Impact of Bio-inoculation with Halotolerant Rhizobacteria on Growth, Physiological, and Hormonal Responses of Durum Wheat Under Salt Stress

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Abstract

Soil salinity and water scarcity are major factors limiting plant productivity. Under these conditions, the use of plant growth-promoting rhizobacteria (PGPR) is a promising tool to mitigate the negative effects of stress in plants. Our study aims to determine the effect of two bacteria selected on the basis of their high halotolerance and ability to promote plant growth. Phylogenetic analysis and total 16S rDNA sequences showed that the first strain used (BSSM328) belongs to the genus *Halomonas*, and the second strain (BSSM27) belongs to the genus *Kushneria*. The effects of these two strains are evaluated by monitoring some growth (Shoot and Root length, Shoot and Root Dry Weight), physiological, biochemical, and hormonal (Abscisic acid, Jasmonic acid, Salicylic acid) parameters in the absence and presence of salt stress (100 and 200 mM NaCl). The results obtained showed that the growth of durum wheat was significantly stimulated by inoculation while salt inducedan inhibitory effect proportional to the severity of salt stress. Both strains significantly reduced proline levels, relative elec-trolyte leakage, and abscissic and jasmonic acid content when exposed to salt stress. Salicylic acid content varied with NaCl concentration and strain used. In contrast, they significantly increased total chlorophyll content and decreased carotenoid content. The reduction in total antioxidant capacity and malondialdehyde levels under these conditions was not significant. Both strains used showed great potential to suppress the effects of salt stress on durum wheat growth, with a superior effect for strain BSSM27. Therefore, they could be used as bio-inoculants to improve wheat performance and productivity in aridand semi-arid regions.

Keywords Durum wheat · PGPR · Salt stress · Phytohormones · Inoculation

Introduction

Due to climate change, salinity in the last decade has become a major problem in the world, especially in aridand semi-arid regions. Salinization in these regions is more critical due to decreased precipitation and increased temperatures resulting in high evaporation and transpira- tion, which cause the accumulation of salts in the soil. It is also caused by agricultural practices, including inadequate water management, excessive use of chemical fertilizers, and seawater intrusion **(**Jha et al. 2019**)**.

Approximately 2000 hectares per day of irrigated landin arid and semi-arid areas across 75 countries have been degraded by salinization over the past 20 years **(**IAEA2021**)**.Currently, estimations indicate that 20–50% of irri- gated soils worldwide are affected by salinization **(**FAO 2022**).**

In the Mediterranean region, soils affected by salinityare estimated at 1 million ha and it is the main cause of desertification **(**Machado and Serralheiro 2017**)**. Algeriais one of the Mediterranean countries where agricultural land is largely affected by this phenomenon of salinization **(**Boudibi et al. 2021**)**.

Salinity has a negative impact on germination, growth and development of plants, leading to losses in produc- tivity (Hernández 2019). It acts mainly by imposing an osmotic stress which appears from the beginning of the stress and which has an immediate effect on the avail- ability of water, thus, limiting its absorption, leading toa dehydration of the plant and a loss of cellular turgor (Acosta-Motos et al. 2017). Salinity also acts through its ionic component, which occurs later. In fact, the accu-mulation of Na^+ and Cl^- ions in the tissues, in additionto their toxicity, leads to a disturbance of mineral nutri-tion. The consequences of this ionic and osmotic stress are reflected in the development of secondary oxidative stress (Narsing Rao et al. 2019).

In Algeria, durum and soft wheat, as well as barley, are essential food resources for the population. According to statistics from the Algerian Ministry of Agriculture and Rural Development (MADR 2017), the area reserved for cereal cultivation was 3.3 million hectares. The produc-tion of durum wheat using 40% of these areas is currently insufficient due to soil and climatic conditions associated with poor control of cultivation techniques.

Compared to other cereals, durum wheat is considereda sensitive plant to salt stress (Munns and Gilliham 2015). Indeed, salt has adverse negative effects on the growth anddevelopment of durum wheat. This is mainly due to theirinability to exclude NaCl from their tissues (Roy et al. 2014). To improve crop productivity, farmers often use chemi- cal fertilizers, which, by accumulating in the soil, intensify its salinization. The current, cheaper, and ecological alternative is the use of rhizosphere bacteria that have the ability to reduce the negative effects of salt (Etesami and Beattie 2018). These bacteria called PGPR act through different mechanisms: (i) acting on phytohormones by synthesizing indole acetic acid (IAA)(Myo et al. 2019) or reducing ethylene production through the activity of the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase (Gupta and Pandey 2019), (ii) facilitating nutri- ent uptake through different mechanisms: production of siderophores that chelate iron (Kumar et al. 2018), solu- bilization of mineral phosphorus (Kadmiri et al. 2018),or nitrogen fixation (Etesami and Beattie 2018), and (iii) by forming biofilms around the roots (Haque et al. 2022) and exopolysaccharides (Talebi Atouei et al. 2019). In addition, PGPRs can also increase water use efficiency by modulating transpiration and stomatal conductance and decreasing reactive oxygen species content in inoculated plants (Vejan et al. 2016).

Several groups of PGPR have been reported for improving seedling growth, such as Bacillus, Pseudomonas, Rhizobium, and Oceanobacillus (Afzal et al. 2017; Albdaiwi et al. 2019). Several studies have been conducted to investigate the contribution of plant inoculation with halotolerant PGPRs to miti-gating salinity damage. Indeed, Albdaiwi et al. 2019; Shahid et al. 2022 reported restorative effects on

wheat growth after inoculation with halotolerant bacteria. However, all studies reported in the literature have focused on morpho-physiologi- cal parameters as well as osmoprotectant accumulation. Little attention has been paid to the combined effect of PGPR and salt stress on changes in endogenous phytohormone levels.

Moreover, to our knowledge, no study has been conducted on the use of *Kushneria* sp. as a PGPR for wheat growth under salt stress conditions and its ability to suppress the deleterious effects of salt stress. This is the originality of this study.

The objective of this study is for the first time to iso-late halotolerant bacteria associated with a halophilic plant (*Halocnemum strobilaceum*) grown on saline soil, exam- ine their PGPR attributes, and finally identify them by 16S rRNA sequencing. In a second step, the effect of salt stress on the growth of durum wheat and the contribution of inoculation with *Kushneria* sp. and *Halomonas* sp. on the avoidance of deleterious effects of salt stress were evaluated. The third objective is to better understand the mechanisms involved in the response to salt and inoculation.

Materials and Methods

Bacterial Strain Isolation and Characterization

The bacterial strains used in this study were isolated from the rhizosphere and roots of *Halocnemum strobilaceum*,(*Salicornia strobilacea*), a spontaneous halophyte plant from Chott Melghir in Biskra (34°15′00″N, 6°19′00″E).

Rhizospheric bacteria were isolated by suspending 1 g of rhizospheric soil in 9 mL of sterile physiological water (NaCl 0.9%). Dilutions ranging from 10–1 to 10–4 were pre- pared. Root-associated bacteria were also isolated. The root surface was sterilized by immersion in ethanol and HgCl2, followed by several rinses in sterile-distilled water. Roots were macerated in sterile physiological water (0.9% NaCl). 100 µl of each dilution and root macerate were spread on Petri dishes containing Trypticase Soy Agar medium (TSA 1/10) supplemented with different concentrations of NaCl (0, 0.8, 1.2, 1.8, 2.5 M). Plates were incubated at 30 °C and colony formation was monitored.

Colonies were examined morphologically for shape, size, appearance, texture, pigmentation, and optical proper- ties (opaque, translucent, transparent). Colonies of different morphotypes were selected, purified by successive plating on TSA with 0.8 M NaCl, and stored in a 40% glycerol solution at -80 °C until further use.

Preliminary characterization was undertaken by Gram determination, enzyme search (catalase, oxidase, urease, nitrate reductase, gelatinase, amylase), carbohydrate fermentation using an API gallery, indole production, methyl red and Voges-Proskauer reactions, and H2S formation.

