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ORIGINAL PAPER

The Application of the Plant Growth-Promoting Endophyte *Pantoea* Sp. YSD J2 Alleviates the Salt Stress of *Cyperus Esculentus* L. Var Sativus

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Abstract

Purpose The primary aim of this study was to explore the key physiological and metabolic responses of tigernut inoculated with the plant growth-promoting endophyte (PGPE) *Pantoea* sp. YSD J2 under varying NaCl concentrations.

Methods The salt tolerance, colonization ability, and growth-promoting potential of YSD J2 were evaluated at different NaCl concentrations. The physiological and metabolic responses of tigernut inoculated with YSD J2 were also analyzed.

Results YSD J2 exhibited remarkable salt tolerance and colonization ability under various NaCl levels, producing several growth-promoting substances, including indole-3-acetic acid (IAA) (2.20–23.23 μg mL^{-1}), siderophores (1.00–3.50%), exopolysaccharides (EPS) (2.00–29.76 mg mL^{-1}), biofilm (6.00–44.00 mg mL^{-1}), phosphate solubilization (1.00–32.00 μg mL^{-1}), and potassium solubilization (1.00–2.60). Inoculation with YSD J2 significantly enhanced plant growth under salt stress, improving shoot height (10.95–16.00%), root length (0.82–14.51%), shoot weight (52.02–143.57%), root weight (52.78–107.00%), relative water content (0.51–8.48%), and tiller number (33.33–283.33%). YSD J2 inoculation also increased chlorophyll content (31.87–118.18%), elevated the activities of peroxidase (POD) (6.74–21.90-fold), superoxide dismutase (SOD) (5.60–12.19%), and catalase (CAT) (25.00–52.63%), reduced malondialdehyde (MDA) levels (9.69–22.02%), and enhanced the accumulation of osmotic substances, including soluble sugar (18.93–45.05%), soluble protein (3.08–59.56%), and proline (10.86–12.94%). Additionally, YSD J2 inoculation reduced endogenous Na^+ accumulation while increasing K^+ and Ca^{2+} absorption.

Conclusion These results suggest that YSD J2 is a promising PGPE capable of improving plant growth, photosynthetic pigment content, antioxidant enzyme activities, osmotic regulation, and ion homeostasis, thereby mitigating the detrimental effects of salt stress in tigernut.

Keywords Tigernut · PGPE · Salt stress · Plant growth · Physiological and metabolic responses

1 Introduction

Soil salinization, a global phenomenon exacerbated by climate change, significantly impacts plant growth, physiological processes, and chemical composition (Marroquin et al. 2023; Belaouni et al. 2022). Currently, salinity threatens over 833 million hectares of land worldwide (Yue et al. 2022). Common strategies to mitigate salt stress and enhance crop yields include the application of chemical fertilizers and the cultivation of salt-tolerant crops. However, these approaches have inherent limitations and fail to fundamentally improve soil quality or sustainably increase crop yields (Dey et al. 2021).

Plant growth-promoting endophytes (PGPE) may play a crucial role in enhancing plant growth, increasing nutrient uptake, protecting plants from soil-borne pathogens, and

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improving crop tolerance to salt stress (Gupta et al. 2022). PGPE colonize the internal tissues of plants, directly aiding them by improving the absorption of soil nutrients such as nitrogen, iron, and phosphorus (P), and providing metabolites synthesized by the bacteria, including indole-3-acetic acid (IAA) and hydrogen cyanide (HCN) (Zhao et al. 2019; Damo et al. 2022). They also activate the plant's antioxidant system, enhancing the activity of antioxidant enzymes (El-Esawi et al. 2019) and promoting the accumulation of osmotic regulators (Akram et al. 2017). Furthermore, PGPE help regulate water balance by selectively absorbing K^+ while excluding Na^+, thereby maintaining a favorable K^+/Na^+ ratio (Islam et al. 2016). They stabilize hormone levels (Pandey et al. 2020) and secrete exopolysaccharides (EPS) to form biofilms that mitigate Na^+ toxicity (Liu et al. 2022). Numerous studies have demonstrated that PGPE, including *Pantoea* spp., *Achromobacter* spp., *Arthrobacter* spp., *Bacillus* spp., *Sphingomonas* spp., *Enterobacter* spp., and *Pseudomonas* spp., can enhance plant resistance to salt stress (Amini Hajiabadi et al. 2022; Dong et al. 2019; Li et al. 2016). Additionally, specific PGPE strains have been shown to improve the salt tolerance of various crop species (Chu et al. 2019).

Cyperus esculentus L. var. sativus (tigernut) is the second most abundant species in the Cyperaceae family (Pascual-Seva et al. 2021). Tigernut tubers are rich in essential components such as fats, proteins, starch, and dietary fiber, making them highly valued for their nutritional and health benefits (Asare et al. 2020; Zhang et al. 2023). Additionally, tigernut stems have significant applications in food, cosmetics, animal husbandry, and medicine due to their diverse biological functions and pharmacological activities (Ma et al. 2022; Zhang et al. 2022; Jing et al. 2020). Owing to its nutritional value and health-promoting properties, tigernut has gained considerable attention (da Costa Neto et al. 2019; Oyetoro et al. 2019; E Duman et al. 2019).

Although endophytic bacteria generally employ conserved mechanisms to induce plant salt tolerance, such as activating antioxidant pathways or modulating ion transport, the strain-specific molecular mechanisms underlying these effects remain poorly characterized. This research gap arises from the diverse genetic and metabolic profiles of different endophytic strains, which may mediate unique interactions with host plants under saline conditions. In our previous study, the endophytic bacterium *Pantoea* sp. YSD J2 (YSD J2), isolated from tigernut stems, demonstrated multiple growth- and metabolism-promoting abilities (Wang et al. 2022). However, the functional relevance of these traits to salt stress tolerance in tigernut remains unaddressed, as no studies have investigated the role of PGPE in this crop under saline environments.

Leveraging the established salt tolerance-promoting roles of PGPE in cereals and the documented PGP traits of YSD J2, we hypothesize that YSD J2 inoculation confers salt stress resilience to tigernut through four mechanisms: first, enhancing nutrient uptake efficiency and phytohormonal homeostasis under NaCl stress; second, amplifying antioxidant enzyme activity to scavenge reactive oxygen species (ROS) and mitigate oxidative injury; third, promoting the accumulation of osmolytes to maintain cellular turgor pressure and ion equilibrium; and fourth, regulating ion homeostasis through selective Na^+ exclusion and K^+ retention to optimize the K^+/Na^+ ratio in plant tissues. To test these hypotheses, we designed a controlled experiment where tigernut plants were subjected to gradient NaCl concentrations with or without YSD J2 inoculation. The study objectives are: (1) to characterize the salt-adaptive PGP traits of YSD J2 under saline conditions; (2) to quantify the impact of YSD J2 inoculation on tigernut growth parameters under salt stress; (3) to profile the effects of YSD J2 on photosynthetic metabolites, osmoprotectants, and antioxidant enzyme activities; and (4) to elucidate the molecular and physiological mechanisms underlying YSD J2-mediated salt tolerance in tigernut.

2 Materials and Methods

2.1 Salt Tolerance Measurement and Morphological Changes of *Pantoea* Sp. YSD J2

Strain YSD J2 is an endophytic bacterium isolated from the stem of tigernut in 2019, identified as *Pantoea* sp. (Wang et al. 2022), and the GenBank entry number of the 16 S rDNA gene is OK090942. The salt tolerance of YSD J2 was assessed through multiple approaches. First, the strain was cultured in LB medium supplemented with NaCl at concentrations of 170, 500, 1000, 1500, 2000, 2500, and 3000 mM. After 24 h of incubation at 37 °C, the optical density at 600 nm (OD_{600}) was measured (Kumar et al. 2021). The metabolic activity under salt stress was further evaluated using a resazurin assay (Yuanye, China). Briefly, 100 µL of resazurin solution (0.1 mg mL^{-1} in PBS) was added to 1 mL of bacterial culture (with LB medium containing resazurin but no bacteria as a negative control), followed by incubation at 37 °C for 30 min in the dark. The fluorescence was then measured using a microplate reader at 530 nm excitation and 590 nm emission wavelengths. To determine viable cell counts, both undiluted and 100-fold diluted bacterial suspensions were spread on LB agar plates containing corresponding NaCl concentrations. After 24 h of incubation at 37 °C, colony-forming units (CFU) were enumerated. Concurrently, morphological changes in YSD J2 were observed

using transmission electron microscopy (TEM). The treated bacterial cells were embedded, thin-sectioned, and placed on carbon support films. After drying, the samples were analyzed using a TEM microscope (Talos L120C G2) (Zhang et al. 2024).

2.2 PGP Features Production in *Vitro*

YSD J2 was inoculated into different media containing NaCl concentrations of 170, 500, 1000, 1500, 2000, 2500, and 3000 mM to evaluate its PGP traits. IAA production was assessed following the method of Wang et al. (2022), using media supplemented with tryptophan. Phosphate (P) solubilization capacity was determined using Pikovskaya's medium enriched with 0.2 mg mL^{-1} lecithin (organic phosphate) and 5 mg mL^{-1} $Ca_3(PO_4)_2$ (inorganic phosphate), as described by Wang et al. (2022). EPS production was evaluated according to Sharma et al. (2023). Potassium (K) solubilization and siderophore production were detected using Aleksandrov agar medium and MKB medium, respectively, as outlined by Wang et al. (2022). Biofilm production was quantified by measuring wet weight, following the method of Trulear et al. (1982).

