Research Article

Mitigation of Salinity Stress in Maize (*Zea mays* L.) Using the Endophytic Fungus GR7

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Abstract | Endophytic fungi are gaining recognition as natural allies in agriculture, capable of enhancing crop tolerance against environmental stresses. In this study, the potential of the endophytic strain GR7, isolated from *Senna occidentalis* L., was examined for its ability to counteract the negative effects of salinity on maize (*Zea mays* L.) seedlings. Plants were cultivated in sterilized soil for 21 days under controlled conditions and subjected to six treatment groups: (i) untreated control, (ii) 100 mM NaCl, (iii) 150 mM NaCl, (iv) GR7 inoculation alone, (v) GR7 + 100 mM NaCl, and (vi) GR7 + 150 mM NaCl. Measurements included shoot and root length, fresh biomass, and dry biomass, alongside spectrophotometric assessment of physiological and biochemical parameters. These comprised chlorophyll, indole-3-acetic acid (IAA), salicylic acid (SA), flavonoids, phenolics, proteins, soluble sugars, ascorbic acid oxidase, peroxidase, and antioxidant activity. The findings demonstrated the growth-promoting ability of GR7. Under normal conditions, inoculated plants showed a 64.3% increase in shoot length and a root length extension of 20.33 cm compared with uninoculated seedlings. Even under severe salt stress (150 mM NaCl), GR7-treated seedlings maintained superior growth, reaching 35.7 cm in shoot length, 4.8 g in fresh biomass, and 0.36 g in dry biomass. Biochemical evaluations mirrored these results, showing increased levels of chlorophyll (64.65 mg/g), IAA (502.43 μg/g), SA (6.38 mg/g), flavonoids (206.14 μg/g), and proteins (317.48 μg/g) in inoculated seedlings. These outcomes highlight the role of GR7 in enhancing photosynthetic pigments, regulating phytohormones, and strengthening metabolic processes, thereby promoting both normal growth and tolerance under salinity stress. Moreover, enhanced peroxidase activity and elevated radical scavenging capacity highlighted the fungus's role in bolstering antioxidant defenses. Overall, these results underline the dual advantage of GR7, stimulating robust plant growth under favorable conditions and mitigating growth inhibition under saline stress. The fungus appears to exert its protective role through improved metabolic activity and strengthened antioxidant machinery, enabling maize seedlings to maintain physiological balance even in the face of osmotic and ionic stress. By integrating growth promotion with biochemical resilience, GR7 presents itself as a viable candidate for development into a bioinoculant that can enhance crop productivity in salt-affected agricultural systems. Future studies focusing on the molecular mechanisms behind GR7–plant interactions, as well as field-scale validation under variable soil salinity levels, will be essential to translate these findings into practical applications. Such work could pave the way for eco-friendly, fungal-based technologies that not only safeguard maize yields but also contribute to sustainable farming practices in stress-prone environments.

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Introduction

Maize (*Zea mays L.*), a cornerstone of global agriculture, is widely cultivated as a staple food, a primary source of animal feed, and an important industrial raw material (Afzal *et al*., 2005; Asif *et al*., 2025). Despite its immense economic and nutritional value, maize production is increasingly threatened by various abiotic stresses. Among these, soil salinity stands out as one of the most detrimental factors, severely limiting germination, growth, and yield potential (Sajid *et al*., 2023). The problem is further intensified by climate change, poor irrigation management, and unsustainable farming practices, which collectively contribute to the accumulation of soluble salts in agricultural soils (Alka *et al*., 2020). Addressing salinity stress in maize is, therefore, not only vital for securing crop productivity but also essential for ensuring food security in the face of global environmental challenges (Shrivastava and Kumar, 2015; Farooq *et al*., 2015; Alka *et al*., 2020; Irshad *et al*., 2025). Approximately 20-22% of the world's irrigated croplands are affected by soil salinity, with projections suggesting this area may expand by 30-50% by 2050 due to climate change and unsustainable irrigation practices. This salinization process severely compromises soil health and reduces crop yields by an estimated 20-40% in affected regions (Munns and Tester, 2008; Khan *et al*., 2024). Salinity stress negatively impacts plants through two primary mechanisms: (1) osmotic stress that reduces water availability, and (2) ionic toxicity from excessive sodium (Na^+) and chloride (Cl^-) accumulation that disrupts cellular functions (Khan *et al*., 2018a; Manan *et al*., 2025). The osmotic phase limits water uptake, while the ionic phase results in the accumulation of toxic ions, such as Na^+ and Cl^-, disrupting cellular homeostasis and metabolic processes (Munns and Tester, 2008; Amaregouda *et al*., 2010; Zhu, 2016; Sajid *et al*., 2023). Beyond its primary osmotic and ionic effects, salinity stress provokes oxidative damage through ROS overaccumulation (Ragel *et al*., 2015). The resulting oxidative burst, if not effectively scavenged by the coordinated action of the ASC-GSH cycle enzymes and antioxidant metabolites, leads to widespread cellular dysfunction through peroxidative degradation of lipids, carbonylation of proteins, and oxidative DNA damage (Gill and Tuteja, 2010; Yadav *et al*., 2010; Subhan *et al*., 2024).

Conventional strategies to address salinity stress, such as breeding salt-tolerant cultivars and optimizing irrigation systems, are often resource-intensive, time-consuming, and only partially effective (Fageria, 2010; Shakir *et al*., 2023a; Zhu *et al*., 2023). Growing environmental concerns have intensified the search for sustainable solutions to enhance crop stress tolerance. Endophytic fungi, which colonize plant tissues symbiotically, offer an eco-friendly approach by improving host resilience through multiple mechanisms: (1) enhanced antioxidant activity, (2) stress hormone modulation, and (3) osmolyte production (Gill and Tuteja, 2010). These adaptive benefits make them valuable tools for sustainable agriculture in stress-affected regions (Rodriguez *et al*., 2008; Redman *et al*., 2011; Chaudhary, 2022). Beneficial endophytic fungi like Piriformospora indica and Trichoderma spp. enhance plant growth and salinity tolerance through multiple mechanisms, including phytohormone production (e.g., IAA), improved nutrient acquisition, hormonal regulation, and activation of antioxidant defense systems (Khan *et al*., 2016; Waqas *et al*., 2012; Egamberdieva *et al*., 2017; Chaudhary, 2022). These beneficial microbes not only enhance plant growth but also stimulate the buildup of osmoprotectants like proline and soluble sugars, along with secondary metabolites such as flavonoids and phenolics. Together, these compounds play a crucial role in alleviating oxidative and ionic damage triggered by salinity stress, thereby strengthening the plant's adaptive capacity (Gill and Tuteja, 2010; Zhang *et al*., 2020; Afzal *et al*., 2005). The current study investigates the potential of endophytic fungi to enhance salinity tolerance in maize (*Zea mays*) by evaluating their effects on: (1) growth parameters, (2) physiological adaptations, and (3) antioxidant defense systems. The findings will provide critical insights for developing fungal-based biostrategies to improve maize cultivation in saline-affected agricultural systems.