Screening of Salt Tolerance and EPS Production

Bacterial isolates were screened for halotolerance or haloph- ily using TSA 1/10 medium at different NaCl concentrations (0.8, 1.2, 1.8, 2.5, 3.5, 4.5, and 5 M). For exopolysaccharide production, the strains were grown on Yeast Extract Sucrose Agar medium (YESA) supplemented with 20 $g.L^{-1}$ sucrose. The slime produced by the colonies on this medium is an indicator of the production of bacterial EPS.

Screening of Plant Growth-Promoting Activities of the Isolates

In this study, the investigation of PGPR attributes of isolates was limited to IAA production and inorganic phosphate solubilization. Indole acetic acid production was determined using the slightly modified method of Bric (Bric et al. 1991): bacterial isolates were grown in Trypticase Soy Broth

medium (TSB) supplemented with 0.8 M NaCl and 0.2% L-Tryptophan. After incubation in the dark at 30 °C with continuous agitation (150 rpm) for 2 days, 2 mL of Salkowski's reagent (50 mL of 35% HClO4 supplemented with 1 mL of 0.5 M FeCl3) was added to 2 mL of the bacterial cultures. The mixture was kept in the dark for 10 min. The development of pink color indicates the production of IAA. The negative control is the medium containing tryptophan without bacteria.

Inorganic phosphate solubilizing ability was performed by spot inoculation of bacterial isolates on NBRIP (National Botanical Research Institute Phosphorus)-modified agar medium containing 5 g/L tricalcium phosphate Ca3(PO4)2 and 0.8 M NaCl. The formation of transparent halo around the bacterial colonies after 7 days of incubation at 30 °C is an indication of phosphate solubilization activity. The phosphate solubilization index (PSI) of isolates was determined according to the following equation: PSI = total halo zone diameter/colony diameter (Morales et al. 2011). All of these tests are performed in triplicate.

Molecular Identification by 16S rRNA Gene and Phylogenetic Analysis

Based on the results obtained (data not shown), two bacteria were selected and identified by 16S rRNA sequencing. For this objective, the strains were grown in TSB1/10 supplemented with 0,8 M NaCl at 30 °C and centrifuged at 10,000 rpm for 3 min to recover the bacterial cells. After the DNA extraction by thermic protocol, the 16S ARNr genes were amplified by PCR using the universal primers fD1 and S17 (Weisburg et al. 1991; Pawlowski and Holzmann 2002). PCR products were sequenced using the standard Sanger sequencing technique by GENEWIZ (Beckman). The resulting sequences were blasted on the National Center for Bio- technology Information server (http://blast.ncbi. nlm.nih. gov/Blast.cgi) and then compared to the most similar neigh- boring phylogenetic sequences retrieved from GenBank databases with Ref Seq accession numbers. The phylogenetic analysis of the 16S ARNr sequences of bacterial isolates with bacterial reference sequences identified in the BLAST search was carried out using the MEGA-X software. The sequences were aligned using the embedded Muscle algorithm to build a phylogenetic tree by calculating distance matrices for neighbor-joining (NJ) analysis with the Kimura two-parameter model and a bootstrapping analysis with 10,000 replicates to test the robustness of internal branches.

Assessment of Plant Growth-Promoting Ability in Durum Wheat

Durum wheat (Triticum durum Desf. Var. MBB) seeds were provided by the ITGC (Institut technique des grandes cultures) of Setif-Algeria. These were surface sterilized by washing with ethanol (70°) for 1 min, followed by three washes with sterile-distilled water. The seeds were then treated with a solution of sodium hypochlorite (NaOCl, 5%) for 5 min, followed by six successive washes with sterile water to remove all traces of the disinfectant. Bacterial cultures of the two selected strains, BSSM328 and BSSM27 on TSB 1/10 with 0.8 M NaCl were centrifuged (5000 rpm for 10 min at 4 °C) and the pellets recovered. After 3 washes in physiological water, the pellets were re-suspended to obtain an OD of 0.6–0.8 (106 CFU. mL−1) at 600 nm. Twenty µl of each bacterial suspension was placed in a Petri dish on YESA (Yeast Extract Sucrose Agar) medium with 2% sucrose and incubated at 30 °C to promote the pro- duction of exopolysaccharides.

The previously sterilized seeds were coated with the bacterial must be produced by the two strains BSSM328 and BSSM27 for 1 h and then aseptically dried. The untreated seeds were used as a control.

Experimental Design

The experiment was set up as a completely randomized design with two factors: salt stress with three levels of salt (0, 100, and 200 mM NaCl) and bacterial inoculation with three treatments (uninoculated pots containing untreated seeds (control), inoculated pots containing BSSM328-coated seeds and inoculated pots containing BSSM27-coated seeds) at 12 seeds per pot. Salt stress was applied prior to sowing by pre-watering the soil with one of three solutions (0, 100, and 200 mM NaCl). After coleoptile emergence (unfolded leaves), each plant was inoculated with a volume of 10 ml of bacterial solution (OD600: 0.6–0.8). The experiment was conducted under laboratory conditions: artificial light (4000 Lux) /darkness 16 h/8 h and temperatures ranging from 25 to 32 °C for 21 days. Each pot was watered every other day with distilled water to 80% of its field capacity. Each treatment was performed in triplicate.

Growth Measurements

At the end of the experiment, after 21 days of sowing, seedlings were harvested from each treatment. Growth was assessed by measuring the following parameters for each plant: shoot length (SL), root length (RL), shoot fresh weight (SFW), root fresh weight (RFW), shoot dry weight (SDW), and root dry weight (RDW). Plant tolerance to salt stress is estimated by calculating the salt tolerance index (STI) as follows: STI = SDW S/SDWNS × 100, where SDW S is the shoot dry weight of a stressed plant, and SDWNS is the shoot dry weight of a non-stressed plant.

Relative Water Content

The relative water content of the leaves indicates the cell turgidity and reflects the water status of the plant. It was evaluated according to Clarke and McCaig (1982), using the following formula

RWC (%) = (FW - DW)/(TW - DW) × 100.

Three collected leaves are weighed immediately to obtain their fresh weight (FW) and then placed in Petri dishes filled with distilled water and placed in the dark in a cold location. After 24 h, the leaves are removed, dried, and weighed again to obtain the total turgor weight (TW). Finally, the samples are placed in an oven set at 65 °C for 48 h and weighed to obtain their dry weight (DW).

Measurement of Membrane Integrity

Membrane integrity was assessed by measuring rela- tive electrolyte leakage (REL), membrane stability index (MSI), and malondialdehyde content (MDA).

Relative electrolyte leakage (REL) was estimated according to Pike et al. (1998) using the following formula: REL (%) = (EC/ET) × 100. About 200 mg of leaves were immersed in 20 mL of distilled water at 25 °C for 4 h. The initial electrical conductivity of the medium (EC) was then recorded. Leaf tissues were destroyed by autoclaving at 120 °C for 20 min to release all cellular electrolytes and then refreshed to 25 °C, and the final electrical conductivity was measured (ET).

Membrane stability index (MSI) was calculated using the formula given by Sairam (1994):

MSI = $1 - (EC/ET) \times 100\%$.

Malondialdehyde (MDA) content was determined fol- lowing the methodology of Hodges et al. (1999) and calcu- lated using the following formula: MDA (nmol/mL) = 106 × (A-B/57000).

$A = (Abs_{532+TBA} - Abs_{600+TBA}) - (Abs_{532-TBA} - Abs_{600-TBA})$ and $B = (Abs_{440+TBA} - Abs_{600+TBA}) \times 0.0571$, where Abs is the absorbance at 440, 532, and 600 nm and TBA corresponds to the solution with (+) or without (−) thiobarbituric acid.