2.3 Microscopic Observation of the Root Structure

Tigernut seeds (Zhongyousha No. 1) used in this study were obtained from Shanghai Jiao Tong University, China. Intact, uniformly sized, and fully developed seeds were surface-sterilized by immersing them in 70% ethanol for 10 min, followed by treatment with 5% NaClO for 5 min. Subsequently, the seeds were thoroughly rinsed with sterile distilled water and air-dried to remove any residual moisture. Batches of 10 seeds were prepared: one group was inoculated with YSD J2 by immersion in a bacterial suspension (10^8 spores mL^{-1}) for 2 h, while the control group (CK) was untreated. Both groups were placed on filter paper-lined plates moistened with sterile distilled water and incubated in the dark at 25 °C for 7 days. After germination, the seedlings were transferred to tubes containing 10 mL of nutrient solution agar with NaCl concentrations ranging from 0 to 500 mM and further incubated in a culture chamber at 25 °C for 7 days. Root structures were examined using a Nikon ECLIPSE E100 upright microscope (Masmoudi et al. 2021).

2.4 Experimental Design and Treatments in the Pot Experiment

Tigernut (cultivar Zhongyousha No. 1) were collected on March 15th, 2024, from Shanghai Jiao Tong University (121° 29′ 21.88″ E, 31° 24′ 18.97″ N), Shanghai, China. The experiment was conducted on January 5th, 2024, in a greenhouse maintained at 25–30 °C with tap water irrigation and no fertilizer application. Surface-sterilized seeds (treated with 70% ethanol for 10 min and 5% NaClO for 5 min) were germinated in sterile containers lined with cotton and filter paper. After the seedlings developed their third leaf, uniformly sized seedlings were selected and transplanted into pots filled with a 2:3 (v/v) mixture of vermiculite and sandy soil. The plants were grown in a controlled environment chamber at 25 °C with 60% relative humidity and a 14 h light/10 hours dark photoperiod. Bacterial suspensions were prepared by incubating YSD J2 in 250 mL of LB medium in a 500 mL shake flask at 37 °C until the culture reached 1×10^8 CFU mL^{-1}. The cells were harvested by centrifugation at 5000×g for 5 min and resuspended in an equal volume of sterile water to create the bacterial suspension.

Based on the root hair morphology of tigernut under different NaCl concentrations, a NaCl tolerance threshold of 500 mM was established for the pot experiments. A factorial design (3 × 2) with five replicates was implemented, resulting in 30 pots (1 seedling/pot; 1.6 L; basal diameter 10 cm; top outer diameter 15.5 cm; top inner diameter 14.5 cm; height 13 cm). The design included three NaCl treatment levels (0, 300, and 500 mM) and two bacterial treatment levels (water: CK; YSD J2 bacterial suspension: YSD J2). When tigernut plants reached the four-leaf stage, NaCl treatment was initiated daily, gradually increasing by 100 mM each day to prevent salt shock until the final concentration of 500 mM was achieved. Intermediate concentrations of 100 mM, 200 mM, 300 mM, 400 mM, and 500 mM were sequentially applied, as shown in Fig. S1. Seventy-two hours after reaching the target NaCl concentration, the bacterial suspension was applied for inoculation. This process was repeated at five-day intervals, with a dose of 20 mL administered five times. Samples were collected five days after the final inoculation.

2.5 Colonization Analysis of Tigernut Tissues

To evaluate the colonization of strain YSD J2 in tigernut tissues, rhizosphere soil, roots, and stems were collected 30 days after inoculation with the YSD J2 suspension. For rhizosphere soil sampling, surface weeds were removed, and soil within 2 mm of the root surface was collected (DeAngelis et al. 2009). For root and stem sampling, adhering soil and debris were removed by washing with sterile water. The tissues were surface-sterilized by sequential treatment with 5% NaClO for 3 min, 75% ethanol for 3 min, and 95% ethanol for 30 s, followed by rinsing 3–5 times with sterile water (Liotti et al. 2018). To confirm the absence of culturable microorganisms on the sterilized tissues, they were placed on LB agar plates and incubated at 37 °C for 24 h.

The plant tissues were then homogenized in sterile water and incubated at 37 °C with shaking at 180 rpm for 1 h. Serial dilutions of the homogenate were plated on LB agar medium and incubated at 37 °C for 24 h, after which colony counts were recorded. Re-isolated colonies were stored in LB medium supplemented with 30% glycerol at -80 °C for subsequent 16 S rDNA sequencing to confirm their identity as *Pantoea* sp.

2.6 Analysis of Plant Morphology and Metabolites Related To Osmotic Stress

After harvesting, several plant morphological traits were measured, including shoot height, root length, tiller number, fresh weight (FW), dry weight (DW), and relative water content (RWC). To determine RWC, the FW of stem samples was recorded. The samples were then submerged in water at 4 °C for 24 h to obtain their turgid weight (TW). Subsequently, the stems were oven-dried at 80 °C for 72 h, and their DW was measured. RWC was calculated using the formula described by Billah et al. (2021). The contents of chlorophyll (Chl), proline (Pro), soluble sugar (SS), and soluble protein (SP) were quantified using corresponding assay kits (Cominbio, China), following the manufacturer's instructions.

2.7 Analysis of Plant Antioxidant Enzyme Activity

Fresh tigernut stems were excised, immediately frozen in liquid nitrogen, and ground into a fine powder using a mortar. The powder was transferred to test tubes and stored at -80 °C until analysis. The activities of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) were quantified using enzyme assay kits (Cominbio, China). Additionally, malondialdehyde (MDA) levels were measured using an MDA assay kit (Cominbio, China), following the manufacturer's instructions.

2.8 Analysis of Na^+, K^+ and Ca^{2+} Contents

Dried plant stems, roots, and soil were ground into a fine powder, mixed with 0.1 N nitric acid (w/v), and heated at 70 °C for 5–6 days. The concentrations of Na^+, K^+, and Ca^{2+} were determined using flame emission spectroscopy (Gond et al. 2015).

2.9 Statistical Analysis

The significance of differences among treatments was evaluated using one-way analysis of variance (ANOVA), with statistical significance defined as $p \leq 0.05$. Figures were generated using GraphPad Prism 8.0.2 software (GraphPad Software, Boston, Massachusetts, USA). To comprehensively analyze the data, principal component analysis (PCA) was performed using CANOCO 5.0 software, and cluster analysis was conducted using the Metware Cloud, a free online platform for data analysis. These analyses were used to elucidate the relationships between treatment factors and growth-related physiological parameters, identifying key factors through which YSD J2 enhances the salt tolerance of tigernut.

3 Results

3.1 The in Vitro PGP Traits of YSD J2 Exposed To Salt Stress

To evaluate the salt tolerance of *Pantoea* sp. YSD J2, we measured its PGP traits under increasing NaCl concentrations (Table 1). The strain produced IAA, EPSs, biofilm,

Table 1 Production of plant growth-promoting (PGP) features by *Pantoea* sp. YSD J2 under salt stress. The production of PGP traits, including OD_{600}, phosphate and potassium solubilization, indole-3-acetic acid (IAA), biofilm, exopolysaccharides (EPS), and siderophore, was measured under different NaCl concentrations (170, 500, 1000, 1500, 2000, 2500, and 3000 mM NaCl). Each condition was tested with $n = 3$ independent biological replicates, and the data are presented as mean ± standard error (SE). Same letters in a column indicate no significant differences between means according to the Duncan's multiple range test (at $p \leq 0.05$)

PGP features		Salt concentrations (mM)						
		170	500	1000	1500	2000	2500	3000
OD_{600}		1.81 ± 0.10	1.75 ± 0.20	1.80 ± 0.07	1.00 ± 0.05	0.10 ± 0.01	0.08 ± 0.01	0.07 ± 0.03
IAA (μg mL^{-1})		16.00 ± 0.10^{c}	23.23 ± 1.00^{a}	17.65 ± 0.90^{b}	11.56 ± 0.30^{d}	5.34 ± 0.06^{e}	3.25 ± 0.04^{f}	2.20 ± 0.02^{g}
Phosphate solubilization	Lecithin (μg mL^{-1})	17.00 ± 0.20^{c}	24.00 ± 0.90^{a}	18.00 ± 0.30^{b}	13.00 ± 0.05^{d}	6.00 ± 0.10^{e}	2.00 ± 0.50^{e}	1.00 ± 0.00^{f}
	$Ca_3(PO_4)_2$ (μg mL^{-1})	25.00 ± 1.00^{b}	32.00 ± 0.40^{a}	26.00 ± 0.09^{c}	20.00 ± 0.05^{d}	14.00 ± 0.05^{e}	12.00 ± 0.09^{e}	11.00 ± 0.30^{f}
Siderophore production (%)		3.39 ± 0.06^{a}	3.50 ± 0.10^{a}	3.40 ± 0.10^{a}	3.20 ± 0.02^{b}	3.00 ± 0.07^{c}	2.00 ± 0.07^{d}	1.00 ± 0.03^{e}
Potassium solubilization		2.48 ± 0.06^{a}	2.60 ± 0.10^{a}	2.50 ± 0.04^{a}	2.30 ± 0.20^{b}	2.00 ± 0.01^{c}	1.50 ± 0.02^{d}	1.00 ± 0.03^{e}
Biofilm production (mg mL^{-1})		44.00 ± 1.00^{a}	37.50 ± 0.90^{b}	15.00 ± 0.60^{c}	6.00 ± 0.20^{d}	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}
EPS (mg mL^{-1})		18.00 ± 0.10^{c}	29.76 ± 1.00^{a}	20.15 ± 0.07^{b}	16.00 ± 0.20^{d}	10.00 ± 0.10^{e}	6.00 ± 0.010^{f}	2.00 ± 0.09^{g}

P, K, and siderophores, which are key factors for enhancing plant growth under salt stress. Maximum biofilm yield occurred at 170 mM NaCl, while peak production of IAA, P, K, siderophores, and EPSs was observed at 500 mM NaCl. All PGP traits gradually declined at higher concentrations (1000–3000 mM), indicating progressive metabolic inhibition under extreme salinity. These results demonstrate that YSD J2 maintains functional PGP traits at agriculturally relevant salt levels (≤500 mM NaCl), with optimal performance in IAA and EPS production. The decline beyond 500 mM NaCl defines the strain's functional limits in highly saline conditions.

