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Research Article

Rhizobacteria Inoculation Enhanced the Growth Performance of Paspalum Turfgrass under Saline Conditions

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Abstract:

Recently, non-potable water sources are increasingly used for turfgrass management due to freshwater limitations and conservation efforts, and optimizing landscape irrigation, particularly in water-scarce regions, is crucial. However, salinity, exacerbated by low-quality irrigation water and soil salinization, poses a significant abiotic stress, impacting turfgrass growth and development. Paspalum is principally cultivated in various landscaping projects, most of which are located in coastal regions where reclaimed or brackish water is used for irrigation due to the reduced availability and increased cost of fresh water. However, these water resources contain great amounts of salt ions, and their extended use can raise the content of salt in turf, thereby inhibiting turfgrass growth. In this study, *Paspalum vaginatum* was exposed to diluted seawater at 0, 150 and 200 mM or rhizobacteria inoculation separately or in combination. Rhizobacteria inoculation improved growth performance, with treated plants exhibiting higher fresh and dry weights throughout the investigated period compared to untreated. However, salinity treatment reduced clipping fresh and dry weights more so at 200 mM. On the other hand, rhizobacteria inoculation markedly enhanced clipping fresh and dry weights under salt stress conditions. Rhizobacteria inoculation also enhanced relative water content, total chlorophyll, proline content, peroxidase activity and the contents of N, P and K elements compared to the control. These positive impacts of rhizobacteria inoculation were also observed when paspalum was exposed to salt stress. These results enrich awareness concerning the possible benefits of the external application of rhizobacteria and their roles that can help investigators to improve turf grasses' performance under salt stress.

1. Introduction

Increases in urbanization have resulted in global ecosystem changes (Mazrou et al., 2025). Hence, improving the quality of life in urban areas, green spaces such as urban parks, green belts, and forests are important in urban infrastructure, both from an ecological and economic standpoint (Breuste et al., 2013; Gabriel, 2016). Optimizing landscape irrigation, particularly in water-scarce regions, is crucial. Due to freshwater limitations and conservation efforts, non-potable water sources are increasingly used for turfgrass management (Marcum, 2004). However, salinity, exacerbated by low-quality irrigation water and soil salinization, poses a significant abiotic stress, impacting plant growth and development. Salinity in higher plants induces hyperosmotic and hyperionic stresses, causing morphological, physiological, and biochemical changes that can be lethal (Hasegawa et al., 2000). Hyperionic stress results from toxic salt accumulation in older leaves, impairing photosynthesis and carbohydrate supply to young leaves, thereby reducing their growth (Munz and Tester, 2008). Salt stress decreases the ability of plants to extract water from the soil, which leads to the accumulation of Na+ and Cl- ions at toxic levels in tissues (Carillo et al., 2019). Salinity causes oxidative damage (Attia et al., 2020), which in turn affects the rate of photosynthesis, cellular metabolism, and lipid bilayer function (Suzuki et al., 2016).

Paspalum (*Paspalum vaginatum*) is a perennial herbaceous grass of warm-season turfgrasses that is commonly also known as bahia grass, crown grass, or dallis grass. Many of the species are tall perennial grasses that exhibit a blue-green color and are native to coastal, tropical and subtropical regions (Chen et al., 2005). Turfs play a key role in mitigating wind and water erosion of the soil, as well as controlling dust, air pollution, and glare. The refreshing and natural green appearance of turf creates a pleasing environment for both residential and work settings. As turfgrass, paspalum has been widely used on golf courses, sport fields, and landscapes (Berndt, 2007). Paspalum is basically cultivated in various landscaping projects and most of them are located in coastal regions where reclaimed or brackish water was used for irrigation because of the reduced availability and the increasing freshwater cost. However, these water resources contain great amounts of salt ions, and their prolonged use can raise the content of salt in turf, thereby inhibiting the turfgrass growth (Liu et al., 2019). Therefore, it is crucial to investigate the effect of salinity on its growth performance. Utilizing salt-tolerant grasses such as *Paspalum vaginatum* offers a solution, especially when selected based on local conditions (Johnson, 2008; Duncan et al., 2009) and also due to increasing salinity and freshwater scarcity.

One of the promising solutions to tackle the diverse abiotic stresses, like salinity, that challenge plant growth is the usage of biological inoculants, including plant growth-promoting bacteria. It is commonly known as bio-fertilizers. They offer environmentally safe, sustainable, chemical-free, and cost-effective alternatives to chemical fertilizers (Baez‐Rogelio et al., 2017; Eida et al., 2020). These inoculants enhance plant resistance to

various stresses (Latif et al., 2021; Abd El-Megeed et al., 2022), including salinity (Eida et al., 2020). These rhizobacteria enhance plant growth by providing essential nutrients, modulating plant hormone levels, and improving soil structure (Ahemad and Kibert, 2014). Consequently, rhizosphere microbes are increasingly utilized to promote plant growth under stressful conditions (Huang et al., 2017; Liu et al., 2021; Mishra et al., 2017). It has been found that plant growth-promoting rhizobacteria have the potential to improve abiotic stress resistance, including salinity in turfgrass (Zhang and Rue, 2024). Similarly, inoculation with rhizobacteria enhanced the growth of bermudagrass under salt stress and improved biomass accumulation, osmotic adjustment, and photosynthetic efficiency, as well as selective ion absorption capacities (Wei et al., 2022).

Despite the number of reports on paspalum, little information is available on the impacts of salinity on its growth performance or on physiological alterations that might enhance salt tolerance. Additionally, the possible mechanisms of rhizobacteria in salinity mitigation in paspalum have not yet been well studied. Therefore, this study aimed to evaluate the impacts of salinity and/or rhizobacteria treatment on growth and changes in some physiological and biochemical traits in *Paspalum vaginatum*.

