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Effect of combined treatment with *Bacillus amyloliquefaciens* H-2-5 and illite on mitigating salt stress in *Panicum miliaceum*

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Abstract

Soil salinity is a significant environmental challenge that negatively affects crop yield. Growth-promoting bacteria (GPB), such as *Bacillus amyloliquefaciens* H-2-5, offer a promising biological approach to enhance plant tolerance under saline conditions. This study investigates the beneficial role of *B. amyloliquefaciens* H-2-5, in combination with the clay mineral illite, in alleviating salt stress in *Panicum miliaceum*. Under 150 mM NaCl stress, the combined treatment significantly improved plant growth, increasing shoot and root lengths by 44.3% and 40.9%, respectively, compared to untreated stressed plants. The treatment enhanced relative water content (RWC) (18.8%) and chlorophyll concentration (36.3%), indicating improved water status and photosynthetic capacity. Moreover, elevated levels of flavonoids (57.1%) and polyphenols (27.3%) reflected the stimulation of antioxidant responses, while reduced superoxide dismutase (SOD) and DPPH activities suggested lowered oxidative damage. Elemental analysis revealed reduced sodium uptake (35.7%) and increased potassium accumulation (20.6%), resulting in a more favorable Na⁺/K⁺ ratio. These findings highlight the significant contribution of *B. amyloliquefaciens* H-2-5 as a GPB that not only promotes plant growth but also strengthens physiological and biochemical mechanisms against salt-induced stress, supporting its potential as a sustainable tool for improving crop resilience in saline environments.

Keywords *Bacillus amyloliquefaciens* H-2-5, Proso millet, NaCl stress, Antioxidants, DPPH, Ion homeostasis

Introduction

Salinity is one of the important abiotic stresses that adversely affect crop production and global food security. At present, over 62 million hectares, or 20% of the world's agrarian farmland, is affected by salinity, with projections indicating a potential increase of 50% by 2050 [1]. Salt stress disrupts a range of physiological, metabolic, and biochemical processes in plants including photosynthesis, respiration, and transpiration [2]. The high concentrations of salt affect the osmotic balance, thereby impairing water uptake by plant roots [3]. In addition, salt stress triggers osmotic and oxidative stress, due to the formation of reactive oxygen species (ROS). These ROS can

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cause oxidative damage by oxidizing proteins, deactivating enzymes, peroxidizing lipids, altering nucleic acids, and triggering programmed cell death (PCD) [4]. To mitigate salt stress, plants regulate their hormone concentration, produce osmolytes, and regulate the K^+/Na^+ ratio, crucial for stomatal conductance and nitric oxide (NO) signaling. Furthermore, Plants may also modify photosynthetic activity and enhance the activity of antioxidant enzymes to improve salt resilience [5].

Proso millet (*Panicum miliaceum* L.), small-seeded (grass resemble) crops, is usually grown in arid and semi-arid regions [6]. However, they are an essential staple in diverse geographical areas as food and fodder for humans and livestock [7]. Due to their resilience to climatic variations, they can grow in different environmental conditions. As compared to other grains, they contain micro and macronutrients and bioactive components [7]. Their resistance to different abiotic stresses such as drought, salinity, heat and light renders them ideal for investigating stress-responsive traits at the physiological, cellular and molecular levels [8]. Therefore, it is necessary to create strategies to improve crop tolerance. Sustainable agricultural production depends on the selection and promotion of salt-tolerant plants. The reclamation of saline soils using traditional and chemical methods is both time-consuming and costly [9]. Due to the significance of proso millet for food security, scientists are investigating solutions, such as bioaugmentation with plant growth-promoting rhizobacteria (PGPR) and the application of illite to alleviate salt stress in proso millet plants. PGPR inhabits the rhizospheric zone and can mitigate the adverse effects of salinity and enhance agricultural productivity by maintaining ionic balance, turgor pressure and osmotic stability, and soil fertility in salt-affected areas [10]. PGPR contribute to plant development through nitrogen fixation, reduced ethylene levels, synthesis of phytohormones and siderophores, provision of pathogen defence, and nutrient solubilisation [11]. Halotolerant PGPR supports plant growth by producing exopolysaccharides (EPS) and osmolytes, as well as by enhancing the activity of antioxidant enzymes and proteins [12], thereby regulating Na^+ accumulation and ROS in saline environments [13]. Soils contain a variety of clay minerals. Illite, a clay mineral, is particularly beneficial for agriculture because of its high ion exchange properties and nutrient retention capabilities [14]. The clay mineral illite is characterized by its 2:1 crystal structure and the molecular formula ' $(K_{1-1.5}Al_4[Si_{7-6.5}Al_{11-1.5}O_{20}](OH)_4$ ', which is notable for its high potassium content [15, 16]. These rhizobacteria facilitate root development and exudate secretion, integral to weathering activities. Exudates from roots promote bacterial proliferation and indirectly aid in the liberation of nutrients from minerals [17]. Applying weathering bacteria to soil or plants,

mainly when used with crushed rocks, can enhance soil fertility and increase crop yields. This method is more sustainable than conventional fertilization techniques and may contribute to reducing the negative impact of agriculture on climate change [17]. Many studies are available on PGPR, but the combined application of PGPR and illite is insufficient. Keeping this view in mind, this study aimed to evaluate the synergistic effects of *B. amyloliquefaciens* H-2-5 and illite on *Panicum miliaceum* under salt-stress conditions. The research evaluated key morphological and physio-biochemical responses by modulating plant growth traits, chlorophyll, antioxidants, and ion homeostasis to determine their potential for enhancing crop resilience in saline conditions.