Materials and Methods

Endophytic fungi collection

The endophytic fungal strain used in this study was obtained from the Plant-Microbe Interaction Laboratory, Department of Botany, Abdul Wali Khan University, Mardan, Pakistan, where it had been originally isolated and preserved in the culture collection. For experimental use, the strain was sub-cultured to obtain fresh cultures and maintained on potato dextrose agar (PDA) slants at 4 °C until further use (Ghodpage *et al*., 2008; Zhao *et al*., 2020).

Preparation of fungal inoculum

The endophytic fungal isolate GR7 was mass-cultivated by inoculating it into Czapek broth medium, followed by incubation at 27 °C in a shaking incubator set at 120 rpm for six days (Khan *et al*., 2018a). After incubation, the mycelial biomass was collected through filtration, rinsed thoroughly with sterile distilled water, and homogenized to prepare the inoculum for subsequent soil application (Gupta *et al*., 2018).

Plant growth experiments

Growth conditions and experimental design: Maize (*Zea mays*) seeds were surface-sterilized through sequential treatments: (1) 70% ethanol (v/v) for 2 min, followed by (2) three rinses with autoclaved distilled water (121°C, 15 min) to ensure complete removal of epiphytic microorganisms (Ullah *et al*., 2024a). Surface-sterilized seeds were sown in sterilized plastic pots (15 cm diameter × 12 cm height) containing 300 g of autoclaved (121°C, 15 psi for 30 min) sandy loam soil (pH 7.2, EC 1.8 dS/m). Plants were maintained in a growth chamber under controlled conditions: 25±2°C Day/18±2°C night temperatures, 60% relative humidity, and 18-h photoperiod (300 μmol m^2s^1 PAR) (Jarak *et al*., 2012).

Salinity stress treatments

To investigate the combined and individual effects of salinity stress and GR7 inoculation on maize growth, six experimental treatments were designed, each replicated three times to ensure reliability of the data. The first set of seedlings was maintained as an untreated control. The second and third groups were exposed to salinity stress by irrigation with 100 mM and 150 mM NaCl, respectively. In the fourth treatment, seedlings were grown in soil amended solely with GR7 fungal biomass at a concentration of 1 g per 100 g of soil. The fifth and sixth treatments involved a combination of GR7 inoculation with subsequent exposure to 100 mM and 150 mM NaCl, respectively. Salinity stress was introduced seven days after germination and maintained for a duration of 21 days, following the method described by Kader *et al*. (2002).

Growth parameter measurements

Following 21 days of treatment, maize seedlings were carefully uprooted and subjected to morphological assessment. Growth parameters, including shoot length and root length, were measured to the nearest millimeter using a calibrated ruler to ensure precision. Fresh biomass was recorded immediately after harvest with an analytical balance (±0.0001 g sensitivity). For dry biomass estimation, plant samples were placed in a hot-air oven at 70 °C for 72 hours, or until a constant weight was obtained, as outlined by Kumar *et al*. (2021). These measurements provided accurate insights into the effects of GR7 inoculation and salinity stress on the overall growth performance of maize seedlings.

Biochemical analyses of maize seedlings

The physiological and biochemical attributes of *Zea mays* seedlings exposed to GR7 inoculation, salinity, and waterlogging stress were quantified using standard spectrophotometric protocols (Ullah *et al*., 2025c). Total chlorophyll content was extracted in 80% acetone and quantified by recording absorbance at 645 nm and 663 nm. Auxin production, specifically indole-3-acetic acid (IAA), was measured through the Salkowski reagent method, in which culture filtrates were incubated in darkness before recording absorbance at 540 nm. Salicylic acid (SA) levels were assessed by reaction with 0.1% ferric chloride, followed by absorbance at 540 nm (Kumar, 2022). Flavonoid accumulation was determined using the $NaNO_2$–$AlCl_3$–NaOH colorimetric assay with quercetin as a standard, recording absorbance at 415 nm. Total phenolic content was estimated through the Folin–Ciocalteu method, with absorbance recorded at 650 nm and gallic acid serving as a reference compound (Reddy *et al*., 2023). Protein concentration was evaluated using the Lowry method at 650 nm with bovine serum albumin (BSA) as a standard, while soluble sugars were determined by the phenol–sulfuric acid method at 490 nm, calibrated against a glucose standard (Khan *et al*., 2018b). Enzymatic assays were also performed to evaluate oxidative stress responses. Ascorbic acid oxidase (AAO) activity was quantified by monitoring decreases in absorbance at 290 nm following substrate addition, whereas peroxidase activity was estimated at 420 nm using guaiacol as the substrate (Redman *et al*., 2011; Shakir *et al*., 2023b). The antioxidant potential of seedlings was assessed by the DPPH radical scavenging assay, in which extracts were incubated with DPPH solution for 30 minutes at 25 °C, and absorbance was recorded at 517 nm. Radical scavenging capacity was calculated using the equation $\%DPPH = (1 - AE/AD) \times 100$, where AE and AD represent the absorbance of the extract and blank, respectively (Ullah *et al*., 2018, 2024). Collectively, these biochemical analyses provided a detailed profile of the physiological adjustments induced by GR7 inoculation. The results underscored the fungus's role in improving photosynthetic efficiency, enhancing osmolyte accumulation, and strengthening antioxidant defenses under both salinity and waterlogging stress. Such findings reinforce the potential application of GR7 as a bioinoculant capable of promoting crop resilience in challenging environments (Monib *et al*., 1979).

Statistical analysis

Each experimental treatment was conducted in three independent replicates to ensure accuracy, reproducibility, and reliability of the findings. The collected data sets were subjected to one-way analysis of variance (ANOVA) to assess the effects of fungal inoculation and salinity stress on maize growth and biochemical attributes. ANOVA was selected because it is a robust statistical tool for identifying whether significant differences exist among the means of multiple treatment groups, particularly in factorial experiments where several factors interact. Following ANOVA, post hoc mean comparisons were performed using Duncan's multiple range test (DMRT), a widely used method in plant physiology and microbiology studies due to its effectiveness in distinguishing treatment-specific variations with higher sensitivity. The threshold for statistical significance was set at $p < 0.05$, ensuring that only differences with a probability of error less than 5% were considered meaningful. All experimental data are presented as mean values along with their corresponding standard error (SE) to provide a clear representation of variability among replicates. This statistical approach not only validates the consistency of the results but also enhances the reliability of the interpretations drawn from the study, thereby supporting robust scientific conclusions.