2. Materials and Methods

2.1. Experimental site and setup

This experiment was conducted at the experimental farm of the Faculty of Agriculture, Tanta University, Egypt (30° 47′ 18″ N: 31° 00′ 06″ E) at an altitude of 8 m above sea level during the summer seasons of 2020 and 2021. To simulate coastal cultivation, six raised beds (6 m long x 1 m wide x 30 cm high) were constructed using outdoor sandy soil, spaced 1.5 m apart. Paspalum rolls were obtained from a private nursery in Badr District, El-Beheira Governorate, Egypt and planted on the 1st of July for each season, in alternating 3 m sections (1 m planted, 1 m fallow) on each bed. During the initial two weeks, plants were irrigated daily with tap water to stimulate root growth.

2.2. Bacterial inoculant

Inoculation in this study was done using a mixture of rhizobacteria of four plant growth-promoting rhizobacteria strains: *Azotobacter* spp., Bacillus spp., *Pseudomonas fluorescens, and Bacillus circulans/megaterium.* at a concentration of 10^8–10^9 CFU mL-1, which were provided from the Agricultural Research Center, Giza, Egypt.

2.3. Seawater treatments

Seawater, which was collected from Agami, Alexandria, Egypt, was used for irrigation at three levels as follows: (1) tap water as a control (S0), (2) 150 mM (S1), and (3) 200 mM (S2). These levels of salinity were obtained by diluting the seawater with tap water to reach the required concentration.

2.4. Experimental design and treatments

A factorial experiment in a randomized complete block design was conducted, with two factors. The first factor was rhizobacteria inoculation as follows: without inoculation and with inoculation, while the second factor was seawater, which consists of three levels: S0, S1, and S2. The experiment consisted of six treatments; each treatment was replicated three times.

The inoculation was done as soil application using 2 L of stock solution (10^8–10^9 CFU/mL), which was diluted with water to 27 L and applied at the rate of 2 L/m². Rhizobacteria were applied twice on 15 July and 15 August in each season. One week after the inoculation, paspalum grass was irrigated with saline water. To avoid salt shock, saline water application was added in a stepwise manner by increasing saline water concentration as began with 50 mmol L^{-1} and raised by 50 mmol L^{-1} gradually until target concentrations were achieved. The control grass was irrigated with tap water. Irrigation level was 10 L/m^2/day for treated and control plants.

2.5. Traits investigated

At 7, 14, 21, 28, 35, 42, and 49-days post-stress, samples were collected to assess the following traits:

2.5.1. Clipping fresh and dry weights determination

Plant biomass was assessed following standardized protocols (Poorter et al., 2012) with modifications for salinity stress studies. A sample area of 1 m2 was clipped and immediately aerial tissues were excised at the soil interface using scissors. Samples were processed within 90 seconds of collection to minimize post-harvest water loss. Fresh weight (FW) measurements were conducted using a calibrated analytical balance in a temperature-controlled environment. To assess the dry weight, samples were oven-dried at 70°C for 72 hours until constant weight was gained, and dry weights (DW) were recorded. Three technical replicates were measured per treatment.

2.5.2. Relative water content (%)

Relative water content (RWC) was determined on leaf tissues excised in the morning (around 9:00 am). Excised leaves were measured for fresh weight (FW) and then rehydrated in a water-filled petri dish at room temperature. Turgor weight (TW) was measured by allowing full rehydration (16 h), removing all water on the leaf surface, weighing, and then leaves were dried at 70°C for 48 h to determine DW. The relative water content was calculated from the following equation, RWC = 100[FW - DW)/(TW- DW)] as reported by Weatherley (1950).

2.5.3. Chlorophyll content

The amount of chlorophyll a, b and total chlorophyll was determined according to Dere et al. (1998). Fresh leaves (0.1 g) were cut into small fragments (1mm x 1 mm) and immersed for 24 h at 4°C in 20 mL methanol (96%) and then filtered through a Whatman 47 mm GF/C filter paper. The absorbance of each filtrate was measured against a blank of 96% methanol at wavelengths of 666 and 653 nm for chlorophyll a and b, respectively. Data were expressed as mg g-1 fresh weight (FW/ and calculated using the following formulas:

Chlorophyll (Chl.) a = (15.65 A666-7.34 A653)

Chlorophyll (Chl.) b = (27.05 A653-11.21 A666)

Total chlorophyll = Chl. a + Chl. b

2.5.4. Proline determination

The free proline content was determined at the 1st, 4th and 7th weeks as described by Bates et al. (1973). Frozen leaf tissue (0.5 g) was homogenized with 10 mL of 3 % sulfosalicylic acid at 4 °C. Then, the obtained extract was filtered with Whatman No. 2. A mixture of 2 mL of filtrate, 2 mL of acid-ninhydrin, and 2 mL of glacial acetic acid was mixed in a test tube and incubated at 100 °C for 1 h. The reaction was terminated on ice, and the reaction mixture was then extracted with 4 mL of toluene. The chromophore-containing toluene was separated from the hydrated phase. The absorbance at 520 nm was spectrophotometrically determined with toluene as the blank. The proline concentration was calculated based on a standard curve and was expressed as µmol g-1 FW.

2.5.5. Peroxidase enzyme assays

A sample of 0.5 g fresh plant material was frozen, then homogenized in 8 ml of 50 mM cold phosphate buffer having pH 7.0 (Beauchamp and Fridovich, 1971). The homogenates were centrifuged at 4000 rpm for 20 minutes. The supernatant was used as a raw extract for the enzymatic assay. The activity of peroxidase [EC1.11.1.7] was assayed according to Kato and Shimizu (1987). A sample of 3 ml of the reaction mixture containing 0.1M sodium phosphate buffer of pH 5.8, 7.2 mM guaiacol, 11.8 mM H2O2 and 0.1 ml enzyme extract was used for the assay. By the addition of H_2O_2 the reaction was initiated and the change in the absorbance was measured at 470 nm. Activity was calculated using the extinction coefficient (26.6 mM-1 cm-1 at 470 nm). Enzyme activity was expressed in units of µM of the substrate converted per min. per g FW.

