Plant Research Journal, 01(01), 38-42 (2025)

Research Article – Plant Microbe Interaction

Efficacy of *Bacillus subtilis* to enhance the growth of maize under saline conditions

Masooma Saleh, Basharat Ali*

Institute of Microbiology and Molecular Genetics, University of the Punjab, Lahore-54590, Pakistan

*Corresponding author(s): Tel.: +92-300-4297937; Email: basharat.ali.mmg@pu.edu.pk

Received: 28-11-2025
Accepted: 13-12-2025
Published: 13-12-2025

Abstract

This study investigates the role of auxin-producing *Bacillus subtilis* in promoting the growth of *Zea mays* (L.) under saline conditions. Plant growth under bacterial inoculations was assessed through rooting assays and pot experiments. The results showed that the bacterial strains produced a significant amount of auxin at different concentrations of L-tryptophan. At 0 µg/mL, strain M-1 produces auxin at approximately 128 µg/mL, and at 500 µg/mL, strain M-2 produces 174 µg/mL of auxin. In pot trials, shoot length increased by 69% with the M-1 strain at 0 mM NaCl compared to the respective control. When salt treatment was given, the increase in shoot length for M-2 was around 26% at 100 mM NaCl, 26.3% at 300 mM NaCl, and 10% at 500 mM NaCl, over respective control. At 0 mM NaCl, the M-1 and M-2 strains produced a fresh weight of 30.7%, as compared to the control. At 100 mM and 500 mM NaCl, the fresh weight of M-2 was recorded as 71.4% and 66.6%, respectively. At 300 mM NaCl, M-1 showed an improved wet weight of 65%. Similarly, at 0 mM NaCl, the M-1 and M-2 strains recorded an approximately 1-fold increase in dry weight compared to the control. However, at a higher salinity level (500 mM NaCl), the dry weight increased by around 16% compared to the respective control. The results of this study showed that the strains of *B. subtilis* have the potential to mitigate the salinity of maize plants under saline conditions.

Keywords: Auxin production, *Bacillus* subtilis, Indole-3-acetic acid, Plant growth-promoting rhizobacteria, Salt stress

Key message: *Bacillus subtilis can mitigate the salt stress of maize plants.*

Abbreviations: NaCl (Sodium Chloride), **IAM** (Indole-acetamide Pathway), **IAA** (Indole-3-Acetic Acid), **PGPB** (Plant Growth-Promoting Bacteria), **IAM** (Indole-Acetamide Pathway), **IPA** (Indole-Pyruvate Pathway, **PSB** (Phosphate-Solubilizing Bacteria), **BCAs** (Biocontrol Agents)

Citation: Saleh, M., Ali, B. (2025). Efficacy of Bacillus subtilis to enhance the growth of maize under saline conditions. Plant Research Journal, 01(01), 38-42.

1. Introduction

Maize is an annual plant belonging to the grass family. It is one of the easiest crops to pollinate manually (Badu-apraku and Fakorede, 2017). In Pakistan, maize is one of the top three cereal crops, along with wheat and rice. Total maize production was 7,800,000 tons in 2020 (Azeem *et al*, 2022). Challenges reported in maize farming include pest and disease infestation, absence of tolerant cultivars, substandard quality seeds, low soil fertility, insufficient crop rotation, and restricted intercropping practices. The use of beneficial microorganisms may act as a stress-protecting agent for plants, offering advantageous alternatives for sustainable and eco-friendly agriculture (Azeem *et al*., 2022). The rhizosphere is the layer of soil affected by the root and contains a significantly higher concentration of bacteria compared to the adjacent bulk soil. The rhizosphere is defined as the soil zone surrounding plant roots, teeming with diverse species and regarded as one of Earth's most intricate ecosystems. Free-living soil bacteria known as "plant growth-promoting rhizobacteria" invade the root's rhizosphere and improve the plants' capacity to absorb nutrients. Microbes use several processes, such as siderophore synthesis, phosphate solubilization, phytohormone synthesis, and the inhibition of phytopathogenic fungi, to promote plant growth. Many bacterial genera, such as *Pseudomonas*, *Bacillus*, *Enterobacter*, *Rhizobium*, *Bradyrhizobium*, and *Xanthomonas*, have been identified as potential phytohormone-producing rhizobacteria that can enhance plant growth (Javorekova *et al*., 2020). Only a small percentage of the bacteria in the rhizosphere microbiome are proven to be harmful to plants; the majority, known as plant growth-promoting rhizobacteria (PGPR), interact favorably with the plant, boosting its development and survival. These helpful bacteria are essential to sustainable crop production because they increase plant development, make nutrients available to plants, prevent disease growth, and enhance soil structure. Additionally, they are utilized in the bioremediation of contaminated soils and mineralize

organic contaminants. *B. subtilis* is one of several species of the *Bacillus* genus that have been identified as biological control agents and plant growth promoters. (Bolivar-Anilo, 2021).

