in increased root proliferation, allowing the plants to produce more roots. Plants with more roots have easier access to nutrients and water. Fe is a fundamental component of enzymes and proteins and involved in energy transfer within plant, nitrogen fixation and enters in root cells. These factors may contribute to an increase in leaf length.[14] Kaur and Singh[15] reported increment in grains number per plant with Fe application, over control. It might be attributed to increase in supply of photosynthates to sink due to higher chlorophyll content and photosynthesis due to more availability of micronutrients by foliar sprays during growth period of the crop.[16] Results of current study demonstrated that, plants inoculated with Pc3 showed maximum increment in protein content of grains while, DS4and Tr3 inoculated plants exhibited improved chlorophyll and sugar content with foliar spray of 100 and 200µg/ml Fe respectively, over control. Kong et al.[17] reported increment in soluble sugar content of wheat by the inoculation of PGPR. Similarly, in a study, an elevation in protein content was noted in inoculated maize plant with PGPR.[18] PGPR used as bio-fertilizers in this work have shown to exhibit positive results for other growth promoting attributes. They might colonize the plant roots and increased plant growth without experiencing damage due to mineral toxicity. Auxin enhances root surface area by producing secondary roots and promotes nutrition status of plant, results in improved growth and yield of plant. In acidic and basic soil, due to strong bonding with calcium and magnesium, phosphate is reduced in the plant roots but bacterial isolates secrete certain enzymes that disrupt this linkage and help to improve the phosphate availability to the plants. These biological entities have the ability convert organic nitrogen to ammonia thus, act as natural biofertilizer for plant growth improvement.[17] In current study, Tr3 and Pc3worked efficiently in growth experiments hence, proved their potential to be worked as bio-fertilizers. The constant availability of Fe to the wheat plants during their lifecycle is the reason for the high Fe content in plants. In this work, foliar application method of Fe was more effectual than soil.The possible reason might be that foliar sprayed Fe is easily absorbed by the leaves and transferred to phloem, which directly affects metabolism and promotes growth. Hence, synergistic utilization of Fe micronutrient and bio-fertilizers has proven to be a promising and cost effective approach to upgrade Fe content, yield and quality of wheat plants.

CONCLUSION

Malnutrition has become a leading concern globally due to intake of micronutrients deficient diet that has an influential

impact on human health. The agricultural scientific community is being challenged by this alarming situation because of the expanding world population and augmenting food demand. To cope with this problem, biofortification of cereal crops is emerging as a sustainable solution to address Fe deficiency. Based on the results presented in this study, it is recommended that treatment of food crops such as wheat with micronutrients and bio-fertilizers, specifically foliar application, is an effective green strategy to deal with Fe insufficiency issues and produce micronutrients dense food, that not only minimizes pollution induced by the use of chemical fertilizers but also eco-friendly and can be adopted by farmers especially in developing nations.

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Conflict of Interest:

Authors declare no conflict of interests.

Author's contribution:

- Rida Rasheed performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, designed study, conducted the experiments and drafted the manuscript.
- Ambreen Ahmed analyzed the data, contributed reagents/materials/analysis tools, authored or reviewed drafts of the paper, approved the final draft, designed study, provided chemicals, checked and revised the manuscript.

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Table 1: Atomic Absorption Spectrometric Analysis of *Triticum aestivum* L. leaves.

Sr. no.	Treatments	Control	Fe (ppm)			
			[100µg/ml]		[200µg/ml]	
			Soil	Foliar	Soil	Foliar
1	Control	0.91	2.12	1.28	1.56	1.14
2	Pc3	1.19	2.01	2.02	2.20	2.12
3	Tr3	1.13	1.91	2.07	1.93	2.24
4	FS1	1.2	2.04	1.94	2.01	2.15
5	DS4	1.01	1.97	2.02	1.91	2.03
6	FS2	1.24	1.78	2.18	2.12	1.79

[Pc3=*Proteus mirabilis*, Tr3=*Jeotgalicoccus aerolatus*, FS1=*Burkholderia cepacia*, DS4=*Pseudomonas aeruginosa*, FS2= *Pseudomonas* sp.]