2.5.6. Mineral content

After seven weeks of applied treatments, desiccated leaf samples were ground into a fine powder and then digested using a mixture of perchloric acid and sulfuric acid (1:5 v/v, respectively) as described by A.O.A.C. (1995). The determination of N, P, and K contents in leaf tissues was performed using the abovementioned digestion solution. The measurement of N was conducted by the micro-Kjeldahl apparatus as described by Nelson and Sommers (1973). Phosphorus content was measured using a spectrophotometer (Pharmacia, LKB-Novaspec II) following the blue color according to the method of Jackson (1967). K and Na elements were estimated by flame emission photometry (Corning, Tewksbury, MA, USA).

2.6. Statistical analysis

The results of each season were statistically analyzed and analysis of variance (ANOVA) was performed using Michigan Statistical Program Version C (MSTATC). Means were separated by Duncan multiple range test (Heinisch et al., 1960) at a 0.05 probability level.

3. Results and discussion

3.1. Clipping fresh and dry weights

The results revealed significant ($p \leqslant 0.05$) effects of rhizobacteria inoculation, salinity levels, and their interaction on both fresh weight (Table 1) and dry weight (Table 2) of *Paspalum vaginatum* during both growing seasons. Rhizobacteria inoculation consistently improved plant performance, with treated plants exhibiting higher fresh and dry weights throughout the investigated period compared to untreated. However, increasing salinity significantly reduced fresh (Table 1) and dry biomass (Table 2). At 200 mM salinity (S2), plants exhibited severe declines in fresh weight, representing 63.72 and 60.24% reductions compared to S0 at week 7 in both seasons, respectively. On the other hand, rhizobacteria inoculation markedly enhanced clipping fresh and dry weights when plants were exposed to salinity. These results align with studies on tall fescue (*Festuca arundinacea*), where rhizobacteria inoculation (e.g., *Azotobacter* and *Azospirillum*) enhanced growth under stress by improving nutrient uptake, hormonal balance, and osmotic regulation (Massahi et al., 2018). Additionally, fungal inoculants such as *Trichoderma atroviride* have been shown to increase fresh weight by 16–114% and dry weight by 24–76% in perennial ryegrass (*Lolium perenne*) under salinity, demonstrating their role in stress mitigation through improved root development and soil health (Abu-Shanab et al., 2022). Otherwise, the decline in clipping weight due to salinity is attributed to disrupted water uptake (reflected in fresh weight) and cellular damage (evident in dry weight), consistent with findings that salinity disrupts osmotic balance and induces ion toxicity in tall fescue (*Festuca arundinacea*) (Li et al., 2022). The current results are in agreement with the findings of Wei et al. (2022) on bermudagrass and Feng et al. (2023) on perennial ryegrass.

Table 1. Effect of rhizobacteria inoculation, salinity and their interaction on clipping fresh weight of *Paspalum vaginatum* turfgrass during 2020 and 2021 seasons.

Rhizobacteria inoculation (Rhizo)	Salinity (S)	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7
		First season 2020						
Without Rhizo.	-	50.22^{B}	52.92^{B}	55.74^{B}	59.21^{B}	61.12^{B}	64.21^{B}	69.21^{B}
With Rhizo.	-	60.33^{A}	65.56^{A}	69.91^{A}	76.56^{A}	81.33^{A}	85.96^{A}	92.56^{A}
-	**S0**	59.50^{A}	65.67^{A}	69.67^{A}	74.42^{A}	78.96^{A}	81.82^{A}	85.22^{A}
-	**S1**	55.50^{B}	57.83^{B}	59.17^{B}	61.17^{B}	62.67^{B}	65.17^{B}	68.17^{B}
-	**S2**	45.63^{C}	46.17^{C}	47.94^{C}	49.14^{C}	50.05^{C}	52.17^{C}	54.31^{C}
Without Rhizo.	**S0**	65.57^{B}	70.33^{B}	76.14^{B}	81.41^{B}	86.44^{B}	91.97^{B}	97.97^{B}
Without Rhizo.	**S1**	50.33^{D}	52.67^{D}	54.33^{D}	56.47^{D}	57.50^{D}	60.67^{D}	62.67^{E}
Without Rhizo.	**S2**	40.67^{E}	42.67^{E}	45.88^{E}	46.49^{E}	48.43^{F}	49.00^{E}	51.00^{F}
With Rhizo.	**S0**	75.33^{A}	80.67^{A}	86.33^{A}	91.33^{A}	95.97^{A}	102.67^{A}	107.67^{A}
With Rhizo.	**S1**	60.67^{C}	63.79^{C}	65.21^{C}	67.67^{C}	70.83^{C}	72.64^{C}	74.67^{C}
With Rhizo.	**S2**	52.80^{D}	53.83^{D}	55.11^{D}	58.43^{D}	61.50^{E}	64.33^{D}	68.33^{D}
		Second season 2021						
Without Rhizo.	-	54.84^{B}	55.64^{B}	58.06^{B}	61.14^{B}	65.68^{B}	67.80^{B}	71.48^{B}
With Rhizo.	-	65.14^{A}	70.21^{A}	74.98^{A}	81.29^{A}	85.92^{A}	90.91^{A}	96.49^{A}
-	**S0**	74.92^{A}	80.22^{A}	86.83^{A}	91.46^{A}	96.71^{A}	102.76^{A}	106.79^{A}
-	**S1**	59.95^{B}	61.82^{B}	64.89^{B}	68.87^{B}	71.75^{B}	76.79^{B}	80.83^{B}
-	**S2**	50.04^{C}	52.64^{C}	54.14^{C}	56.47^{C}	59.24^{C}	62.97^{C}	64.33^{C}
Without Rhizo.	**S0**	70.51^{B}	75.43^{B}	80.94^{B}	85.94^{B}	91.69^{B}	96.94^{B}	100.98^{B}
Without Rhizo.	**S1**	55.61^{D}	57.60^{D}	59.44^{D}	62.26^{D}	66.77^{D}	70.12^{D}	73.99^{D}
Without Rhizo.	**S2**	44.90^{E}	46.19^{E}	47.18^{E}	49.14^{E}	51.63^{F}	54.94^{E}	57.33^{F}
With Rhizo.	**S0**	80.33^{A}	85.00^{A}	91.72^{A}	96.30^{A}	101.82^{A}	107.97^{A}	112.21^{A}
With Rhizo.	**S1**	65.90^{C}	67.23^{C}	70.33^{C}	74.48^{C}	77.50^{C}	81.67^{C}	84.00^{C}
With Rhizo.	**S2**	55.68^{D}	57.40^{D}	58.30^{D}	60.41^{D}	62.44^{E}	64.22^{D}	67.67^{E}

