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Dual PGPR-AMF Inoculation Offsets Salinity Stress Impact on the Fodder Halophyte *Sulla carnosa* by Concomitantly Modulating Plant ABA Content and Leaf Antioxidant Response

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Abstract

Salt-tolerant microbes are known to mitigate various biotic and abiotic stresses in plants. However, the intimate mechanisms involved, as well as their effects on the production of signaling molecules associated with the host plant–microbe interaction remain largely unknown. The present work aimed to investigate the role and potential uses of arbuscular mycorrhizal fungi (AMF) *Rhizophagus intraradices* and/or halotolerant plant growth-promoting rhizobacteria (PGPR) *Bacillus subtilis* in improving plant growth, functional biochemical synthesis and signaling of endogenous abscisic acid during plant response to short- and long-term salt stress in the forage halophyte *Sulla carnosa*. Plant growth attributes and biochemical traits were determined at 2 different time intervals (45 and 60 d after transplanting time) when salinity was raised from 100 to 200 mM NaCl. *S. carnosa* showed significant reduction in dry biomass in response to NaCl stress at the second harvest (200 mM NaCl); however inoculating plants with *B. subtilis* alone or associated with *R. intraradices* offset salt impact. Leaf electrolyte leakage was significantly increased by salinity but was significantly reduced following dual microbial inoculation. The applied bacterial inoculants also mitigated oxidative stress as reflected by the higher activities of catalase (APX) and superoxide dismutase (SOD) antioxidant enzymes and reduced H_2O_2 level. Inoculation with *B. subtilis* and *R. intraradices* upregulated 9-cisepoxycarotenoid dioxygenase 1 (*NCED1*) and *SOD* genes expression in *S. carnosa* plants upon salinity treatment. Furthermore, dual AMF-PGPR -inoculated plants accumulated significantly higher levels of abscisic acid (ABA) in both leaves and roots than non-inoculated and single inoculated plants under salinity stress at both harvest times, thereby accounting for their higher salt tolerance of salt-challenged *S. carnosa* plants. As a whole, the use of halophytic plants associated with beneficial soil microorganisms could improve the effectiveness of biological methods for saline soil rehabilitation. At the mechanistic level, ABA might represent a key player in the attenuation of salt impact in inoculated plants.

Keywords *Sulla carnosa* · Salt stress · AMF · PGPR · Abscisic acid · *NCED1*

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Introduction

Soil and/or water salinization is a global and spreading environmental problem especially under the ongoing climate changes. Salinity stress imposes several physiological and metabolic constraints on plants, impairing plant growth through defects in the genetic regulation of cellular pathways (Gupta and Huang 2014). In particular, halophytic plants can adapt to saline environments as they possess unique mechanisms enabling their survival and thriving in harsh saline habitats (Mann et al. 2023). There is also increasing evidence that salt stress tolerance of halophytes is also related to complex ecological processes involving key players, the

microorganisms inhabiting roots and rhizosphere, or the so-called plant microbiome (Wang et al. 2024).

The rhizosphere is the key microhabitat for interactions among plants, soil, and microorganisms, serving as a complex ecosystem inhabited by numerous microbes, including plant growth-promoting rhizobacteria (PGPR), which improve plant behavior when challenged with biotic and abiotic stresses (Sharma et al. 2021). Studies have shown that PGPR such as *Glutamicibacter*, *Bacillus pumilus*, *Bacillus subtilis*, *Pantoea agglomerans,* and *Pseudomonas* sp. efficiently enhance salt stress tolerance of some halophytes like *Phragmites australis, Hordeum marinum, Casuarina obesa,* and *Suaeda fruticosa* through the accumulation of osmolytes (proline, trehalose, and glycine betaines), maintaining the ion balance, the activation of plant's antioxidative enzymes, reducing reactive oxygen species, and the synthesis of exopolysaccharides (EPS), ACC deaminase, indole-3-acetic acid (IAA), and siderophores (Ilangumaran and Smith 2017; Sunita et al. 2020; Kumawat et al. 2022; Hidri et al. 2023; Metoui-Ben Mahmoud et al. 2024). Furthermore, arbuscular mycorrhizal fungi (AMF), play a key role as important root symbionts, enhancing plant nutrient uptake and resistance to several abiotic stress factors. AMF confer better salt tolerance and fitness in host plants by mediating critical physiological processes, such as accumulating osmolytes, facilitating water and nutrient uptake, maintaining ion balance, protecting plants from oxidative damage, and increasing photosynthetic ability (Dashtebani et al. 2014; Dastogeer et al. 2020; Pan et al. 2020a). According to Pan et al. (2020b), AMF can mitigate salt stress damage in plants by enhancing their salt tolerance and improving energy efficiency. Thus, AMF and PGPR are among the most effective and best studied soil microorganisms which can enhance the performance of plants under salt stress condition.