Proline Content

Proline content was determined in leaf tissue as described in Bates et al. (1973) modified by Vives-Peris et al. (2017). In 5 mL of 3% sulfosalicylic acid, 50 mg of dry sample was ground. The samples were centrifuged at 4000 rpm for 20 min at 4 °C. The supernatant was mixed with glacial acetic acid, ninhydrin reagent (625 mg ninhydrin in 15 mL glacial acetic acid), and 10 mL 6 M orthophosphoric acid in a 1:1:1 ratio (v:v:v). The reaction mixture was heated to 100 °C for 1 h in a water bath, cooled, and centrifuged again for 5 min at 2000 rpm at 4 °C. A volume of 4 mL of toluene was added to the supernatant and mixed vigorously for 15–20 s. The absorbance of the organic layer was measured at 520 nm.

Total Antioxidant Capacity (TAC)

Antioxidant capacity was measured according to Re et al. (1999) by the Trolox equivalent antioxidant capacity (TEAC) assay using ABTS (2,2-azobis-ethyl benzothiazoline-6-sul- phonic) radical. Then, 50 mg of fresh plant material was ground in 1 mL of methanol, centrifuged at 12,500 rpm during 12 min, dried with speed-vacuum, re-suspended in 200 µL of 80% methanol and put in the ultrasonic for 10 min, and finally filtered the suspension through a 0.22 um filter.

A volume of 10 µl of the extract was added to 990 µl of an ABTS and K2O8S2 solution having an absorbance of 0.7 at 734 nm. The absorbance of the mixture was also read 3 min later. A standard curve was constructed with different concentrations of Trolox. The antioxidant capacity was expressed in mM Trolox equivalent.

The percentage inhibition of ABTS was calculated according to the following formula Adedapo et al. (2008):

Inhibition (%) = 100 × [(Abs C − Abs W)]/(Abs C)

Abs C: Absorbance of the ABTS radical + methanol, Abs W: Absorbance of ABTS radical + extract.

It has been reported that the higher the inhibition value (% ABTS), the higher the antioxidant capacity of the samples.

Pigments Analysis

About five leaves are weighed and placed in 2 ml of acetone. After 24 h of maceration in the dark, absorbance was measured at 470 nm, 663 nm, and 647 nm. Total chlorophyll and carotenoid content were calculated from the equations of Lichtenthaler (1987).

Phytohormones Analysis

The contents of abscisic acid (ABA), salicylic acid (SA), and jasmonic acid (JA) were determined at the shoot level of the seedlings using the method of Durgbanshi et al. (2005). Extraction of phytohormones was performed on two hundred milligrams of shoot homogenized to a fine pow- der in water using ball equipment. The pH of the extracts was adjusted to 2.8–3.2 with acetic acid and fractionated twice with diethyl ether. The supernatants were evaporated under vacuum in a concentration centrifuge at room temperature. The dry residue was re-suspended in 500 µL of a 90:10 (v/v) water:methanol solution and filtered through 0.22 µm PTFE filters. Twenty µl of this solution was injected into the HPLC–MS system. [2H6]-ABA, dehydro-Jasmonic acid, and [13C6]-salicylic acid were also injected into the HPLC–MS system. Results were processed using Masslynx v4.1 software, and phytohormone levels were quantified using a standard curve prepared with commercial standards.

Statistical Analysis

Statistical analyses were performed with Stat-Software XLstat. Data were analyzed using one- and two-way ANOVA with two factors (inoculation and salt stress) and represented the means of three replicates

standard deviation (SD). A Tukey's post hoc test ($p \leq 0.05$) was used when significant differences were detected between treatments. Principal component analysis (PCA) and agglomerative hierarchical clustering (AHC) were performed to identify possible correlations between the different parameters studied and the distinct responses of the different bacterial treatments.

Results

Phenotypic, Biochemical and Genotypic Characterization

The selected isolates (BSSM328 and BSSM27) are Gram-negative, motile, non-spore forming, and strictly aerobic bacteria. The cells are in the form of rods grouped in pairs and in chains.

On TSA supplemented with 0.8 M NaCl, isolate BSSM27 forms circular, smooth, mucoid, opaque colonies with a convex elevation. The colonies of isolate BSSM328 are smooth, shiny, opaque, and whitish. All other biochemical and physiological characters of these 2 isolates are shown in the Table 1.

Analysis of 16S rRNA sequencing data from both isolates performed using the blast tool revealed that both isolates belonged to the phylum Proteobacteria, class Gammapro- teobacteria. BSSM328, isolated from rhizospheric soil, is identified as a Halomonas genus, and BSSM27, isolated from the root of *Halocnemum strobilaceum* (*Salicornia strobilacea*), belongs to the genus Kushneria. The phylogenetic tree of BSSM328 and BSSM27 was generated by comparison with the sequences of related reference bacterial species generated in MEGAX, based on the sequence alignment of the 16S rRNA gene using the neighbor-joining method (Fig. 1).

Assessment of Plant Growth-Promoting Traits

Both bacterial isolates produce EPS and IAA, solubilize inorganic phosphate and exhibit high salt tolerance (Table 2).

Effect of Inoculation and Salt Stress on Growth

In the absence of salt stress, inoculation with BSSM328 and BSSM27 strains induced a significant increase in all growth parameters compared to non-inoculated plants, as shown by the increase in SL, RL, and RDW for plants inoculated with both strains and SDW for plants inoculated with the BSSM27 strain.

Salt stress, on the other hand, induced significant reductions in all growth parameters in both non-inoculated and inoculated plants with the two bacterial strains. However, it should be noted that these reductions are proportional to the severity of the salt stress and that all the reductions recorded for all the parameters are less important in the inoculated plants compared to the non-inoculated plants, whatever, the severity of the salt stress (Table 3). This reflects the protective effect of inoculation against the negative impact of salt stress.

The comparison of the percentages of stimulation of the parameters also shows the specific effect of each strain, which is a function of the salt concentration. Indeed, at 0 mM NaCl, strain BSSM27 performed better as it induced an increase of 33.2%, 70.71%, 57.69%, and 192.85%, respectively, in SL, RL, SDW, and RDW compared to an increase of 23.63%, 113.10%, 38.46%, and 121.42% for plants inoculated with strain BSSM328. To assess the effect of salt stress on different plant organ growth, the RDW/SDW ratio was calculated.

The results showed that salinity decreased this ratio for all treatments, particularly non-inoculate seedlings (Table 3), indicating that the roots are more tolerant to salt stress than shoots (RDW/SDW > 1).

In the presence of salt, the BSSM27 strain always per- forms better, with stimulation percentages often higher than those induced by the BSSM328 strain.

The calculation of the salt tolerance index (STI) clearly showed the inhibitory effect of salt since the STI recorded was 76.92% and 38.46% for uninoculated plants under 100 mM and 200 mM NaCl respectively. In contrast, the STI of plants inoculated respectively with BSSM328 and BSSM27 strains is 130%, 145% at 100 mM, and 110% and 240% at 200 mM. This clearly demonstrates that inoculation has removed the negative effects of salt stress on the growth of durum wheat. There restorative effect of these two strains is greatest at 200 mM NaCl compared to 100 mM especially for BSSM27.

Relative Water Content

In the absence of salt stress, inoculation with strains BSSM328 and BSSM27 non-significantly (4 and 5.63%) improved plant water status. Under salt stress, the relative water content of inoculated plants was significantly improved by both strains used. This increase is about 19%, 14% for plants inoculated with BSSM328, and 22% and 23% for plants inoculated with the BSSM27 strain at 100 mM and 200 mM NaCl respectively.

Membrane Integrity

In the absence of salt stress, inoculation with BSSM328 and BSSM27 strains reduced relative electrolyte leakage by 18% and 20%, respectively, and reduced MDA levels by 57 and 46%.

Salt stress, for its part, induced an increase in relative electrolyte leakage and MDA accumulation in the leaves of all stressed plants compared to unstressed, concomitant with a decrease in the membrane stability index (MSI). These variations were significant only for REL and MSI at the 200 mM salt level (Fig. 2). In non-inoculated plants, this increase is in the range of 17% and 105% for relative electrolyte leakage and 33% and 59% for MDA levels at 100 and 200 mM NaCl, respectively. In contrast, MSI dropped from 78.54% to 56.08% at 200 mM NaCl.