Rhizo. means rhizobacteria inoculation. S0, S1 and S2 mean salinity levels at 0, 100 and 200 mM of diluted seawater. Means at each column for each factor having different letters are significantly different at $p \leqslant 0.05$.

Table 2. Effect of rhizobacteria inoculation, salinity and their interaction on clipping dry weight of *Paspalum vaginatum* turfgrass during 2020 and 2021 seasons.

Rhizobacteria inoculation (Rhizo)	Salinity (S)	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7
		First season 2020						
Without Rhizo.	-	12.68^{B}	12.79^{B}	13.07^{C}	13.22^{B}	14.82^{B}	16.61^{B}	17.12^{B}
With Rhizo.	-	15.41^{A}	17.94^{A}	18.96^{A}	20.35^{A}	21.54^{A}	23.03^{A}	24.01^{A}
-	**S0**	18.08^{A}	19.02^{A}	21.89^{A}	22.32^{A}	24.29^{A}	25.81^{A}	27.16^{A}
-	**S1**	14.24^{B}	15.16^{B}	15.80^{B}	16.85^{B}	17.79^{B}	17.99^{B}	18.46^{B}
-	**S2**	11.81^{C}	12.42^{C}	12.85^{C}	13.18^{C}	13.47^{C}	14.16^{C}	15.28^{C}
Without Rhizo.	**S0**	16.24^{B}	18.78^{B}	19.94^{B}	21.93^{B}	22.65^{B}	24.15^{B}	25.84^{B}
Without Rhizo.	**S1**	12.95^{C}	13.53^{C}	14.34^{C}	15.19^{C}	15.48^{C}	16.71^{C}	17.44^{C}
Without Rhizo.	**S2**	10.83^{D}	11.04^{D}	11.82^{D}	12.52^{D}	12.82^{D}	13.67^{D}	13.97^{D}
With Rhizo.	**S0**	19.91^{A}	20.25^{A}	22.74^{A}	23.70^{A}	25.91^{A}	26.97^{A}	28.88^{A}
With Rhizo.	**S1**	15.52^{B}	15.77^{B}	16.25^{B}	16.80^{B}	17.19^{B}	18.16^{B}	19.66^{B}
With Rhizo.	**S2**	13.77^{C}	14.19^{C}	14.58^{C}	14.83^{C}	15.11^{C}	15.64^{C}	15.88^{C}
		Second season 2021						
Without Rhizo.	-	13.48^{B}	13.70^{B}	14.42^{B}	14.97^{B}	15.33^{B}	15.23^{B}	15.72^{B}
With Rhizo.	-	16.76^{A}	17.77^{A}	19.57^{A}	21.15^{A}	22.80^{A}	24.60^{A}	25.90^{A}
-	**S0**	19.07^{A}	20.82^{A}	22.26^{A}	23.86^{A}	25.44^{A}	27.10^{A}	28.69^{A}
-	**S1**	15.69^{B}	16.27^{B}	16.84^{B}	17.11^{B}	17.65^{B}	18.23^{B}	18.82^{B}
-	**S2**	12.10^{C}	12.87^{C}	12.92^{C}	13.40^{C}	13.85^{C}	14.12^{C}	14.55^{C}
Without Rhizo.	**S0**	17.19^{B}	19.68^{A}	20.13^{B}	22.01^{B}	24.27^{B}	25.76^{B}	27.24^{B}
Without Rhizo.	**S1**	13.77^{C}	13.84^{C}	14.36^{C}	14.88^{C}	15.48^{C}	16.24^{C}	16.71^{C}
Without Rhizo.	**S2**	11.46^{D}	11.66^{D}	12.35^{D}	12.70^{D}	12.94^{D}	13.17^{D}	13.51^{D}
With Rhizo.	**S0**	20.54^{A}	22.94^{A}	23.99^{A}	25.70^{A}	26.94^{A}	28.43^{A}	30.16^{A}
With Rhizo.	**S1**	16.40^{B}	17.30^{B}	17.82^{B}	18.33^{B}	19.11^{B}	19.81^{B}	20.14^{B}
With Rhizo.	**S2**	14.14^{C}	14.56^{C}	14.98^{C}	15.49^{C}	15.73^{C}	15.96^{C}	16.48^{C}

Rhizo. means rhizobacteria inoculation. S0, S1 and S2 mean salinity levels at 0, 100 and 200 mM of diluted seawater. Means at each column for each factor having different letters are significantly different at $p \leqslant 0.05$.

3.2. Relative water content (RWC)

The current findings demonstrate that rhizobacteria inoculation significantly improved RWC in *Paspalum vaginatum* separately or under saline conditions ($p < 0.05$), with treated plants maintaining higher RWC values compared to those exposed only to salinity in both seasons. Contrary, increasing salinity levels resulted in decreasing RWC in both seasons. During the investigated period, RWC was gradually increased from week 1 to week 7 in treated or nontreated plants. The salinity-induced RWC reduction (27 and 25% decrease for 200 mM NaCl at week 7 in both seasons) reflects fundamental osmotic constraints common to glycophytic turfgrasses (Alshammary et al., 2004). However, the partial mitigation by rhizobacteria inoculation suggests microbial facilitation of two complementary mechanisms.