Results

Effect of GR7 endophytic fungus and salinity stress on the growth of Zea mays

Maize seedlings were cultivated in plastic pots containing 300 g of sterilized soil and maintained under controlled conditions for a period of 21 days. Six experimental treatments were established: (i) untreated control, (ii) 100 mM NaCl stress, (iii) 150 mM NaCl stress, (iv) GR7 fungal inoculation alone, (v) GR7 + 100 mM NaCl, and (vi) GR7 + 150 mM NaCl. The GR7 inoculum was applied at a rate of 1 g fungal biomass per 100 g of soil. At harvest (day 21), seedlings were evaluated for morphological attributes, including shoot and root length, as well as fresh and dry biomass. In addition, biochemical responses were assessed spectrophotometrically to provide insights into the physiological adjustments associated with fungal inoculation and salt stress. A comprehensive summary of the observed effects is presented in Figure 1. Seedlings inoculated with GR7 displayed enhanced growth compared to uninoculated plants under both normal and saline conditions. GR7 application resulted in significant increases in shoot and root lengths, as well as biomass accumulation. Notably, the GR7 + 150 mM NaCl treatment group demonstrated marked resilience, exhibiting higher shoot length, fresh weight, and dry weight relative to seedlings exposed to salinity stress alone. These findings suggest that GR7 plays a dual role, stimulating growth under optimal conditions while simultaneously alleviating the detrimental effects of salt stress.

Effect of Gr7 and salinity on shoot and root length of Zea mays

The GR7 endophytic fungus enhanced shoot length (SL) in maize seedlings compared to uninoculated plants (Figure 2). Inoculation with GR7 promoted growth, increasing shoot length by 64.3% relative to the control. Furthermore, GR7-inoculated seedlings exhibited improved tolerance to salinity, maintaining higher shoot length under both 100 mM and 150 mM NaCl stress compared to non-inoculated stressed plants (Figure 1). In contrast, seedlings without GR7 exposed to salinity showed a progressive decline in shoot length, with the greatest reduction (33.87%) observed at 150 mM NaCl. Salinity stress also significantly affected root length (Ullah and Shakir, 2023). However, association with GR7 mitigated this effect, as inoculated seedlings under salt stress exhibited longer roots than their uninoculated counterparts (Figure 3). The maximum root length (20.33 cm) was recorded in GR7-treated seedlings, exceeding that of the control, while the shortest roots (10.9 cm) were observed in seedlings subjected to 100 mM NaCl, followed by those treated with 150 mM NaCl (Ullah *et al.*, 2025a, b).

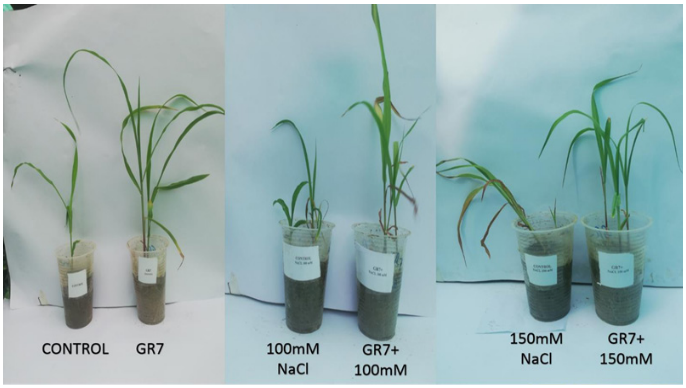

Figure 1: *Growth promotion and salinity stress alleviation in Zea mays seedlings by GR7 endophytic fungus. The GR7 endophytic fungus, originally isolated from Senna occidentalis L., was evaluated for its role in promoting growth and mitigating NaCl-induced stress in maize seedlings. Seedlings were grown for 28 days under controlled room temperature conditions with an 18-hour photoperiod. Treatments included the application of GR7 fungal biomass to the soil, which was used to assess its effect on growth performance and stress tolerance.*

Effect of GR7 and salinity on fresh and dry weight of Zea mays

Fresh weight analysis revealed three distinct response patterns: (a) GR7-inoculated plants showed a 21.5% increase over controls (4.83 vs 3.98 g), (b) GR7+salinity combinations partially mitigated stress effects, and (c) non-inoculated salt-stressed plants exhibited dose-dependent biomass reduction (r^2 = 0.94 for NaCl concentration-weight correlation). The 17.2% higher fresh weight in GR7+150mM NaCl plants versus salt-stressed controls (4.12 vs 3.51 g)

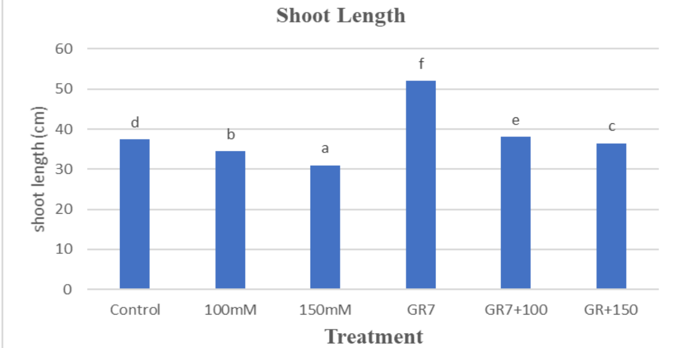

Figure 2: *Shoot length of Zea mays seedlings in response to endophytic fungus GR7 under control and salinity stress. Stress treatments of 100 and 150 indicate 100 mM and 150 mM NaCl, respectively. GR7 biomass was incorporated into the soil at 1 g per 100 g. Data represent mean values of three replicates ± SE. Bars sharing different letters are significantly different at p < 0.05.*

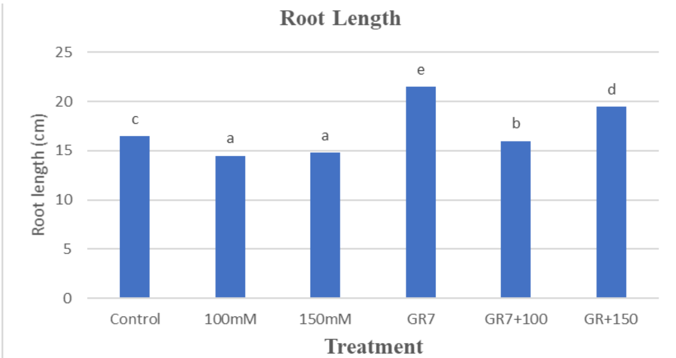

Figure 3: *Root length of Zea mays seedlings grown with or without GR7 under control and salinity stress conditions. Salinity treatments of 100 and 150 indicate 100 mM and 150 mM NaCl, respectively. GR7 inoculum was applied at 1 g fungal biomass per 100 g of soil. Values represent mean ± SE of three independent replicates. Bars sharing different letters denote significant differences at p < 0.05.*