Materials and methods

Isolation, identification, and screening of PGPR's isolate with seed germination assay

The bacterial isolates utilised in this study, specifically *B. amyloliquefaciens* H-2-5, *B. tequilensis* (KNPO3), and *Acinetobacter pittii* YNA40, were previously isolated, characterised, and thoroughly documented in our previous study [18–20]. Initially, proso millet seeds (Ibaekchal millet, Danong Co., Ltd.) were sterilised with 2.5% NaOCl and subsequently immersed in the broths of the three selected bacterial isolates at 10^8 CFU/mL concentration. In contrast, the control petri dish was prepared using only distilled water and LB medium. Following a 6-hour shaking incubator, approximately 20 seeds were transferred onto a 'double-layered' sterile filter paper within a Petri dish. The seeds were then allowed to germinate and grow for 7 days under controlled conditions '(14 h light/10 h dark, 28°C/24°C, 60% relative humidity, and a light intensity of $250 \mu\text{mol m}^{-2} \text{s}^{-1}$)'. The total length of seedlings was measured on the 10th day.

Experimental design and treatment combination

The investigation was performed in a greenhouse (controlled conditions) at Kyungpook National University, Republic of Korea. *B. amyloliquefaciens* H-2-5 isolates were cultivated in Luria-Bertani (LB) medium for five days at 30 °C in a shaking incubator at 200 rpm and the culture was adjusted to 10^8 CFU/mL. Proso millet seeds were subjected to surface sterilisation using the previously described method. One hundred seeds were sown in small pots containing autoclaved horticultural soil and kept in a greenhouse chamber where the temperature was consistently maintained at 30 ± 2 °C. The soil composition included peat moss '(13–18%), perlite (7–11%), coco-peat (63–68%), 90 mg/kg NH_4^+ , 205 mg/kg NO_3^- , 350 mg/kg P_2O_5 , and 100 mg/kg K_2O ', with a pH range of 5–7, and electrical conductivity not exceeding 1.2 S/m. The experimental treatments were organised into two main categories: (A) No-stress: (i) Control (Only distilled

water), (ii) Illite, (iii) H-2-5, (iv) Illite + H-2-5 (**B**) Salt stress: (i) Control (150 mM NaCl), (ii) Illite, (iii) H-2-5, (iv) Illite + H-2-5. After two weeks, the illite group was treated with 2 g of illite per pot. The microbial treatment involved diluting the culture medium, incubating at 30 °C for three days, and applying 50 mL/pot through soil drenching. Seven days later, salt stress was induced by administering 50 mL/pot of 150 mM NaCl for five days. On the 28th day, plant growth parameters were evaluated before harvesting. The samples were then stored at -80 °C for further biochemical analysis.

Measurement of plant growth traits, leaf relative water content (RWC), chlorophyll and flavonoid content

Plant morphological characteristics, including shoot and root lengths and fresh biomass, were evaluated using a measuring scale and a digital balance. RWC was determined according to the method outlined in [21]. Firstly, fresh leaf samples were randomly selected, and their fresh weights (fw) were recorded. The leaves were then immersed in distilled water for 6–8 h to determine their turgid weights (tw), followed by a drying oven at 75–80 °C for 6–8 h. The RWC was calculated using the equation:

$$\text{RWC} = (\text{fw} - \text{dw}) / (\text{tw} - \text{dw}) \times 100$$

Leaf chlorophyll content was assessed using the method described by Butts et al. [22] using a CCM-300 Chlorophyll Content Meter (Opti-Sciences, USA). The ratio of the red fluorescence emission at 700 nm to the far-red fluorescence at 735 nm was measured.

Total flavonoid content was determined using a previously described method [23]. Leaves were extracted with 80% MeOH and crude extract (100 µL) combined with a solution containing MeOH (500 µL), aluminium chloride (50 µL) (10%), 50 µL NaOH (1 M), and distilled H₂O (300 µL) for 30 min (room temperature). Total flavonoid content was assessed spectrophotometrically (510 nm), with quercetin serving as the standard.

Quantification of antioxidants (SOD, polyphenol) and DPPH scavenging activity

The enzymatic activity of superoxide dismutase (SOD) was assessed using a modified method [24], and the SOD activity was measured using the equation: $[(1 - (A - B/C)) \times 100]$. The methodology outlined by Adhikari et al. [25] was slightly modified to assess the DPPH free radical scavenging activity. The 0.05 g freeze-dried ground sample was shaken with 100% methanol for 6 h and centrifuged. 0.1% of freshly prepared DPPH was mixed with the resulting supernatant and incubated in the dark at room temperature for 30 min. The absorbance was measured at 517 nm using a spectrophotometer (Multiskan

GO; Thermo Fischer Scientific, Finland). The DPPH radical scavenging was calculated using the formula, $[1 - (X1 - X2) / X3] \times 100$, 'where X1 is the absorbance of the sample extract with DPPH, X2 is the absorbance of the sample extract with methanol, and X3 is the absorbance of DPPH with methanol'.

Additionally, the total polyphenol content was quantified using the protocol by Adhikari et al. [25]. A freeze-dried ground sample (0.05 g) was diluted with absolute MeOH and centrifuged, and the supernatant was combined with 1 ml of 2% Na₂CO₃. A 50 µl of Folin-Ciocalteu reagent (1 N) was added and incubated in the dark condition for 30 min (room temperature). Absorbance was measured using a spectrophotometer (Multiskan GO; Thermo Fischer Scientific) at 750 nm with gallic acid as the standard.