3.2 Biphasic Salt Adaptation in YSD J2

To elucidate the salt tolerance mechanism of YSD J2, we systematically analyzed its survival capacity and associated physiological adaptations. The strain exhibited a dose-dependent response to NaCl stress, with colony-forming ability transitioning from too-numerous-to-count at ≤1500 mM NaCl to a well-defined subpopulation at ≥2000 mM NaCl, demonstrating 0.025%, 0.01%, and 0.005% survival rates at 2000, 2500, and 3000 mM NaCl respectively (Fig. 1A). Metabolic activity assays corroborated this pattern, showing that while most cells experienced severe metabolic suppression (≤2.1% resazurin activity at 2000 mM NaCl), a small fraction maintained viability (Fig. 1B, C). TEM revealed progressive structural modifications in YSD J2 cells along increasing salinity gradients. Under moderate salinity conditions (≤500 mM NaCl), the cells maintained intact membrane structures while secreting abundant extracellular polymeric substances. When salinity reached intermediate concentrations (1000–1500 mM NaCl), gradual structural disorganization became apparent in the cells. Remarkably, even under extreme salt stress (≥2000 mM NaCl), a subpopulation of cells with preserved cellular integrity could still be observed (Fig. 2, S2). These collective findings demonstrate that YSD J2 employs a sophisticated dual-phase adaptation strategy, combining population-wide physiological adjustments with selective maintenance of a stress-resistant subpopulation to ensure survival across extreme salinity ranges.

3.3 Effects of YSD J2 Inoculation on Root Morphology of Tigernut Under Salt Stress

To investigate the potential protective effects of YSD J2 inoculation on root architecture under salt stress conditions, we conducted microscopic observations of tigernut seedlings subjected to varying concentrations of stress treatment. The results demonstrated that non-inoculated control plants exhibited characteristic salt damage symptoms, including inhibited root hair development and structural abnormalities. In contrast, YSD J2 inoculation alleviated salt-induced root hair deformation, particularly under 500 mM NaCl stress where inoculated plants showed visibly reduced curling and shortening of root hairs relative to untreated controls (Fig. 3). These qualitative observations suggest that

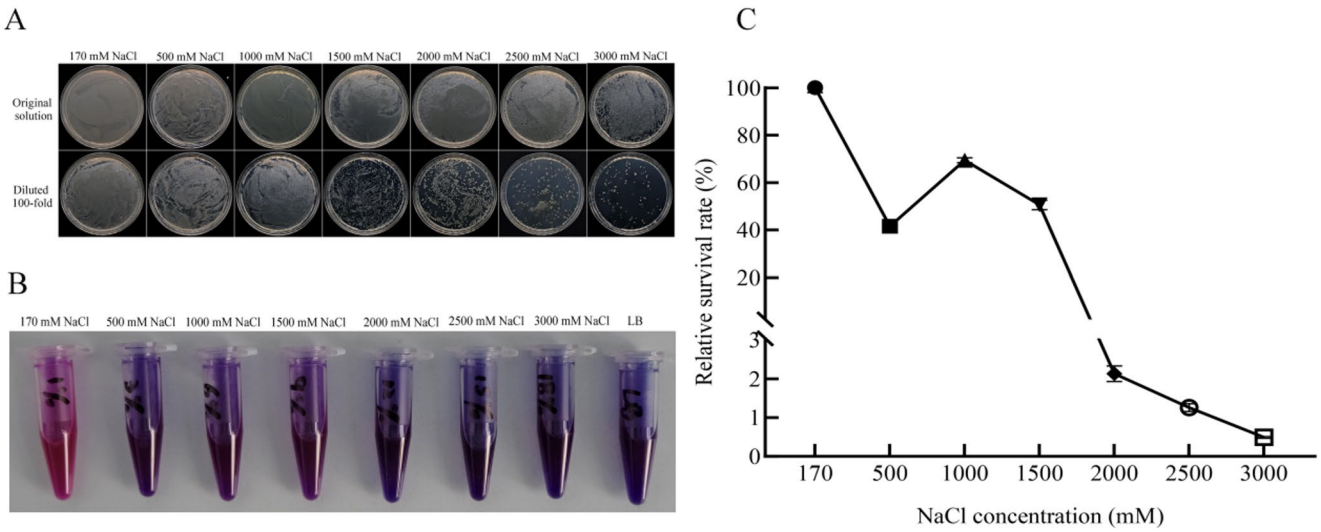


Fig. 1 Responses of *Pantoea* sp. YSD J2 to salt stress across salinity gradients. (**A**) Dilution plating images showing colony formation of *Pantoea* sp. YSD J2 in undiluted bacterial solution and 100-fold diluted solution under different NaCl concentrations (170, 500, 1000, 1500, 2000, 2500, and 3000 mM NaCl). (**B**) Visualization of cell viability via resazurin assay, showing the activity status of *Pantoea* sp. YSD J2 under the tested NaCl concentrations. The LB group served as a negative control, containing LB medium and resazurin only, whereas all other groups were inoculated with the YSD J2 strain. (**C**) Relative survival rates of *Pantoea* sp. YSD J2 based on resazurin assay, with values presented under each NaCl concentration (relative to the 170 mM NaCl group, set as 100%). All experiments were performed with $n = 3$ independent biological replicates. Data are presented as mean ± standard error (SE)

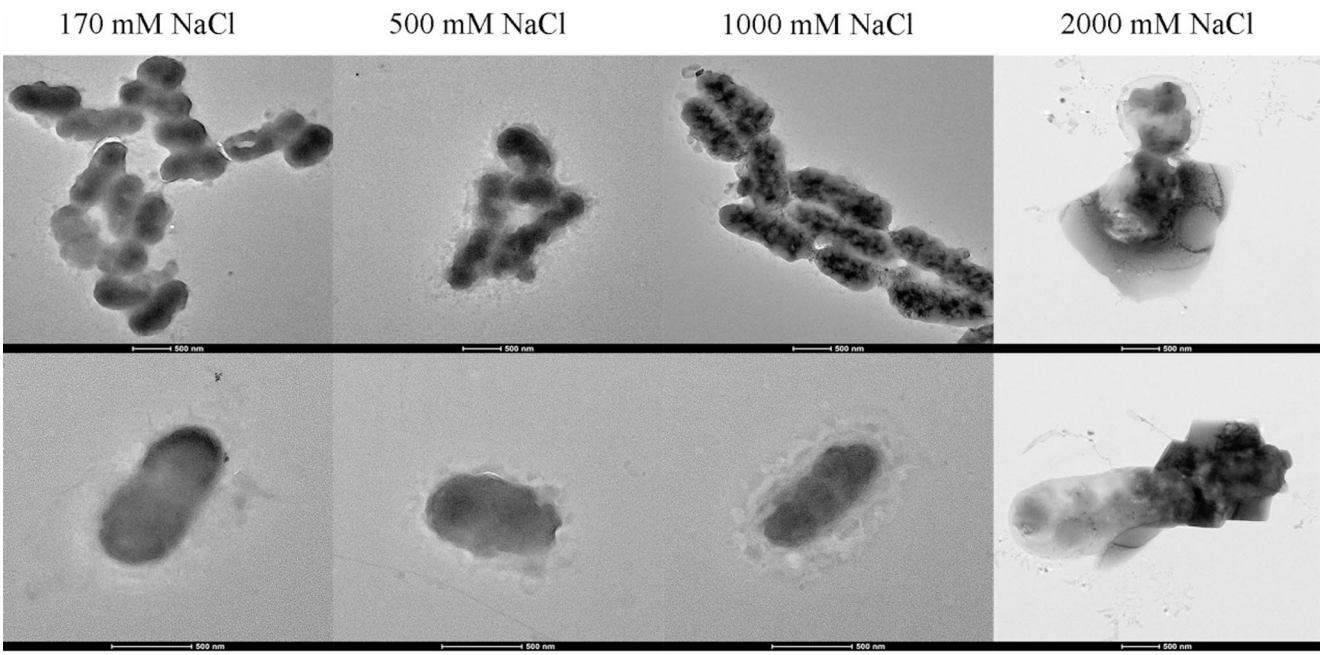


Fig. 2 Morphological changes in *Pantoea* sp. YSD J2 induced by salt stress. Transmission electron microscopy (TEM) images of *Pantoea* sp. YSD J2 cells exposed to different NaCl concentrations (170, 500, 1000, and 2000 mM NaCl). Each condition was tested with $n = 3$ independent biological replicates, and representative images are shown. The scale bar in all TEM images represents 500 nm

YSD J2 inoculation may help mitigate some of the adverse effects of salt stress on root morphology.