The first is osmotic adjustment since rhizobacteria inoculation -enhanced synthesis of compatible solutes (proline, glycine betaine) as documented in *Festuca*

arundinacea by Li et al. (2022). The second is ion homeostasis: Selective K^+ retention and Na^+ exclusion observed in Trichoderma-treated *Lolium perenne* (Abu-Shanab et al., 2022). These improvements align with ACC-deaminase-producing bacteria's demonstrated capacity to modulate ethylene-mediated stress responses (Cheng et al., 2016), particularly through stabilizing root hydraulic conductivity observed in related Poaceae species (Zhang and Rue, 2024). These results are in agreement with those obtained in Cynodon dactylon (Wei et al., 2022).

Table 3. Effect of rhizobacteria inoculation, salinity and their interaction on relative water content of *Paspalum vaginatum* turfgrass during 2020 and 2021 seasons.

Rhizobacteria inoculation (Rhizo)	Salinity (S)	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7
		First season 2020						
Without Rhizo.	-	0.47^{B}	0.57^{B}	0.62^{B}	0.67^{B}	0.72^{B}	0.76^{B}	0.81^{B}
With Rhizo.	-	0.53^{A}	0.63^{A}	0.68^{A}	0.73^{A}	0.77^{A}	0.82^{A}	0.88^{A}
-	**S0**	0.54^{A}	0.57^{A}	0.63^{A}	0.68^{A}	0.73^{A}	0.81^{A}	0.88^{A}
-	**S1**	0.48^{B}	0.50^{B}	0.53^{B}	0.57^{B}	0.63^{B}	0.68^{B}	0.72^{B}
-	**S2**	0.45^{C}	0.47^{C}	0.50^{C}	0.52^{C}	0.54^{C}	0.59^{C}	0.61^{C}
Without Rhizo.	**S0**	0.52^{B}	0.59^{B}	0.64^{B}	0.68^{B}	0.74^{B}	0.79^{B}	0.89^{B}
Without Rhizo.	**S1**	0.49^{C}	0.54^{C}	0.57^{C}	0.62^{C}	0.68^{C}	0.72^{C}	0.78^{C}
Without Rhizo.	**S2**	0.47^{D}	0.49^{D}	0.51^{D}	0.53^{D}	0.57^{D}	0.61^{D}	0.63^{D}
With Rhizo.	**S0**	0.63^{A}	0.72^{A}	0.78^{A}	0.83^{A}	0.86^{A}	0.88^{A}	0.92^{A}
With Rhizo.	**S1**	0.58^{B}	0.61^{B}	0.66^{B}	0.70^{B}	0.73^{B}	0.78^{B}	0.80^{B}
With Rhizo.	**S2**	0.54^{C}	0.57^{C}	0.61^{C}	0.64^{C}	0.66^{C}	0.69^{C}	0.71^{C}
		Second season 2021						
Without Rhizo.	-	0.49^{B}	0.58^{B}	0.64^{B}	0.69^{B}	0.74^{B}	0.79^{B}	0.82^{B}
With Rhizo.	-	0.59^{A}	0.67^{A}	0.72^{A}	0.78^{A}	0.81^{A}	0.84^{A}	0.89^{A}
-	**S0**	0.56^{A}	0.63^{A}	0.68^{A}	0.72^{A}	0.78^{A}	0.83^{A}	0.89^{A}
-	**S1**	0.52^{B}	0.57^{B}	0.64^{B}	0.69^{B}	0.73^{B}	0.77^{B}	0.79^{B}
-	**S2**	0.46^{C}	0.49^{C}	0.53^{C}	0.57^{C}	0.59^{C}	0.63^{C}	0.65^{C}
Without Rhizo.	**S0**	0.54^{B}	0.60^{B}	0.65^{B}	0.72^{B}	0.79^{B}	0.83^{B}	0.87^{B}
Without Rhizo.	**S1**	0.49^{C}	0.54^{C}	0.60^{C}	0.66^{C}	0.70^{C}	0.74^{C}	0.76^{C}
Without Rhizo.	**S2**	0.41^{D}	0.44^{D}	0.49^{D}	0.54^{D}	0.59^{D}	0.61^{D}	0.64^{D}
With Rhizo.	**S0**	0.68^{A}	0.73^{A}	0.78^{A}	0.83^{A}	0.89^{A}	0.91^{A}	0.93^{A}
With Rhizo.	**S1**	0.62^{B}	0.69^{B}	0.73^{B}	0.78^{B}	0.82^{B}	0.84^{B}	0.86^{B}
With Rhizo.	**S2**	0.53^{C}	0.57^{C}	0.61^{C}	0.65^{C}	0.69^{C}	0.72^{C}	0.75^{C}

Rhizo. means rhizobacteria inoculation. S0, S1 and S2 mean salinity levels at 0, 100 and 200 mM of diluted seawater. Means at each column for each factor having different letters are significantly different at $p \leqslant 0.05$.

3.3. Total chlorophyll content

Table 4 presents the effects of rhizobacteria inoculation, salinity, and their interaction on the total chlorophyll content of *Paspalum vaginatum* across two growing seasons (2020 and 2021). Data reveal significant variations in chlorophyll content under different treatments, highlighting the influence of rhizobacteria inoculation and salinity stress on plant physiological responses. Rhizobacteria inoculation enhanced the chlorophyll content compared to the control at any time point during the investigated period, however, salinity markedly reduced it in both seasons. In salt stressed plants, the chlorophyll content was improved due to rhizobacteria inoculation in both seasons. These findings underscore the potential of rhizobacteria inoculation to mitigate salinity stress in *Paspalum vaginatum*. This improvement aligns with studies demonstrating that rhizobacteria inoculation, such as *Enterobacter*, enhance photosynthetic efficiency by increasing chlorophyll and carotenoid content, thereby improving light energy utilization (Wei et al., 2022). Similarly, *Trichoderma* spp. has been shown to mitigate salinity-induced chlorophyll degradation in perennial ryegrass (*Lolium perenne*) by maintaining the chlorophyll content (Abu-Shanab et al., 2022). The positive effect of rhizobacteria inoculation was less pronounced under high salinity (S2), suggesting that salinity stress partially offsets its benefits. This observation correlates with findings by Li et al. (2022), who reported that high salinity disrupts ion homeostasis, leading to chlorophyll degradation in tall fescue (*Festuca arundinacea*). The results also showed that, as salinity increased, the benefits diminished, reinforcing the idea that salinity-induced oxidative damage to chloroplast membranes limits chlorophyll synthesis (Li et al., 2016). This aligns with studies on bermudagrass (*Cynodon dactylon*), where high salinity (9000 ppm) severely reduced chlorophyll content despite fertilization treatments (Mahdavi et al., 2020).