In inoculated plants, MDA and REL levels always remained lower than in non-inoculated plants. At 100 mM NaCl, the reduction rates of REL and MDA in the leaves of BSSM328 and BSSM27 inoculated plants are 12% and 21% and 63% and 38%, respectively. At 200 mM NaCl, these reductions reach 32% and 51% for relative leakage of electrolytes and 50% and 40% for MDA in plants inoculated with BSSM328 and BSSM27, respectively.

It seems that the two strains act differently to preserve membrane integrity: BSSM328 reduces more MDA levels while BSSM27 reduces the relative leakage of electrolytes. Inoculation with both bacterial strains increased MSI values, especially at 200 mM NaCl where significant differences were recorded compared to the non-inoculated control. The increase recorded was higher in seedlings inoculated with BSSM27 (40.71%) than those inoculated with BSSM328 (25.44%).

Proline and Total Antioxidant Capacity

In the absence of salt stress, inoculation did not have an apparent effect on the proline content of seedlings (Fig. 3A). Application of salt stress, on the other hand, caused a highly significant ($p < 0.001$) increase in proline content in all inoculated and non-inoculated plants. However, these increases were greater in the non-inoculated plants. They are about 28.56% at 100 mM and 53.54% at 200 mM in non-inoculated plants.

It is interesting to note that, unlike REL and MDA, both bacterial strains used induced a similar effect on proline content.

In seedlings inoculated with BSSM328 and BSSM27 strains, a reduction of 16% and 20% and 21% and 25% is recorded at 100 mM and 200 mM NaCl, respectively. In the absence and presence of salt stress, the antioxidant potential of inoculated plants was higher than that of non- inoculated plants. These non-

significant increases were in the range of 14% to 23% at 100 mM and 12% to 27% at 200 mM (Fig. 3B).

Pigment Production

In uninoculated plants, salt stress induced a decrease in the total chlorophyll content of leaves by about 22.4% at 100 mM and 35.4% at 200 mM. At the same time, carotenoid pigment contents were increased very significantly ($p < 0.05$) with salt stress (Table 4). Inversely, inoculation with strains BSSM328 and BSSM27 induced an increase in total chlorophyll content and a reduction in carotenoid content both in the presence and absence of salt. These variations are more drastic at 200 mM NaCl especially in plants inoculated with BSSM328 compared to BSSM27. Indeed, inoculation with BSSM328 significantly increases the total chlorophyll content by 17%, 40%, and 56% compared to 4%, 14%, and 28% for the BSSM27 strain at 0 mM, 100 mM, and
200 mM respectively.

The ratio of carotenoids to total chlorophyll increased with the intensity of salt stress, especially in uninoculated plants. Indeed, the highest ratios are recorded in the most stress sensitive plants and the lowest ratios in the most tolerant plants.

Phytohormone Production

Endogenous levels of the phytohormones abscisic acid (ABA), salicylic acid (SA), and jasmonic acid (JA) were changed by salt stress and bacterial inoculation (Fig. 4).

In non-inoculated plants, salt at all levels induced a significant increase in leaf ABA (Fig. 4A) and jasmonic acid (Fig. 4C) content. In contrast, inoculation resulted in a significant reduction of ABA and jasmonic acid contents both in the absence and presence of salt stress. These results indicate that the two strains and the salt stress at the two used concentrations would act in a similar way on these two phytohormones. However, it should be noted that the action of salt and inoculation is more drastic on jasmonic acid than on abscisic acid. Indeed, the rates of change for ABA vary from 19 to 58% while for JA they vary from 89 to 530%. In the absence of salt stress, inoculation with BSSM328 induced a very consistent increase of about 897%, whereas BSSM27 caused a slight decrease (29%) in SA content. Unlike ABA and JA, the effect of salt and inoculation on SA levels is strain and salt stress severity dependent.

Correlations Between Parameters Studied and Distinct Responses of the Different Bacterial Treatments with Principal Component Analysis (PCA) and Agglomerative Hierarchical Clustering (AHC)

Agglomerative hierarchical clustering (AHC) clearly showed the distribution of parameters according to the type of inoculation and the severity of salt stress.

For the parameters, two clusters were distinguished (Fig. 5A) revealing the strong negative correlations existing between their parameters. The first cluster (V1) grouped the parameters REL, MDA, TAC, PROLIN, ABA, JA, and carotenoid, reflecting the positive correlation existing between them. The second group (V2) includes the parameters SL, RL, SDW, RDW, RWC, total chlorophyll (TCHL), and SA, positively correlated with each other. Treatments were grouped into three groups according to their level of salt tolerance (Fig. 5B): the first group (T1) of the most salt-sensitive individuals, comprising uninoculated seedlings affected by both salt concentrations (C1, C2) and plants inoculated with BSSM328 stressed with 200 mM NaCl (A2). The second group (T3), representing the best performing plants, included treatments inoculated with both strains in the absence of salt stress (A0 and B0). The third group (T2) representing the plants with an intermediate tolerance includes the plants not inoculated

in the absence of salt stress (C0), the plants inoculated with BSSM328 at 100 mM (A1) and the plants inoculated with BSSM27 at 100 and 200 mM NaCl (B1 and B2).

This hierarchization prioritization allowed us to rank the different treatments in relation to their response to salt stress and inoculation with the two strains. A PCA was performed to reveal the relationship between the studied parameters and the treatment groups (Fig. 5C). The two factorial axes F1 and F2, describing the correlations between the variables, hold 76.36% of the total information, with 76.82% for the F1 axis and 9.56% for the F2 axis.

The PCA confirmed the strong positive or negative cor- relation of the parameters between them and the division of the different treatments into three distinct groups (Fig. 5C). The results also showed that the individuals most affected by salt stress (T1), marked by a reduction in growth param- eters, an increase in relative electrolyte leakage, MDA, pro- line, carotenoids, ABA, and JA content, and by a reduction in total antioxidant capacity, were strongly and positively associated with the variables of cluster V1 and negatively associated with the variables of cluster V2. The opposite observations are noted for cluster T3, grouping the best per-
forming plants.

PCA showed the benefit of inoculation both in the absence and presence of salt stress, the effect being strain dependent.

Discussion

The present work focused on the impact of inoculation of two halotolerant PGPR strains isolated from southern Algeria on the growth of durum wheat under salt stress. Salt stress at both concentrations induced a classical response in the growth of durum wheat seedlings that was reflected in a reduction of all growth parameters related to length and dry and fresh biomass of both aerial and root parts (Ali et al. 2017; Emami et al. 2020). Several authors showed that growth decreased in pea and maize with an increase in salinity (Zafar et al. 2020; Alvi et al. 2022).