3.4 Ability of YSD J2 To Colonize Tigernut Tissues

To evaluate the colonization efficiency and potential endophytic capacity of YSD J2 in tigernut tissues under salt stress, rhizosphere and internal tissue re-isolation assays were conducted. Rhizosphere CFU counts increased with NaCl concentrations up to 300 mM but decreased significantly at 500 mM NaCl. YSD J2 maintained robust rhizosphere colonization across all salt levels, with densities exceeding 6×10^7 CFU g^{-1} rhizosphere soil (Fig. 4A). For internal tissues, YSD J2 was consistently recovered from surface-sterilized roots and stems, with CFU counts $> 3.7 \times 10^4$ CFU g^{-1} fresh matter (Fig. 4B and C). Under 0–300 mM NaCl, root CFU counts increased with salinity regardless of inoculation but declined at 500 mM NaCl. Notably, inoculated plants exhibited higher root and stem CFU than non-inoculated controls (Fig. 4B and C). These results demonstrate that YSD J2 maintains stable colonization under salt stress, with significantly higher densities in rhizosphere soil (6.4×10^7–6×10^7 CFU g^{-1}) than in internal tissues (roots: 3.9×10^4–3.7×10^4 CFU g^{-1} fresh matter; stems: 3.1×10^5–2.5×10^5 CFU g^{-1} fresh matter). Recovery from surface-sterilized tissues suggests potential endophytic colonization, with this evidence supporting the capacity of YSD J2 to colonize internal plant tissues.

3.5 Effects of Inoculation with YSD J2 on Tigernut Growth Under Salt Stress

To assess the potential of *Pantoea* sp. YSD J2 in alleviating salt stress in tigernut, we conducted pot experiments examining its effects under varying NaCl concentrations (0, 300, and 500 mM). Compared to non-inoculated controls (CK), YSD J2 inoculation demonstrated remarkable growth-promoting effects across all tested salt concentrations. Key growth parameters including tiller number, shoot height, root length, RWC, and biomass accumulation showed significant improvements in inoculated plants. Most notably, at 300 mM NaCl, YSD J2 increased tiller number by 283.33% relative to CK (Fig. 5A). Biomass production was substantially enhanced, with shoot weight increasing by 52.02% (0 mM), 119.26% (300 mM) and 143.57% (500 mM), and root weight by 52.78% (0 mM), 107.00% (300 mM) and 82.14% (500 mM) compared to CK (Fig. 5C and F). The protective effects of YSD J2 were particularly evident at high salinity (500 mM NaCl), where inoculated plants maintained normal growth while CK plants showed severe growth inhibition (Fig. S3). YSD J2 also increased RWC by 8.48% (Fig. 5D) and root length by 14.51% (Fig. 5E) at 500 mM NaCl compared to CK. These results clearly demonstrate that YSD J2 inoculation can effectively mitigate salt stress in tigernut plants, with particularly strong protective effects under moderate to high salinity conditions (300–500 mM NaCl). The strain's ability to maintain plant growth across

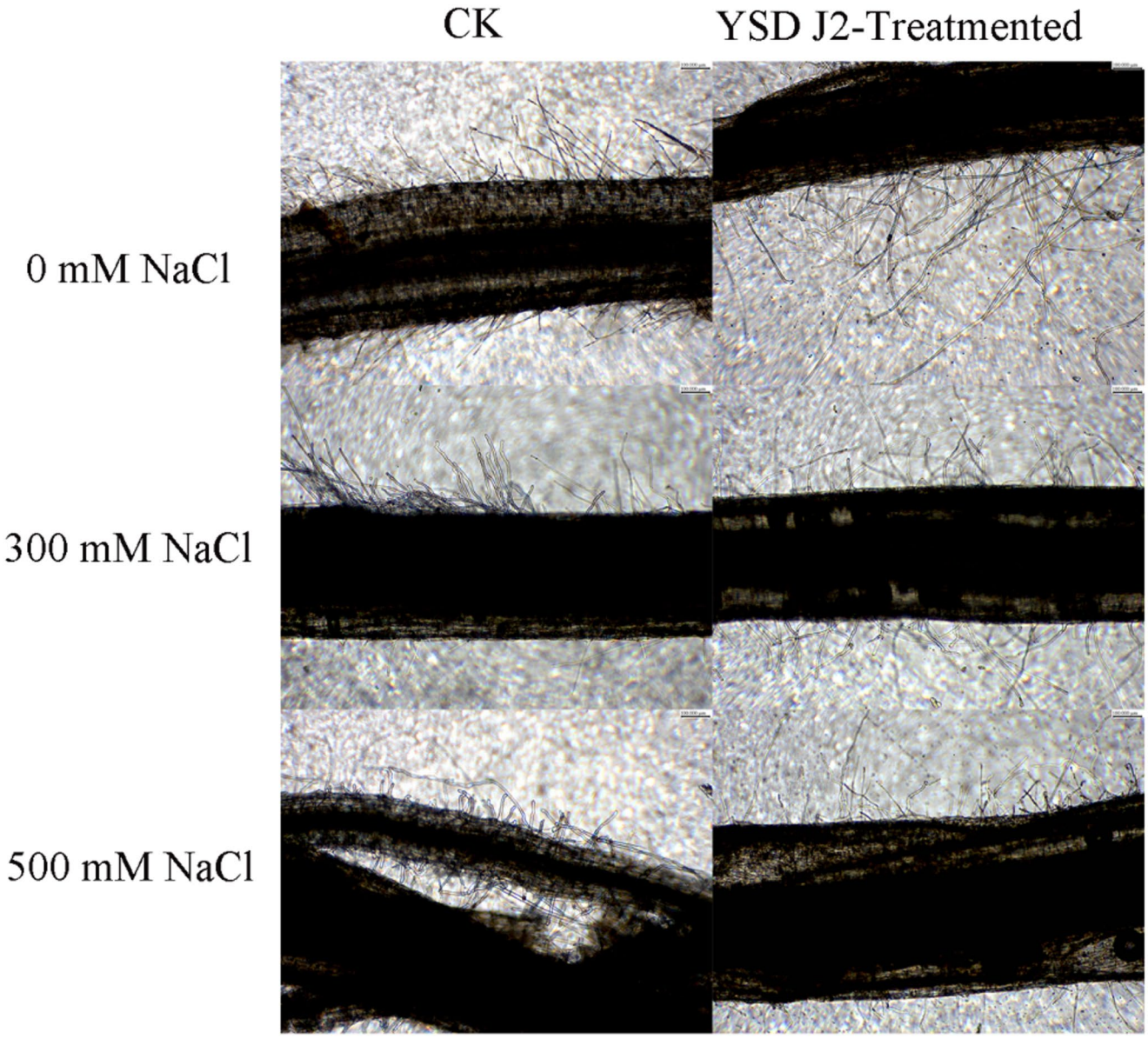


Fig. 3 Microscopic observation of root hair structure in the mature zone of tigernut seedlings. Root hairs of uninoculated (CK) and *Pantoea* sp. YSD J2-inoculated tigernut seedlings were observed under different NaCl concentrations (0, 300, and 500 mM NaCl). For each condition, 10 seeds per group (CK and YSD J2) were used, and the experiment was repeated with $n = 3$ independent biological replicates. Representative images are shown. Scale bars are indicated in the images

this salinity range suggests its potential for improving crop productivity in salt-affected soils.