It has also been documented that microorganisms can modulate the biosynthesis of various endogenous plant hormones (such as abscisic acid, jasmonic acid, salicylic acid, brassinosteroids, indole-3-acetic acid, cytokinins, ethylene, gibberellic acid, and strigolactones) to resist salt stress conditions (Ilangumaran and Smith 2017; Barnawal et al. 2017; Yoo et al. 2019; Diagne et al. 2020; Yu et al. 2020; Khan et al. 2021). Beneficial soil microbes can regulate the status of plant hormones as one of their efficient mechanisms to improve plant tolerance under stress conditions. Phytohormones are chemical messengers that affect the ability of plants to acclimatize to varying environments (Porcel et al. 2014). They play a key role in the ability of plants to adapt to changing environmental conditions by mediating growth and development, including source/sink transitions and nutrient allocation (Peleg and Blumwald 2011). Thus, hormonal regulation contributes in several ways to plant stress responses and adaptation (Shaterian et al. 2005; Waadt et al. 2022).

Typically, abscisic acid (ABA) has a wide range of physiological functions in higher plants, such as regulating plant growth and development, and mediating plant defense response to a range of stresses, including responses to drought and salt stress (Sah et al. 2016). ABA is considered as a root-derived signaling compound that enables plants' survival under adverse environmental conditions such as salt stress (Keskin et al. 2010). ABA accumulation, induced by stress signals, activates ABA-dependent signaling pathways by regulating the expression of numerous genes responsive to salt stress (Fernando and Schroeder 2016; Yi et al. 2019). It has found that ABA can induce the expression of genes encoding ion transporters, thus enhancing the ion-selective absorption and contributing to the transfer of Na^+ from the cytoplasm to the vacuole or discharging it out of the plant (Osakabe et al. 2014). An increase in endogenous ABA content also induces the expression of genes encoding antioxidant enzymes, thereby increasing the activity of several important antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) (Jiang and Zhang 2002). A plethora of studies have highlighted the critical roles of ABA in reducing stomatal conductance and inducing the expression of genes responsible for various osmoprotectant syntheses such as glycine betaine and proline (Jung et al. 2008; Antoni et al. 2011).

Sulla carnosa is a halophytic legume with potential to be used for soil rehabilitation, ground cover and livestock forage in arid and semi-arid salt-affected areas (Saddhe et al. 2020). The growth of *S. carnosa* on these degraded soils may be related to the plant ability to associate with halotolerant PGPR and/or AMF that improve the plant nutrition (Hidri et al. 2019). Indeed, these beneficial microorganisms constitute means of adapting plants to salt stress conditions. In this regard, PGPR and/or AMF play an important role in elicitation of defense mechanisms under salt stress conditions by modulating plant hormone especially ABA, however, reports examined beneficial microorganism-mediated regulation of ABA metabolism for enhancing stress tolerance in halophytic plants are scarce.

The main goal of this study was to investigate the capacity of salt-tolerant plant-growth-promoting bacteria (PGPR) (*Bacillus subtilis*) isolated from a salt-affected area and an arbuscular mycorrhizal fungus (AMF) (*Rhizophagus intraradices)* adapted to saline soils to regulate ABA metabolism for enhancing antioxidant capacity and stress tolerance in the indifferent halophyte *S. carnosa* Desf (that grows on salt-affected and non-salt-affected soils).

Materials and Methods

Plant, Microorganisms, and Inoculation Treatments

The plant used in this study was habitat indifferent halophyte (that grows on salt-affected and non-salt-affected soils) *Sulla carnosa* Desf; an endemic Tunisian extremophile species growing in semi-arid and arid regions with alkaline-saline soil conditions. *S. carnosa* seeds were collected from 'Sidi Khlif' (35°30′24.7'' N 10°38′26.5'' E).

The PGPR bacterium strains *Bacillus subtilis* (GenBank Accession no. MK089520), used in this study was isolated from rhizospheric soil from the salt-affected area in Soliman Sebkha (located at 36° 42′ 03.500" N, 10° 26′ 00.800' E, approximately 30 km south of Tunis, within a semi-arid bioclimatic zone) and was selected on the basis of its plant growth-promoting attributes, like its indole acetic acid (IAA) and siderophore production and phosphate solubilization (Hidri et al. 2019). The isolated strain was grown in 250 ml of Luria–Bertani (LB) liquid medium at 28 ± 2 °C with shaking at 180 rpm for 48 h. One milliliter of pure bacterial culture containing 10^8 CFU mL^{-1} was applied to the appropriate pot at transplanting and again 15 days later around the plant in the soil.

The AM fungi used was a strain of *Rhizophagus intraradices* (Schenck and Smith 1982) EEZ 58 (Ri), which was obtained from the collection of 'Estación Experimental del Zaidín' (CSIC, Granada, Spain). The AM fungal inoculum was obtained from an open pot culture of *Zea mays* as the host plant and maintained by storage in polyethylene bags at 4 °C containing soil, spores, mycelia, and infected root fragments. The inoculum was added at the germination stage and at transplanting time, just below seedlings. Non-inoculated control plants received a filtrate of the inoculum, in order to provide a general microbial population free of AM propagules.

