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Synergistic Effects of Arbuscular Mycorrhizal Fungi and Rhizobacteria on Wheat Triticum aestivum L. Salinity Toleranc

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Abstract: This work compares and synergistically tests the plant growth promoting rhizobacterium bacteria (Bacillus cereus) and arbuscular mycorrhiza fungus (Rhizophagus irregularis) in diminishing of the severe salinity stress (200 mM NaCl) in wheat (Triticum aestivum L.) under controlled conditions. Four treatments were tested - control, PGPR, AMF and a combined consortium with 5 replicates each. Growth attributes (length of shoots and roots, dry biomass, leaf area) and important physiological and biochemical indicators (chlorophyll content, proline accumulation, relative water content, and superoxide dismutase activity) were measured. Salinity stress resulted in a large decrease (40-50%) in the experimental parameters used in the study for the untreated control caused by osmotic imbalanced and the toxic to Na+. Inoculation with B. cereus increased plant performance by 23-45%, which was explained by an increased synthesis of phytohormones (IAA) and the formation of exopolysaccharides. R. irregularis inoculation resulted in more substantial improvements (30-64%) by developing extraradical hyphal networks that enhanced the uptake of N, P, and K. The combination of PGPR and AMF showed the strongest effect with the improvements ranging from 47-86% i.e. 69% increase in dry biomass, 86% increase in SOD activity, 40% decrease in Na+ accumulation and 40% increase in proline levels. These results point to the great synergistic possibility of PGPR--AMF co-inoculation as an eco-efficient approach to improve tolerance of wheat under high salinity stress.

Keywords: AMF, PGPR, salinity, *Triticum aestivum*, microbial synergism.

INTRODUCTION

Soil salinity is among a critical abiotic stress constrain agitation around the globe, which impact nearly one-fifth of irrigated lands with a tendency of growth due to unsustainable irrigation practices, climate change and secondary salinization processes [Rengasamy, P. 2010]. High levels of salts, especially sodium chloride (NaCl), cause great osmotic and ionic stresses which disturb water absorption, nutrient equity, and cellular homeostasis in plants [Munns, R., & Tester, M. 2008]. Wheat (Triticum aestivum L.), one of the cereal crops with a strategic importance, accounting for about 20% of the world's energy production as a source of food, is very sensitive to salinity especially when the soil electrical conductivity exceeds 8 dS/m [Abbood, H. A., & Salman, H. A. 2024]. Under such conditions plants generally exhibit decreased production of chlorophyll, membrane integrity, inhibition of root elongation and high concentrations of decline of biomass accumulation, and in photosynthetic performance [Shabala, S. 2013]. Therefore, development of sustainable, biologically based strategies for improvement of wheat tolerance to salinity is of urgent research priority.

In recent years, more and more attention has been paid to the application of beneficial soil microorganisms, in particular plant growth-promoting rhizobacteria (PGPR) and arbuscular mycorrhizal fungi (AMF), as environmentally sound means to increase plant resilience under saline conditions [HUSSEIN, A. A. 2024]. PGPR such as Bacillus cereus colonize the rhizosphere and promote plant growth in several different ways such as by the synthesis of phytohormones (e.g. indole-3 acetic acid), synthesis of exopolysaccharides (EPS), better nutrient solubilization and by influencing enzymes that respond to plant stress [Glick, B. R. 2012]. EPS secretion is part of protective biofilms that are developed around roots that limit Na^+ influx, stabilize the soil structure and maintain root hydration under osmotic stress [Ashraf, M. F. M. R., & Foolad, M. R. 2007]. These mechanisms are collectively found beneficial for root development, chlorophyll retention, water status of leaves, and antioxidant activity of salt stressed plants.

Similarly, AMF, especially Rhizophagus irregularis; develop symbiotic relationships with roots forming an extensive network of extraradical hyphae giving the effective absorptive surface area a more than eighty-fold increase [Smith, S. E., & Smith, F. A. 2011]. This network optimizes the management of the uptake of essential nutrients such as Nitrogen, Phosphorus and Potassium, while at the same time driving down the accumulation of toxic ions [Evelin, H. *et al.*, 2009]. AMF symbiosis also stimulates synthesis of Osmo protectants (e.g. prone), activity of very important antioxidant enzymes (e.g. superoxide

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dismutase, SOD), which reduces oxidative damage under salt stress [Porcel, R. *et al*., 2012].

