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Research Article

Effects of zinc solubilizing bacteria on the growth dynamics of wheat germplasm

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Abstract

Zinc is an essential micronutrient crucial for optimal plant growth and development, influencing various physiological processes such as enzyme function, protein synthesis, and photosynthesis. However, a significant portion of inorganic zinc applied to agricultural soils becomes insoluble, limiting its availability for plant uptake. This deficiency presents a challenge in zinc-deficient regions, resulting in reduced crop yields and nutritional imbalances. A pot experiment was conducted during 2022–23 to assess the growth-promoting potential of three zinc solubilizing bacterial strains: Pseudomonas lurida (DJ4), Pseudomonas aeruginosa (DJ5), and Pseudomonas fluorescens (DJ13) across eight wheat varieties under greenhouse conditions. The experiment employed a completely randomized design with two factors. Results indicated that all tested zinc solubilizing bacteria and the chemical fertilizer $ZnSO4$ significantly enhanced growth parameters compared to the control. Notably, the maximum increases in shoot length (38.81±0.35 cm) and root length (21.22±2.17 cm) were observed with $ZnSO_4$ application and Pseudomonas aeruginosa (DJ5) in wheat varieties Sirin and Atta Habib, respectively. Furthermore, the highest root fresh weight (3.26±0.024 $g\cdot plant^{-1}$) and dry weight (0.356±0.024 $g\cdot plant^{-1}$) were recorded with Pseudomonas fluorescens (DJ13) inoculation in wheat variety Sirin whereas Pak-China recorded the highest shoot-to-root ratio (SRR) of 4.02±0.01 after inoculation with Pseudomonas lurida (DJ4). These findings suggest that zinc solubilizing bacteria effectively mobilize zinc for plant uptake, enhancing growth and potentially maximizing wheat production in Khyber Pakhtunkhwa

Keywords: Growth; Khyber Pakhtunkhwa; Micronutrient; Pseudomonas aeruginosa (DJ5); Pseudomonas fluorescens (DJ13); Pseudomonas lurida (DJ4); Varieties; Wheat; Zinc solubilizing bacteria

Introduction

Wheat is one of the major crops cultivated in South Asia, playing a critical role in feeding a substantial portion of the global population **[1, 2]**. As an essential staple, wheat is vital for maintaining global food security, ranking sixth in terms of calories and protein consumption worldwide **[3]**. In 2023, Pakistan produced over 26 million metric tons of wheat, underscoring its significance as an agricultural nation where 22.35% of the GDP originates from agricultural activities **[4, 5]**. The priority in sustainable agriculture is to implement innovative techniques that require fewer inputs for the efficient production of food and fiber **[6]**. Conducting trials to minimize the use of costly chemical fertilizers is crucial for optimizing farming practices sustainably. Rhizospheric bacterial species associated with wheat rhizomes enhance nutrient assimilation and provide defense against soil-borne phytopathogens [7].

Zinc (Zn) is a necessary micronutrient for plant growth and development, with concentrations typically ranging from 5 to 100 mg kg^{-1} [8]. It plays a vital role in numerous physiological processes, including protein synthesis, lipid metabolism, nucleic acid formation, chlorophyll synthesis, and enzyme activation involved in auxin and glucose metabolism [9]. Furthermore, zinc contributes to plant resilience in varying climatic conditions, helping them withstand temperature fluctuations and drought [10]. According to the FAO, over fifty percent of the world's soils are affected by zinc deficiency, primarily due to its association with naturally occurring mineral forms such as zincite and zinc sulfide, which are often unavailable to plants [11, 12].

Addressing zinc deficiency typically involves applying inorganic fertilizers however; this approach can have negative environmental impacts and result in significant amounts of fertilizer being rendered unavailable to plants. Zinc-solubilizing bacteria can mitigate this issue by secreting organic acids into the soil that solubilize zinc cations and lower soil pH [13]. These microorganisms may also enhance zinc availability through chelation processes involving anions [14]. Other mechanisms contributing to zinc solubilization include the production of siderophores and proton-oxidoreductive systems on chelated ligands and cell membranes [15-17]. Certain plant growth-promoting bacteria (PGPR), such as *Bacillus aryabhattai*, *Rhizobium* strains, and *Pseudomonas*, have shown promising role in increasing zinc content and promoting growth in plants [18-20]. Numerous studies have demonstrated that zinc-mobilizing PGPR significantly enhance the yields of cereal crops like maize, wheat, and rice [21]. To meet the demands of the growing global population, it is essential to increase crop yields through the application of PGPR that improve nutrient availability and soil fertility [22, 23]. This study reports on the growth promotion effects of three zinc-solubilizing bacteria on eight wheat varieties.