Greenhouse Experiment

The experiment had a factorial design with three factors (microbial inoculation, salt stress, and harvesting times) and tenfold replication, giving a total of eight treatments: (1) non-saline non-inoculated plants (control plants, C), (2) saline non-inoculated plants (S), (3) non-saline inoculated plants with the AMF *R. intraradices* (Ri), (4) non-saline inoculated plants with *B. subtilis* (Bs), (5) non-saline dual-inoculated plants with AMF and *B. subtilis* (Ri + Bs) (6) saline inoculated plants with the AMF *R. intraradices* (S + Ri), (7) saline inoculated plants with *B. subtilis* (S + Bs), (8) saline dual-inoculated plants with AMF and *B. subtilis* (S + Ri + Bs). There were ten replicates of each treatment resulting in a total of 80 pots, each with one plant.

A loamy soil (Alfisol Xeralf Rhqdoxeralf) (Soil Survey Staff 1975) was collected from the province of Granada (Spain) during the spring of 2014. After sieving through a 5 mm mesh, the soil was mixed with quartz sand (< 1 mm) in a 1:1-volume ratio, and sterilized by steaming at 100 °C for 1 h over 3 consecutive days. Basic physico-chemical properties (pH, EC, organic C, available N, P, and K) of the experimental soil were analyzed prior to the pot experiments (Table 1). Pots were filled with 500 g of this soil/sand mixture. The experiments were conducted in a greenhouse at 24/20 °C (day/night), with 16 h/8 h photoperiod, a relative humidity of 50–60%, and an average photosynthetic photon flux density of 800 µmol m^{-2} s^{-1}, as measured with a light meter (LICOR, model LI-188B, Lincoln, NE, USA).

Plants were sampled to assess changes in growth attributes, physiological and biochemical traits at 2 different time intervals. One month after transplanting *S. carnosa* seedlings to pots, plants were watered three times per week with 50 mL of either a 0 mM or 100 mM NaCl solution. A first harvest on half of the plants was performed after two weeks of salt treatment. The remaining plants were then challenged for two weeks with 200 mM NaCl salinity before performing the final harvest.

Measured Parameters

Biomass Production

After each harvest, the plants were separated into their root and shoot components, and their fresh weights were promptly measured; then a part were stored at − 80 °C for biochemical/gene analyses and the other part was dried in a forced-air oven (70 °C) for 48 h and weighed to determine dry weights.

Table 1 Physico-chemical properties of experimental soil

Characteristics	Values
pH	8.1
EC (dS m^{-1})	200 S cm^{-1}
Textural Class	Loamy soil
Organic matter (%)	15 g kg^{-1}
Total nitrogen (%)	1.9
Extractable potassium (mg kg^{-1})	6.9
Total phosphorus (mg kg^{-1})	1
Available phosphorus (mg kg^{-1})	0.027

Stomatal Conductance

One day prior each harvest, stomatal conductance was measured 2 h after the lights were turned on by using a porometer system (Porometer AP4, Delta-T Devices Ltd, Cambridge, United Kingdom) following the user manual instructions. Measurements were taken in the second-youngest leaf on five plants in each treatment.

Electrolyte Leakage (EL)

The second youngest leaf was collected from plants under each treatment and thoroughly washed with deionized water to remove electrolytes adhering to the surface. Leaf was then submerged in 10 mL of distilled water and incubated at room temperature on a rotary shaker for 3 h, and the electrical conductivity of the solution (C0) was determined with a conductivity meter COND 510 (Porometer AP4, Delta-T Devices Ltd, Cambridge, United Kingdom). Samples were autoclaved at 120 °C for 20 min and the final electrical conductivity (CF) was noted. The conductivity of distilled water was also measured and referred to as CW. The percentage of electrolyte leakage was defined as follows:

$$EL\,(\%) = \left[(C0-CW)/(CF-CW)\right] \times 100$$

Antioxidant Enzyme Activities

Fresh leaf samples (0.5 g) were ground in liquid nitrogen to prevent any proteolytic damage. The enzymes were extracted in 5 mL of extraction buffer (50 mM phosphate buffer (pH 7.5), 0.1 mM EDTA, 1% polyvinylpyrrolidone), centrifuged at 15 000x*g* for 20 min (Sharma et al. 2021; Aroca et al. 2003). The supernatant was used to determine the enzyme activity.

APX (EC: 1.11.1.11) activity was measured by the method of Nakano and Asada (1981). The reaction mixture consisted of 50 mM potassium phosphate buffer (pH = 7.0), 0.5 mM ascorbic acid, 1.2 mM H_2O_2, 0.1 mM EDTA and enzyme extract. The reaction was initiated with the addition of H_2O_2 and the decrease in absorbance was measured at 290 nm for 2 min. The specific activity was calculated with an extinction coefficient of 2.8 mM^{-1} cm^{-1}.