These include the ability to boost nutrient uptake by plants and improve their resistance to abiotic stress, as well as the synthesis of growth regulators and the alteration or release of hormones including auxin, ethylene, gibberellins, abscisic acid, and cytokinins. Additionally, certain bacteria have the capacity to solubilize phosphorus, promote N2 fixation, and create an antagonistic environment against other phytopathogens. Certain bacterial strains have been discovered that can shield maize plants from the damaging effects of salt (Katsenios, 2022).

The indole-3-acetic acid (IAA) is the most common endogenous auxin, which plays a role in root development and elongation. IAA is a common product of L-tryptophan metabolism by several microorganisms, including plant growth-promoting bacteria (PGPB). IAA biosynthesis can occur through various pathways, including the indole-acetamide pathway (IAM), the indole-pyruvate pathway (IPA), or the tryptamine pathway (Navid *et al*., 2024).

In maize plants, PGPR also facilitates nitrogen fixation. The capacity of Acinetobacter sp., Klebsiella pneumoniae, and *Bacillus* sp. to fix nitrogen was assessed. The insoluble soil phosphate can be dissolved by PGPR into a form that plants can use. The term "phosphate-solubilizing bacteria, PSB" refers to this particular group of PGPR. Examples of PSB linked to maize include Sphingobium sp., *Bacillus subtilis*, *Pseudomonas* sp., and *Paenibacillus* sp. By secreting metabolites, antagonistically inducing systemic resistance, and competing for nutrients and space, rhizobacteria also serve as biocontrol agents (BCAs), aiding in the management of phytopathogenic microorganisms that reside in the soil.

This study's primary goal was to screen PGPR for critical characteristics. The capacity of *B. subtilis* strains to generate auxin with or without L-tryptophan was examined *in vitro*. Additionally, the strains were examined using the *in vitro* rooting assay and their biochemical activity. Lastly, to assess the effect of bacteria on vegetative parameters, pot trials were also carried out in the wire house under *in vivo* settings.

2. Materials and Methods

2.1 Bacterial strains

Strains of *B. subtilis* were obtained from the Institute of Microbiology and Molecular Genetics' microbial collection. To get pure cultures, the strains were frequently re-cultured on Luria-Bertani (LB) agar plates.

2.2 Biochemical Characterization

The streak plate method was used to isolate bacterial colonies. Gram and spore staining were performed for morphological characterization. The biochemical attributes of the strains were evaluated by performing catalase, citric acid utilization, TSI, indole, oxidase, nitrate, and urease tests.

2.3 Colorimetric evaluation of bacterial synthesis of IAA

For auxin quantitative analysis, two concentrations, 0 and 1000 µg/ml of L-tryptophan, were used in triplicate. For one strain, four flasks containing 15 mL L-broth were prepared and autoclaved. About 0.2 g of L-tryptophan was dissolved in 20 mL of autoclaved distilled water to create a stock solution. L-broth was mixed with 1.5 ml of stock L-tryptophan to achieve a 500 µg/ml concentration. The appropriate *Bacillus* strain was added to each flask. For three days, culture flasks were incubated at 37 °C and 120 rpm on a rotatory shaker.

2.4 Rooting assay under *in vitro* conditions

After streaking on L-agar plates, bacterial cultures were created and cultured at 37 °C for the entire night. Each of the 14 test tubes (1.5×15 cm) received 10 ml of distilled water, which was subsequently autoclaved (HIRAYAMA-HVE-50) to sanitize them. The preparation of a McFarland standard (turbidity comparable to 2.0) involved combining 9.8 ml of 1% sulfuric acid (H_2SO_4) with 0.2 ml of 1% barium chloride ($BaCl_2$). Using an inoculating loop, each bacterial cell was extracted from the cultivated plates and placed into sterile Falcon tubes filled with autoclaved water. A cell density of roughly 6.0 × 10^8 CFU/mL was achieved by adjusting the turbidity of each microbial culture to meet the McFarland 2 threshold.