Though both the use of PGPR and AMF have significant benefits, new studies have shown that the two designs have better synergistic effects when used jointly [Savastano, N., & Bais, H. 2024]. By co inoculating, nutrient acquisition, osmotic adjustment, and antioxidants defense pathways can be modulated simultaneously to achieve greater strength in mitigating the stress as in fig.1 [Kumawat, K. C. *et al*., 2021]. In addition, AMF may positively contribute to the maintenance and functioning of the root-associating microhabitats displayed in root exudation patterns, as well as AMF can positively impact root colonization by the known means of facilitating the presence and activity of the target bacterium, PMFR, [Toljander, J. 2006]. It is therefore not surprising that microbial consortia frequently translate into enhanced biomass, chlorophyll concentrations, water conditions and ion regulation over inoculation with single-microbes.

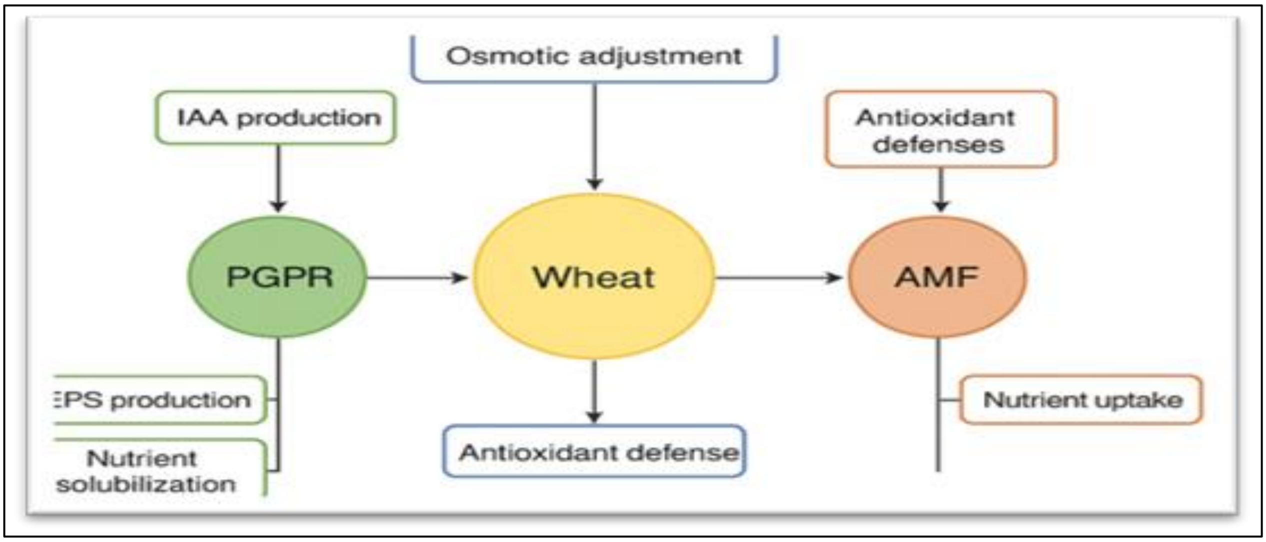

Figure 1: Hypothetical scheme of the synergistic effect of the synergy between PGPR and AMF to increase .salt stress tone in wheat (12)

There has been the need to focus on sustainable agricultural technologies, the individual and combined effectiveness of Bacillus cereus (PGPR), and Rhizophagus irregularis (AMF) in high salinity environments. In this regard, this research undertakes an investigation of the degree to which these microorganisms; used either in isolation or as a synergetic consortium, alleviate the adverse effects of the extreme salt stress (200 mM NaCl) on the growth and physiological functioning of wheat under a controlled condition. It is expected that the findings can be used to develop bioinoculants which are cost effective and environmentally friendly to be used in saline and arid agriculture systems.

MATERIALS AND METHODS

Plant Material and Experimental Input:

The model crop in the study was wheat (Triticum aestivum L.). Two microbial inoculants were used namely the plant growth-promoting rhizobacterium Bacillus cereus (PGPR) and the arbuscular mycorrhizal fungus Rhizophagus irregularis (AMF). The growth media was a mixture of sterilized sand–vermiculite (3:1, v/v), which was added with NaCl (100- 200 M) to provide salinity stress. Additional materials included LB agar for bacterial cultivation and standard reagents for chlorophyll and enzymatic assays [Moseler, A. M. 2017].

Isolation and Preparation of *Bacillus cereus* (PGPR)

Rhizosphere soil samples (10 g; 0–15 cm depth) were collected from saline wheat fields and suspended in 90 mL sterile phosphate-buffered saline (PBS, pH 7.0). The suspension was vortexed for 10 minutes to detach associated bacteria, followed by serial dilution to 10^{-6}. To selectively recover spore-forming bacteria, the diluted samples were optionally heat-treated at 80 °C for 10–20 minutes.