Elemental composition analysis

To assess the elemental composition of the plant samples, the method described in [26] was employed. Finely powdered freeze-dried samples (0.5 g) were digested with 2 mL of nitric acid (100%), and the resulting solution was subjected to a series of procedures which include heating, evaporation, and filtration. The resultant solution was subsequently analysed using inductively coupled plasma emission spectroscopy (Optima 7900DV, PerkinElmer, USA).

Statistical analysis

The experiments were conducted individually three times, each with three replications. All measurements were performed independently across biological replicates, and data from each replicate were collected and analyzed separately without pooling. This approach was used to ensure accurate estimation of variability and statistical significance. One-way analysis of variance (ANOVA) was performed using OriginPro 2024 software to assess the differences among treatments, followed by Tukey's HSD test at a significance level of $p < 0.05$. Graphical visualizations were created using GraphPad Prism (v. 9.5.1, San Diego, CA, USA).

Results

Screening of different bacterial isolates based on their seedling length

In the germination assay conducted on proso millet, three bacterial isolates were used: such as *B. amyloliquefaciens* H-2-5, *B. tequilensis* (KNPO3), and *Acinetobacter pittii* YNA40 were applied to the petri dishes. As illustrated in Fig. 1, these isolates markedly enhanced the total seedling length of the germinated seed compared to that of the control group, which was not inoculated with any bacterial isolates. Notably, *B. amyloliquefaciens* H-2-5 exhibited the most pronounced effect, increasing

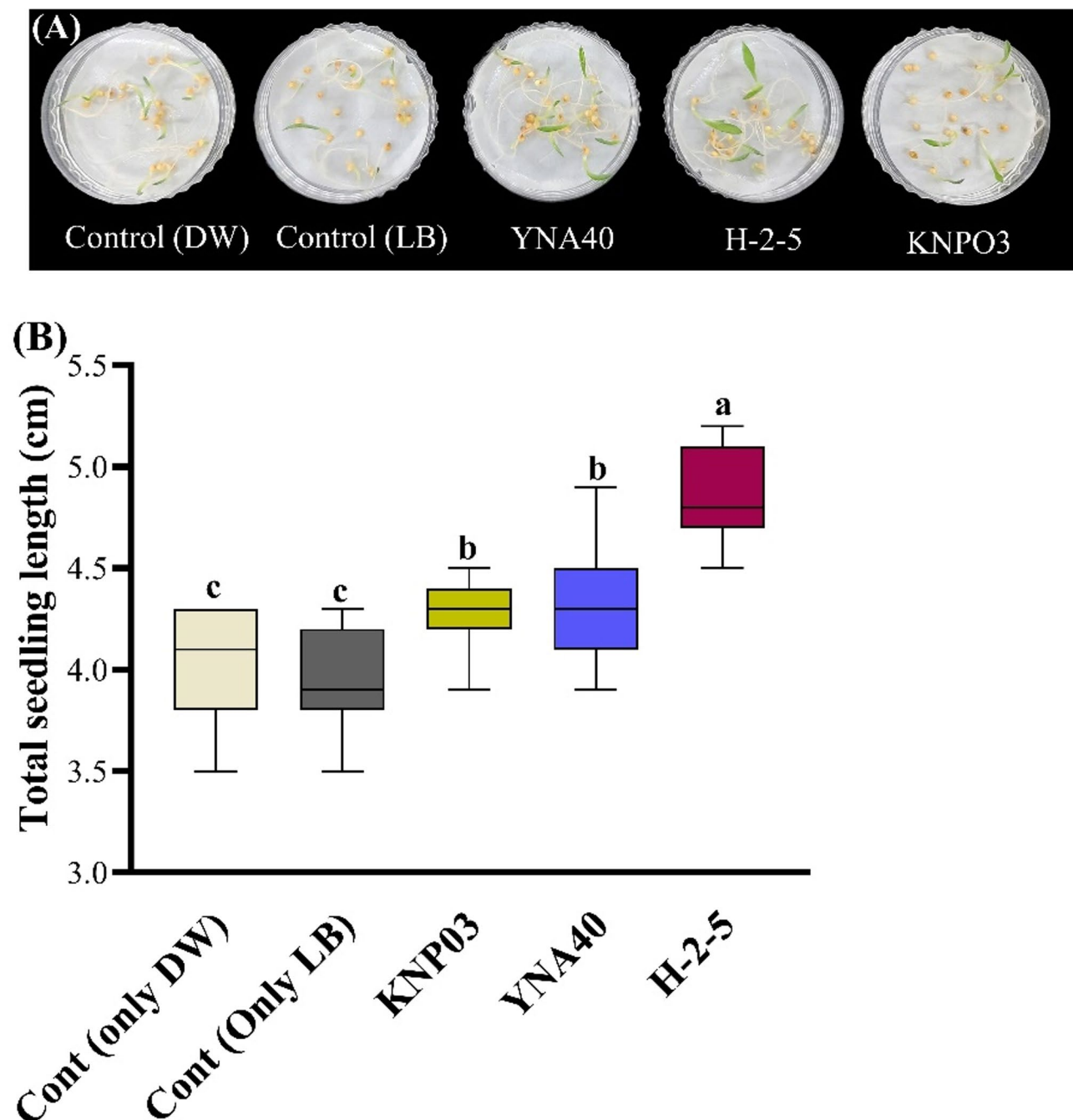


Fig. 1 The effects of three distinct bacterial isolates on the germination of proso millet. **(A)** Seeds were in Petri dishes, and the **(B)** total seedling length was evaluated. Data are expressed as means $\pm$ standard deviation ($n=3$). Statistical significance was determined using one-way ANOVA followed by Tukey's HSD test ($p<0.05$); different letters indicate significant differences among treatments

the total seedling length by 22.4% and 12.1%, compared to the controls treated with distilled water and (DW) and Luria- Bertani (LB) medium, respectively. Consequently, *B. amyloliquefaciens* H-2-5 was selected for further study because of its superior performance (highest seedling length).