2.5 Pot trials under *in vivo* conditions

Two bacterial strains were used *in vivo* pot experiments. Autoclaved distilled water was used to create bacterial cultures. The bacterial suspensions' turbidity was changed to match the previously mentioned cell density. Following the previously described procedure, maize seedlings were surface sterilized with 0.1% mercuric chloride ($HgCl_2$) and then treated with the made bacterial suspensions. The control group consisted of seeds treated with autoclaved distilled water. Before being sown, sterile seeds were immersed in a bacterial suspension with the required density. At the Institute of Microbiology and Molecular Genetics, 19-by-20-cm pots were filled with soil and set up in a wire house in a natural setting. Ten treated seeds were planted in each pot with a 10-cm gap between them using sterile forceps. After giving each container, a little water to establish initial hydration, the seeds were watched for germination and sprouting. (Raheem *et al*, 2018).

2.6 Statistical analysis

“Analysis of variance (ANOVA) was performed on data from auxin production, in-vitro assay, and pot trials. Means were compared using Duncan's multiple range test, $p \leq 0.05$” by using SPSS 20 software.

3. Results

3.1 Morphological and Biochemical Characterization

Microscopic examination revealed that all isolates retained the crystal violet stain, confirming a Gram-positive reaction.

Bacterial smears were subjected to endospore staining using malachite green. Microscopy revealed the presence of green-stained endospores within red-colored vegetative cells, indicating successful differential staining. The results of catalase, oxidase, citrate, and urease tests were +ve, and the results of TSI, indole, and nitrate tests were -ve.

3.2 Colorimetric evaluation of bacterial synthesis of IAA

The bacterial strains generated notable amounts of auxin *in vitro* both with and without the precursor L-tryptophan. Overall, increasing L-tryptophan concentrations also stimulated auxin production by bacterial strains. For instance, strain M-1 and M-2, respectively, showed 28% and 74% increases in auxin levels at 500 µg L-tryptophan as compared to the respective controls at 0 µg/ml L-tryptophan (Fig. 1).

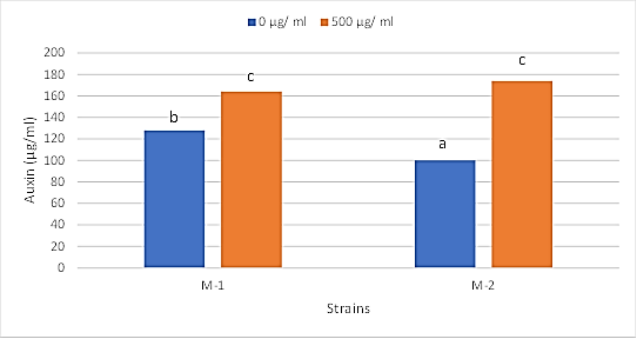

Fig.1. Production of bacterial auxin at two distinct L-tryptophan concentrations (0 µg/ml and 500 µg/ml). The mean of three replicates is shown by the bars. Using Duncan's Multiple Range Test (DMRT), different letters indicate significant differences between treatments ($p \leq 0.05$).

3.3 Rooting assay

In the rooting assay, strains showed a statistically significant increase in shoot length at different salt concentrations, with the most promising response observed at 0 mm NaCl, where M-1 recorded a 69% increase over the control. At 100 mM NaCl, the strain showed around 31% percent increase. The rooting response is illustrated in Fig.2.

Fig.2. Effect of different salt concentrations on plant growth after six weeks of seed germination.

3.4 Pot trials under *in vivo* conditions

Comparing the bacterial-treated seeds to the control, the results revealed a considerable improvement in every measured parameter (Fig.3). In comparison to the control, strain M-1 considerably lengthened the shoots of maize plants at 0 mM NaCl (Fig.4). Bacterial strains somewhat reduced salt stress at 100 and 300 mM. However, the strains produced findings that were statistically comparable to the control at a higher salinity level (500 mM).