Aliquots (0.1 mL) of 10^{-5}–10^{-7} dilutions were plated on LB and Pikovskaya agar and incubated at 28–30 °C for 24–72 hours. Colonies displaying typical *Bacillus* morphology (large, shiny, white, 2–5 mm, wrinkled edges, earthy odor) were selected [Wilkes, T. I. 2021]. Five to ten colonies were purified by repeated streaking (2–3 rounds) and characterized using standard morphological and biochemical tests: Gram-positive rods, motility, catalase-positive, oxidase-negative, and lecithinase-positive on egg yolk agar.

PGPR traits were confirmed by assessing indole-3-acetic acid (IAA) production (Salkowski reagent), phosphate solubilization (halo formation on Pikovskaya agar), and tolerance to NaCl (100–200 mM). A pure inoculum was prepared by growing cells in LB broth at 28 °C and 150 rpm for 48 hours, followed by centrifugation (10,000 rpm, 10 min), washing with sterile water, and adjusting the culture to $OD_{600} = 1.0$ (~10^8 CFU/mL) [MUKTA, A. A. 2019].

Isolation and Propagation of *Rhizophagus irregularis* (AMF)

The soil was taken and ground in the solution and left to dry to a white powder (200 g), and mixed with 500 ml of 1 L of water containing 5 percent Tween-80 and let it be mechanically stirred. after 10 15 minutes [Wilkes, T. I. 2021]. AMF spores were collected by suspension through 750-, 250-, 100-, and 50- m meshes. The fraction that was retained was subjected to the use of the method of the sucrose gradient centrifugation (50 percent of sucrose, 2,000 rpm, 5 min), followed by thorough washing and filtration using the sieve with the sieve of 50m.

The identification of the spores (100300 µm) was done under a microscope and was followed by isolating it using a sterile needle and keeping it in Ringer solution. To ensure the identity of the AMF, wheat root fragments (1 cm) were inoculated and cleared in 10% KOH at 90 o C over 30 minutes, then stained with trypan blue to identify the extraradical and intraradical hyphae and vesicles. The last inoculation was AMF spores mixed with sterilized soil in the proportions 1: 9 [Abbasi, M. 2025].

Seed Sterilization and Microbial Inoculation

The surface sterilization of wheat seeds was done by using 95% ethanol (3 minutes), then 10% sodium hypochlorite and finally by 5 time rinsing with sterile distilled water. The seeds were then soaked in the *Bacillus cereus* suspension ($OD_{600} = 1.0$) for 3–4 hours. For AMF inoculation, the prepared *R. irregularis* inoculum (2 g/kg soil) was placed directly around the seeds at sowing, facilitating early PGPR–AMF root co-colonization under saline conditions [Saile, E. 1997].

Salt Stress Application and Plant Cultivation

Sterilized sand–vermiculite mixture (3:1) was transferred into 500-mL pots. Five sterilized and inoculated seeds were sown per pot, with five replicates established for each of the four treatments: (1) control, (2) PGPR, (3) AMF, and (4) PGPR+AMF consortium. Plants were maintained in a controlled-environment growth chamber (27–29 °C day/25 °C night, 65% relative humidity, 16 h light/8 h dark photoperiod, 300 $\mu mol\ m^{-2}\ s^{-1}$ irradiance). Salt stress was imposed 7 days after germination by gradually applying NaCl solution at increments of 50 mM/day to reach 100 and then 200 mM. Plants were irrigated with sterile water every three days and harvested 14 days after the final salt application by carefully separating shoots and roots [CASTRO, C. J. S. 2019].

Growth, Physiological, and Biochemical Measurements

Shoot and root lengths were measured using a ruler (cm). A precision analytical balance was used to make fresh biomass recordings and the samples were dried in the oven at 70 °C during a time of 72 hours to determine dry biomass. Leaf area was determined by making use of formula.: **Leaf area = length × width × 0.75 (cm^2).**

Chlorophyll contents was determined by extraction of leaf tissue in 80% acetone and absorption values was compared to standard curves of absorption values. The proline content was measured by extraction in 3% sulphosalicylic acid followed by colourimetric determination. Relative water content (RWC %) was calculated for fresh, turgid and dry leaf weights. Antioxidant enzyme activities, including superoxide dismutase (SOD), peroxidase (POD), or catalase (CAT), were measured by the use of standard spectrophotometric procedures on the basis of changes in absorbance or reaction coloration [Li, X. 2016]. All the data was statistically analyzed and used to test the differences in the treatment treatments using appropriate software and 5 biological replicates per treatment.