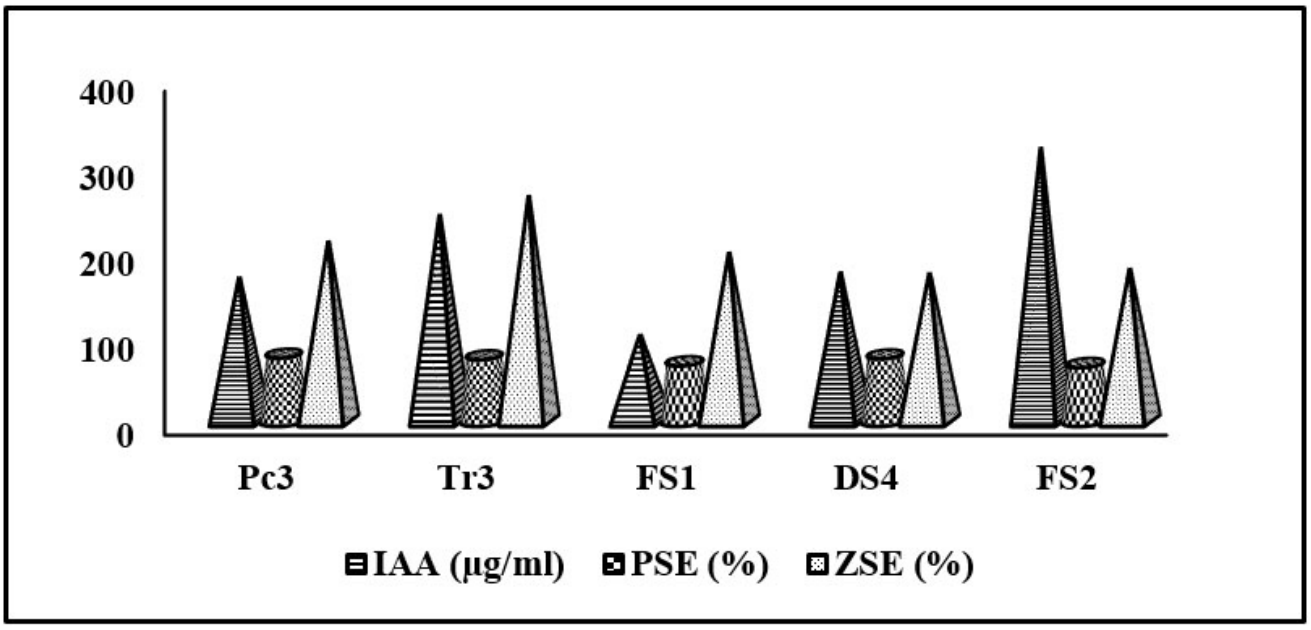

Figure 1: Plant growth promoting attributes of bacterial inoculants. [IAA=Indole-acetic acid, PSE= Phosphate solubilization efficiency, ZSE= Zinc solubilization efficiency, Pc3=*Proteus mirabilis*, Tr3=*Jeotgalicoccus aerolatus*, FS1=*Burkholderia cepacia*, DS4=*Pseudomonas aeruginosa*, FS2= *Pseudomonas* sp.]