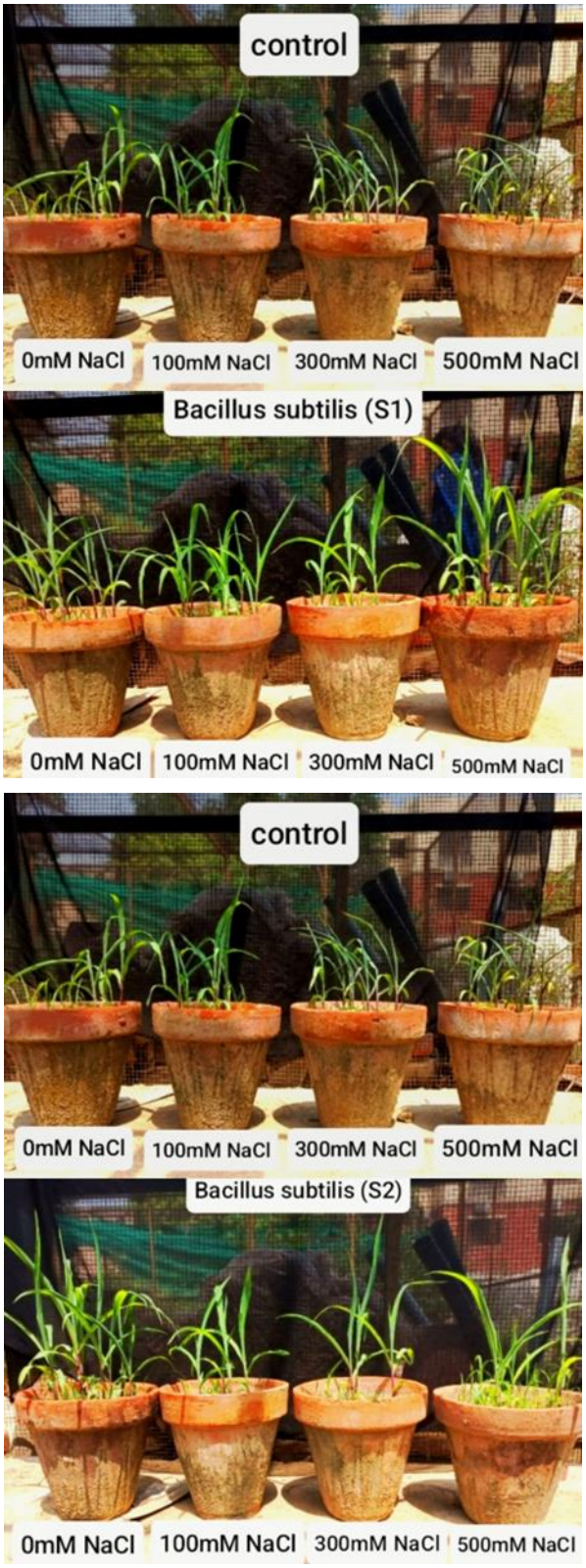

Fig.3. Effect of bacterial inoculations on the growth of maize under salt stress in pot trials.

Under bacterial inoculations at 0 mM NaCl, a notable improvement in fresh weight was noted (Fig.5). Strains M-1 and M-2 considerably reduced the effects of salt stress on plants at 100 mM NaCl. There were slight increases in fresh weight at 300 and 500 mM NaCl. In comparison to the control, bacterial strains exhibited a minor improvement in the growth parameter for dry weight at 100 mM (Fig.6). Nevertheless, bacterial strains had statistically similar outcomes to the control at greater salinities.

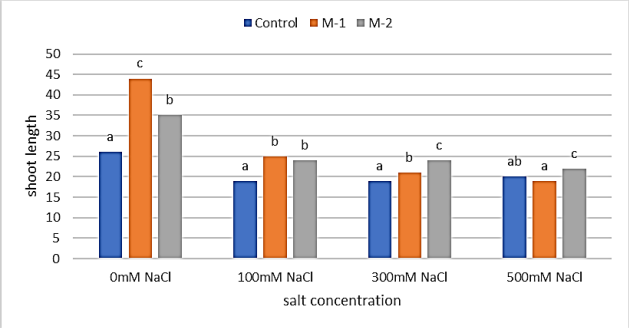

Fig.4. Effect of bacterial inoculation on shoot length (cm) of *Z. mays* (L.), 8 weeks after seed germination in different salt concentrations. Mean of three replicates is represented by bars. "Different letters showed significant differences among the treatments by Duncan's Multiple Range Test, p≤0.05".