Total SOD (EC 1.15.1.1) activity was determined following the method of Burd et al. (2000) by monitoring spectrophotometrically the inhibition of the photochemical reduction of nitroblue tetrazolium (NBT) at 560 nm. The reaction mixture was comprised of 50 mM potassium phosphate buffer (pH = 7.8), 13.32 mM methionine, 65 µM nitro blue tetrazolium (NBT), 13.3 µM riboflavin and the enzyme extract.

Enzymatic activities were expressed as units per mg of protein (Bradford 1976), using bovine serum albumin (BSA) as a standard.

Hydrogen Peroxide Content

Leaf hydrogen peroxide (H_2O_2) content was determined spectrophotometrically according to Patterson et al. (1984), with slight modifications as described previously by Aroca et al. (2003). 200 mg of fresh leaves weight were homogenized in a cold mortar with 5 mL of 5% (w/v) TCA containing 0.1 g of activated charcoal and 1% (w/v) PVPP then centrifuged at 18,000×*g* for 10 min. The supernatant was filtered through a Millipore filter (0.22 µm). To the supernatant (130 µL), 1.2 mL of 100 mM potassium phosphate buffer (pH 8.4) and 0.6 mL of the colorimetric reagent was added. The colorimetric reagent was freshly prepared by mixing 1:1 (v/v) of 0.6 mM potassium titanium oxalate and 0.6 mM 4–2 (2-pyridylazo) resorcinol (disodium salt). Samples were incubated at 45 °C for 1 h and absorbance was taken at 508 nm against 5% TCA as blank.

Gene Expression Analysis by Real-Time RT-PCR (qPCR)

The expression of *S. carnosa* 9-cis-epoxy carotenoid dioxygenase (*NCED1*), and SOD genes was studied by real-time PCR (qPCR) by using iCycler (Bio-Rad, Hercules, California, United States of America).

S. carnosa leaves were collected and frozen in liquid nitrogen at the final harvest. Total RNA was isolated from samples by phenol/chloroform extraction, according to the method described by Kay et al. (1987). Concentration and purity were evaluated in a NanoDrop ND1000 spectrophotometer (NanoDrop-Technologies Inc.) and the integrity was evaluated by gel electrophoresis. The RNA was treated with RQ1 DNase (Promega, Madrid, Spain), purified through a silica column using the NucleoSpin RNA Clean-up kit (Macherey–Nagel, Hoerd, France), and stored at -80°C until use.

Gene specific primers for *NCED1* (forward: 5′- CAACAC AACTACAACCGCCA -3′, reverse: 5′- AGGGAGTGTTTG GAGAGAGC TT-3′) and *SOD* (forward: 5′- GGTGATGAT GGAACCGCAAA -3′, reverse: 5′- CCCTGCAGACCAATA ATGCC -3′) were used in quantitative reverse transcription-polymerase chain reaction (qRT-PCR).

PCR conditions were 3 min incubation at 95 °C to activate the hot-start recombinant Taq DNA polymerase, followed by 32 cycles of denaturation for 30 s at 95 °C, 30 s at 58 °C, and 30 s at 72 °C, where the fluorescence signal was measured.

Three independent biological replicates per treatment were performed and each real-time PCR reaction was done in triplicate. The first strand of cDNA was synthesized using the iScript cDNA synthesis kit (Bio-Rad), according to the manufacturer's instructions. The relative gene expression levels were calculated using the comparative $2^{-\Delta Ct}$ method (Livak and Schmittgen 2001), with actin as an endogenous control (Porcel et al. 2014).

Abscisic Acid Content

ABA concentration was analyzed in shoot and root extracts using high performance liquid chromatography electrospray-mass spectrometry (HPLC–ESI–MS/MS). ABA extraction and purification were carried out using the method described by Bacaicoa et al. (2009), but using 0.2 g of frozen shoot and root tissue (previously ground to a powder in a mortar with liquid nitrogen) instead of 0.5 g. ABA was quantified by HPLC–ESI–MS/MS using an HPLC device (2795 Alliance HT; Waters Co., Milford, Massachusetts, United States of America) coupled to a 3200 Q TRAP LC/MS/MS System (Applied Biosystems/MDS Sciex, Ontario, Canada), equipped with an electrospray interface, using a reverse-phase column (Synergi 4 mm Hydro-RP 80A, 150 mm × 2 mm, Phenomenex, Torrance, Canada). A linear gradient of methanol (A) and 0.5% acetic acid in water (B) was used: 35% A for 1 min, 35% to 95% A in 9 min, 95% A for 4 min, and 95% to 35% A in 1 min, followed by a stabilization time of 5 min. The flow rate was 0.20 mL min^{-1}, the injection volume was 40 µL and column and sample temperatures were 30 and 20°C, respectively. The detection and quantification of ABA was performed by multiple reaction monitoring (MRM) in the negative-ion mode, employing multilevel calibration curves with deuterated hormone as an internal standard. For further details see Bacaicoa et al. (2009). The source parameters were curtain gas 25 psi, GS1 50 psi, GS2 60 psi, ions spray voltage − 4000V, CAD gas medium, and temperature 600°C.