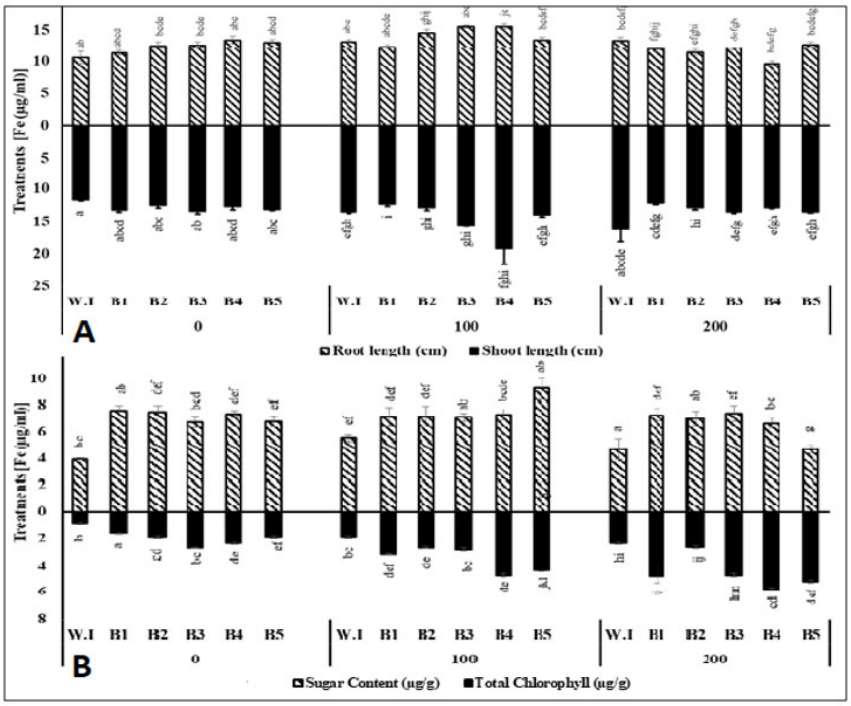

Figure 2: Impact of bacterial isolates (W.I-Without Inoculation, B1-*Pseudomonas aeruginosa*, B2- *Burkholderia cepacia*, B3-*Pseudomonas* sp, B4-*Jeotgalicoccus aerolatus* and B5- *Proteus mirabilis*) on the root length, shoot length [A],sugar content and total chlorophyll content [B] of *Triticum aestivum*L. grown under laboratory conditions using 100 and 200µg/ml Fe. Different letters indicate significant differences between treatments using the Duncan's multiple range test (P ≤ 0.05).

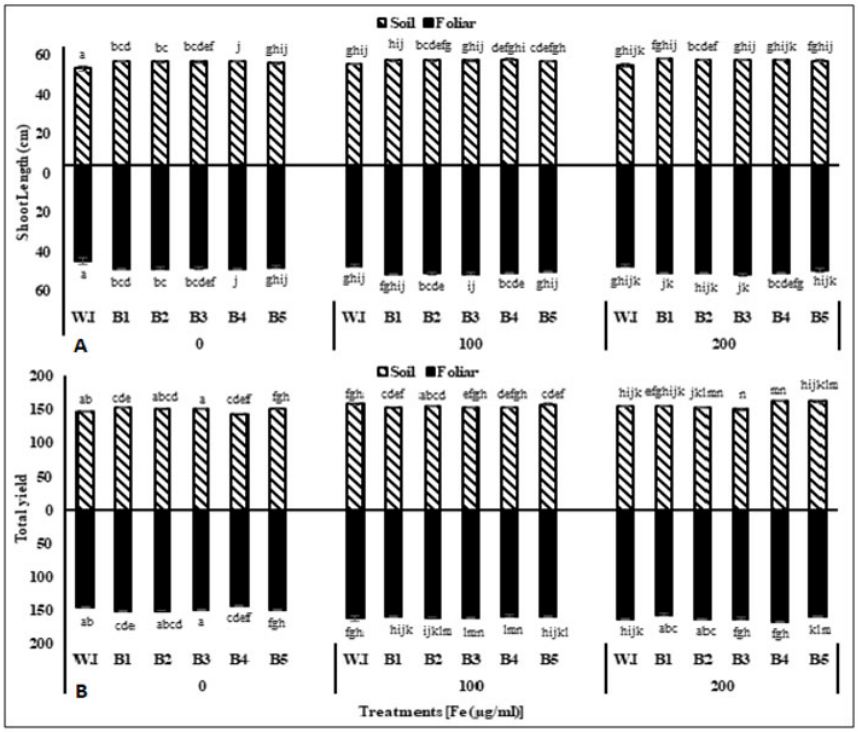

Figure 3: Impact of bacterial isolates (W.I-Without Inoculation, B1-*Pseudomonas aeruginosa*, B2- *Burkholderia cepacia*, B3-*Pseudomonas* sp, B4-*Jeotgalicoccus aerolatus* and B5- *Proteus mirabilis*) on the shoot length [A] and total yield [B] of *Triticum aestivum*L. grown under wire house conditionsusing 100 and 200µg/ml Fe via soil and foliar application.Different letters indicate significant differences between treatments using the Duncan's multiple range test (P ≤ 0.05).