Effect of illite and H-2-5 on the morphology of the proso millet plant under salt stress

Figure 2 illustrates the effect of illite and H-2-5 on the morphology of the proso millet plant exposed to salt stress. Without stress, application of illite, H-2-5, and their combination (illite + H-2-5) resulted in an increase in shoot length (SL) by 8.3%, 13.3%, and 17.4%, respectively, compared to non-stressed control (NC) plants

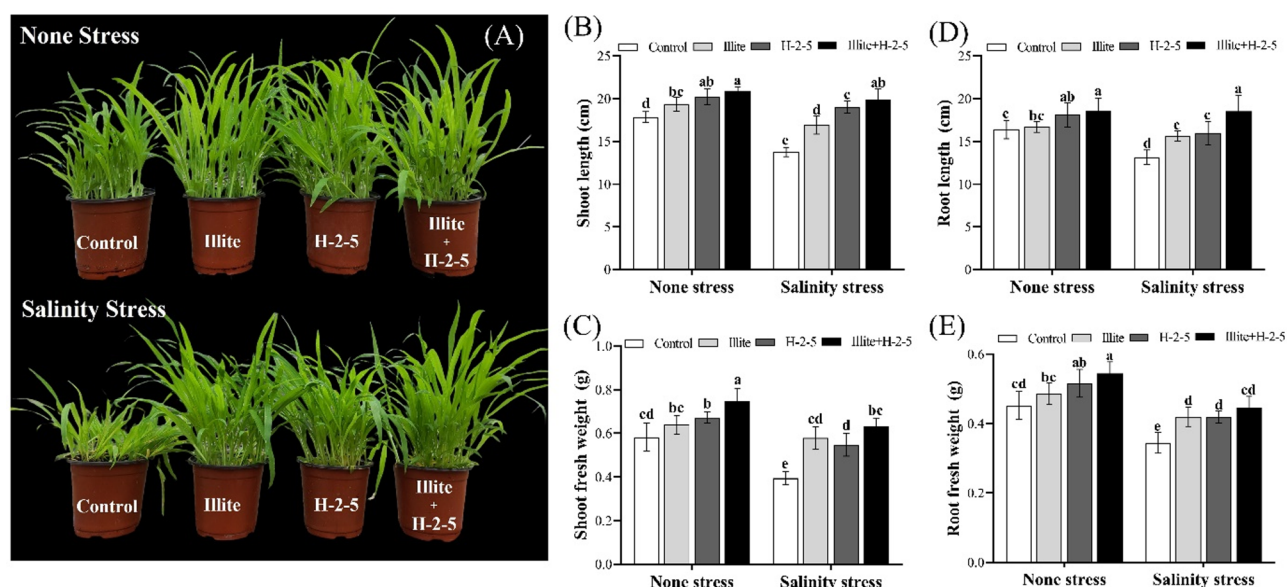


Fig. 2 The impact of illite and H-2-5 on proso millet plant under salt stress, showing various morphological aspects, such as (A) the representative appearance of the plant, (B) shoot length, (C) shoot fresh weight, (D) root length, and (E) root fresh weight. Data are expressed as means $\pm$ standard deviation ($n=3$). Statistical significance was determined using one-way ANOVA followed by Tukey's HSD test ($p < 0.05$); different letters indicate significant differences

(Fig. 2B). Conversely, salt stress led to a notable reduction in SL (22.9%) compared to the NC. However, under salt stress, the application of illite, H-2-5, and illite + H-2-5 significantly increased SL by 22.9%, 38.0%, and 44.3%, respectively, relative to the salt-stressed control (SC). Similarly, under non-stress conditions, illite, H-2-5, and illite + H-2-5 significantly increased root length (RL) by 1.95%, 10.6%, and 13.7%, respectively, compared to NC plants (Fig. 2D). In contrast, salt stress significantly decreased RL (19.6%) compared to NC plants. However, under salt stress, the application of illite, H-2-5 and illite + H-2-5 significantly enhanced RL by 18.7%, 21.4%, and 40.9%, respectively, compared to the SC. Without stress, illite, H-2-5, and illite + H-2-5 significantly increased shoot fresh weight by 10.0%, 15.5%, and 28.2%, respectively, compared to the NC plants. Under salt stress, the shoot fresh weight (SFW) was significantly reduced by 32.3% compared to that in the NC plants. However, following the application of illite, H-2-5 and illite + H-2-5, SFW enhanced considerably by 46.7%, 39.08%, and 59.9%, respectively, compared to the SC (Fig. 2C). Root fresh weight (RFW) increased after the aforementioned treatment by 7.5%, 14.1%, and 20.3%, respectively, compared to the NC plants. Conversely, salt stress significantly reduced the RFW (23.9%) compared to the NC plants. However, the application of illite, H-2-5 and illite + H-2-5 significantly improved the RFW by 22.1%, 22.0%, and 29.6%, respectively, compared to the SC plants (Fig. 2E).