Statistical Analysis

Statistical analysis was performed using SPSS 20.0 statistical program (SPSS Inc., Chicago, IL, United States of America). Mean values were compared by Duncan's test and the differences were considered significant at $p < 0.05$. Correlation analysis was performed using XLSTAT software v. 2014 (Addinsoft, Paris, France).

Results

Biomass Production

Salinity levels imposed statistically significant effects on the shoot dry biomass of non-inoculated plants at different harvesting times, with a marked decrease under high salinity in the second harvest which was reduced by *ca.* 39% compared to those of the control (Fig. 1 and Fig. 2). Regarding root and whole plant dry weights of salt-affected non-inoculated plants, significant differences between the salinity levels were observed at the second harvest, which was 56% and 39% lower than that of the control plants, respectively (Fig. 1).

Globally, microbial inoculated plants were found healthier than non-inoculated plants especially at the second harvesting time despite dual-inoculated plants with *B. subtilis* and *R. intraradices* showed more enhanced shoot and whole plant dry biomass at both harvesting times (Fig. 1). Plants inoculated with *B. subtilis* showed higher root dry weight under saline and non-saline conditions at the second harvest compared to control and stressed non-inoculated plants. Data regarding plant growth attributes indicated that dual microbial inoculation (*B. subtilis* and *R. intraradices*) consistently increased the observed parameters as compared to control plants in saline conditions at both harvesting points (Fig. 1 and Fig. 2).

Stomatal Conductance and Electrolyte Leakage

Data of stomatal conductance and electrolyte leakage attributes demonstrated variations in *S. carnosa* plants response driven by salinity stress and microbial inoculation either alone or in combination (Fig. 3) at both harvesting times.

R. intraradices alone or associated with *B. subtilis* markedly enhanced the stomatal conductance as compared to plants grown under salt-free conditions at the first harvesting time (Fig. 3a). At the second harvest, bacterial strain *B. subtilis* alone or associated with *R. intraradices* was found highly efficient for observed stomatal conductance as compared to *R. intraradices* alone and non-inoculated plants (Fig. 3a). Salt stress had statistically significant impact on stomatal conductance of inoculated and non-inoculated plants at the second harvesting time. However, no significant differences between different inoculations were observed under salt stress conditions at both harvesting stage (Fig. 3a).

The differences of electrolyte leakage were significant between salinized and non-salinized as well as between inoculated and non-inoculated plants at different plant growth stages (first and second harvest) (Fig. 3b). Non-inoculated and inoculated *S. carnosa* plants showed a significant increase in electrolyte leakage under saline stress condition