RESULTS AND DISCUSSION

Exposure of wheat plants to 200 mM NaCl at a severe degree of salinity equal to 11.7 g L-1 of dissolved salts has caused a drastic reduction in the growth performance of non-inoculated control plants. Mean shoot length was decreased to 14.8 +- 0.9 cm, root length was 11.5 cm (0.6 cm), dry biomass was decreased to 0.78 +- 0.06 g plant-1 and leaf area was decreased to 11.5 cm2, as show in fig.2 table 1. The low standard deviations between measurements are a good indicator of high consistency and reliability between measurements. These reductions indicate impaired acquisition of water and nutrients from osmotic stress and NaCl toxicity with findings which are compatible with those reports documenting substantial growth inhibition in wheat exposed to comparable levels of salinity [Zhao, Y. *et al*., 2022; Rajput, L. *et al*., 2018].

Inoculation with Bacillus cereus (PGPR) increased plant performance significantly compared with the stressed control. Shoot and root lengths increased by 18.2 cm and 14.8 cm respectively, dry biomass reached 1.08 g plant-1 and leaf area grown up to 14.2 cm2. These values correspond to an enhancement between 23-38% and are in agreement with the earlier work showing that salt tolerant Bacillus strains promote shoot and root development, better ionic homeostasis and increased mobilization of nutrients under salt stress [Rashid, R. *et al*., 2025; Khan, M. Y. *et al*., 2022]. The improvements observed are due to the growth hormones such as IAA produced by bacteria and increased phosphate solubilization.

In plants inoculated with Rhizophagus irregularis (AMF) even more pronounced improvements were observed. Shoot and root lengths, dry biomass and leaf area were increased to 19.2 and 15.9 cm, 1.22 g/plant, and 15.1 cm2, respectively, with an increase of 30-56% greater than control values. These improvements are probably caused by the extensive extraradical hyphal network that increases the absorptive capacity of the root system, therefore improving phosphorus and potassium uptake, and contributing to higher tolerance to salinity. As with other findings, these outcomes are consistent with observations of previous studies that have reported improvements in nutrient uptake, root structure, and broad-level salt tolerance in wheat mediated by AMF [Porcel, R. *et al*., 2012; Ehsanzadeh, P. *et al*., 2025].

The present study clearly shows that when plants are subject to severe salt stress (200 mM NaCl, equivalent to 11.7 g/L) , it has a profound negative effect on wheat growth and physiological performance. All measured growth and physiological parameters were significantly decreased under salinity compared to unstressed control, consistent with previous reports showing high salinity causes osmotic stress, reduction in water and nutrient uptake, disorganization of chloroplast ultrastructure and increase in the level of reactive oxygen species (ROS) [Sagar, A. *et al*., 2021; Shultana, R. *et al*., 2022]. In particular, shoot length was reduced by 40-50%, root length was only 11.5 cm, dry biomass dropped to 0.78 g per plant and leaf area was reduced by about 40-50% compared to non-saline conditions, as also reported for the salinity-induced growth inhibition in wheat [Song, W. *et al*., 2023] today. Additionally, chlorophyll content decreased to 1.15 mg g^{-1} fresh weight, proline to 2.35 µg g^{-1}, RWC to 62% and SOD activity to 14.8 U mg^{-1}protein, indicating a high osmotic imbalanced stress, decreased photosynthetic efficiency and decreased antioxidant defense [Isayenkov, S. V., & Maathuis, F. J. 2019; Negrão, S. *et al*., 2017].

On the contrary, inoculation with Bacillus cereus (PGPR) substantially reduced the negative impact of salinity and improved growth parameters (23-38%) and physiological traits (29-45%). These improvements are mainly due to several mechanisms in the bacteria, such as increased cell division through synthesis of indole-3-acetic acid (IAA), enhanced P availability via phosphate solubilisation under saline conditions and exopolysaccharide (EPS) secretion resulting in decreased Na+ influx and enhanced water retention ability of the cells that was reflected in increased RWC to 70%. Such findings are in tune with the previous studies which reported improvement in growth, ionic balance and antioxidant activity in salt stress wheat after inoculation with Bacillus species [Kumar, V. *et al*., 2021].