Inoculation with the two bacterial strains retained in this work improved the growth of durum wheat in the absence of stress but also mitigated the depressive effect of salt stress on growth parameters. These results are in perfect agreement with the findings of Albdaiwi et al. (2019), who reported similar results after inoculation of durum wheat with strains related to Halomonas sp. Indeed, the calculation of the tolerance index indicates that non-inoculated plants are the most susceptible since the SIT is the lowest under salt stress, while plants inoculated with BSSM27 are found to be the most tolerant with the highest SIT, reflecting the better performance of BSSM27 compared to BSSM328. The mechanisms by which these PGPRs improve plant growth are multiple. It should be noted that the BSSM328 and BSSM7 strains used are capable of solubilizing inorganic phosphate, producing exopolysaccharides and a crucial phytohormone, which is IAA. Several authors have shown that the genera Halomonas and Kushneria are PGPR characterized by IAA and EPS production, ACC deaminase, siderophore, and phosphate solubilizing (Kara- mat et al. 2018; Yañez-Yazlle et al. 2021). This difference in performance between the two strains used would be related at least in part to the greater amount of IAA produced by *Kushneria sp*. compared to that produced by *Halomonas sp*. IAA, which is the main auxin in plants, is involved in modulating plant growth by acting on cell division and stimulating stem elongation and root branching (Abbas et al. 2018; Vimal et al. 2019) by an increase in cell wall synthesis (Kadmiri et al. 2018). The calculation of the RDW and SDW ratio shows that inoculation stimulates root extension while salt stress affects it negatively. Indeed, abiotic stresses such as salinity cause changes in root extension, a process in which phytohormones play an important role (Vacheron et al. 2013). Inoculation, for its part, improves plant growth by acting mainly on this hormonal balance (Shultana et al. 2020; Khan et al. 2020). This

stimulation of the extension of the root system is responsible for the better mineral and water nutrition of the plant and, therefore, for the better growth. The relative water content is a good indicator of the water status of the cells and reflects the extent of the effect of salt stress. Its reduction reflects the loss of cell turgor, which will cause growth inhibition. Inoculation of wheat seedlings resulted in an increase in relative leaf water content as reported by other authors (Albdaiwi et al. 2019; Ilyas et al. 2020; Haroon et al. 2022). Salt, in contrast, caused a reduction in this RWC which remains, however, higher in inoculated plants compared to non-inoculated plants. This suggests that water uptake is maintained at a sufficient level to avoid dehydration of plant tissues. These results also reveal a positive correlation ($r = 0.667$) between relative water content (RWC) and aerial plant growth (SDW). Our results agree with those of Cherchali et al. (2019) and Ji et al. (2020), who report that the presence of these exopolysaccharide-producing bacteria improves soil structure and, consequently, soil porosity, which facilitates the movement of water and nutrients from the soil to the plant. These EPS, which adhere to soil particles, act as the best matrix for the retention of soil moisture, thereby protecting the roots from desiccation. These EPS by sequestration of toxic Na^+ and Cl^- ions will remove the plant from the action of salt stress (Talebi Atouei et al. 2019; Kumar et al. 2020). In addition, the work of Ali et al. (2017) and Shultana et al. (2020) explained the increase in RWC of inoculated plants by an improved pro- duction of phytohormones, especially IAA auxins that enhanced root growth.

Relative electrolyte leakage (REL), MSI, and MDA levels are parameters indicative of cell membrane dysfunction as a consequence of oxidative stress resulting from applied salt stress. The results obtained show that salt induced an alteration of the integrity of the membranes, which is reflected by an increase in cellular permeability (REL), which would result from the peroxidation of membrane lipids, which is favorable to the accumulation of toxic ions Na^+ and Cl^- in the cell. Similar results are reported by other authors (Shultana et al. 2020; Shahid et al. 2022). Inoculation of wheat plants with both PGPRs reduced MDA levels and increased membrane stability compared to values recorded in the leaves of non-inoculated plants, indicating a stabilizing effect of the cell membranes by the inoculums. These results are consistent with those of other authors who report the same observations in inoculated durum wheat plants (Cherchali et al. 2019; Shahid et al. 2022; Haroon et al. 2022). Authors suggest that PGPRs would protect the integrity of cell membranes from the detrimental effect of salt by stimulating the synthesis of membrane constituent lipids and/or reducing the unsaturation of their fatty acids (Dimkpa et al. 2009).

The total antioxidant activity is more reduced in inoculated plants compared to non-inoculated plants, especially for Kushneria sp. strain BSSM27. This could be explained by the fact that inoculated plants would be less affected by salt stress than non-inoculated plants, as reported by Shahid et al. (2022) who indicate that salt stress is not perceived by plants inoculated with EPS-producing bacteria. Other authors reported that inoculation would protect seedlings from the effects of stress by inducing a reduction in antioxidant activities in both stressed and unstressed seedlings (Ayub et al. 2020; Neshat et al. 2022) via hormones and/or EPS that these PGPRs produce (Jaszek et al. 2014).

Salt stress generates osmotic stress, hence, the need to ensure osmotic adjustment through the synthesis of a large number of compatible solutes, including proline. Its main role would be to maintain cell turgor by osmotic adjustment, which would allow it to maintain physiological processes such as photosynthesis and growth. In addition to its role as an osmolyte, proline exerts a stabilizing effect on proteins, membranes, acts as an antioxidant (Sallam et al. 2019), can serve as a source of carbon and nitro- gen under stress, and also acts as a signaling molecule to induce ABA accumulation, affect related gene expression, and regulate plant growth under salt stress (Marusig and Tombesi 2020). Proline accumulation results from induction and/or activation of enzymes catalyzing its biosynthesis, inhibition of its degradation, or decreased utilization as an amino acid in protein synthesis.

Inoculation of durum wheat seedlings reduced proline content. These results are in agreement with those of Zilaie et al. (2022) and Haroon et al. (2022), who suggested that inoculated plants are protected by PGPRs; therefore, proline accumulation decreases. In addition, Karamat and Ahmed (2018) propose that the use of *Kushneria marisflavi* CSE9 activates a mechanism responsible for salt stress alleviation that is different from the stimulation of proline synthesis.

According to Arif, (2015), the accumulation of ROS in chloroplasts leads to the degradation of their membranes; carotenoids then take over to protect chlorophyll from ROS action. Kheirizadeh Arough et al. (2016) reveal that the main reason for the decrease in chlorophyll could be their degradation by reactive oxygen species (ROS). Indeed, a relationship has always been established between growth reduction by salt stress and chlorophyll levels (Chen et al. 2022). Similar results have already been reported by other authors (Abdel Latef et al. 2020; Tiwari et al. 2020). The reduction in chlorophyll levels is related to the increase in chlorophyllase activity (Szafranska et al. 2017), to the competition for glutamate, which is the common pre- cursor of proline and chlorophyll, and to the fact that Mg^{2+} ions which enter the composition of chlorophyll compete with Na^{+} ions as a result of the disturbance of mineral nutrition (Ali et al. 2017). In addition, all inoculated plants had higher levels of total chlorophyll than the non-inoculated control. This clearly shows that inoculation lifts the inhibitory effects of salt by preserving these chlorophyll pigments (Yasmin et al. 2020; Khan et al. 2021).

Carotenoids are considered antioxidants (Rahman et al. 2020), so their increase would be as a result of the installation of oxidative stress in non-inoculated plants. These results are similar to those in the case of durum wheat seed- lings inoculated with Pseudomonas fluorescens under salt stress. The highest ratios of chlorophyll and carotenoids are recorded in the most sensitive plants (not inoculated) and the lowest ratios in the most tolerant plants (inoculated). Thus, it would appear that this ratio is a marker of salt tolerance.

Phytohormones play a critical role in the ability of plants to acclimate to various environments (Khan et al. 2019). Salinity-induced growth reduction has been widely associated with altered hormonal balance (Fahad et al. 2015). ABA plays a primary role in acclimation to osmotic stress through regulation of stomatal movement that limits transpiration and water loss (Acosta-Motos et al. 2017; Hsu et al. 2021) and modulation of expression of genes related to osmotic stress resistance (Ding et al. 2014). Salicylic acid and jas- monic acid are pivotal in the interplay between stress signaling pathways that serve as a link between the perception of environmental stresses and the generation of physiological and biochemical responses involved in the management of stress tolerance (Asif et al. 2022).

The increase in ABA levels concomitant with a reduction in RWC in uninoculated stressed seedlings suggests that endogenous ABA accumulation is a consequence of the water deficit that occurs after the application of salt stress as reported by Zhang et al. (2006). Inoculation that preserves the water status of stressed seedlings decreases their ABA levels (Khan et al. 2021), especially in the case of *Kushneria sp*. strain.