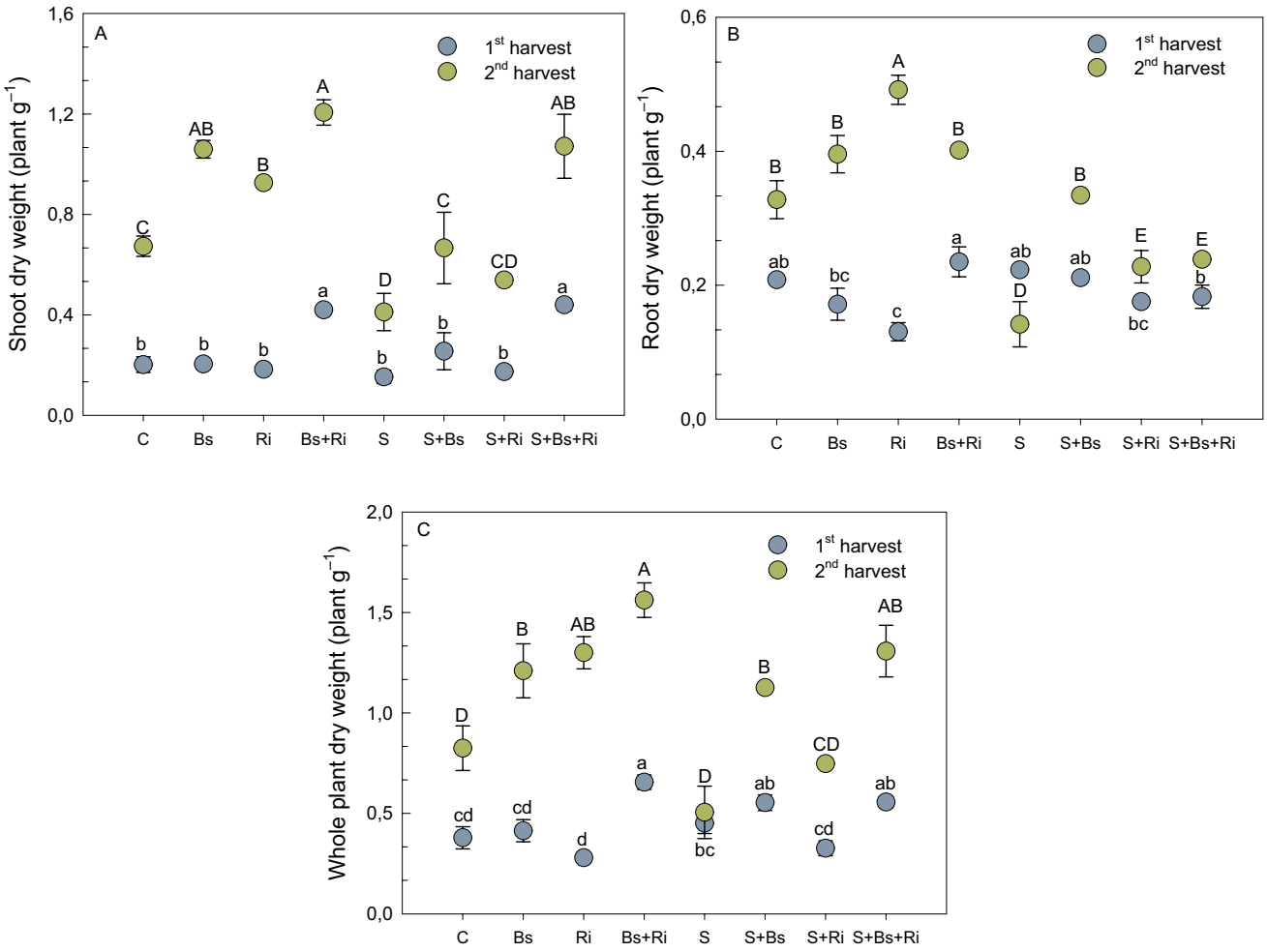


Fig. 1 Effect of inoculation with *R. intraradices* and/or *B. subtilis* on shoot dry weight (**A**), root dry weight (**B**), and total dry biomass (**C**) of eight-week-old *S. carnosa* plants challenged or not during 2 weeks with moderate salinity (100 mM NaCl) and during 2 other weeks with high salinity (200 mM NaCl). Data represent the mean ± SD of eight replicates and different letters indicate a significant difference ($p \leq 0.05$) using Duncan's test

at each plant growth stage (first and second harvest), as compared to their respective control plants (Fig. 3b). However, when comparing inoculated and non-inoculated plants, salinity stress markedly affected non-inoculated control plants at each stress level (Fig. 3b). Moreover, inoculation with AMF *R. intraradices* reduced electrolyte leakage under salinized condition at the first harvest time (− 42%), and at the second harvest when associated with *B. subtilis* compared to non-inoculated plants (− 32%) (Fig. 3b).

Hydrogen Peroxide Content and Antioxidant Enzyme Activities

Statistical analysis of the data showed significant effects of induced salinity levels, microbial inoculations and plant growth stages on hydrogen peroxide content and antioxidant enzyme activities of *S. carnosa* plants.

Leaf H_2O_2 level progressively increased with progression in plant growth (first and second harvest) under varying levels of induced soil salinity (Fig. 4a). H_2O_2 accumulation was significantly higher in non-inoculated plants compared to inoculated plants at the second harvest (Fig. 4a). Furthermore, *R. intraradices* with *B. subtilis* proved to be more effective with the least extent of H_2O_2 formation at each harvest under both saline and non-saline conditions (Fig. 4a).

The activities of ascorbate peroxidase (APX) and superoxide dismutase (SOD) were determined in the leaves of *S. carnosa* plants under different salinity levels and microbial inoculations at two harvesting times as presented in Fig. 4b and Fig. 4c. Under salt-free condition, SOD activity was significantly low in non-inoculated plants while inoculated plants showed high activity at both harvesting times (Fig. 4b). In contrast to non-inoculated plants,