3.6 Effects of Inoculation with YSD J2 on Tigernut Pigment Content Under Salt Stress

Chlorophyll pigments play a vital role in maintaining photosynthetic efficiency and plant productivity under abiotic stress. To evaluate YSD J2's protective effects on photosynthetic apparatus, we analyzed chlorophyll content in tigernut plants exposed to varying NaCl concentrations (0-500 mM). The results showed a clear protective effect of YSD J2 inoculation on chlorophyll preservation under salt stress. Non-inoculated control plants (CK) exhibited progressive chlorophyll degradation with increasing salinity, with Chl a content declining by 12.06–42.86%, Chl b by 7.20-21.76%, and total chlorophyll by 10.06–43.07% across the tested salinity range (Fig. 6A-C). The most severe reduction occurred at 500 mM NaCl, indicating substantial damage to photosynthetic machinery under extreme salinity conditions. In contrast, YSD J2 inoculation significantly mitigated these salt-induced pigment losses. Compared to CK plants, inoculated specimens maintained substantially higher chlorophyll levels, with Chl a content

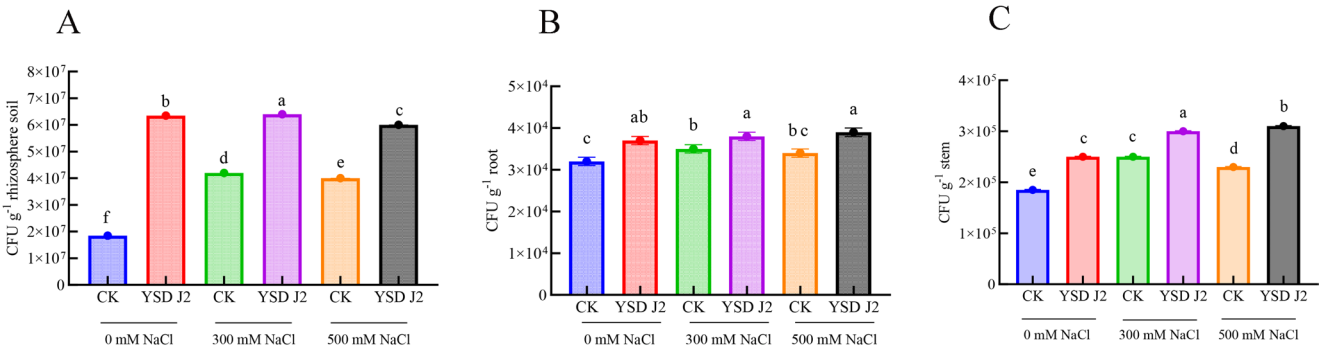


Fig. 4 Colonization ability of *Pantoea* sp. YSD J2 in tigernut plants under salt stress. (**A**) rhizosphere soil colonization, (**B**) root internal tissue penetration, and (**C**) stem internal tissue penetration of *Pantoea* sp. YSD J2 at different NaCl concentrations (0, 300, and 500 mM NaCl). Tigernut plants were grown under three NaCl concentrations (0, 300, and 500 mM) and two bacterial treatments (CK and YSD J2) in a factorial design (3 × 2) with $n = 5$ replicates per treatment combination (1 seedling/pot). Different letters indicate significant differences at $p \leq 0.05$ within the treatments at each NaCl concentration or among NaCl concentrations for each treatment. Data are presented as mean ± standard error (SE)

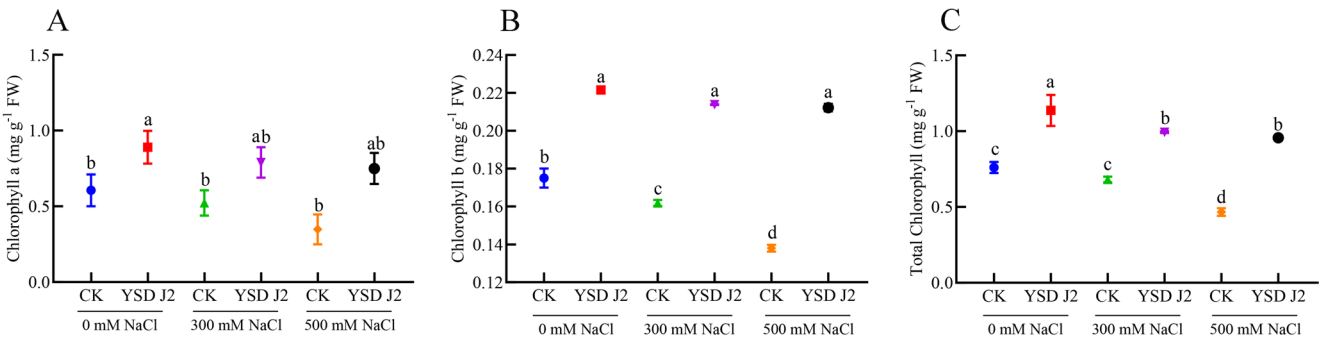


Fig. 5 Effect of *Pantoea* sp. YSD J2 on plant growth attributes: (**A**) number of tillers; (**B**) shoot height; (**C**) shoot weight; (**D**) relative water content; (**E**) root length; (**F**) root weight. Tigernut plants were grown under three NaCl concentrations (0, 300, and 500 mM) and two bacterial treatments (CK and YSD J2) in a factorial design (3 × 2) with $n = 5$ replicates per treatment combination (1 seedling/pot). Different letters indicate significant differences at $p \leq 0.05$ within the treatments at each NaCl concentration or among NaCl concentrations for each treatment. Data are presented as mean ± standard error (SE)

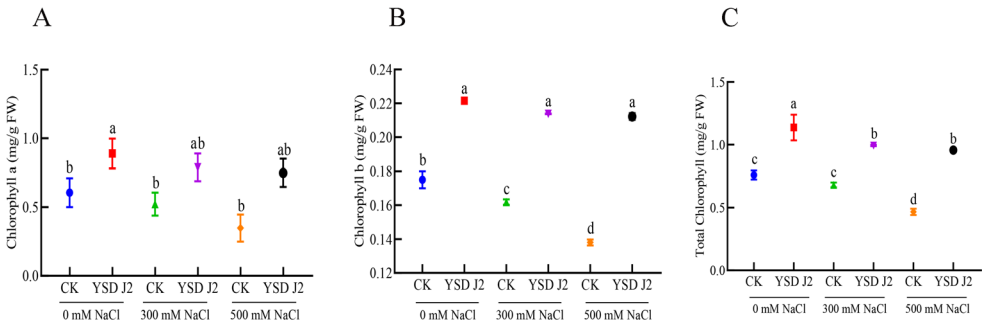


Fig. 6 Effect of *Pantoea* sp. YSD J2 on plant pigment contents (per fresh weight, FW): (**A**) chlorophyll a; (**B**) chlorophyll b; (**C**) total chlorophyll. Tigernut plants were grown under three NaCl concentrations (0, 300, and 500 mM) and two bacterial treatments (CK and YSD J2) with $n = 5$ replicates per treatment combination (1 seedling/pot). Different letters indicate significant differences at $p \leq 0.05$ within the treatments at each NaCl concentration or among NaCl concentrations for each treatment. Data are presented as mean ± standard error (SE)

showing increases of 47.30-117.09%, Chl b levels rising by 27.00-55.73%, and total chlorophyll content improving by 31.87-118.18% across all salinity treatments. Notably, the protective effects became more pronounced at higher salt concentrations. Under 500 mM NaCl stress, YSD J2 inoculation increased Chl a and total chlorophyll contents by 117.09% and 118.18%, respectively, compared to non-inoculated controls. These findings demonstrate YSD J2's ability to effectively mitigate salt-induced chlorophyll degradation, highlighting its potential application for improving crop performance in saline soils.

3.7 Effects of Inoculation with YSD J2 on Tigernut Osmoregulation Under Salt Stress

To investigate YSD J2's role in osmotic adjustment, we analyzed key osmolytes (proline, SS, and SP) in tigernut plants under salt stress (0-500 mM NaCl). The results revealed that non-inoculated control plants (CK) exhibited a 19.25–35.64% elevation in proline content with increasing salinity, while YSD J2 inoculation further enhanced proline accumulation by 10.86–12.94% across all treatments (Fig. 7A). Soluble sugar content showed a concentration-dependent response, with YSD J2 increasing levels by 18.93% and 45.05% at 0 and 300 mM NaCl respectively, but causing a 32.84% reduction at 500 mM NaCl compared to CK (Fig. 7B). The most pronounced effect was observed in SP content at 300 mM NaCl, where inoculation induced a 59.56% increase, with more modest effects (3.08–5.05% increase) at other concentrations (Fig. 7C). These distinct patterns of osmolyte accumulation demonstrate YSD J2's ability to fine-tune osmotic adjustment in tigernut, with particularly strong regulatory effects at moderate salinity levels (300 mM NaCl), suggesting its potential for optimizing plant stress responses in saline environments.

3.8 Effects of Inoculation with YSD J2 on Tigernut ROS Clearance Under Salt Stress

To assess YSD J2's antioxidant capacity, we analyzed ROS-scavenging enzyme activities and oxidative damage markers in tigernut under salt stress (0-500 mM NaCl). The results demonstrated that YSD J2 inoculation significantly enhanced the plant's antioxidant defense system while reducing oxidative damage. Salt stress induced progressive activation of antioxidant enzymes in control plants (CK), with CAT activity increasing by 58.33-133.33% across the tested salinity range (Fig. 8A). YSD J2 inoculation further boosted CAT activity by 25.00-52.63%, indicating enhanced H_2O_2 detoxification capacity. SOD activity showed a biphasic response, peaking at 300 mM NaCl before declining at 500 mM in CK plants, while YSD J2 treatment maintained elevated SOD levels at all concentrations (Fig. 8B). The most substantial enzymatic response were observed in POD activity, where inoculated plants exhibited 6.74-21.90-fold increases compared to CK, with maximum stimulation at 500 mM NaCl (Fig. 8C). Corresponding with these enzymatic changes, YSD J2 inoculation reduced MDA accumulation by 9.69–22.02% at 300–500 mM NaCl compared to controls, with maximal reduction (22.02%) at 500 mM NaCl (Fig. 8D). These results indicate that YSD J2 enhances oxidative stress tolerance in tigernut under salt stress conditions.