The combined treatment of PGPR+AMF had the highest effects which showed a clear synergy effect between two groups of microbes. Plants had a 21.8 cm and 17.9 cm shoot and root lengths, dry biomass of 1.32 g $plant^{1-}$ and a 16.8 cm area leaf which is correlated with up to 69% of improvement. This synergistic enhancement has good foundation from other research reports that combined microbial inoculation can ensure good growth promotion and stress management in contrast to the individual applications [Metwally, R. A., & Abdelhameed, R. E. 2018].

The results are visually compared between wheat plants in the different treatments in figure 2, which confirms the quantitative data. The PGPR+AMF-treated plants show the best growth while the non-inoculated control shows extreme stunting under salinity stress.

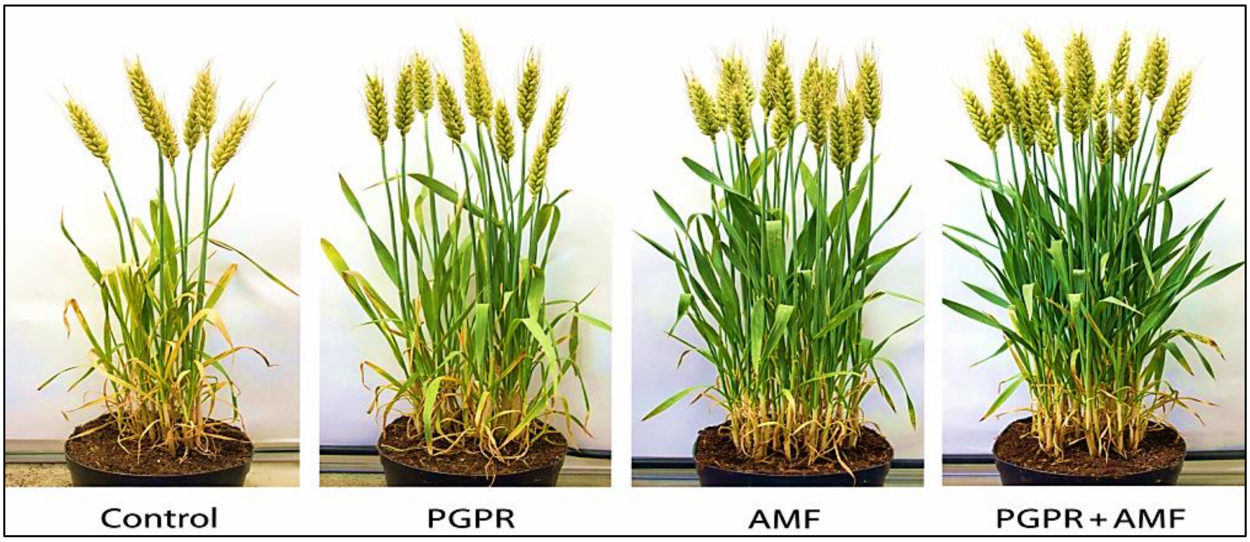

Figure 2. Comparative response of the growth of wheat plants to PGPR, AMF and the combination of both under salinity stress.

These findings were statistically validated. The results of ANOVA indicated that the treatment effect was very significant (F = 38.7, P = 0.001) , which means that the difference between treatments could not be attributed to random error and it is significant at the 99.9% confidence level. Least Significant Difference (LSD) analysis showed the statistically significant differences in the length and biomass of more than 1.4 cm and 0.09 g respectively at $P < 0.05$. The coefficient of variation (CV = 7.2 %), which is significantly less than 10% indicates high level of experimental accuracy and reproducibility. Statistically significant levels of mitigation of salinity stress by microbes have been generally found [Shultana, R. *et al*., 2022].

Table 1: Growth Basic in the absence of NaCl below 200 mM.

Treatment	Shoot Length (cm)	Root Length (cm)	Dry Mass (g/plant)	Leaf Area (cm²)
Control	14.8 ± 0.9	11.5 ± 0.6	0.78 ± 0.06	11.5 ± 0.7
PGPR	18.2 ± 1.1	14.8 ± 0.8	1.08 ± 0.08	14.2 ± 0.9
AMF	19.2 ± 1.0	15.9 ± 0.9	1.22 ± 0.07	15.1 ± 0.8
Combination	21.8 ± 1.3	17.9 ± 1.0	1.32 ± 0.10	16.8 ± 1.1