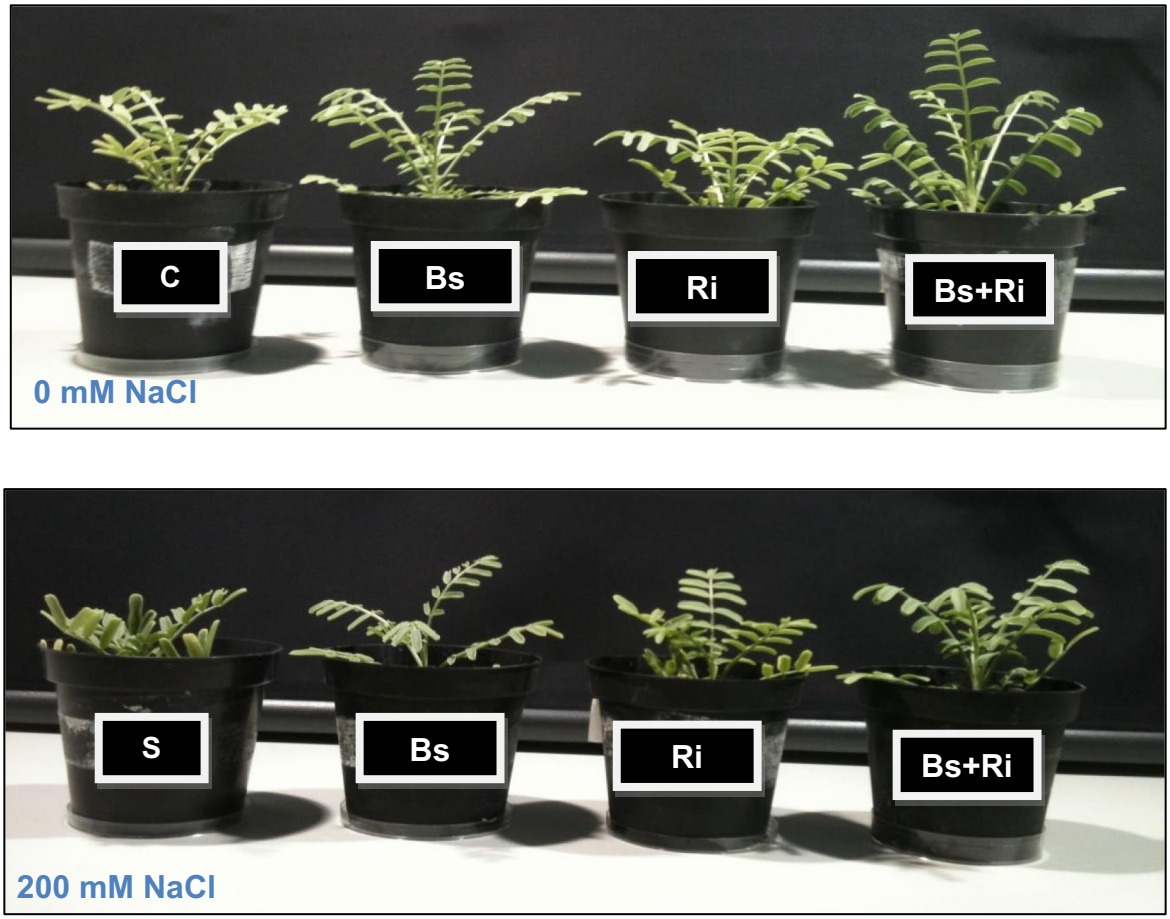


Fig. 2 Phenotypic comparison of non-inoculatad (Control) and inoculated (*R. intraradices* and/or *B. subtilis*) *S. carnosa* plants challenged or not during 2 weeks with moderate salinity (100 mM NaCl) and during 2 other weeks with high salinity (200 mM NaCl)

inoculated plants appeared tolerant against increasing levels of salinity stress with succeeding growth stages whereas microbial inoculation significantly improved SOD activity under salinized condition at the second plant growth stage compared to non-inoculated plants (Fig. 4b). It is worth mentioning that dual inoculation led to higher SOD activity for salinized and non-salinized plants at the second harvest.

APX activity showed a significant increase in non-inoculated plants on exposure to salt stress compared to their performance at both harvest stages. At first harvest, single microbial inoculation with *B. subtilis* or *R. intraradices* increased APX activity under salt-free conditions. However, no significant difference between inoculated and non-inoculated plants was observed under saline stress conditions. These differences were more pronounced at the second harvest for all combinations of salinity stress and bacterial inoculations in *S. carnosa* plants. Indeed, a stronger increase in APX activity was observed with dual microbial inoculation at second harvest.

Plant Abscisic Acid Content

Salinity levels imposed statistically significant effects on shoot ABA content of non-inoculated plants at both harvesting times with progressive increase toward high salinity stress. Dual microbial inoculation (*R. intraradices* with *B. subtilis*) consistently increased shoot ABA content as compared to control plants in saline and non-saline conditions at both harvesting points (Fig. 5a).

No significant effect of salinity stress on root ABA content of *S. carnosa* plants was observed at both harvest times (Fig. 5b). At the first harvest, inoculated *S. carnosa* plants showed statistically significant decrease in root ABA content under salt-free condition, whereas non-significant difference between inoculated and un-inoculated plants with *B. subtilis* alone or combined with *R. intraradices* was computed under salt stress conditions. Yet, these differences were more pronounced at the second harvest for all combinations of salinity stress and microbial inoculations in *S. carnosa*. Statistically, a stronger increase in root ABA content was observed with *B. subtilis* or *R.*