3.9 Effects of Inoculation with YSD J2 on Tigernut Ion Uptake Under Salt Stress

To evaluate the impact of YSD J2 on ion regulation under salt stress, we analyzed Na^+, K^+ and Ca^{2+} accumulation in tigernut roots, stems, and soil under 0–500 mM NaCl conditions. Non-inoculated control plants (CK) exhibited severe ionic imbalance, with Na^+ content increasing 5.82- to 18.72-fold in plant tissues and soil, while K^+ and Ca^{2+} levels decreased by 23.03–73.50% (Figs. 9A–C). YSD J2 inoculation significantly mitigated these effects, reducing Na^+ accumulation by 4.21–43.57% and enhancing K^+ retention by 5.26–35.72% in plant tissues compared to CK. The most pronounced improvements were observed in Ca^{2+} homeostasis, where inoculated plants maintained 15.63–185.19% higher levels than CK, particularly in stems at 0 mM NaCl (185.19% increase). Additionally, YSD J2 improved K^+/Na^+ ratios by 25.44–108.71% in plant tissues and 78.84–87.34% in soil under 300–500 mM NaCl stress (Fig. S4). These findings demonstrate that YSD J2 inoculation enhances ion

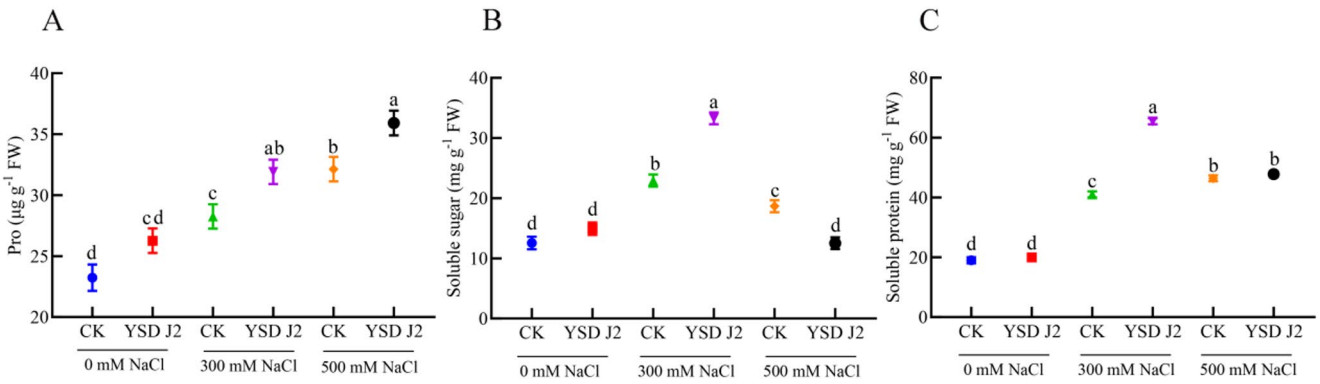


Fig. 7 Impact of inoculation with *Pantoea* sp. YSD J2 on osmotic stress metabolites (per fresh weight, FW). (**A**) proline (Pro); (**B**) soluble sugar (SS); (**C**) soluble protein (SP). Tigernut plants were grown under three NaCl concentrations (0, 300, and 500 mM) and two bacterial treatments (CK and YSD J2) with $n = 5$ replicates per treatment combination (1 seedling/pot). Different letters indicate significant differences at $p \leq 0.05$ within the treatments at each NaCl concentration or among NaCl concentrations for each treatment. Data are presented as mean ± standard error (SE)

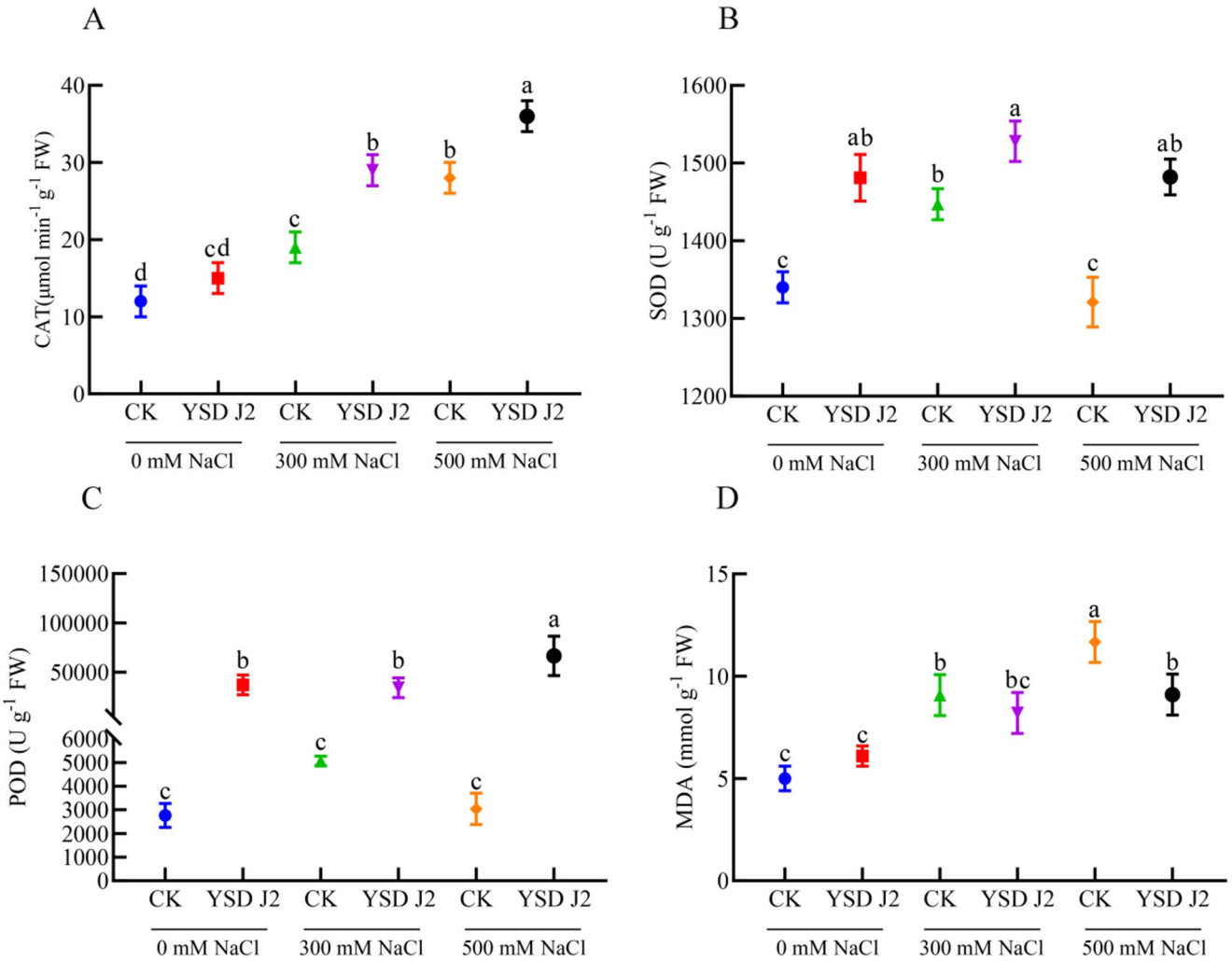


Fig. 8 Impact of inoculation with *Pantoea* sp. YSD J2 on antioxidative enzymatic activities (per fresh weight, FW). (**A**) catalase (CAT) activity; (**B**) superoxide dismutase (SOD) activity; (**C**) peroxidase (POD) activity; (**D**) malondialdehyde (MDA) content. Tigernut plants were grown under three NaCl concentrations (0, 300, and 500 mM) and two bacterial treatments (CK and YSD J2) with $n = 5$ replicates per treatment combination (1 seedling/pot). Different letters indicate significant differences at $p \leq 0.05$ within the treatments at each NaCl concentration or among NaCl concentrations for each treatment. Data are presented as mean ± standard error (SE)

selectivity in tigernut, reducing Na^+ toxicity while preserving essential K^+ and Ca^{2+} uptake under salt stress.

3.10 Principal Component Analysis

To systematically assess YSD J2's effects on salt tolerance mechanisms, we conducted PCA and cluster analysis of key physiological parameters. The PCA results showed that the PC1 and PC2 accounted for 79.7% of the total variance, effectively capturing the major response patterns (Fig. 10A). PC1 exhibited strong positive loadings for photosynthetic pigments (Chl a, Chl b, total Chl) and growth parameters (shoot weight, root weight), while PC2 was predominantly associated with stress response indicators (CAT activity, proline, and soluble protein content). Under non-stress conditions (0 mM NaCl), YSD J2-inoculated plants displayed markedly higher PC1 scores for photosynthetic and growth-related traits compared to controls. Under salt stress conditions (300–500 mM NaCl), a distinct response pattern emerged in inoculated plants. These plants showed negative PC1 scores for MDA content, coupled with positive PC2 scores for antioxidant enzymes and osmoprotectants. This pattern indicates a coordinated physiological response involving both oxidative stress mitigation and osmotic adjustment. Cluster analysis further supported these findings, with YSD J2-treated plants forming clearly separated groups characterized by enhanced antioxidant enzyme activities (POD, SOD, CAT), elevated osmoprotectant levels (proline, SP), and reduced lipid peroxidation (MDA content) (Fig. 10B). The clustering patterns were particularly pronounced under moderate to high salinity conditions (300–500 mM NaCl), demonstrating the treatment's effectiveness under stress

conditions. The multivariate analyses demonstrate that YSD J2 inoculation coordinately regulates multiple physiological pathways to enhance salt tolerance in tigernut, particularly under moderate-to-high salinity conditions.