Effect of illite and H-2-5 on the leaf RWC, chlorophyll content and antioxidative enzymatic activity

In stress-free environments, illite, H-2-5, and their combination (illite + H-2-5) did not produce significant changes in leaf RWC across the treatments (Fig. 3A). However, there was a 17.4% reduction in leaf RWC under salinity stress compared to the NC plants. Notably, illite, H-2-5, and illite + H-2-5 applications resulted in a substantial increase in leaf RWC by 8.4%, 15.8%, and 18.8%, respectively, compared to the SC plants (Fig. 3A). In the absence of stress, the chlorophyll content increased by 14.7%, 15.8%, and 15.7% following the application of illite, H-2-5, and illite + H-2-5, respectively, compared to the NC plants. Conversely, salinity stress significantly reduced this essential pigment by 28.3% compared to the NC plants. Nevertheless, compared to the SC plants, the treatments enhanced the chlorophyll content by 21.2%, 22.0%, and 36.3%, respectively (Fig. 3B). Under non-stressful conditions, applying illite, H-2-5, and illite + H-2-5 did not significantly alter flavonoid contents among the treatments. In contrast, salinity stress led to a considerable decline in the content of flavonoids (36.4%) compared to the NC plants. However, the treatments increased the flavonoid content by 35.71%, 42.8%, and 57.1%, respectively, compared to the SC plants (Fig. 3C).

Without stress, SOD content increased by 16.5%, 30.3%, and 29.1% following the application of illite, H-2-5, and illite + H-2-5, respectively, compared to the NC plants. In contrast, salinity stress significantly elevated SOD activity by 64.7% compared to the non-stress control plant. However, SOD activity was reduced by 6.7%,

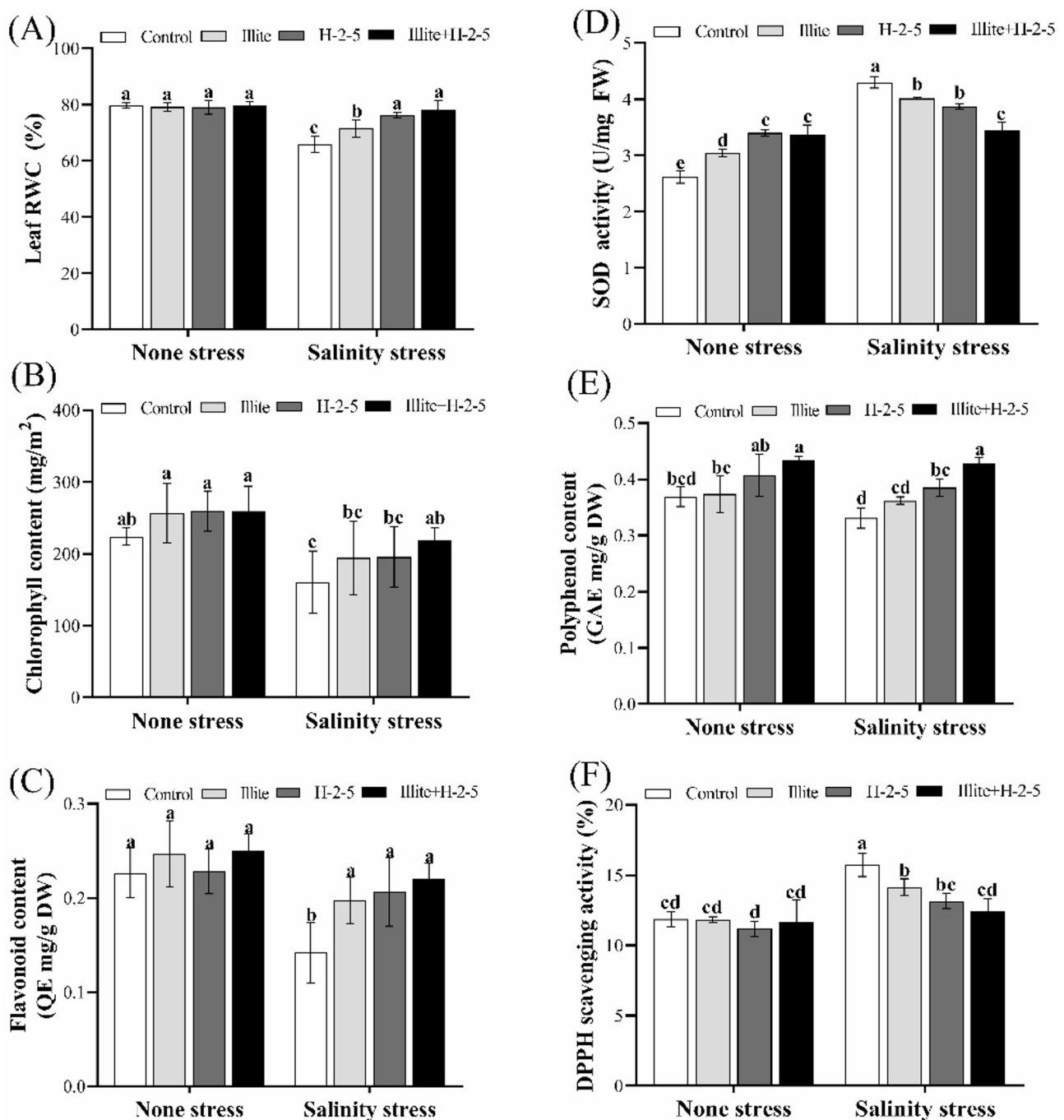


Fig. 3 Effect of illite and H-2-5 on (A) leaf RWC (B) chlorophyll content, (C) flavonoid content, (D) SOD activity, (E) polyphenol content and (F) DPPH scavenging activity under salt stress. Data are expressed as means $\pm$ standard deviation ($n=3$). Statistical significance was determined using one-way ANOVA followed by Tukey's HSD test ($p<0.05$); different letters indicate significant differences