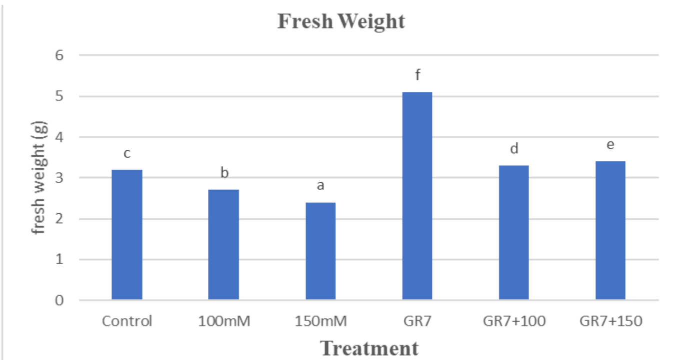

Figure 4: *Comparison of fresh weight in Zea mays seedlings with and without GR7 inoculation under control and salinity stress conditions (100 and 150 mM NaCl). GR7 was added to soil at a rate of 1 g per 100 g. Data are presented as mean ± SE (n = 3). Significant differences among treatments (p < 0.05) are indicated by different letters.*

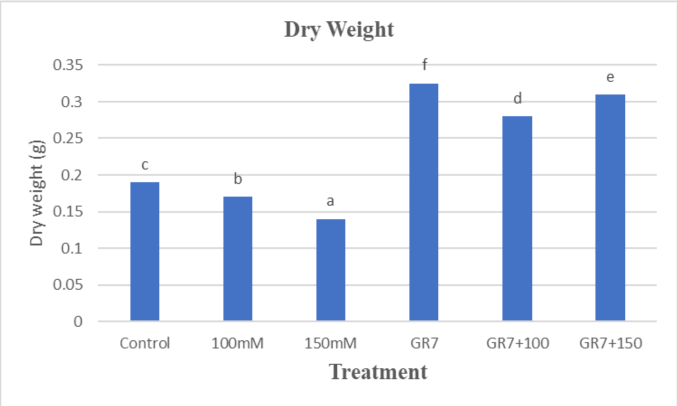

Figure 5: *Comparison of dry weight in Zea mays seedlings with and without GR7 inoculation under control and salinity stress conditions (100 and 150 mM NaCl). GR7 was added to the soil at a rate of 1 g per 100 g. Data are presented as mean ± SE (n = 3). Significant differences among treatments ($p < 0.05$) are indicated by different letters.*

suggests fungal mediation of osmoregulation, possibly through enhanced water retention or osmolyte production under stress conditions. Seedling dry weight measurements demonstrated sensitivity to NaCl toxicity conditions. The seedlings inoculated with the GR7 endophytic fungus demonstrated a significantly higher dry weight (0.32 g) compared to uninoculated control seedlings (Figure 5). This positive effect of GR7 was consistent under both control and salinity-stressed conditions. GR7-associated seedlings recorded greater dry biomass than non-inoculated plants subjected to 100 mM and 150 mM NaCl stress, indicating that fungal inoculation mitigated the inhibitory effects of salinity on biomass accumulation. Under salt stress, uninoculated seedlings exhibited a marked reduction in dry weight, reflecting the detrimental impact of NaCl on plant water balance and metabolic activity. However, inoculation with GR7 not only offset this decline but also enabled seedlings to maintain dry weights close to, or even surpassing, those recorded under non-stressed conditions. This suggests that GR7 contributes to improved osmotic adjustment and nutrient uptake, thereby enhancing the plant's ability to partition assimilates into structural biomass. The increase in dry biomass of GR7-inoculated seedlings under saline conditions highlights the fungus's role in boosting resource-use efficiency and stress resilience. These findings support the concept that GR7 facilitates improved carbon assimilation and allocation, potentially by modulating plant hormonal responses and strengthening antioxidative defense mechanisms. Such effects collectively ensure better maintenance of growth and dry matter production in maize even under high salinity stress. As illustrated in (Figure 4), the fresh weight was measured.

Biochemical analysis of Zea mays treated with GR7 and salinity

Total chlorophyll content: The total chlorophyll content of maize seedlings subjected to GR7 inoculation and varying levels of NaCl stress was quantified spectrophotometrically. GR7 inoculation led to a significant increase in chlorophyll accumulation under both non-stressed and salt-stressed conditions (Ullah *et al*., 2023). The maximum chlorophyll content (59.56 mg/g) was observed in seedlings inoculated with GR7, followed by those treated with GR7 in combination with 150 mM NaCl (Figure 6). By contrast, seedlings exposed solely to 100 mM and 150 mM NaCl also displayed elevated chlorophyll levels compared to the untreated control, though to a lesser extent than the GR7-associated treatments.

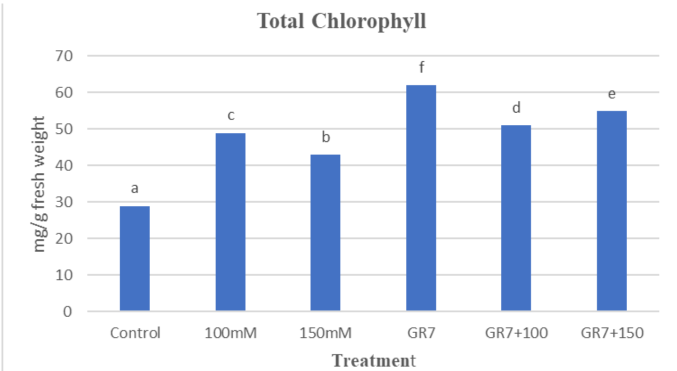

Figure 6: *Comparison of total chlorophyll content in Zea mays seedlings with and without GR7 inoculation under control and salinity stress conditions (100 and 150 mM NaCl). Data are presented as mean ± SE (n = 3). Significant differences among treatments ($p < 0.05$) are indicated by different letters.*

Indole-3-acetic acid content of Zea mays seedlings

The indole-3-acetic acid (IAA) content of maize seedlings treated with GR7 and exposed to varying concentrations of NaCl was quantified spectrophotometrically (Ullah *et al*., 2019a, c). Under non-stress conditions, GR7-inoculated seedlings exhibited lower IAA levels compared to the control. However, under salinity stress, GR7 association significantly enhanced IAA content in seedlings treated with 100 mM and 150 mM NaCl relative to non-inoculated seedlings (Figure 7). In contrast, non-inoculated seedlings subjected to NaCl stress displayed a marked reduction in IAA content compared to the control.