4 Discussion

Pantoea sp. YSD J2 has been previously identified as a promising candidate for biofertilizer development (Wang et al. 2022). This study aimed to evaluate its effectiveness in enhancing the growth, physiological, and antioxidant characteristics of tigernut under salt stress.

Our research highlights *Pantoea* sp. YSD J2's remarkable resilience to salt and its capacity to enhance growth. YSD J2 was selected based on its cell morphology, increased biomass, and plant root hair structure under varying NaCl concentrations (Figs. 2 and 3, and 5). Previous studies have shown that certain microbial strains can withstand high salt stress. For example, *Bacillus licheniformis* MN718157 and *Aspergillus violaceofuscus* MH220545, known for their high salt tolerance, exhibited peak biomass at 50 mM and 100 mM NaCl, respectively (Muthuraja et al. 2022). Similarly, *Azospirillum brasilense*, which enhances plant salt resistance, achieved its highest biomass at 100 mM NaCl (Checchio et al. 2021). Our results suggest that YSD J2 exhibits even greater tolerance potential.

Pantoea sp. YSD J2 showed potential for endophytic colonization in plant tissues (Fig. 4), with rhizosphere colonization consistently exceeding internal tissue colonization. This observation aligns with previous studies on *Bacillus* spp. by Coy et al. (2019) and Durham et al. (2013). The observed increase in rhizosphere CFU under 300–500 mM NaCl in both non-inoculated and inoculated treatments suggests a salt-mediated enrichment of stress-adapted microbiota. In non-inoculated plants, this likely reflects salt-triggered release of endogenous microbes from plant tissues into the rhizosphere, combined with stress-stimulated root exudation that supports microbial proliferation (Fu et al. 2025). Genomic analysis of YSD J2 identified several putative stress-adaptive gene clusters that may contribute to its colonization advantage under salt stress conditions (Wang et al. 2022). These include siderophore biosynthesis genes (*entF*, *entE*, *entB*, *entA*, *entC*), phosphate solubilization genes (*phnG*, *phnH*, *phnI*, *phnJ*, *phnL*, *phnM*, *phnP*, *pat*), IAA synthesis genes (*ipdC*, *aldH*, *aldE*, *iaaH*), spermidine production genes (*speE*, *speA*, *speB*, *speC*, *speD*, *speG*), and trehalose synthesis genes (*otsA*, *otsB*, *treZ*, *treA*, *treB*, *treC*). While these genetic elements theoretically could explain the strain's superior stress tolerance and colonization capacity (Zeng et al. 2017; Glick et al. 2014; Domínguez-Ferreras et al. 2009), it must be emphasized that these functional predictions remain provisional. Future studies could provide direct validation through targeted gene knockout/complementation or stress-responsive transcriptomics. Nevertheless, the observed colonization patterns align conceptually with PGPE strategies where endophytes enhance host stress resilience while gaining ecological advantages (Gagné-Bourque et al. 2015; Santoyo et al. 2016; Grover et al. 2011). The successful re-isolation of YSD J2 from surface-sterilized plants and its maintained colonization under salt stress provide phenotypic evidence supporting its potential as a stress-tolerant PGPE, though the precise molecular mechanisms require further elucidation.

Pantoea sp. YSD J2 demonstrated significant potential to secrete multiple PGP substances under varying NaCl concentrations, as shown in Table 1, with peak production of IAA, P, K, siderophores, and EPS observed at 500 mM NaCl. PGP bacteria are well-known for producing phytohormones such as auxins, EPS, and siderophores, which play critical roles in enhancing plant development and stress tolerance (Durán et al. 2014). However, previous studies have shown that high salt concentrations often hinder the biosynthesis of these metabolites (Nadeem et al. 2016), though YSD J2 exhibited resilience by maintaining robust PGP activity under moderate salt stress, a trait consistent with other salt-tolerant *Pantoea* strains. Under 500 mM NaCl conditions, YSD J2-inoculated seedlings displayed improved growth, root hair structure and density compared to non-inoculated controls, likely due to synergistic mechanisms: IAA promoted root hair density and elongation, siderophores alleviated iron limitation while competing with pathogens, EPS enhanced soil-water retention to improve osmotic stress tolerance, and P and K solubilization genes increased the bioavailability of insoluble nutrients in saline soils. These findings align with previous research on *Pantoea* spp., particularly *P. agglomerans* strains, which have been shown to enhance plant growth under salt stress through multiple mechanisms, including IAA production, P and K solubilization, and EPS synthesis (Cherif-Silini et al. 2019; Gond et al. 2015; Sun et al. 2020). Recent studies have further supported these findings, highlighting the potential of *P. agglomerans* in mitigating salt stress through similar physiological processes (Lu et al. 2021; Gupta et al. 2023; Nawaz et al. 2023). Collectively, YSD J2's ability to sustain PGP substance secretion, nutrient mobilization, and stress resilience under salt stress highlights its potential as an effective biofertilizer agent for promoting plant growth in saline environments.

Our in *vivo* study demonstrates that YSD J2-inoculated plants under salt stress exhibited significant improvements in growth parameters, including RWC, number of tillers, and shoot/root biomass, while showing mitigated chlorophyll loss in stems compared to uninoculated controls.

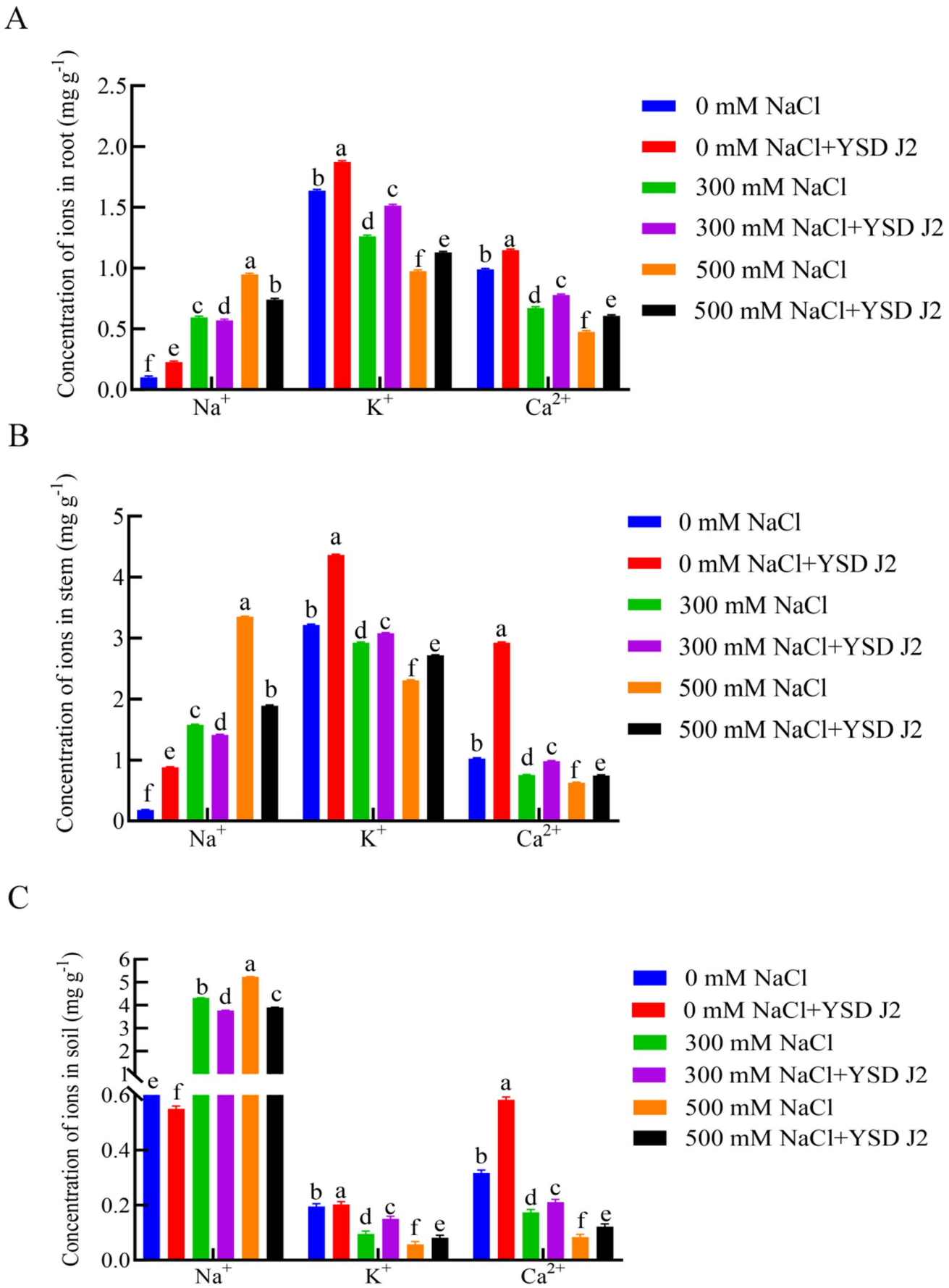

A
Concentration of ions in root (mg g-1)
2.5
2.0
1.5
1.0
0.5
0.0
Na+
K+
Ca2+
0 mM NaCl
0 mM NaCl+YSD J2
300 mM NaCl
300 mM NaCl+YSD J2
500 mM NaCl
500 mM NaCl+YSD J2
B
Concentration of ions in stem (mg g-1)
5
4
3
2
1
0
Na+
K+
Ca2+
0 mM NaCl
0 mM NaCl+YSD J2
300 mM NaCl
300 mM NaCl+YSD J2
500 mM NaCl
500 mM NaCl+YSD J2
C
Concentration of ions in soil (mg g-1)
6
5
4
3
2
1
0.6
0.4
0.2
0.0
Na+
K+
Ca2+
0 mM NaCl
0 mM NaCl+YSD J2
300 mM NaCl
300 mM NaCl+YSD J2
500 mM NaCl
500 mM NaCl+YSD J2