Materials and Methods

Bacterial source

Three Rhizobacteria bacterial strains, *Pseudomonas lurida* (DJ4), *Pseudomonas aeruginosa* (DJ5) and *Pseudomonas fluorescens* (DJ13), selected from the Biochemistry Laboratory (IBGE), were previously characterized by a high zinc solubilization under in vitro conditions and plant growth-promoting potential [24].

Wheat growth promotion by bacterial strains

Wheat growth promotion by the three selected bacterial strains was checked in a pot experiment in the greenhouse of the Institute of Biotechnology and Genetic Engineering, The University of Agriculture Peshawar, Pakistan. Eight wheat genotypes (Atta Habib, Sirin, Pak-China-17, Zincol-16, Shahkar-13, Khaista, Pir Sabak-13, and Pir Sabak-15) were used during this experiment. The experiment was arranged in a completely randomized design with three replications. The recommended doses of nitrogen (120 kg ha^{-1}), phosphorus (90 kg ha^{-1}), and potassium (60 kg ha^{-1}) were applied in the form of urea, single superphosphate (SSP), and sulphate of potassium (SOP), respectively. Half doses of N and full doses of P and K were applied at the time of sowing, while remaining N was applied at the booting stage with irrigation water. Routine plant management and irrigation were followed throughout the experiment.

Treatments, inoculum preparation, and seed inoculation

Zn solubilizing bacterial strains DJ4, DJ5 and DJ13 were used for determining the plant growth promotion characteristics. The treatment combination included inoculation with the three rhizobacterial strains or pots supplemented with 15 kg·ha^{-1} $ZnSO_4.7H_2O$ **[25]**. The control plants were grown under similar conditions but were not supplemented with bacteria or Zn fertilizer. Rhizobacterial cultures were initiated in an aluminium foil-covered flask containing LB medium in an orbital shaker at 120 rpm at 28±2°C in the dark until an OD600 of 0.8-1.0 was obtained **[26]**. Approximately 5.0 ml of the bacterial culture was harvested by centrifugation and suspended in 1% agar, mixed with 100 g surface sterilized wheat seeds of different genotypes, and air dried in a laminar flow hood. Eight seeds were planted, and five plants were maintained in each pot.

Evaluation of plant performance

A pot experiment was conducted to evaluate the effect of Zn application and bacterial inoculation on wheat plant growth promotion potential. The performance was evaluated after 30 days of growth by recording shoot and root length and fresh and dry weight of shoot and root.

Results and Discussion

The statistical analysis of the interaction between treatments and wheat varieties on shoot length revealed no significant effects. However, the most notable variation in shoot length 30 days after emergence was observed in the Sirin variety, which achieved a maximum shoot length of 38.81±0.35 cm following $ZnSO_4$ supplementation. This was closely followed by the Atta Habib variety, which recorded a shoot length of 38.11±1.51 cm after inoculation with DJ13. In contrast, the minimum shoot lengths were recorded for both Sirin and Khaista under control conditions, measuring 26.33±1.83 cm and 26.33±0.92 cm, respectively (Fig. 1). Additionally, four outliers were identified within the interquartile range (25-75%). Notably, Atta Habib exhibited a significantly reduced shoot length of 29.10±1.20 cm under control conditions, while Pak-China-17 demonstrated significantly lower growth after DJ4 inoculation (30.49±1.25 cm). Conversely, Pir Sabak-2013 achieved a significantly higher shoot length of 35.34±0.60 cm following DJ13 inoculation but showed reduced growth under control conditions (27.72±0.35 cm). These findings align with previous studies **[27, 28]**, which reported enhanced shoot length associated with zinc-solubilizing bacteria (ZSB) inoculation. Furthermore, significant interactions between treatment and variety were observed concerning root length, indicating that these factors collectively influence plant growth dynamics. The Atta Habib variety exhibited the greatest root length variation 30 days post-emergence, attaining a maximum of 21.22±2.17 cm after DJ5 inoculation, followed by 19.05±0.87 cm after DJ13 inoculation. In contrast, Pir Sabak-2015 recorded the lowest root length at 11.23±0.43 cm under control conditions (Fig. 2). Five outliers were identified from the interquartile range for each variety, reinforcing the variability in response to treatments. Sirin reached a significantly greater root length of 15.16±1.73 cm following DJ4 inoculation but showed reduced growth under control conditions (12.56±0.43 cm). Pak-China-17 demonstrated significantly higher root length after $ZnSO_4$ supplementation (16.89±1.30 cm), while Khaista achieved a maximum root length of 14.72±1.56 cm after DJ4 inoculation but lower growth under control conditions (9.53±0.43 cm). These results corroborate findings from **[29, 30]**, which indicated that various plant growth-promoting rhizobacterial species enhance root length. The analysis of shoot fresh weight (SFW) further underscores the impact of treatments on wheat genotype performance, revealing that the highest SFW was recorded in Pir Sabak-2013 at 7.79±0.10 g·plant^{-1} after DJ13 inoculation, followed closely by Pir Sabak-2015 at 7.70±0.57 g·plant^{-1} following $ZnSO_4$ supplementation