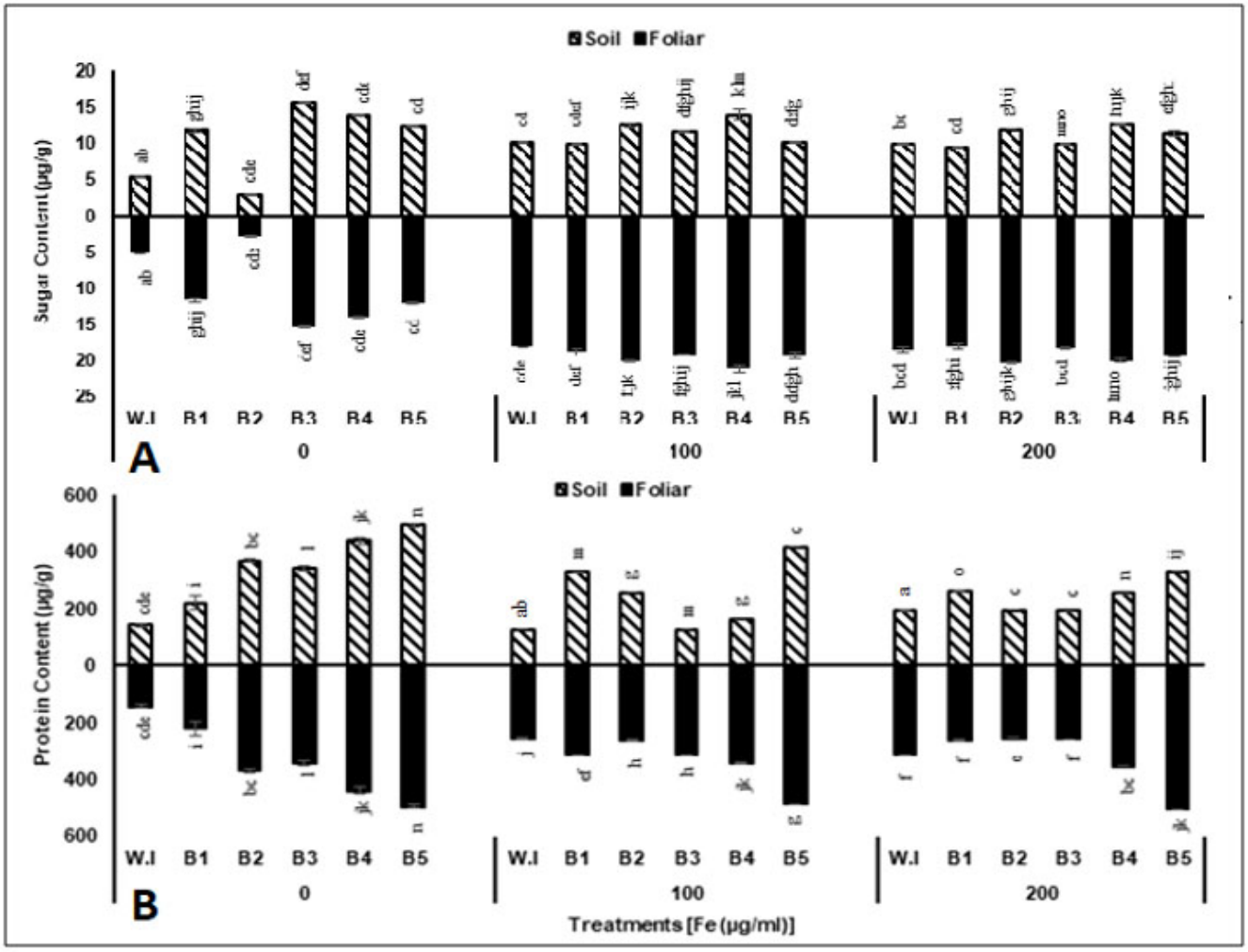

Figure 4: Impact of bacterial isolates (W.I-Without Inoculation, B1-*Pseudomonas aeruginosa*, B2- *Burkholderia cepacia*, B3-*Pseudomonas* sp, B4-*Jeotgalicoccus aerolatus* and B5- *Proteus mirabilis*) on the sugar content [A] and protein content [B] of *Triticum aestivum*L. grown under wire house conditions using 100 and 200µg/ml Fe via soil and foliar application. Different letters indicate significant differences between treatments using the Duncan's multiple range test (P ≤ 0.05).