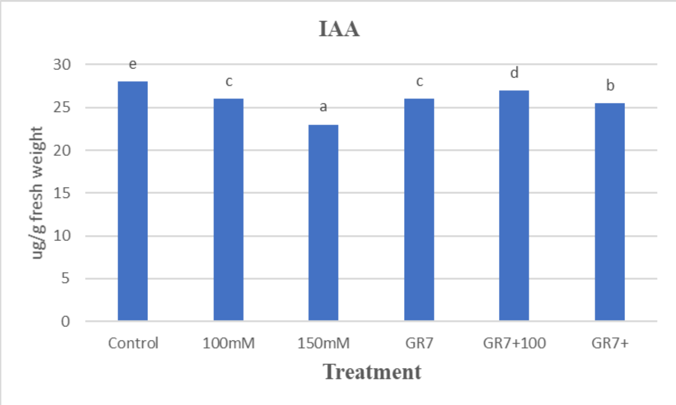

Figure 7: *Comparison of indole-3-acetic acid (IAA) content in Zea mays seedlings with and without GR7 inoculation under control and salinity stress conditions (100 and 150 mM NaCl). Data are presented as mean ± SE (n = 3). Significant differences among treatments ($p < 0.05$) are indicated by different letters.*

SA content of Zea mays seedlings

The salicylic acid (SA) content of maize seedlings inoculated with GR7 and exposed to varying concentrations of NaCl was quantified spectrophotometrically (Ullah *et al*., 2018b; 2019b). GR7-inoculated plants consistently displayed higher SA accumulation under both control and salt-stress conditions compared to their non-inoculated counterparts. The highest SA content (4.57 mg/g) was observed in seedlings treated with GR7 alone (Figure 8).

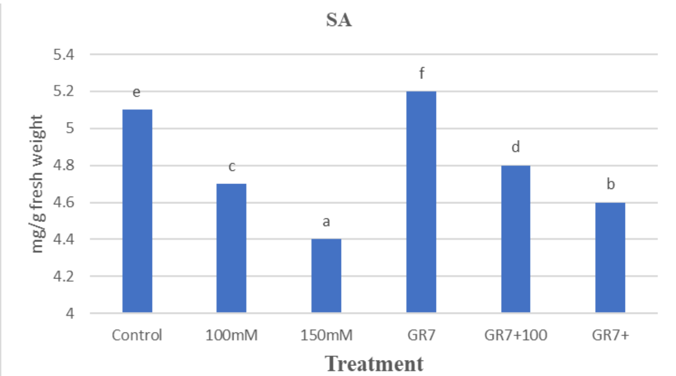

Figure 8: *Comparison of salicylic acid (SA) content in Zea mays seedlings with and without GR7 inoculation under control and salinity stress conditions (100 and 150 mM NaCl). Data are presented as mean ± SE (n = 3). Significant differences among treatments ($p < 0.05$) are indicated by different letters.*

In contrast, seedlings exposed to NaCl stress without fungal inoculation exhibited a pronounced reduction in SA levels relative to the untreated control. This reduction highlights the detrimental effect of salinity on the endogenous accumulation of defense-related metabolites. Interestingly, the presence of GR7 appeared to mitigate this negative effect, maintaining significantly higher SA content even under 150 mM NaCl stress. These findings suggest that GR7 inoculation enhances SA biosynthesis or signaling in *Zea mays*, which may play a pivotal role in priming the plant's defense responses and improving tolerance to salinity stress. The elevation of SA levels in inoculated plants under both normal and stress conditions reinforces the role of endophytic fungi as key modulators of host biochemical pathways.

Flavonoid content of Zea mays seedlings

The flavonoid content of maize seedlings treated with GR7 and subjected to different concentrations of NaCl was quantified spectrophotometrically. GR7-inoculated seedlings exhibited a significant increase in flavonoid accumulation under salinity stress, particularly at 100 mM and 150 mM NaCl. The highest flavonoid content (113.80 µg/g) was observed in seedlings inoculated with GR7 and exposed to 150 mM NaCl (Figure 9). Similarly, non-inoculated seedlings also showed elevated flavonoid levels under NaCl stress compared to the control (Ullah *et al*., 2025).

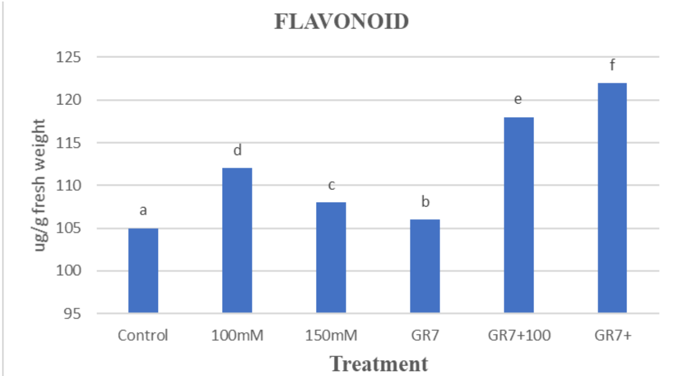

Figure 9: *Comparison of flavonoid content in Zea mays seedlings with and without GR7 inoculation under control and salinity stress conditions (100 and 150 mM NaCl). Data are presented as mean ± SE (n = 3). Significant differences among treatments ($p < 0.05$) are indicated by different letters.*

Phenol contents of Zea mays seedlings

The phenolic content of *Zea mays* seedlings subjected to GR7 inoculation and varying levels of NaCl stress was quantified spectrophotometrically. GR7-treated seedlings exhibited a pronounced increase in phenolic compounds under both non-stressed and 150 mM NaCl conditions. The maximum phenolic concentration (1.47 mg/g) was observed in GR7-inoculated plants exposed to 150 mM NaCl (Figure 10). In contrast, non-inoculated seedlings also showed an elevation in phenolic levels under 150 mM NaCl stress, though the increase was less prominent than in GR7-associated plants. The host SA contents remained unchanged when exposed to 100mM NaCl stress according to Samantaray (2024). These results suggest that GR7 not only promotes basal phenolic metabolism but also strengthens the host plant's ability to accumulate phenolics under severe salt stress. This enhancement of phenolic compounds, known for their antioxidant and stress-protective properties, may represent a crucial mechanism by which GR7 improves salinity tolerance in *Zea mays*.