(Fig. 3). Conversely, the lowest SFW was noted in Sirin after DJ5 inoculation at 4.25±0.09 g·plant^{-1}, with no outliers identified within the interquartile range across any wheat varieties, indicating consistent responses among genotypes under similar treatment conditions. These findings are consistent with previous research that highlighted increased SFW in wheat due to bacterial treatments [31]. Moreover, the interaction between treatment and variety significantly affected shoot dry weight (SDW), where maximum SDW was observed in Pir Sabak-2015 at 0.775±0.097 g·plant^{-1} after ZnSO$_4$ supplementation (Fig. 4). In contrast, Sirin exhibited the lowest SDW at 0.412±0.022 g·plant^{-1} under control conditions, highlighting that treatment conditions can drastically influence biomass accumulation in different wheat varieties. Two outliers were noted within the interquartile range: one for Pir Sabak-2013 after DJ13 inoculation (0.753±0.028 g·plant^{-1}) and another under control conditions (0.514±0.018 g·plant^{-1}). These results align with studies indicating that ZSB inoculation significantly enhances SDW [32, 33]. The analysis of root fresh weight (RFW) also revealed critical insights into genotype responses to treatments; Pak-China-17 exhibited maximum variation across treatments with Sirin achieving the highest RFW at 3.26±0.024 g·plant^{-1} following DJ13 inoculation, while Pak-China-17 recorded the lowest RFW at 1.61±0.018 g·plant^{-1} after DJ5 inoculation (Fig. 5)Four outliers were identified from the interquartile range across different treatment conditions for each variety; notably, Atta Habib showed higher RFW at 2.93±0.319 g·plant^{-1} after DJ13 inoculation, while Sirin displayed both higher (3.26±0.024 g·plant^{-1}) and lower RFW values (1.83±0.282 g·plant^{-1}) depending on treatment conditions. In addition to RFW, root dry weight (RDW) analysis demonstrated significant interactions between treatment and variety as well; maximum RDW was observed in Sirin at 0.356±0.024 g·plant^{-1} and Zincol-2016 at 0.347±0.009 g·plant^{-1} following DJ13 inoculation (Fig. 6). Conversely, Pir Sabak-2015 recorded the lowest RDW at 0.170±0.009 g·plant^{-1} under control conditions; four outliers were noted for higher RDW among Atta Habib, Sirin, Zincol-2016, and Shahkar-13 post-DJ13 inoculation while three outliers with lower RDW were identified for Atta Habib, Sirin, and Zincol-2016 post-DJ4 inoculation. These results suggest that wheat varieties exhibit a synergistic relationship with DJ13 inoculation; however, DJ4 appears to exert an antagonistic effect leading to reduced RDW outcomes that resonate with previous studies exploring plant growth-promoting interactions through various mechanisms [34]. Furthermore, significant variations were observed in the shoot-to-root ratio (SRR) among wheat varieties under different treatment conditions (Fig. 7). After a growth period of 30 days post-emergence, maximum SRR variation was noted in Atta Habib and Sirin varieties; Pak China achieved the highest SRR of 4.02±0.01 following DJ4 inoculation due to inhibited root growth while Sirin exhibited the lowest SRR of 1.82±0.05 after DJ4 treatment due to enhanced root development. Three outliers were identified within the interquartile range for these wheat varieties. Although Pak China's confidence interval ranged from 2.25 to 2.75, DJ4 application severely restricted its root growth resulting in an elevated SRR above this range; similar observations were made for Pir Sabak-2015 post-DJ4 and Zincol-2016 post-DJ5 treatments. The root-to-shoot ratio is often analyzed to elucidate competition for water and nutrients; thus it serves as an essential indicator of plant health [35].