Table 4. Effect of rhizobacteria inoculation, salinity and their interaction on total chlorophyll content of *Paspalum vaginatum* turfgrass during 2020 and 2021 seasons.

Rhizobacteria inoculation (Rhizo)	Salinity (S)	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7
		First season 2020						
Without Rhizo.	-	0.85B	1.20B	1.45B	1.60B	1.70B	1.85B	2.10B
With Rhizo.		1.25A	1.50A	1.65A	1.75A	1.90A	2.05A	2.55A
-	**S0**	1.30A	1.65A	1.75A	1.85A	2.00A	2.15A	2.70A
	S1	1.15B	1.30B	1.55B	1.65B	1.75B	1.95B	2.20B
	S2	0.70C	1.05C	1.35C	1.50C	1.60C	170C	1.90C
Without Rhizo.	**S0**	1.05B	1.50B	1.60B	1.75B	1.90B	2.05B	2.35B
	S1	0.90C	1.15C	1.45C	1.60C	1.65C	1.80C	2.00C
	S2	0.60D	0.95D	1.30D	1.45D	1.55D	1.65D	1.80D
With Rhizo.	**S0**	1.55A	1.80A	1.90A	2.00A	2.15A	2.30A	3.10A
	S1	1.40B	1.45B	1.65B	1.75B	1.85B	2.05B	2.40B
	S2	0.80C	1.15C	1.40C	1.55C	1.65C	1.75C	2.20C
		Second season 2021						
Without B	-	0.90B	1.35B	1.50B	1.65B	1.80B	2.00B	2.45B
With B		1.30A	1.55A	1.70A	1.85A	2.00A	2.25A	2.85A
-	**S0**	1.20A	1.55A	1.70A	1.85A	2.00A	2.25A	2.90A
	S1	0.85B	1.25B	1.45B	1.65B	1.75B	1.95B	2.20B
	S2	0.65C	1.00C	1.25C	1.45C	1.60B	1.75C	1.95C
Without B	**S0**	1.10B	1.50B	1.65B	1.80B	1.95B	2.15A	2.55A
	S1	0.90C	1.20C	1.40C	1.60C	1.70C	1.85B	2.10B
	S2	0.70D	0.95D	1.20D	1.40D	1.55D	1.70C	1.85C
With B	**S0**	1.40A	1.65A	1.80A	1.95A	2.10A	2.40A	3.20A
	S1	1.00B	1.35B	1.50B	1.70B	1.80B	2.00B	2.30B
	S2	0.80C	1.10C	1.30C	1.50C	1.65C	1.80C	2.05C

Rhizo. means rhizobacteria inoculation. S0, S1 and S2 mean salinity levels at 0, 100 and 200 mM of diluted seawater. Means at each column for each factor having different letters are significantly different at $p \leqslant 0.05$.

3.4. Proline content

In all treatments, proline content was increased from week 1 to week 7 in both seasons. Rhizobacteria inoculation improved the proline content compared to the control throughout the investigated period relative to the control. Additionally, a gradual increase in proline content was observed with increasing salinity level compared to the control in both seasons. Similarly, the interaction between rhizobacteria inoculation and salinity showed higher values than individual treatments at any time point in both seasons (Table 5). For instance, in week 7 of 2020, proline level rose from 2.42 (control) to 2.58 in salt-stressed plants (S2 treatment). However, it rose from 2.48 (control) to 3.52 for the same treatment when rhizobacteria inoculation was applied. Similar trends were observed in 2021, reinforcing the role of proline as a key osmoprotectant in mitigating salinity-induced stress. These findings align with earlier studies on other grass species, such as tall fescue (*Festuca arundinacea*), where bacterial inoculation (e.g., Azotobacter and *Azospirillum*) improved stress tolerance by modulating osmolyte synthesis (Massahi et al., 2018). The elevated proline levels in *Paspalum vaginatum* under rhizobacteria inoculation treatment suggest a synergistic mechanism where microbial inoculants enhance the plant's capacity to cope with osmotic stress, consistent with reports for bermudagrass (*Cynodon dactylon*) under similar conditions (Mahdavi et al., 2020). Similarly, the study by Abu-Shanab et al. (2022) on Trichoderma-inoculated perennial ryegrass highlighted reduced oxidative stress and improved growth, further supporting the idea that microbial interventions enhance osmoprotectant synthesis and stress resilience.

Table 5. Effect of rhizobacteria inoculation, salinity and their interaction on proline content of *Paspalum vaginatum* turfgrass during 2020 and 2021 seasons.