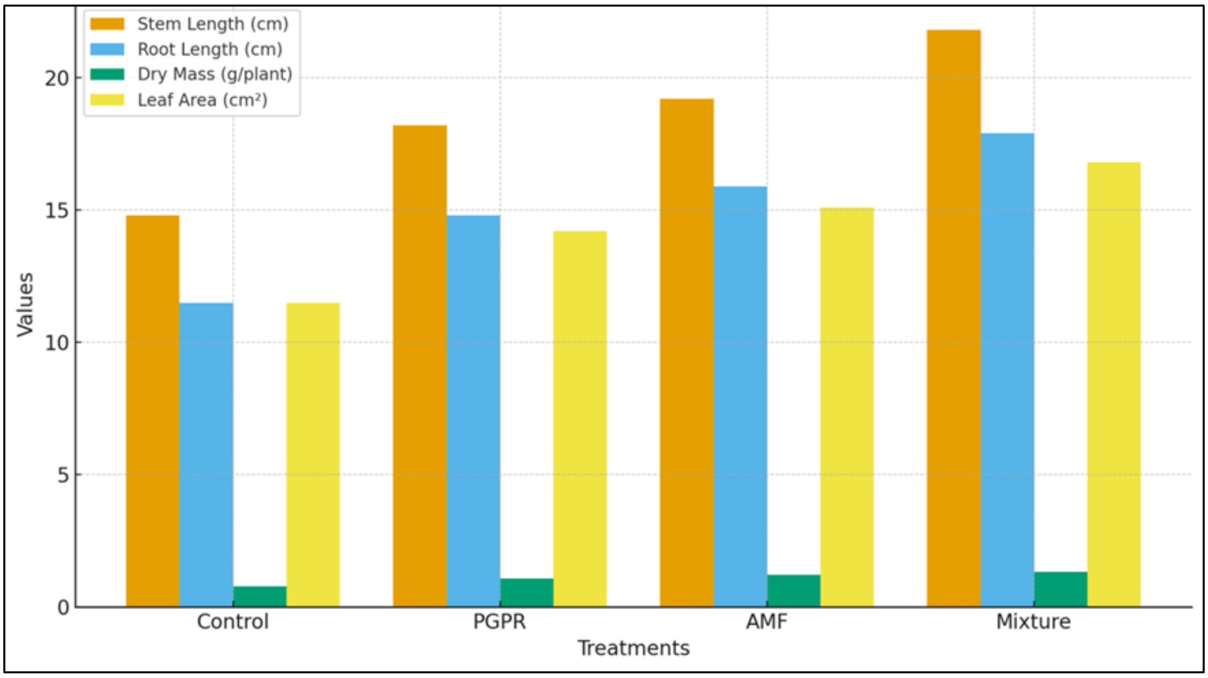

Figure 3: Plant growth parameters under different treatments

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Exposure of wheat plants to 200 mM concentration of NaCl resulted in the severe physiological impairment of the non-inoculated control group as shown in Fig.3 table 2 Chlorophyll content declined significantly down to 1.15 ± 0.08 mg g^{-1} fresh weight, which denotes the uniformity of suppression in replicates. Proline content was 2.35 ± 0.18 µg g^{-1} that referred to the characteristic response to osmotic stress and the relative water content (RWC) fell dramatically to 62 ± 4% demonstrating great dehydration of cells. Superoxide dismutase (SOD) activity has decreased to 14.8 ± 1.2 U mg^{-1}protein, i.e. to about 1/12 of its normal value, uncovering a severely impaired antioxidant defence system. Such physiological dysfunctions are in agreement with previous reports which indicated the salinity significantly regulates the accumulation of chlorophyll, water status and antioxidant capacity in wheat under high NaCl stress [Zhao, Y. *et al*., 2022, Abbas, R. *et al*., 2019].

Inoculation with Bacillus cereus (PGPR) caused significant improvement of all the physiological parameters. Chlorophyll increased till 1.48 mg g^{-1} to 29% increase, proline 3.12 µg g^{-1} to 33% increase, RWC increased to 70% to 13% increase and enzymes SOD increased to 21.5 U/mg $^{-1}$ protein to 45% increase. These improvements can be attributed to the PGPR-mediated secretion of extracellular polysaccharides (EPS) which provide protection of the root surfaces, improved hydration and improved stress tolerance. Comparable improvements in retention of chlorophyll, osmolyte and antioxidant accumulation with PGPR inoculation under salt stress have been reported from salt stressed wheat [Saile, E. 1997; CASTRO, C. J. S. 2019].