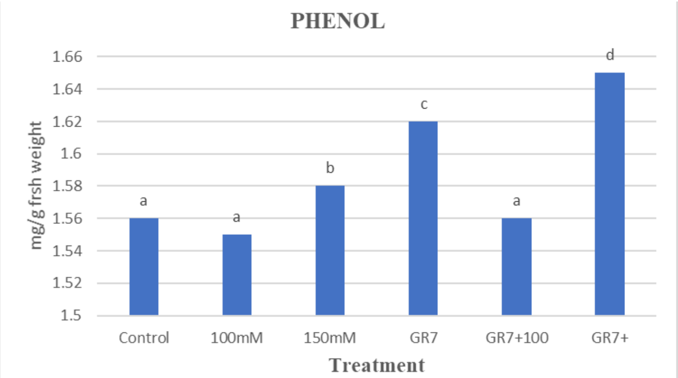

Figure 10: *Comparison of phenolic content in Zea mays seedlings with and without GR7 inoculation under control and salinity stress conditions (100 and 150 mM NaCl). Data are presented as mean ± SE (n = 3). Significant differences among treatments ($p < 0.05$) are indicated by different letters.*

Protein content

Protein levels in *Zea mays* seedlings subjected to GR7 inoculation and salinity stress were determined spectrophotometrically. GR7-associated seedlings exhibited markedly higher protein contents under both 100 mM and 150 mM NaCl stress compared to uninoculated seedlings. The maximum protein concentration (380.22 µg/g) was recorded in GR7-inoculated seedlings exposed to 150 mM NaCl (Figure 11). In contrast, uninoculated seedlings showed a progressive decline in protein levels with increasing salinity, indicating the detrimental effect of NaCl stress on protein biosynthesis and stability. The ability of GR7 to sustain and enhance protein content under stress conditions suggests that this endophyte may play a vital role in maintaining metabolic activity and cellular function during salt stress. Elevated protein levels in inoculated seedlings likely reflect the activation of stress-responsive proteins, including enzymes and structural proteins, that contribute to improved tolerance and growth performance under adverse conditions.

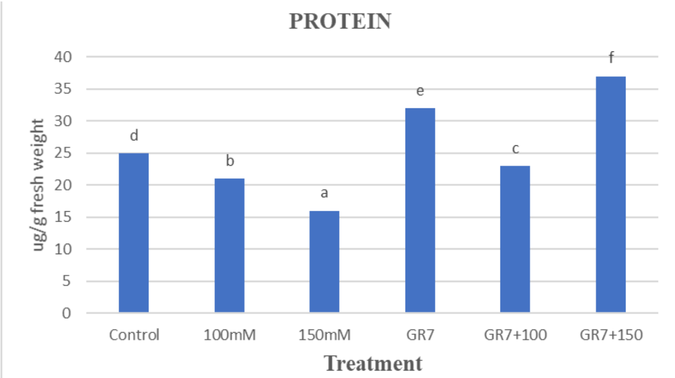

Figure 11: *Comparison of protein content in Zea mays seedlings with and without GR7 inoculation under control and salinity stress conditions (100 and 150 mM NaCl). Data are presented as mean ± SE (n = 3). Significant differences among treatments ($p < 0.05$) are indicated by different letters.*

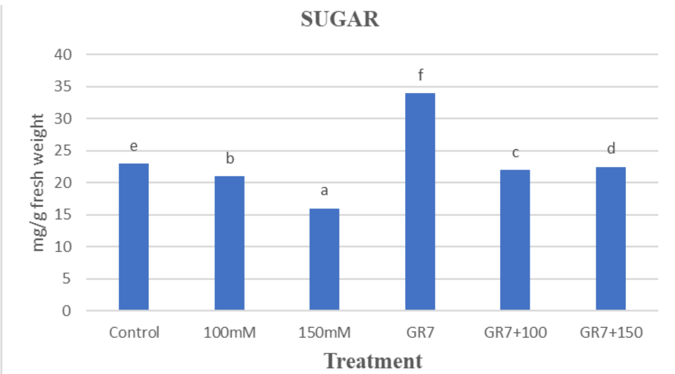

Figure 12: *Comparison of sugar content in Zea mays seedlings with and without GR7 inoculation under control and salinity stress conditions (100 and 150 mM NaCl). Data are presented as mean ± SE (n = 3). Significant differences among treatments ($p < 0.05$) are indicated by different letters.*

Sugar content

The soluble sugar content of *Zea mays* seedlings treated with GR7 under different NaCl stress conditions was also analyzed spectrophotometrically (Ullah *et al.*, 2018). GR7-inoculated seedlings accumulated significantly higher sugar levels than the control under both normal and salt-stressed conditions. The highest sugar concentration (345.49 µg/g) was observed in seedlings inoculated with GR7 (Figure 12). Conversely, uninoculated seedlings subjected to 100 mM and 150 mM NaCl exhibited a marked reduction in sugar content compared with the control. In contrast, uninoculated seedlings subjected to 100 mM and 150 mM NaCl stress displayed a pronounced reduction in sugar levels relative to the control, reflecting the inhibitory effect of salinity on carbohydrate metabolism. The increased sugar accumulation in GR7-associated plants indicates the fungus's role in osmotic adjustment, energy supply, and stress protection, which are essential for maintaining growth and metabolic activity under saline conditions. This suggests that GR7 not only supports biomass production but also enhances the biochemical resilience of maize through improved sugar metabolism.

Ascorbic acid oxidase content of Zea mays seedlings

Ascorbic acid oxidase (AAO) activity in *Zea mays* seedlings subjected to GR7 inoculation and salinity stress was quantified spectrophotometrically. GR7-inoculated seedlings displayed elevated AAO activity under both control conditions and 150 mM NaCl stress compared to uninoculated controls (Ullah *et al*., 2025d). The highest AAO activity (2.63 units) was observed in GR7-associated seedlings under 150 mM NaCl stress (Figure 13). Interestingly, AAO levels declined significantly in GR7-treated seedlings under 100 mM NaCl stress. In non-inoculated plants, AAO activity increased under 100 mM NaCl but decreased sharply under 150 mM NaCl, where the lowest activity (0.88 units) was recorded (Ullah *et al*., 2018g).

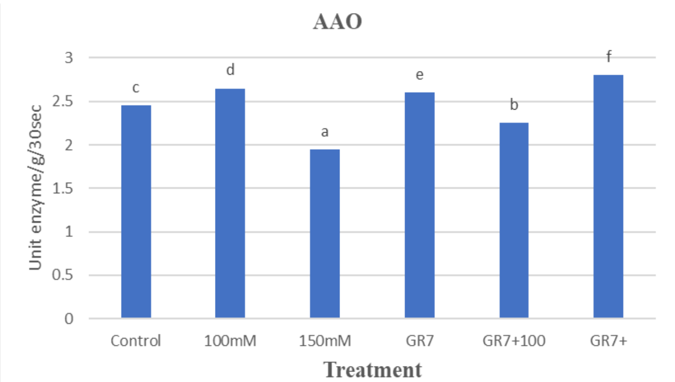

Figure 13: *Comparison of ascorbate oxidase (AAO) activity in Zea mays seedlings with and without GR7 inoculation under control and salinity stress conditions (100 and 150 mM NaCl). Data are presented as mean ± SE (n = 3). Significant differences among treatments ($p < 0.05$) are indicated by different letters.*

Peroxidase contents of Zea mays seedlings

The peroxidase activity of maize seedlings treated with GR7 and exposed to different concentrations of NaCl was measured spectrophotometrically (Ullah *et al*., 2018d). GR7-inoculated seedlings subjected to 100 mM and 150 mM NaCl stress exhibited higher peroxidase activity compared to non-inoculated plants. The maximum peroxidase activity (0.19-unit enzyme/30 s/g) was observed in GR7-inoculated seedlings exposed to 150 mM NaCl (Figure 14). Non-inoculated seedlings also showed an increase in peroxidase activity under salinity stress relative to the untreated control.