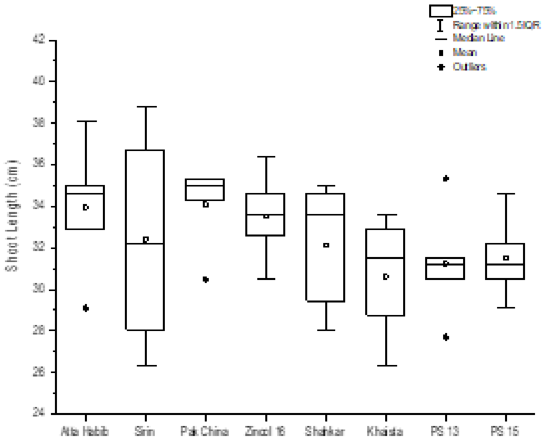

Figure 1: Effect of different treatments on Shoot length (cm) of eight wheat varieties

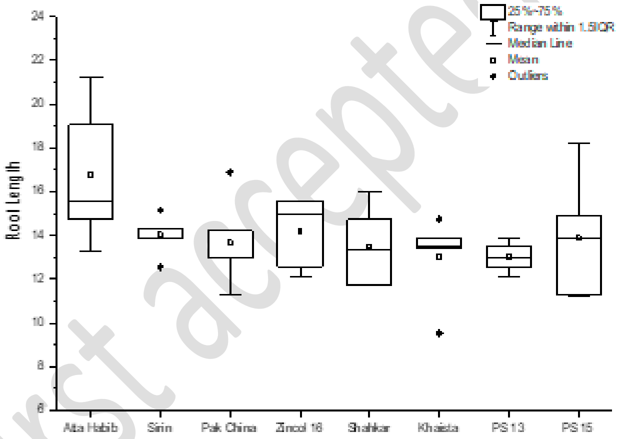

Figure 2: Effect of different treatments on Root length (cm) of eight wheat varieties

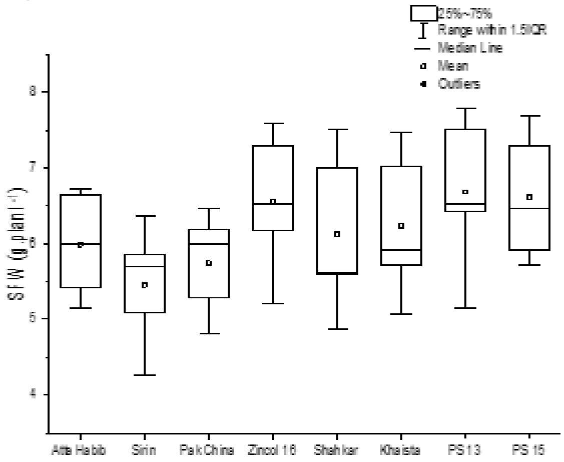

Figure 3: Effect of different treatments on Shoot fresh weight (g·plant^{-1}) of eight wheat varieties

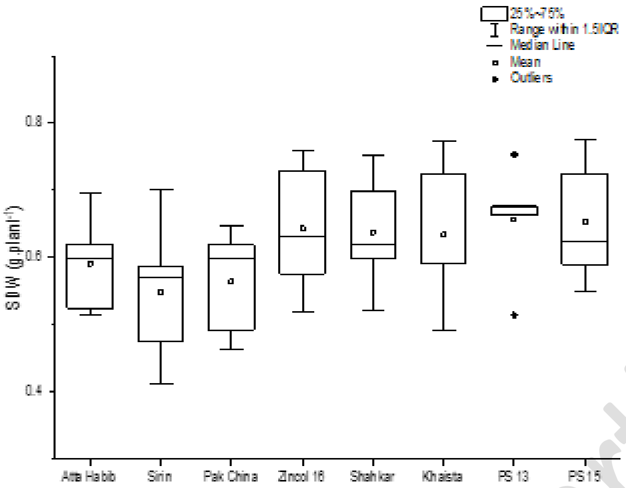

Figure 4: Effect of different treatments on Shoot dry weight (g·plant^{-1}) of eight wheat varieties