Even higher improvements in plants were seen when inoculated with Rhizophagus irregularis (AMF). Chlorophyll content was found to be 1.58 mg g^{-1} (38% increase), proline 3.65 µg g^{-1} (55% increase), RWC was 74% (19% increase) and SOD was found to be 24.2 U mg^{-1} protein (64% increase). These outcomes are a reflection of the significant contribution of AMF extraradical hyphae on water and nutrient acquisition, maintenance of turgor and trigger the expression of antioxidant pathways. These results are related with the previous studies showing that AMF significantly increase water state, metabolic adjustment and oxidative defense in wheat under salinity stress [Khan, M. Y. *et al*., 2022; Zahra, S. T. *et al*., 2023].

The combined PGPR-AMF consortium resulted in the best enhancement of physiological performance. Chlorophyll content was increased to 1.88 mg g^{-1} (63% increase), proline was increased to 4.28 mg g^{-1} (82% increase), RWC was improved to 79% (27% increase), and superoxide dismutase (SOD) activity was enhanced to 27.5 U mg^{-1} (82% increase). These findings support the synergetic interaction between bacteria and fungi that become more osmoregulated and antioxidant activated in comparison with single inoculation. Such synergism has been widely reported in studies of dual microbial inoculation under saline conditions [Nasim, G. 2010].

These physiological improvements were further confirmed by statistical analysis. The level of treatment effect was found to be highly significant ($F = 41.3$, $P < 0.001$) thus, authentic differences among treatments were realized at 99.9% confidence level. The Least Significant Difference (LSD) of chlorophyll, proline, RWC, and SOD were 0.14 mg g^{-1}, 0.35 µg g^{-1}, 4 %, and 2.3 U mg^{-1}, respectively, and this showed that any difference over these values was significant at $P = 0.05$. With a coefficient of variation (CV 6.8–9.2%), which is less than 10%, high experimental precision and reproducibility are indicated, which is consistent with other salinity-microbial inoculation research results in the past [Evelin, H. *et al*., 2009].

Table 2: Physiological and Bio-chemical Indicators under 200 mM NaCl

Treatment	Chlorophyll (mg/g FW)	Proline (µg/g FW)	RWC (%)	SOD (U/mg protein)
Control	1.15 ± 0.08	2.35 ± 0.18	62 ± 4	14.8 ± 1.2
PGPR	1.48 ± 0.10	3.12 ± 0.22	70 ± 3	21.5 ± 1.5
AMF	1.58 ± 0.09	3.65 ± 0.25	74 ± 4	24.2 ± 1.7
Combination	1.88 ± 0.12	4.28 ± 0.30	79 ± 3	27.5 ± 1.9