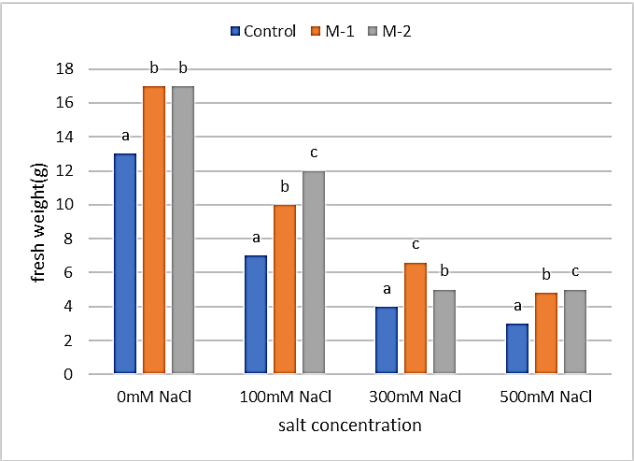

Fig.5. Impact of bacterial inoculation on *Z. mays* (L.) fresh weight eight weeks following seed germination at varying salinity levels. The three replicates' mean was displayed in the bars. "According to Duncan's Multiple Range Test (DMRT), significant differences between the treatments are indicated by different letters, p≤0.05".

4. Discussion

The current study assessed the ability of *B. subtilis* strains to control maize plant development in saline environments. The IAA generation was one of the characteristics of these strains that promoted plant development. A crucial step in the synthesis of IAA is the conversion of L-tryptophan. Therefore, when organisms are fed L-tryptophan, there is typically an increase in the creation of IAA. When tryptophan is present, many bacteria, including Streptomyces, *Bacillus*, and Pseudomonas, produce more IAA (Dashti *et al*, 2021).

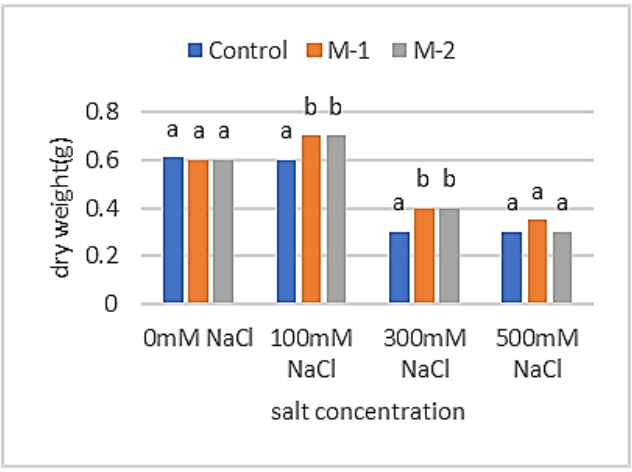

Fig.6. Effect of bacterial inoculation on dry weight of *Z. mays* (L.), "The bars showed the mean of three replicates. Different letters indicate the significant differences among the treatments by Duncan's Multiple Range Test (DMRT), p≤0.05".

Due to their neutralizing impact, the endophytic bacterial strains exhibited the highest tolerance to various salt concentrations (Shah *et al*., 2022). *In vitro*, the auxin production test was conducted with varying L-tryptophan concentrations (0 µg/ml and 500 µg/ml). The M-1 and M-2 strains produced 69% and 34% of the shoot length at 0 mM NaCl, respectively. We have previously demonstrated that increasing L-tryptophan concentrations also resulted in enhanced auxin production by bacteria (Ali *et al*., 2009). The shoot length of M-2 was measured at 26% at 100 mM NaCl, 26.3% at 300 mM NaCl, and 10% at 500 mM NaCl after salt treatment. The M-1 and M-2 strains' fresh and dry weights at various salt concentrations were noted. The impact of bacterial inoculations on plant growth in pot trials was demonstrated by performing a statistical analysis. Plant growth responses under bacterial inoculations were promising as compared to the control. The fresh weight of M-1 and M-2 strains recorded significant improvements at 100 mM and 500 mM NaCl, respectively. Additionally, strains M-1 and M-2 also recorded increases in dry weight of 89.8% at 0 mM NaCl, 16.6% at 100 mM NaCl, 33.3% at 300 mM NaCl, and 16.6% at 500 mM NaCl. Auxin-producing halotolerant rhizobacteria have also been shown to improve plant growth in salt-amended soils (Raheem and Ali, 2015).