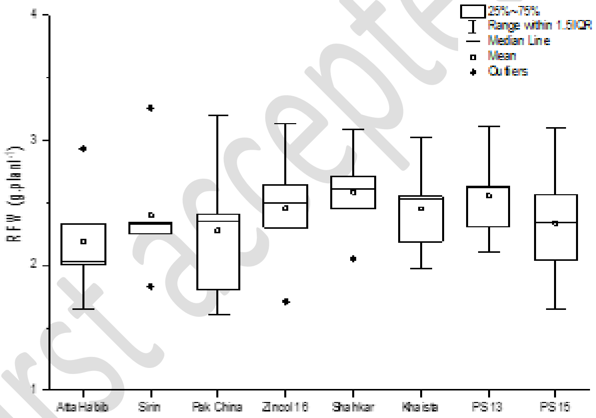

Figure 5: Effect of different treatments on Root fresh weight (g·plant^{-1}) of eight wheat varieties

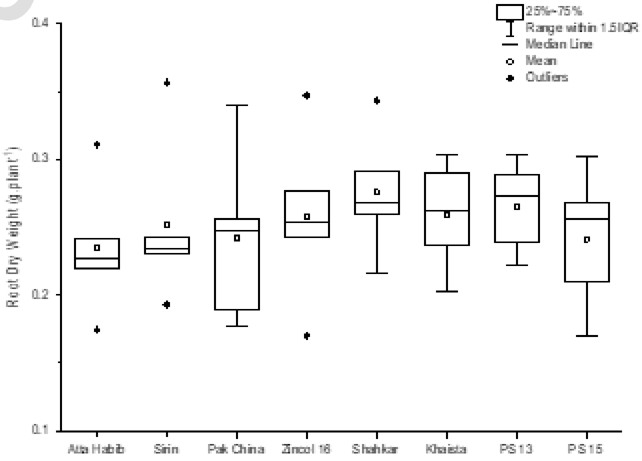

Figure 6: Effect of different treatments on Root dry weight (g·plant^{-1}) of eight wheat varieties

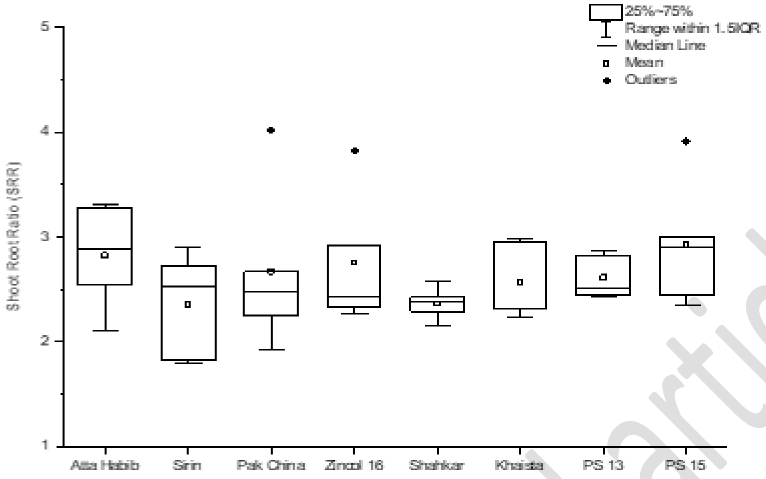

Figure 7: Effect of different treatments on Shoot to root ratio (SRR) of eight wheat varieties

Conclusion

The application of zinc solubilizing bacteria not only supports plant growth but also offers a promising avenue for maximizing wheat production across diverse genotypes, thereby contributing to food security and agriculture resilience in the region. Future research should explore the long term effects of these bacterial inoculations and their interactions with different soil types to further substantiate their efficacy in various agricultural settings.

Authors' contributions

Conceived and designed the experiments: A Iqbal & M Arif, Performed the experiments: S Malik, Analyzed the data: A Iqbal, Contributed materials/ analysis/ tools: I Munir, Wrote the paper: S Malik.

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