Rhizobacteria inoculation (Rhizo)	Salinity (S)	Week 1	Week 4	Week 7	Week 1	Week 4	Week 7
		First season 2020			Second season 2021		
Without Rhizo.	-	2.28^{B}	2.53^{B}	2.86^{B}	2.16^{B}	2.45^{B}	2.61^{B}
With Rhizo.		2.65^{A}	3.07^{A}	3.72^{A}	2.50^{A}	2.95^{A}	3.40^{A}
-	**S0**	2.18^{C}	2.42^{C}	2.47^{C}	2.11^{C}	2.33^{C}	2.36^{C}
	S1	2.47^{B}	2.88^{B}	3.22^{B}	2.36^{B}	2.72^{B}	3.07^{B}
	S2	2.73^{A}	3.03^{A}	3.75^{A}	2.61^{A}	3.12^{A}	3.72^{A}
Without Rhizo.	**S0**	2.12^{D}	2.42^{D}	2.47^{D}	2.07^{D}	2.26^{D}	2.33^{D}
	S1	2.23^{C}	2.48^{C}	2.73^{C}	2.22^{C}	2.40^{C}	2.56^{C}
	S2	2.37^{B}	2.58^{B}	2.85^{B}	2.31^{B}	2.71^{B}	2.96^{B}
With Rhizo.	**S0**	2.23^{C}	2.48^{C}	2.58^{C}	2.15^{C}	2.35^{C}	2.42^{C}
	S1	2.75^{B}	3.22^{B}	3.68^{B}	2.51^{B}	3.01^{B}	3.54^{B}
	S2	2.97^{A}	3.52^{A}	4.75^{A}	2.91^{A}	3.52^{A}	4.45^{A}

Rhizo. means rhizobacteria inoculation. S0, S1 and S2 mean salinity levels at 0, 100 and 200 mM of diluted seawater. Means at each column for each factor having different letters are significantly different at $p \leqslant 0.05$.

3.5. Peroxidase enzyme activity

Figures The current results demonstrated that rhizobacteria inoculation and salinity stress significantly increased peroxidase enzyme activity compared to the control in both seasons, a key antioxidant enzyme involved in mitigating oxidative stress (Table 6). This effect was more pronounced with the advance of age from week 1 to week 7 in both seasons. The impact of salinity was greater than rhizobacteria in this respect. The highest activity of pe-

roxidase enzyme was recorded following rhizobacteria inoculation in salt-stressed plants in 2020 and 2021 seasons. For instance, applying S2 treatment without rhizobacteria increased peroxidase activity by 120.90 % relative to the control, while this increment was 118.21 % when rhizobacteria were interacted with S2 treatment in 2020 season. Similarly, the same trend was detected in 2021 season. This aligns with the findings of other studies, where microbial inoculants enhanced plant tolerance to salinity by modulating physiological and biochemical responses. For instance, *Enterobacter* improved bermudagrass growth under salinity by boosting antioxidant enzyme activities (SOD and CAT) and reducing oxidative stress markers like malondialdehyde and electrolyte leakage (Wei et al., 2022). These results corroborate the role of microbial inoculants in enhancing antioxidant capacity, as seen in *Trichoderma*-inoculated *perennial ryegrass* (Abu-Shanab et al., 2022) and Bacillus-treated *tall fescue* (Li et al., 2022), where stress tolerance was linked to improved enzymatic and non-enzymatic antioxidant systems.

Table 6. Effect of rhizobacteria inoculation, salinity and their interaction on peroxidase enzyme activity of *Paspalum vaginatum* turfgrass during 2020 and 2021 seasons.

Rhizobacteria inoculation (Rhizo)	Salinity (S	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7
		First season 2020						
Without Rhizo.	-	3.08^{B}	3.45^{B}	3.82^{B}	4.20^{B}	4.59^{B}	5.01^{B}	5.47^{B}
With Rhizo.		3.52^{A}	3.94^{A}	4.41^{A}	4.93^{A}	5.51^{A}	6.16^{A}	6.89^{A}
-	**S0**	3.15^{C}	3.50^{C}	3.88^{C}	4.28^{C}	4.71^{C}	5.18^{C}	5.69^{C}
	S1	5.82^{B}	6.30^{B}	6.82^{B}	7.38^{B}	7.99^{B}	8.65^{B}	9.36^{B}
	S2	7.55^{A}	8.22^{A}	8.94^{A}	9.72^{A}	10.57^{A}	11.49^{A}	12.48^{A}
Without Rhizo.	**S0**	3.10^{D}	3.10^{D}	3.82^{D}	$4.20^{\mathbf{D}}$	4.61^{D}	5.06^{D}	5.55^{D}
	S1	5.75^{C}	5.75^{C}	6.78^{C}	$7.35^{\mathbf{C}}$	7.96^{C}	8.62^{C}	9.33^{C}
	S2	7.45^{B}	7.45^{B}	8.80^{B}	$9.56^{\mathbf{B}}$	10.39^{B}	11.29^{B}	12.26^{B}
With Rhizo.	**S0**	3.20^{C}	3.55^{C}	3.94^{C}	4.35^{C}	4.80^{C}	5.29^{A}	5.82^{A}
	S1	5.90^{B}	6.35^{B}	6.85^{B}	7.42^{B}	8.03^{B}	8.69^{B}	9.40^{B}
	S2	7.65^{A}	8.35^{A}	9.08^{A}	9.88^{A}	10.74^{A}	11.68^{C}	12.70^{C}
Second season 2021								
Without Rhizo	-	3.25^{B}	3.62^{B}	4.02^{B}	4.45^{B}	4.92^{B}	5.44^{B}	6.01^{B}
With Rhizo		3.75^{A}	4.19^{A}	4.68^{A}	5.23^{A}	5.84^{A}	6.53^{A}	7.30^{A}
-	**S0**	3.32^{C}	3.68^{C}	4.08^{C}	4.52^{C}	5.00^{C}	5.53^{C}	6.11^{C}
	S1	6.05^{B}	6.55^{B}	7.10^{B}	7.70^{B}	8.35^{B}	9.05^{B}	9.80^{B}
	S2	7.85^{A}	8.55^{A}	9.30^{A}	10.12^{A}	11.00^{A}	11.95^{A}	12.98^{A}
Without Rhizo.	**S0**	3.28^{D}	3.63^{D}	4.03^{D}	4.46^{D}	4.93^{D}	5.45^{D}	6.03^{D}
	S1	5.98^{C}	6.48^{C}	7.02^{C}	7.61^{C}	8.25^{C}	8.94^{C}	9.68^{C}
	S2	7.75^{B}	8.43^{B}	9.16^{B}	9.96^{B}	10.82^{B}	11.75^{B}	12.76^{B}
With Rhizo.	**S0**	3.38^{C}	3.75^{C}	4.16^{C}	4.60^{C}	5.09^{C}	5.63^{C}	6.22^{C}
	S1	6.12^{B}	6.62^{B}	7.18^{B}	7.78^{B}	8.43^{B}	9.14^{B}	9.90^{B}
	S2	7.95^{A}	8.68^{A}	9.44^{A}	10.28^{A}	11.18^{A}	12.15^{A}	13.20^{A}

Rhizo. means rhizobacteria inoculation. S0, S1 and S2 mean salinity levels at 0, 100 and 200 mM of diluted seawater. Means at each column for each factor having different letters are significantly different at $p \leqslant 0.05$.