Fig. 9 Impact of inoculation with *Pantoea* sp. YSD J2 on the mineral ion concentration of different plant tissues. (**A**) concentration of ions in the roots; (**B**) concentration of ions in the stems; (**C**) concentration of ions in the soil. Tigernut plants were grown under three NaCl concentrations (0, 300, and 500 mM) and two bacterial treatments (CK and YSD J2) with $n=5$ replicates per treatment combination (1 seedling/pot). Different letters indicate significant differences at $p \leq 0.05$ within the treatments at each NaCl concentration or among NaCl concentrations for each treatment. Data are presented as mean ± standard error (SE)

These findings align with the *Pantoea* genus' established ability to alleviate abiotic stress through PGP activities (Lorenzi et al. 2022; Luziatelli et al. 2020a, b). The accumulation of proline, soluble proteins, and soluble sugars in YSD J2-inoculated plants likely results from multiple mechanisms. Proline biosynthesis genes (*P5CS*, *P5CR*) may enhance proline synthesis, stabilizing cellular structures and scavenging ROS (Szabados and Savouré 2010). Proline's protective roles in maintaining cellular integrity and mitigating oxidative stress under salt stress have been well-documented (Hayat et al. 2012). Stress-responsive genes encoding LEA proteins and HSPs could promote soluble protein accumulation, enhancing cellular stability (Battaglia et al. 2008). LEA proteins and HSPs are known to stabilize cellular structures and prevent protein denaturation under stress conditions (Jacob et al. 2017). Trehalose synthesis genes (*otsA*, *otsB*, *treZ*) and sucrose metabolism may mediate soluble sugar accumulation, maintaining osmotic balance and energy supply. Additionally, phosphate solubilization genes (*phnG*, *phnH*) and IAA biosynthesis genes (*ipdC*, *aldH*) likely support stress tolerance by improving nutrient uptake and root development. While these mechanisms provide plausible explanations for YSD J2's osmoprotective effects, their specific regulatory pathways and gene expression profiles require further experimental validation. Future studies could focus on profiling these genes and pathways to comprehensively elucidate YSD J2's stress adaptation strategies.

Our research reveals that YSD J2 effectively modifies ion absorption under salt stress conditions. Inoculation with YSD J2 significantly reduced Na^+ levels in the soil, roots, and stems of tigernut across various NaCl concentrations, while enhancing K^+ and Ca^{2+} absorption under both stress-free and NaCl-stress conditions. These findings align with previous studies on PGP bacteria that sustain ion balance in saline environments (Martynenko et al. 2022; Reddy et al. 2017). For instance, *P. agglomerans* was shown to increase the K^+/Na^+ ratio by upregulating ion transport genes (Shilev et al. 2020). YSD J2's ability to reduce Na^+ absorption and enhance K^+ uptake may be linked to its EPS synthesis, which binds Na^+ and limits its availability for plant uptake (Amna et al. 2019). The imbalance in K^+/Na^+ and Ca^{2+}/Na^+ ratios caused by excessive Na^+ accumulation can lead to plant toxicity and oxidative membrane damage due to elevated ROS production (Basu et al. 2023). YSD J2 inoculation improved the K^+/Na^+ ratio, reduced MDA levels, and enhanced the activities of CAT, SOD, and POD, indicating improved antioxidant capacity and membrane stability. This suggests that YSD J2 may induce the expression of antioxidant defense genes, effectively scavenging ROS and reducing oxidative damage. Similar effects were observed with *P. oryzihabitans* AXSao6 and *P. knackmussii* MLR6, which enhanced antioxidant enzyme activities and reduced electrolyte leakage under salt stress (Mellidou et al. 2021; Rabhi et al. 2018). The NaCl concentrations applied in this study were intentionally set higher than typical saline agricultural conditions (20–100 mM) to simulate extreme salt stress and assess YSD J2's efficacy under severe salinity. The results demonstrate YSD J2's potential as a promising candidate for enhancing plant growth under high salinity. Nevertheless, further validation under field conditions, with lower and more variable salinity levels, is essential to establish its practical applicability in agricultural settings.

5 Conclusions

This study demonstrates the potential of *Pantoea* sp. YSD J2 in promoting tigernut growth under salt stress by enhancing biomass accumulation, maintaining ion homeostasis, increasing chlorophyll content, and boosting antioxidant activity, while significantly reducing lipid peroxidation. YSD J2's remarkable salt tolerance and effective colonization capabilities underscore its potential as a biofertilizer for alleviating abiotic stress. However, the study's reliance on controlled laboratory conditions and the lack of detailed molecular insights limit its immediate applicability in real-world agricultural settings. Future investigations should prioritize field trials, mechanistic studies, and exploration of synergistic interactions with other plant growth-promoting agents to fully harness YSD J2's potential for sustainable agriculture.

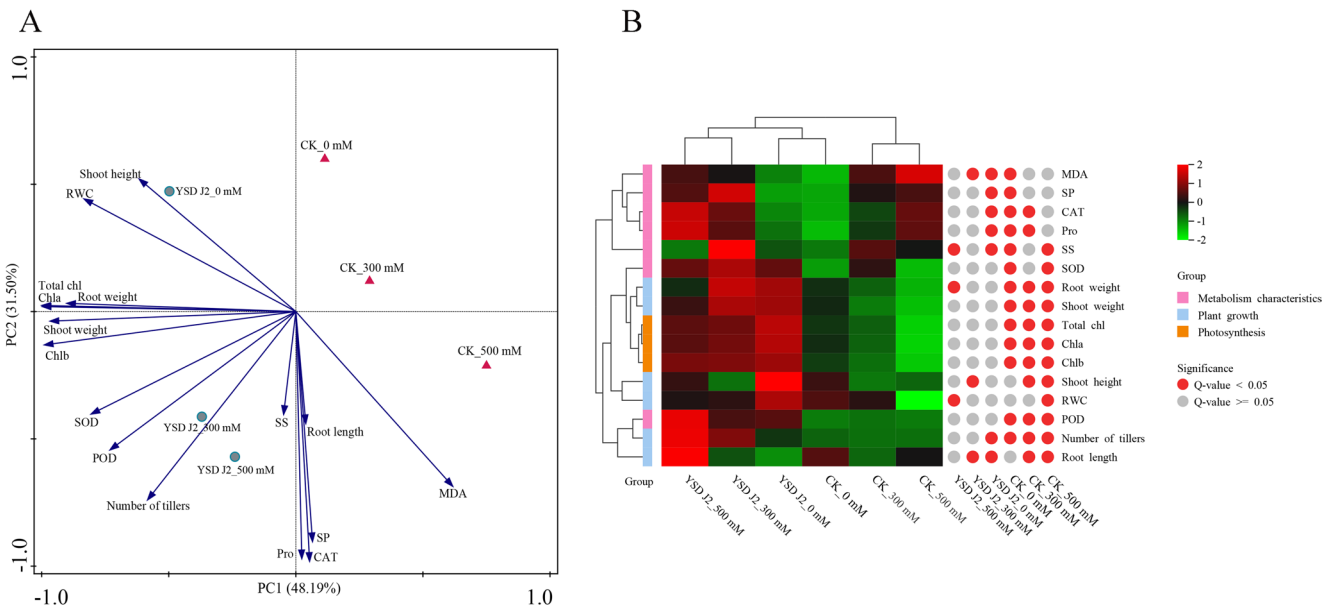


Fig. 10 Principal component analysis (PCA) and cluster analysis used to assess NaCl tolerance of plants enhanced by the strain *Pantoea* sp. YSD J2, based on the growth and metabolism of tigernut. Growth characteristics included shoot height, root length, shoot weight, root weight, chlorophyll a (Chl a), chlorophyll b (Chl b), relative water content (RWC) and number of tillers. Metabolism characteristics included osmotic stress-related metabolites (proline, soluble sugar, soluble protein), lipid peroxidation products (MDA) and antioxidant enzymes, i.e., peroxidase (POD), superoxide dismutase (SOD), catalase (CAT). (A) the markers "CK_0 mM", "CK_300 mM", "CK_500 mM", "YSD J2_0 mM", "YSD J2_300 mM", and "YSD J2_500 mM" indicate different salt and strain *Pantoea* sp. YSD J2 treatments. (B) the color key (green to red) represents the degree of enrichment as z-scores

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Data Availability All data generated or analyzed during this study are included in this paper.

Declarations

Conflict of Interest The authors declare no conflicts of interest.

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