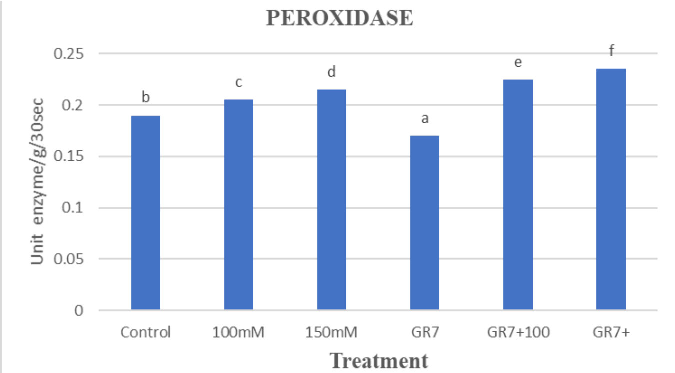

Figure 14: *Comparison of peroxidase activity in Zea mays seedlings with and without GR7 inoculation under control and salinity stress conditions (100 and 150 mM NaCl). Data are presented as mean ± SE (n = 3). Significant differences among treatments ($p < 0.05$) are indicated by different letters.*

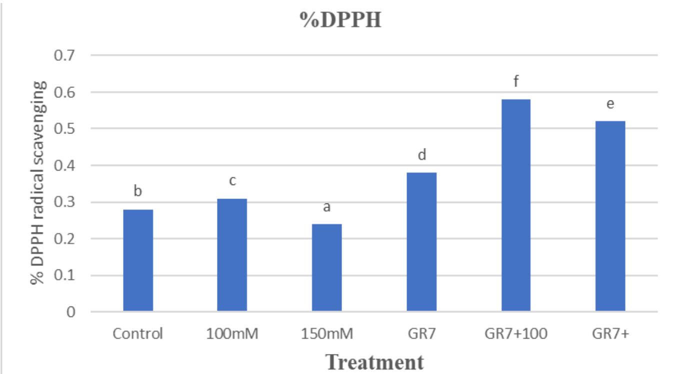

Figure 15: *Comparison of DPPH radical scavenging activity in Zea mays seedlings with and without GR7 inoculation under control and salinity stress conditions (100 and 150 mM NaCl). Data are presented as mean ± SE (n = 3). Significant differences among treatments ($p < 0.05$) are indicated by different letters.*

Radical scavenging activity of Zea mays

GR7-inoculated seedlings exhibited significantly enhanced antioxidant capacity under both control and NaCl-stressed conditions compared to non-inoculated plants ($p < 0.05$). The highest radical scavenging activity (53.42 ± 1.25% DPPH inhibition) was observed in GR7-colonized plants under 100 mM NaCl stress. While antioxidant potential decreased at higher salinity (47.36 ± 1.08% at 150 mM NaCl), it remained 2.3-fold higher than non-inoculated counterparts (20.17 ± 0.89%). Notably, non-inoculated plants showed only marginal increases (29.67 ± 1.12% at 100 mM) before declining sharply at 150 mM stress levels (Figure 15). The 53.42% DPPH radical quenching in GR7+100mM NaCl plants suggests fungal activation of both enzymatic (SOD, CAT) and non-enzymatic (phenolics, ascorbate) systems. The subsequent decline at 150 mM may reflect antioxidant saturation points or differential regulation of specific pathways (e.g., APX vs. GR activity).

Discussion

The present study demonstrates that inoculation with the endophytic fungus GR7 significantly improves the growth and salinity tolerance of maize seedlings. In comparison with non-inoculated controls, GR7-treated plants exhibited significant improvements in shoot and root length, as well as in fresh and dry biomass accumulation, underscoring the fungus's beneficial role in alleviating salt-induced growth inhibition (Yang and Guo, 2018; Ashraf *et al*., 2013). These growth-promoting effects can be attributed to the ability of GR7 to modulate physiological and biochemical pathways that are otherwise severely impaired under saline conditions. Notably, under severe salt stress (150 mM NaCl), GR7-inoculated seedlings retained 64.3% of control shoot length, whereas non-inoculated seedlings suffered a 33.87% reduction in shoot growth, reflecting a pronounced protective effect of the fungus (Brazhnikova *et al*., 2025; Ullah *et al*., 2018e). Such improvements suggest that GR7 plays a pivotal role not only in maintaining plant vigor but also in buffering maize against the detrimental impacts of ionic toxicity and osmotic stress caused by excess salinity (Ashraf *et al*., 2013).

Root length and biomass were also substantially higher in GR7-inoculated maize seedlings, clearly indicating that the endophytic fungus is effective in alleviating the inhibitory impact of salinity on plant growth (Gill and Tuteja, 2010). These observations align well with earlier reports that endophytic fungi can counteract salt-induced growth suppression by promoting ion homeostasis, improving osmotic balance, and fostering a more robust root system architecture (Waqas *et al*., 2012; Khan *et al*., 2016). A well-developed root system not only facilitates efficient water and nutrient uptake under saline conditions but also contributes to greater physiological resilience and overall plant productivity. Biochemical profiling further substantiated the growth-promoting effects of GR7, as significant increases were observed in total chlorophyll, indole-3-acetic acid (IAA), salicylic acid (SA), flavonoids, phenolics, proteins, and soluble sugars in GR7-associated seedlings compared to non-inoculated controls under salt stress. The maximum chlorophyll content (59.56 mg/g) was recorded in GR7-treated seedlings, strongly suggesting enhanced photosynthetic efficiency, a critical factor for sustaining growth and energy balance under adverse conditions (Ashraf and Harris, 2013; Ullah *et al*., 2018f). The elevated levels of IAA and SA further highlight the role of GR7 in modulating phytohormone signaling pathways, thereby strengthening the adaptive responses of maize seedlings to salinity (Tariq *et al*., 2014; Khan *et al*., 2016; Egamberdieva *et al*., 2017). Meanwhile, the increased accumulation of flavonoids and phenolics underscores the contribution of secondary metabolites in scavenging reactive oxygen species (ROS) and functioning as osmoprotectants, ultimately reducing oxidative stress and membrane damage in salt-stressed seedlings (Gill and Tuteja, 2010; Waqas *et al*., 2012). Together, these biochemical modifications reflect a multifaceted protective mechanism mediated by GR7, which not only enhances metabolic stability but also reinforces stress tolerance in maize.