10%, and 20.9%, respectively, compared to the SC plants (Fig. 3D). In stress-free environments, polyphenol content increased by 2.7%, 11.1%, and 19.4% after applying illite, H-2-5, and illite + H-2-5, respectively, compared to the NC plants. On the other hand, salinity stress significantly decreased polyphenol content by 8.3% compared to that in the NC plants. However, the treatments

enhanced polyphenol content by 9.09%, 15.1%, and 27.3%, respectively, compared to the SC plants (Fig. 3E). Without stress, DPPH scavenging activity remained unchanged after applying illite, H-2-5, and illite + H-2-5, compared to the NC plants. In contrast, salinity stress significantly increased this activity by 32.6% compared to the NC plants. However, the treatments decreased DPPH

scavenging activity by 10.0%, 16.4%, and 20.8%, respectively, compared to the SC plants (Fig. 3F).

Effect of illite and H-2-5 on the elemental content

Under stress-free conditions, introducing illite, H-2-5, and illite + H-2-5 did not lead to any notable changes in sodium (Na) content compared to the NC plants. However, there was a marked increase in Na content by 100% under salt stress conditions relative to the NC plants. Remarkably, the application of illite, H-2-5, and illite + H-2-5 resulted in a significant decrease in Na content by 14.3%, 28.6%, and 35.7%, respectively, compared to the SC plants (Fig. 4A). Without stress, applying illite, H-2-5, and illite + H-2-5 significantly boosted the potassium (K) content compared to the NC plants. Conversely, salt stress caused a significant reduction in K content (8.8%) compared to the NC plants. Nonetheless,

the application of illite, H-2-5, and illite + H-2-5 significantly increased the K content by 10.5%, 17.4%, and 20.6%, respectively, compared to the SC plants (Fig. 4B). Without salt stress, the application of illite, H-2-5, and illite + H-2-5 did not significantly alter the Na/K ratio compared to the NC plants. On the contrary, salt stress significantly increased this ratio by 200% compared to the NC plants. However, the application of illite, H-2-5, and illite + H-2-5 significantly reduced this ratio by 33.3%, 50%, and 66.7%, respectively, compared to the SC plants (Fig. 4C).

Discussion

The present study elucidated the synergistic effect of *B. amyloliquefaciens* H-2-5 and illite in mitigating salt stress by enhancing plant morpho-physiology and biochemical responses. Under salt stress, the observed synergistic