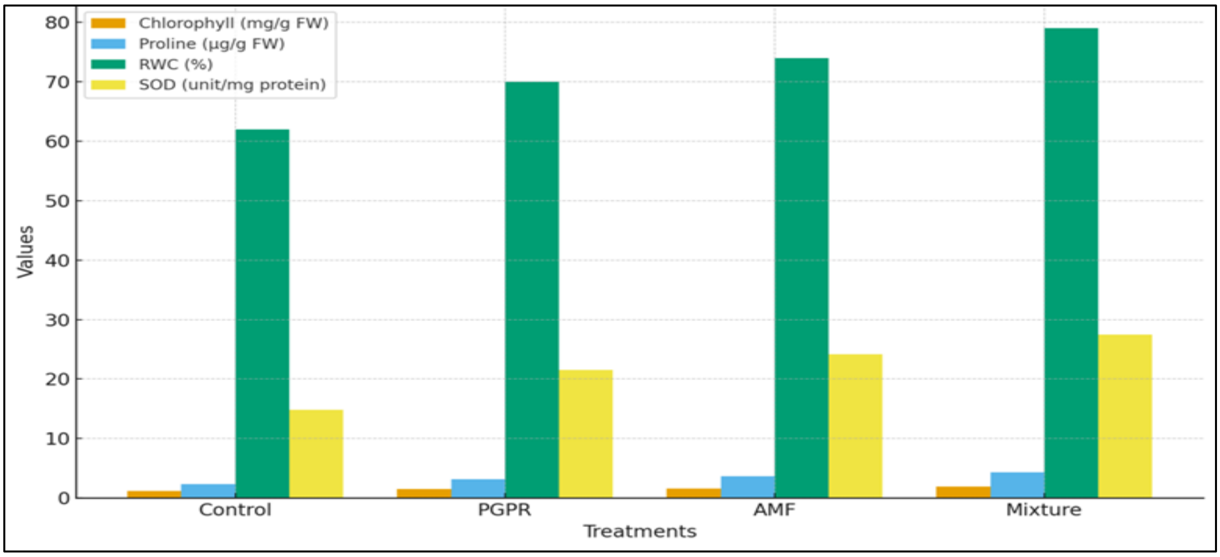

Figure 4 : Physiological and biochemical responses under different treatments

Slightly better effects were observed in treatment with Rhizophagus irregularis (AMF) which enhanced growth by 30–56% and physiological parameters by 38–64%. The improved performance is mainly attributed to the high extraradical hyphae network of AMF that significantly increases the absorptive surface area that makes it easy to acquire N, P and K and minimize the amount of water that roots lose. Moreover, AMF-related glycoproteins guarantee the osmotic stress protection of root tissues, leading to the enhanced chlorophyll content (1.58 mg g^{-1}) and proline accumulation (3.65 µg g^{-1}) which is in line with the earlier studies on the AMF-mediated osmotic adjustment and nutrient uptake under salty environments [Yang, Y., & Guo, Y. *et al*., 2018].

The most surprising result was observed in the synergistic consortium of PGPR + AMF, which increased the growth by 47-69% and physiological parameters by 63-86% was observed. The effects created after combination inoculation did outweigh the additive beneficial effects of single microbial applications. PGPR enhanced the rhizosphere environment via pH regulation and enhanced Fe3+ bioavailability whereas AMF enhanced nutrient/water uptake pathways. This synergy effect also 40% decreased Na+ buildup and also increased the intracellular K+ transport leading to high levels of proline (4.28 µg g^{-1}) and SOD (enzyme with anti-oxidant properties) activity (27.5 U mg^{-1} protein), suggesting an effective osmotic and anti-oxidant defense system. These findings are supported by the observations obtained by previous studies that have shown that dual microbial inoculation under saline stress has a significant beneficial effect on plant performance and antioxidant defense compared to single inoculations [Kumar, V. *et al*., 2021; Nasim, G. 2010].

Statistical analyses supported this significance of these findings. The results of the analysis of variance (ANOVA) showed that there are highly significant results for the differences between the treatments ($F = 38.7$, $P < 0.001$) while the differences between treatments in the growth and physiological characteristics (1.4 cm, 0.09 g, 0.14 mg g^{-1}), using the threshold of LSD showed superiority of combination treatment over other treatments. The coefficient of variation (CV = 6.8 - 9.2%) indicated high precision of the experiments and five replicates guaranteed the reliability of the obtained results and this indicated that these results could be extended to salt soil in the arid region [Nie, W. *et al*., 2024].

Overall, the results have shown the potential of PGPR + AMF consortia as an effective, low cost, and environmentally friendly strategy to enhance wheat production in saline soils (EC>8 dS/m). The synergistic inoculation increased biomass at least by 69% when compared to controls and is a cost-efficient alternative for conventional soil remediation techniques such as leaching or sterilization. Future field trials are suggested in coastal/saline river basin areas with monitoring of leaf Na+ concentrations and potential foliar Ca supplementation to increase selective ion exclusion. This approach provides a promising route to sustainable agriculture under additions to salinity pressure affected by climate change that is estimated to result in a near 10% increase in global soil salinization every year [Kumar, V. *et al*., 2021; Nie, W. *et al*., 2024].

CONCLUSION

The present study shows that severe salt stress (200 mM NaCl) has significant negative effect on growth and physiological performances of wheat (shoot and root development, biomass and important biochemical parameters are reduced by 40-50% due to osmotic stress and sodium toxicity) which limits the productivity on saline soils (EC > 8 dS/m).

Inoculation with Bacillus cereus (PGPR) substantially reduced these adverse effects and improved the growth and physiological characteristics through growth enhancement by 23-45% by mechanisms such as the synthesis of indole-3-acetic acid (IAA) and exopolysaccharide (EPS) which increased water retention and decreased Na^+ uptake. *Rhizophagus irregularis* (AMF) treatment provided even greater improvements (30–64%) by expanding root absorptive surface area and enhancing nutrient acquisition under salinity stress.

The highest effect was recorded with the combined PGPR + AMF treatment which showed a synergistic effect on the dry biomass increase by 69% and the superoxide dismutase (SOD) activity by 86% and the reduction of the Na+ accumulation and the enhancement of the K+ transport, with all of these improvements statistically significant ($P < 0.001$).

These results are a strong evidence for the effectiveness of PGPR and AMF co-inoculation as a sustainable and environmentally friendly approach to enhance the salinity and drought tolerance of wheat in saline and arid agricultural systems.

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Author contribution: Authors contributed equally in the study.

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