JA levels increased proportionally with the intensity of salt stress and showed a similar pattern to ABA. Previous studies concluded that JA is required for ABA accumulation in Arabidopsis thaliana and citrus (de Ollas et al. 2015), which is explained by the positive correlation between ABA and JA. How this hormone regulates ABA synthesis remains unclear. Interestingly, SA levels do not change in the same way as ABA and JA, there are antagonistic interactions between SA and JA and ABA (Khan et al. 2015). SA influences plant physiological and biochemical functions in a dose-dependent manner (Kanval et al. 2022) by stimulating various processes in plants, such as cation uptake and transport, photosynthetic activities, and growth rate (Farhangi- Abriz and Ghassemi-Golezani 2016), and also, the SA pathway induces stomatal opening (Etesami and Beattie 2018). Kushneria sp. BSSM27 and Halomonas sp.

BSSM328 showed variable effects on SA content.
The graphical representation (Fig. 6.) is an illustration of the mechanisms by which the two rhizobacteria BSMM328 and BSSM27 confer salinity tolerance to durum wheat seed- lings. Under salt stress, plants reduce water loss through ABA-controlled stomatal closure and activate protection through osmolyte accumulation (proline) and activation of the antioxidant system (TAC). Membranes are affected by salt stress, resulting in increased levels of REL and MDA associated with decreased membrane stability (MS). These effects lead to a reduction in growth parameters (GP) and an alteration in the photosynthetic machinery (Fig. 6).
Inoculation with PGPRs mitigates the deleterious effects of salt stress, resulting in an improvement in all growth parameters, water status, and membrane stability. PGPRs act by regulating hormone production, improving mineral nutrition, producing EPS capable of sequestering toxic ions (Na+), and preventing soil desiccation due to their high-water retention capacity.
Statistical analysis (PCA and AHC) revealed a negative correlation between REL, MDA, proline, TAC, carotenoid, ABA, and JA parameters of durum wheat seedlings and their salt tolerance level. The inoculation confers to the stressed plants an improved tolerance to salt stress, which is reflected in the improvement of growth parameters (SL, RL, SDW, and RDW), water status (RWC), membrane stabilization (REL and MDA), total chlorophyll, and a reduction of proline, ABA, and JA content in the stressed seedlings. The statistical analysis performed by AHC and PCA permitted the validation of the results obtained.

Conclusion

This study clearly showed that the inoculation of these two PGPRs, *Kushneria sp*. BSSM27 and *Halomonas sp*. BSSM328, strongly mitigated the adverse effects of salt stress on the growth of var MBB durum wheat seedlings by acting at several levels. The first level concerns the stimulation of root extension and the protection of the photosynthetic machinery, which are in favor of the improvement of the growth by better hydric, mineral, and organic nutrition via photosynthesis. The improvement of the water status of durum wheat plants and the stabilization of cell membranes by these PGPRs also contribute to protecting the plants from the effects of osmotic, ionic, and oxidative stress imposed by salt stress. These two bacterial strains used showed variable performance, probably related to their different impacts on the hormonal balance of durum wheat seedlings. *Kushneria sp.* BSSM27, which was used for the first time, showed a beneficial effect on almost all the parameters studied in this work and proved to be more efficient. To have the maxi- mum protective effect, it would be useful to carry out mixed inoculations of these two PGPRs in the future since they are not antagonistic.
The PGPRs used could find their place in biotechnological applications aimed at improving durum wheat yields, especially in arid and semi-arid areas, and at protecting the environment for sustainable development.

Acknowledgements The work published in this article has been supported by the University of sciences and technology, Houari Boumediene (USTHB). The authors want to acknowledge Aurelio Gomez-Cadenas from Department de CiènciesAgràries i del Medi Natural, Universitat Jaume I, (Castellón de la Plana, Spain) for kindly accepting and welcome us to his research laboratory, and Mrs. Marta Pitarch Bielsa for technical assistance with a part of plant analysis, and a special acknowledgment to Thierry Heulin and Wafa Achouak CNRS Research Director for their contribution and collaboration.
Author Contributions MB performed the experiments and wrote the manuscript. SB contributed to the design of the microbiological experi- ments, RM-C contributed in plant analyses, YK and NB contributed

to the supervision of the work and the correction of the manuscript.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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Table 1 Biochemical and physiological characters of BSSM328 and BSSM27 strains

Characters	BSSM328	BSSM27	Characters	BSSM328	BSSM 27
Gram	−	−	D-fructose AC	+	−
Motility	+	+	D-glucose AC	+	−
Endospore forming	−	−	D-mannose AC	+	−
Catalase	+	+	D-melibiose AC	−	−
Oxidase	−	−	D-trehalose AC	+	−
Arginine dihydrolase	+	+	Lactose Maltose Raffinose AC	−	−
Lysine decarboxylase	+	+	Saccharose AC	+	−
Ornithine decarboxylase	+	+	Xylitol Xylose AC	−	−
β-galactosidase	+	+	Adipic acid AS	+	+
Gelatinase	+	+	Arabinose AS	+	+
B-glucosidase	+	−	Capric acid AS	−	+
Protease	+	+	Citrate tri-sodium AS	+	+
Nitrate reductase	+	+	Glucose AS	+	+
Urease	+	+	Malate Maltose Mannitol Mannose AS	+	+
Voges-Proskauer	+	−	N-Acetyl-Glucosamine AS	+	+
H_2S production	−	−	Phenylacetic acid AS	−	+
Methyl α-D-glucopyranose AC	−	−	Potassium gluconate AS	+	+
D-acetylglucosamine AC	−	−	Citrate AS	−	−

(+) positive reaction, (−) negative reaction, *AC* Acidification, *AS* Assimilation

Table 2 Growth-promoting features of the two bacterial isolates BSSM328 and BSSM27

Characters	BSSM328 *Halomonas* sp.	BSSM27 *Kushneria* sp.
EPS production	+	+
Halotolerance	0–3.5 M NaCl	0–2.5 M NaCl
IAA production	+	+ + + +
Phosphate solubilization (PSI)	1.76 ± 0.2	1.84 ± 0.11

Table 3 Effect of inoculation with two halotolerant bacterial strains, BSSM328 and BSSM27, on the shoot and root length ((SL, RL) (cm), shoot and root dry weight (SDW, RDW) (mg.plant−1), salt tolerance index (STI) (%), and relative water content (RWC) (%) in durum wheat under different salinity levels

Treatments		Parameters						
Salt level	Bacteria	SL (cm)	RL (cm)	SDW (mg)	RDW (mg)	RDW/SDW	SIT (%)	RWC (%)
0 mM	C	27.5 ± 0.41b	12.67 ± 2.05b	26.23 ± 4.06bc	28.47 ± 10.84cd	1.06 ± 0.29a	–	84.65 ± 6.14a
	BSSM328	34 ± 0a	27 ± 3.56a	35.53 ± 2.04ab	61.7 ± 12.43ab	1.74 ± 0.32a	–	88.13 ± 3.79a
	BSSM27	36.63 ± 1.14a	21.63 ± 3.56a	41.23 ± 3.07a	82.27 ± 6.43a	2.02 ± 0.32a	–	89.42 ± 2.3a
100 mM	C	18.33 ± 0.47c	8.33 ± 2.62bc	20.37 ± 1.1cd	17.63 ± 0.53cd	0.87 ± 0.043a	79.13 ± 9.84bc	71.38 ± 5.57b
	BSSM328	25.33 ± 3.56b	9.77 ± 0.61bc	26.23 ± 1.76bc	37.73 ± 6.33bcd	1.43 ± 0.14a	128.76 ± 3.48b	84.84 ± 3.27a
	BSSM27	25.37 ± 0.88b	8.83 ± 0.61bc	29.3 ± 1.28bc	46.77 ± 16.03bc	1.58 ± 0.51a	144.61 ± 14.12b	86.82 ± 3.84a
200 mM	C	6.67 ± 0.24e	4.17 ± 0.62c	9.87 ± 0.56e	9.8 ± 0.94d	1 ± 0.15a	38.91 ± 8.51 cd	69.44 ± 2.68b
	BSSM328	12.43 ± 0.85d	6.1 ± 0.73bc	10.77 ± 4.93de	17.8 ± 3.86cd	2.27 ± 1.36a	106.59 ± 45.19bc	79.35 ± 0.47ab
	BSSM27	18.87 ± 0.83c	5.83 ± 0.62bc	24.27 ± 3.8c	28.4 ± 9.01cd	1.18 ± 0.36a	245.43 ± 32.29a	85.43 ± 1.85a