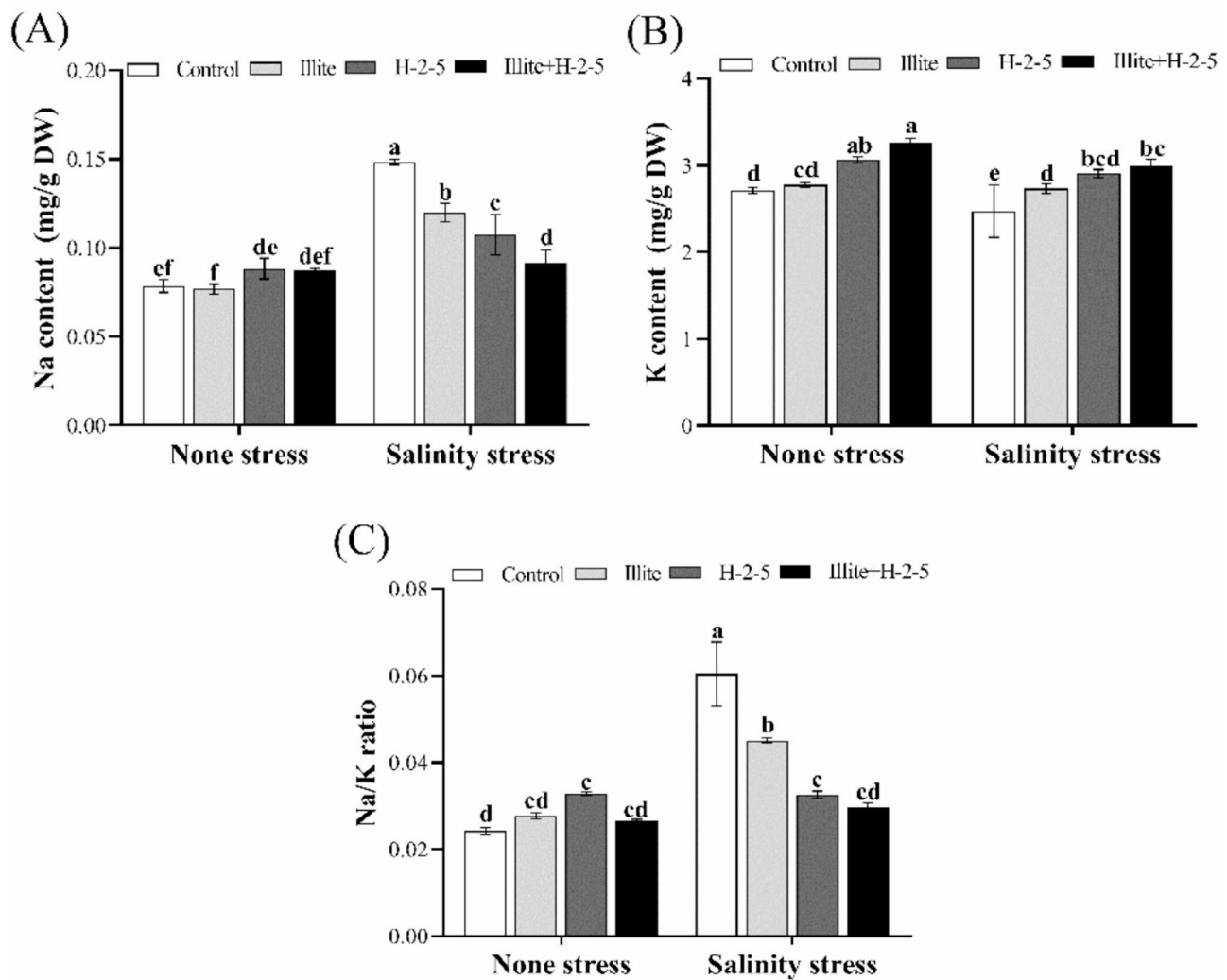


Fig. 4 Effect of illite and H-2-5 on the elemental content of (A) sodium; Na (B) potassium; K and (C) Na/K ratio under salt stress. Data are expressed as means $\pm$ standard deviation ($n = 3$). Statistical significance was determined using one-way ANOVA followed by Tukey's HSD test ($p < 0.05$); different letters indicate significant differences among treatments

interaction of H-2-5 and illite in enhancing plant morphology (i.e. SL, RL, SFW, RFW) is explained. Indole-3-acetic acid (IAA) and exopolysaccharides are produced by *Bacillus* spp. and enable root elongation and nutrient absorption [27]. A clay mineral, illite, has a high surface area and cation exchange capacity (CEC), which allows it to absorb and hold water and nutrients, improving root growth and overall development [28]. Additionally, inoculated plants displayed a decreased leaf RWC, reduced osmotic stress, increased water uptake, and improved growth and yield [29]. Interaction between H-2-5 and illite provided a more favourable rhizospheric environment for plants and improved the ability to withstand salt-induced osmotic stress.

Photosynthesis is a fundamental physiological process in plants that facilitates growth and enhances resilience to environmental stressors [30]. In this study, salt-induced stress resulted in a marked decrease in chlorophyll content. However, plants treated with H-2-5 and illite significantly increased photosynthetic efficiency under salt stress. This enhancement may be attributed to PGPR's modulation of stomatal conductance and enhance the synthesis of chlorophyll precursors [31]. The plant molecule flavonoids serve two functions in plants by promoting growth and development and also defending against both biological and environmental stresses [32]. The enhanced levels of flavonoids lead to increased secondary metabolite production that works as ROS scavengers to protect cellular structures against oxidative damage [33]. The application of H-2-5 along with illite treatment produced synergistic effects that increased flavonoid content in plants during salt stress conditions.

Plants depend on their antioxidant enzymes as they work to minimize the damage caused by ROS [34]. SOD stands as one of the primary enzymes which protects organisms from oxidative stress damage [35]. The addition of H-2-5 together with illite as a treatment reduced SOD enzyme activity. The ability of PGPR to decrease ROS production during salt stress leads to less antioxidant requirement which explains this phenomenon [20]. Plant resistance against ROS increases due to polyphenol accumulation because these strong antioxidants assist in osmotic stress tolerance by destroying toxic radicals and peroxides thus protecting plants from ROS effect [36]. The activities of biosynthetic enzymes enhance plant metabolite levels such as sugars, organic acids, phenols and amino acids through PGPR treatments [37]. Plant ROS levels decreased when H-2-5 and illite were used together under saline conditions according to DPPH scavenging activity measurements. This suggests the lower level of ROS production due to the combined application of H-2-5 and illite. A pivotal approach for mitigating salt stress in plants involves the regulation of ion homeostasis, with a particular emphasis on the Na/K

ratio. In addition to the observed reductions in ROS and antioxidant enzyme activity, several studies have reported that PGPR-induced stress tolerance is closely associated with the transcriptional activation of stress-responsive genes. Although our study did not directly measure gene expression levels, the physiological and biochemical improvements observed align with previous findings where *Bacillus* spp. enhanced salt stress tolerance by modulating key regulatory genes. For instance, inoculation with *Bacillus subtilis* was shown to upregulate SOD, CAT, and APX genes in *Zea mays*, leading to enhanced ROS scavenging capacity and reduced oxidative stress [38, 39]. Similarly, *B. amyloliquefaciens* treatment has been linked to the induction of P5CS (proline synthesis), RD29A (osmotic regulation), and SOS1 (Na⁺ efflux) in *Arabidopsis thaliana* and *Capsicum annuum* under saline conditions [40, 41]. PGPR are also known to influence ion homeostasis by stimulating the expression of high-affinity potassium transporters (HKT1) and vacuolar Na⁺/H⁺ antiporters (NHX1), helping to maintain a favorable Na⁺/K⁺ balance [42, 43]. These previously reported molecular effects are consistent with the improved Na⁺ exclusion, increased K⁺ uptake, and enhanced antioxidant capacity observed in our study. Therefore, it is likely that the application of H-2-5 and illite contributes to stress alleviation not only through direct biochemical modulation but also via activation of stress-responsive genetic pathways. Taken all together, this study demonstrated the combined use of H-2-5 and illite to effectively decrease Na accumulation while enhancing K content within plant tissues (Fig. 5). This adjustment in the Na⁺/K⁺ ratio is vital for maintaining cellular functions, as K plays a crucial role in enzyme activation, osmotic regulation and stomatal function [44]. PGPR assist in elevating the K⁺ level and sustains the K⁺/Na⁺ ratio by facilitating the removal of excess Na⁺ from cells in plants under salt stress [45]. EPS (exopolysaccharides) produced by PGPR during biofilm formation support plant survival under saline conditions. PGPRs are known to produce EPS, which helps plants to survive under saline conditions. Under salt stress, EPS binds with Na⁺ ions, thereby reducing the Na⁺ concentration around the root area and maintaining the ionic equilibrium. Additionally, EPS contribute to soil aggregation, water retention, and metal ions chelation in saline environments [46]. PGPR can also enhance the expression of high-affinity K transporters (HKTs), which preferentially take up K⁺ over Na⁺ [29]. Illite may further promote ion exchange in the soil, thereby reducing Na⁺ availability [28]. These findings highlight the potential of integrating PGPR and soil amendments, like illite, to improve crop resilience in saline environments.