The outcome of this study demonstrated a direct link between L-tryptophan in culture medium and the production of auxin by bacteria. The current study showed that rhizobacteria, particularly *B. subtilis*, significantly enhance vegetative growth parameters in maize. Treated plants exhibited improved shoot length, fresh weight, and dry weight compared to untreated controls. These results highlighted PGPR's potential as an environmentally responsible and sustainable substitute for chemical

fertilizers. Future research should focus on developing more cost-effective PGPR formulations with enhanced shelf life and minimal phytotoxic effects.

Acknowledgements

The University of the Punjab is acknowledged for providing funding for this work.

Author(s) contribution

Conceptualization, BA; methodology, BA; software, BA; validation, BA; formal analysis and investigation, BA; data curation, MS; writing—original draft preparation, MS; writing—review and editing, BA; supervision, BA. All authors have reviewed and approved the manuscript.

Conflict of interest

The authors declare no conflict of interest.

Data availability

All data supporting the findings of this study are available within the paper. We do not have any research data outside the submitted manuscript file.

Declarations

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

References

Ali, B., Sabri, A.N., Ljung, K., & Hasnain, S. (2009). Quantification of indole-3-acetic acid from plant associated *Bacillus* spp. and their phytostimulatory effect on *Vigna radiata* (L.). World Journal of Microbiology and Biotechnology, 25 (3), 519-526.

Azeem, M., Haider, M.Z., Javed, S., Saleem, M.H., & Alatawi, A. (2022). Drought stress amelioration in maize (*Zea mays* L.) by inoculation of *Bacillus* spp. strains under sterile soil conditions. *Agriculture*, 12, 50, 1–21.

Badu-Apraku, B., & Fakorede, M.A.B. (2017). Morphology and physiology of maize. In: *Advances in Genetic Enhancement of Early and Extra-Early Maize for Sub-Saharan Africa*, 33–53.

Bolivar-Anillo, H.J., González-Rodríguez, V.E., Cantoral, J.M., García-Sánchez, D., Collado, I.G., & Garrido, C. (2021). Endophytic bacteria *Bacillus subtilis*, isolated from *Zea mays*, as potential biocontrol agent against *Botrytis cinerea*. *Biology*, 10, 492, 1–26.

Dashti, N., Al-Sarraf, N.Y.A., Cherian, V.M., & Montasser, M.S. (2021). Isolation and characterization of novel plant growth-promoting rhizobacteria (PGPR) isolates from tomato (*Solanum lycopersicum* L.) rhizospherical soil: A novel IAA-producing bacteria. *Kuwait Journal of Science*, 48(2), 1–18.

Javoreková, S., Cinkocki, R., Maková, J., & Hricáková, N. (2020). Isolation and identification of rhizobacteria from maize (*Zea mays* L.) in Luvisols and documentation of their plant growth-promoting traits. *Journal of Microbiology, Biotechnology and Food Sciences,* 10(3), 505-510.

Katsenios, N., Andreou, V., Sparangis, P., Djordjevic, N., Giannoglou, M., Chanioti, S., Kasimatis, C.-N., Kakabouki, I., Leonidakis, D., Danalatos, N., Katsaros, G., & Efthimiadou, A. (2022). Assessment of plant growth-promoting bacteria strains on growth, yield, and quality of sweet corn. *Scientific Reports*, 12(1), 11598.

Navid, S., Tanveer, S. & Ali, B. (2023). Auxin production by *Bacillus simplex* enhanced the growth of *Zea mays* (L.) under in-vitro and in-vivo conditions. *LGU Journal of Life Sciences,* 7(4), 457-471.

Raheem, A., & Ali, B. (2015). Halotolerant rhizobacteria: Beneficial plant metabolites and growth enhancement of Triticum aestivum L. in salt-amended soils. Archives of Agronomy and Soil Sciences, 61, 1691-1705.

Shah, D., Khan, M.S., Aziz, S., Ali, H., & Pecoraro, L. (2022). Molecular and biochemical characterization, antimicrobial activity, stress tolerance, and plant growth-promoting effect of endophytic bacteria isolated from wheat varieties. *Microorganisms*, 10, 21, 1–17.