Data presented are the means of 3 replicate determinations ± SE. Values specified in different letters in every single column indicate significant differences according to Tukey's test ($P \leq 0.05$)

Table 4 Effect of inoculation with two halotolerant bacterial strains, BSSM328 and BSSM27, on total chlorophyll (T CHL) and (B) carotenoids (CAROT) (mg/g) in durum wheat under different salinity levels

Salt level	Bacteria	T CHL (mg/g FM)	CAROT (mg/g FM)	CAROT/TCHL
0 mM	C	125.87 ± 4.99bc	59.25 ± 4.81cd	0.47 ± 0.05cde
	BSSM328	147.13 ± 3.53a	55.27 ± 1.43d	0.37 ± 0.015e
	BSSM27	130.35 ± 1.18ab	57.04 ± 1.55d	0.44 ± 0.01de
100 mM	C	98.13 ± 4.55de	84.06 ± 1.13ab	0.86 ± 0.05b
	BSSM328	137.11 ± 3.78ab	62.31 ± 1.07cd	0.45 ± 0.02de
	BSSM27	112.26 ± 1.59cd	68.39 ± 1.75cd	0.61 ± 0.01cd
200 mM	C	81.32 ± 6.03e	94.2 ± 4.8a	1.17 ± 0.13a
	BSSM328	126.79 ± 9.61bc	63.46 ± 4.9cd	0.5 ± 0.06cde
	BSSM27	103.86 ± 2.67d	71.39 ± 8.23bc	0.68 ± 0.09bc

Data presented are the means of 3 replicate determinations ± SE. Values specified in different letters in every single column indicate significant differences according to Tukey's test ($P \leq 0.05$)

Fig. 1 Phylogenetic tree constructed by using neighbor-joining analysis of two bacterial isolates BSSM328 and BSSM27 based on 16S rDNA sequences and sequences from selected bacteria reference isolates (GenBank accession number for the collected isolates and reference strains). Numbers represent bootstrap values at the nodes based on 1000 replications

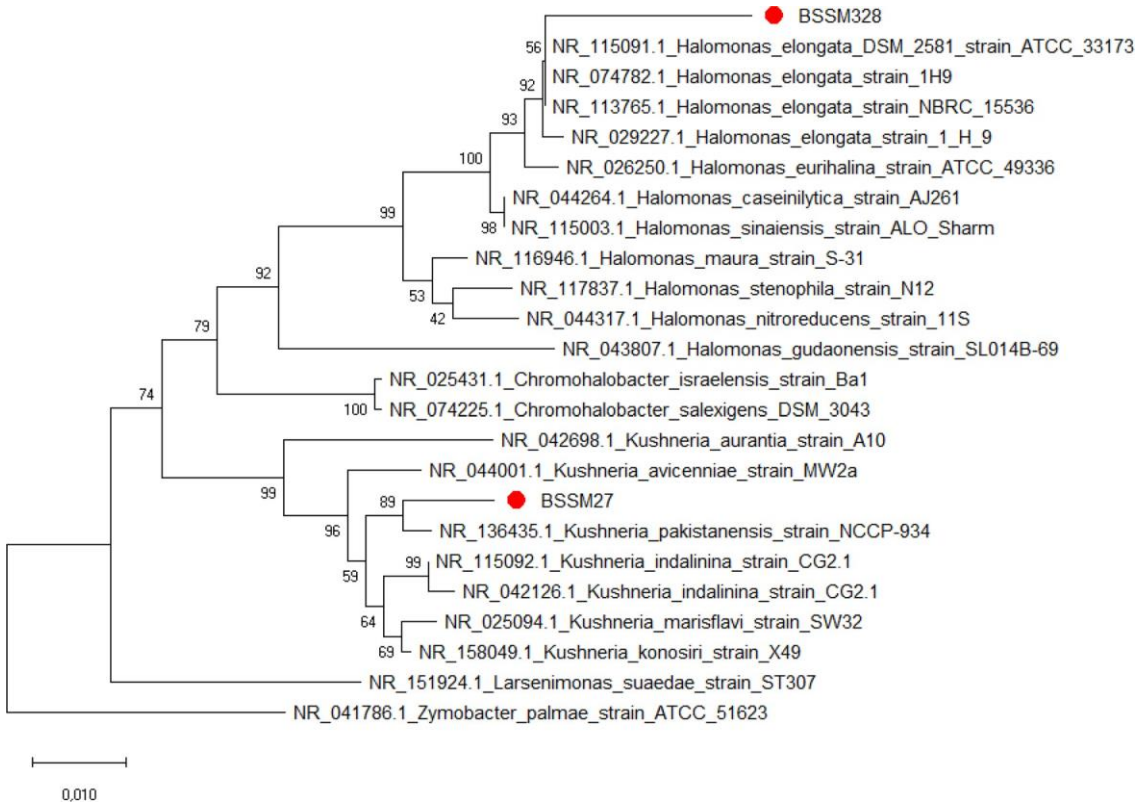

Fig. 2 Effect of inoculation with two halotolerant bacte- rial strains, BSSM328 and BSSM27, on (A) REL (%) and (B) MSI (%) MDA (nmol/g) in durum wheat under different salinity levels. Error bars are the standard deviations of means. Different letters above the bars indicate significant differences at P≤ 0.05.

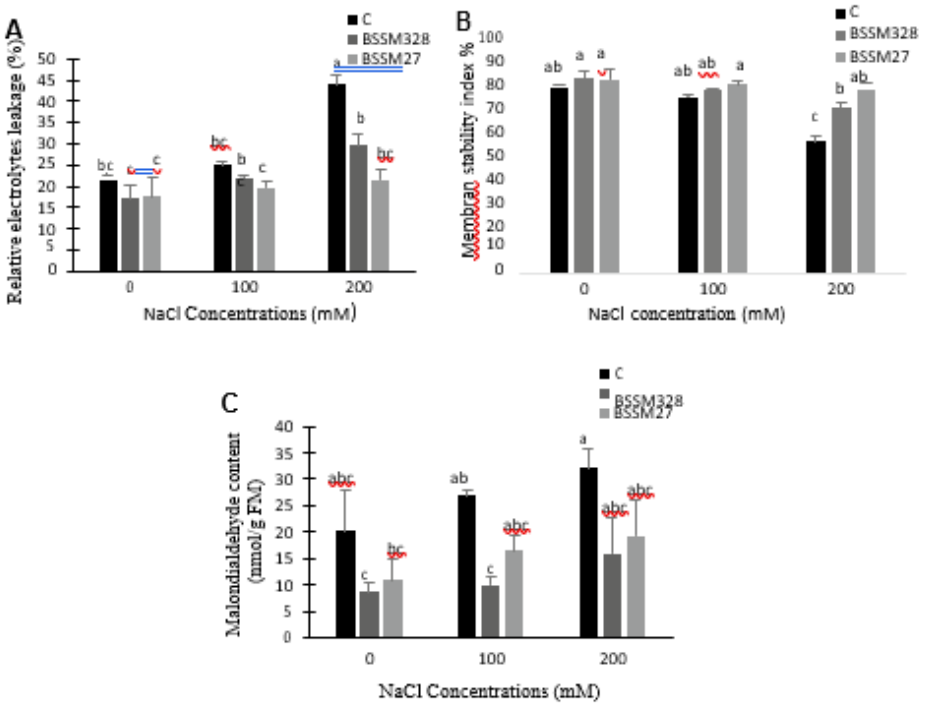

Fig. 3 Effect of inocula- tion with two halotolerant bacterial strains, BSSM328 and BSSM27, on **A** proline content(ug/g) and **B** antioxidant capacity (% inhibition) in durum wheat under different salinity levels. *Errors bars are the standard deviations of means. Different letters above the bars indicate significant differences at P≤ 0.05.*