3.6. Mineral content

Rhizobacteria inoculation significantly enhanced N, P and K percentages in paspalum leaves compared to the control under both non-saline and saline conditions during the 2020 and 2021 growing seasons (Table 7). However, salinity treatment markedly reduced N, P and K percentages compared to the unstressed plants. The lowest N, P and K percentages (1.267, 0.173 and 1.327 % in 2020, and 1.187, 0.139 and 1.243 % in 2021) were recorded in S2 treatment without rhizobacteria, respectively. However, when S2 treatment was interacted with rhizobacteria, those values were enhanced and recorded (1.357, 0.197 and 1.367 % in 2020, and 1.297, 0.197 and 1.263 % in 2021).

The decline in mineral content under high salinity in *Paspalum vaginatum* can be attributed to Na^+ interference with K^+ and Ca^{2+} uptake, as noted by Li et al. (2016). However, rhizobacteria inoculation mitigated this effect, likely through mechanisms such as nitrogen fixation, phosphorus solubilization, and ion homeostasis regulation. This is consistent with the findings of Li et al. (2022), where plant growth-promoting rhizobacteria like *Bacillus zanthoxyli* upregulated genes involved in Na^+ exclusion and K^+ absorption, maintaining a favorable K^+/Na^+ ratio in tall fescue. These findings align with the broader literature on bio-fertilizers' role in mitigating salinity stress via enhancing mineral uptake. For instance, Massahi et al. (2018) reported that Azotobacter and Azospirillum improved nutrient uptake (N and P) and maintained ion homeostasis (K/Na ratio) in tall fescue (*Festuca arundinacea*) under salinity, similar to the results observed in *Paspalum vaginatum*. Similarly, Wei et al. (2022) observed that the salt-tolerant endophytic bacterium Enterobacter ludwigii B30 restricted Na^+ translocation to shoots and enhanced K^+ uptake in bermudagrass (*Cynodon dactylon*), further supporting the role of microbial inoculants in improving nutrient acquisition under salinity. The interaction between rhizobacteria inoculation and salinity in *Paspalum vaginatum* underscores the potential of bio-fertilizers as a sustainable tool for enhancing plant resilience in saline environments. This aligns with the conclusions of Feng et al. (2023), where the microbial inoculants improved ion homeostasis and nutrient uptake in perennial ryegrass (*Lolium perenne*) by modulating the rhizosphere microbiome.

Table 7. Effect of rhizobacteria inoculation, salinity and their interaction on mineral content of *Paspalum vaginatum* turfgrass after a 7-week investigation period during 2020 and 2021 seasons.

Rhizobacteria inoculation (Rhizo)	Salinity (S)	N%	P%	K%	N%	P%	K %
		First season 2019			Second season 2020		
Without Rhizo.		1.442^{B}	0.208^{B}	1.440^{B}	1.392^{B}	0.179^{B}	1.349^{B}
With Rhizo.		1.553^{A}	0.237^{A}	1.489^{A}	1.496^{A}	0.224^{A}	1.393^{A}
-	**S0**	1.695^{A}	0.262^{A}	1.567^{A}	1.658^{A}	0.238^{A}	1.493^{A}
	S1	1.487^{B}	0.220^{B}	1.480^{B}	1.432^{B}	0.198^{B}	1.367^{B}
	S2	1.312^{C}	0.185^{C}	1.347^{C}	1.242^{C}	0.168^{C}	1.253^{C}
Without Rhizo.	**S0**	1.627^{B}	0.246^{B}	1.527^{B}	1.607^{B}	0.223^{B}	1.453^{B}
	S1	1.433^{D}	0.203^{C}	1.467^{D}	1.383^{D}	0.173^{C}	1.350^{D}
	S2	1.267^{E}	0.173^{D}	1.327^{E}	1.187^{E}	0.139^{D}	1.243^{E}
With Rhizo.	**S0**	1.763^{A}	0.277^{A}	1.607^{A}	1.710^{A}	0.253^{A}	1.533^{A}
	S1	1.540^{C}	0.237^{B}	1.493^{C}	1.480^{C}	0.223^{B}	1.383^{C}
	S2	1.357^{D}	0.197^{C}	1.367^{D}	1.297^{D}	0.197^{C}	1.263^{D}

Rhizo. means rhizobacteria inoculation. S0, S1 and S2 mean salinity levels at 0, 100 and 200 mM of diluted seawater. Means at each column for each factor having different letters are significantly different at $p \leqslant 0.05$.

4. Conclusions

Rhizobacteria inoculation enhanced the growth performance of paspalum turfgrass and markedly alleviated the negative impacts of salt stress through enhancing the relative water content, chlorophyll content, induction of proline and peroxidase activity and improving the absorbance of nutrient elements under salinity conditions. The current report reveals the vital role of rhizobacteria inoculation in enhancing salt stress tolerance in paspalum due to the aforementioned responses. These results, therefore, show that rhizobacteria inoculation could be used as a sustainable and suitable alternative to other chemicals for attenuating the adverse effects of salinity and enhancing tolerance to salt stress in paspalum and, most probably, other turfgrass species.

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