Antioxidant enzyme activities were also markedly higher in GR7-inoculated seedlings, with peak ascorbate oxidase (AAO) activity (2.63 units) and peroxidase activity (0.19 units/30 s/g) recorded under 150 mM NaCl stress (Brazhnikova *et al*., 2025; Ullah *et al*., 2018c). This enhanced ROS-scavenging capacity is consistent with previous findings that endophytes upregulate antioxidant enzymes to reduce reactive oxygen species accumulation (Rodriguez *et al*., 2008; Waqas *et al*., 2012; Wege *et al*., 2017). Comparative studies with other endophytes, such as *Piriformospora indica* and *Trichoderma harzianum*, also reveal similar benefits in enhancing salt tolerance through improvements in root and shoot growth as well as photosynthetic efficiency (Yadav *et al*., 2010; Mastouri *et al*., 2010). Notably, the total antioxidant potential of GR7, with up to 53.42% DPPH scavenging activity under 100 mM NaCl, underscores its strong efficacy compared with other endophytes reported in the literature (Gill and Tuteja, 2010; Paramasivam *et al*., 2010; Khan *et al*., 2016; Ullah *et al*., 2018h). This enhanced stress tolerance may be attributed to GR7's unique metabolic capabilities, which could involve the production of novel bioactive compounds or the effective modulation of host signalling pathways (Ashraf and Harris, 2013). Overall, this study demonstrates GR7's potential to strengthen maize resilience under saline conditions, offering valuable implications for sustainable agriculture and food security in stress-affected environments (Chanu *et al*., 2025).

Beyond antioxidant responses, GR7 inoculation appears to influence other critical physiological and biochemical pathways that contribute to stress adaptation. Enhanced accumulation of osmolytes, such as soluble sugars and proline, was observed in inoculated seedlings, which likely contributed to osmotic adjustment and protection of cellular structures under salinity stress (Srivastava *et al*., 2025). These findings are consistent with earlier reports showing that microbial inoculants improve osmolyte balance, thereby maintaining cell turgor and metabolic activity in plants exposed to high salinity (Orlandelli *et al*., 2012; Hu and Qin, (2025).

Furthermore, elevated levels of secondary metabolites, including flavonoids and phenolic compounds, were recorded in GR7-inoculated seedlings. These metabolites are known to function as both antioxidants and signalling molecules, playing dual roles in mitigating oxidative damage and activating stress-responsive pathways (Chanu *et al*., 2025; Wang *et al*, 2023). The increase in these compounds suggests that GR7 not only enhances maize's basal defence mechanisms but may also prime the host for faster or stronger responses upon encountering stress (Naik *et al*., 2025).

Conclusion

The findings of this study demonstrate that inoculation with the endophytic fungus GR7 confers substantial improvements in the growth and salinity tolerance of maize seedlings. Inoculated plants exhibited remarkable enhancements in key morphological attributes, including shoot elongation, root development, and both fresh and dry biomass accumulation, when compared with their non-inoculated counterparts under salt stress. These results highlight the ability of GR7 to mitigate the detrimental effects of salinity by promoting more stable growth and development. The beneficial effects of GR7 appear to be closely linked with its ability to regulate and improve several physiological and biochemical processes within the host plant. GR7-inoculated seedlings displayed higher concentrations of photosynthetic pigments, particularly chlorophyll, which is crucial for sustaining photosynthetic efficiency under stress conditions. Additionally, significant increases were recorded in important plant growth regulators, such as indole-3-acetic acid (IAA) and salicylic acid (SA), alongside secondary metabolites including flavonoids and phenolic compounds. These molecules are known to play key roles in stress adaptation, serving both as signaling mediators and as protectants against oxidative and ionic stress. Alongside these metabolic changes, GR7 also induced marked improvements in the levels of primary metabolites, such as soluble proteins and sugars. These compounds not only contribute to osmotic balance but also support cellular structure and metabolic stability under adverse environmental conditions. Furthermore, the enhanced activities of key antioxidant enzymes, including ascorbic acid oxidase (AAO) and peroxidase (POD), demonstrated the fungus's ability to strengthen the host's antioxidant defense machinery. Such upregulation of enzymatic activity ensured more effective scavenging of reactive oxygen species (ROS), thereby protecting cells from oxidative injury. The higher DPPH radical scavenging capacity observed in GR7-associated seedlings provides additional confirmation of its strong antioxidant potential. This suggests that GR7 inoculation not only fortifies plants against salt-induced oxidative stress but also improves their overall redox balance, contributing to long-term stress resilience.

Recommendations

Based on the present findings, GR7 demonstrates strong potential as a bioinoculant for improving maize growth and mitigating the adverse effects of salinity. Its large-scale application in saline-affected agricultural regions could contribute to enhanced crop productivity and sustainability. To strengthen these outcomes, further field-based evaluations are required across diverse agro-climatic conditions. Moreover, detailed studies on the molecular mechanisms and bioactive metabolites underlying GR7-mediated stress tolerance will provide valuable insights for the development of advanced bioformulations, supporting sustainable crop management practices.

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Novelty Statement

Our findings present the first demonstration of GR7 as a key endophytic fungus conferring salinity tolerance in maize. GR7 inoculation resulted in marked improvements in growth, photosynthetic performance, and antioxidant defense mechanisms under salt stress. Owing to its distinct metabolic traits, GR7 emerges as a promising bioinoculant for advancing sustainable agriculture in saline-prone environments. This research establishes GR7 as a novel endophytic fungus capable of enhancing maize salinity tolerance through improvements in growth, photosynthetic efficiency, and antioxidant defenses. The distinctive metabolic properties of GR7 position it as a valuable bioinoculant for promoting crop resilience and supporting sustainable agricultural practices in salt-affected regions.

Author's Contribution

The research work was conceptualized, and the methodology and software development were carried out by Shakir Ullah. The initial draft of the manuscript was prepared by Rahid Khan. Lubna Shakir and Nazli Rahid were involved in reviewing and refining the manuscript through critical editing. Wasim Khan, Naila Shah, Habiba Ghafoor, and Abdullah provided essential resources and oversaw project administration. All authors have read, revised, and approved the final version of the manuscript for submission.

Generative AI or AI-assisted Technology Statement

The author(s) declare that no Genrative AI was used in the creation of this manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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