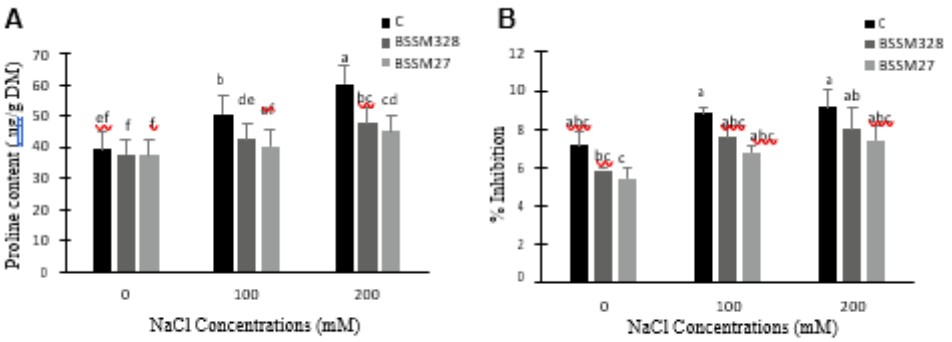

Fig. 4 Effect of inoculation with two halotolerant bacte- rial strains, BSSM328 and BSSM27, on A ABA, B SA, and C JA content (ng/g FM) in durum wheat under different salinity levels. Errors bars are the standard deviations of means. Different letters above the bars indicate significant differences at $P \leq 0.05$.

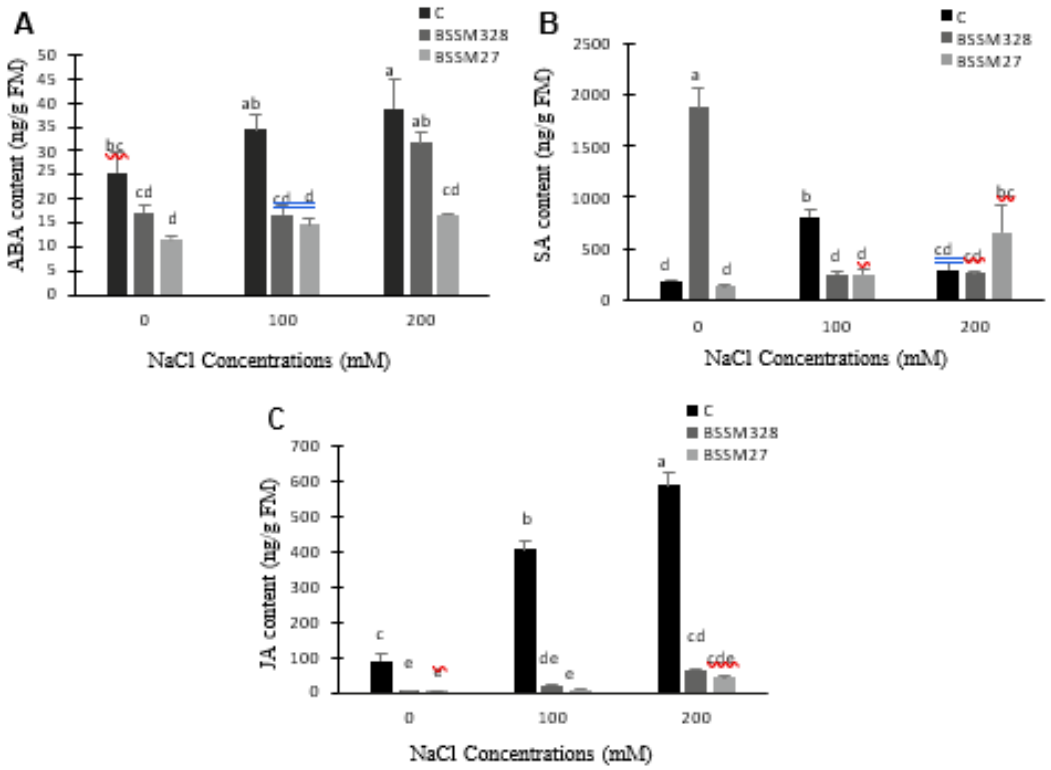

Fig. 5 Agglomerative hierarchical clustering of variables (**A**), of observation (**B**), principal component analysis (**C**) of inoculated and non-inoculated, stressed and non-stressed seedlings. The vari- ables included shoot and root length (SL and RL),shoot dry weight (SDW),root dry weight (RDW), salt index tolerance (SIT), relative water (RWC), relative electrolyte leakage (REL), malondialde- hyde (MDA), total antioxidant capacity (TAC), proline, total chlo- rophyll (TCHL), carotenoid (CAROT), Control (C), BSSM328 (A), BSSM27(B), 0 (0 mM), 1 (100 mM) and 2 (200 mM)

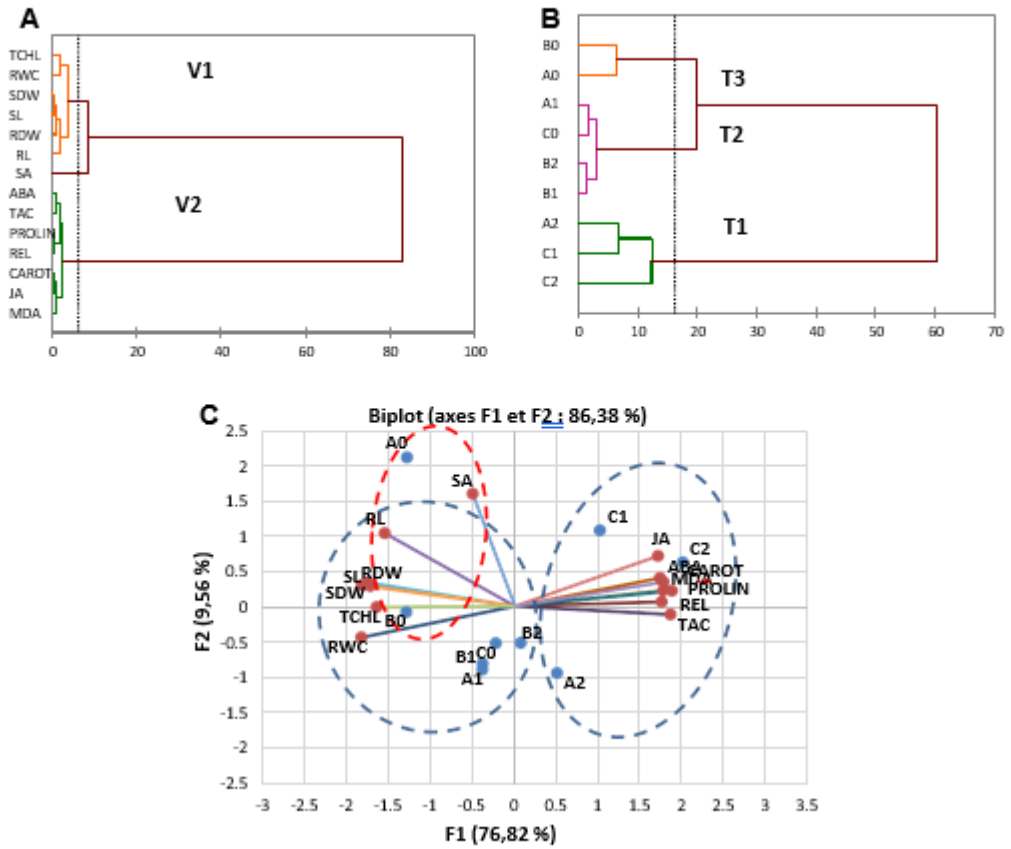

Fig. 6 Mechanisms of PGPR confers salinity tolerance to Wheat. (Blue and purple circles around the roots represent PGPR; blue arrows represent PGPR effects; red arrows represent salt stress effects; green arrows represent the results of each effect of salt stress and PGPR; alternating sign indicates erratic pattern for SA content)