The current study demonstrates that the combined application of *B. amyloliquefaciens* H-2-5, along with illite, serves as an eco-friendly salt stress mitigation

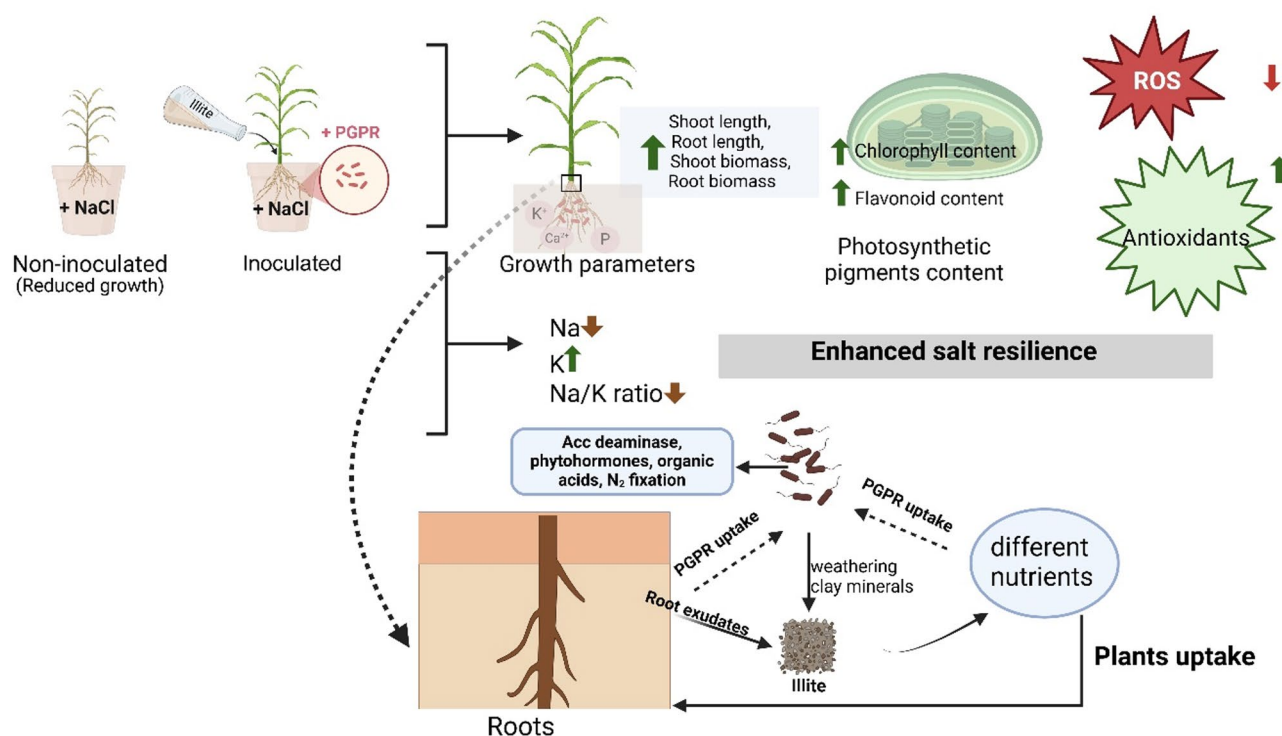


Fig. 5 The mechanistic interaction between *B. amyloliquefaciens* H-2-5 and illite under salt stress in proso millet is illustrated with arrows indicating changes in response. An upward green arrow indicates an increase, while a downward red arrow indicates a decrease

approach for proso millet cultivation. This integrated approach significantly improves plant growth, biomass development, physio-biochemical properties, and stress tolerance through increased chlorophyll levels and strengthened antioxidant capacity. These mechanisms facilitate ion regulation by enabling potassium absorption and reducing sodium ion uptake. *B. amyloliquefaciens* H-2-5, in conjunction with illite, offers a promising environmentally sustainable method to enhance crop production under saline environments. Field trials must be conducted across diverse environments to ensure their compatibility. Further study should focus on elucidating the molecular and genetic mechanisms by which PGPR and minerals assist plants in adapting to salt stress within salt-affected agricultural systems.

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Author contributions

S-M K & Md.I-U-H: Methodology, Investigation, Writing- original draft. J-I K, JR J, MY B & J-Y H: Investigation, Formal Analysis, Validation, Data Curation, Visualization, M-S L & I-J Lee: Manuscript Editing, Review. B-G M: Project administration, Review, Supervision.

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Data availability

Not applicable.

Declarations

Competing interests

The authors declare no conflict of interest.

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