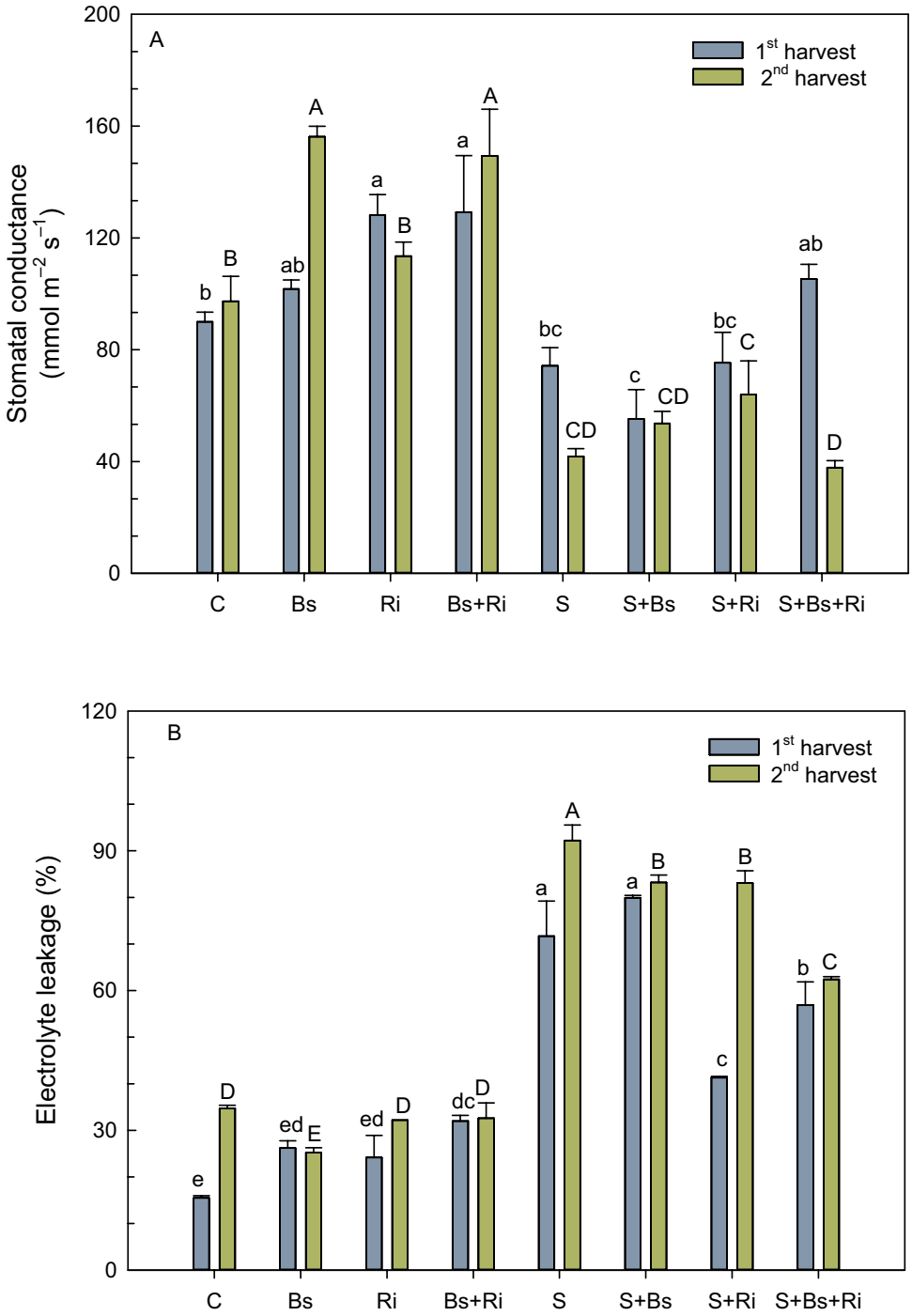


Fig. 3 Effect of inoculation with *R. intraradices* and/or *B. subtilis* on stomatal conductance (**A**) and electrolyte leakage (**B**) of eight-week-old *S. carnosa* plants challenged or not during 2 weeks with moderate salinity (100 mM NaCl) and during 2 other weeks with high salinity (200 mM NaCl). Data represent the mean ± SD of eight replicates and different letters indicate a significant difference ($p \leq 0.05$) using Duncan's test

intraradices inoculation at the second harvest as compared to control non-salinized plants, while higher root ABA content was observed in *R. intraradices* inoculated plants under salt stress conditions at the second harvest (Fig. 5b).

Quantitative Real-Time RT-PCR

The expression of *SOD* and *NCED1* genes was analyzed in leaves of each plant treatment only at final harvesting (Fig. 6). *SOD* gene expression increased in leaves of non-inoculated plants in the presence of salt, while in *R. intraradices* leaves their expression went down as compared to stressed and non-inoculated plants (Fig. 6a). Dual-inoculated plants with *B. subtilis* and *R. intraradices* showed relatively higher *SOD* gene expression compared to *B. subtilis* alone and control plants at each salinity stress levels. However, significant differences among the inoculations were observed at salinity levels of 0 and 200 mM NaCl (Fig. 6a).

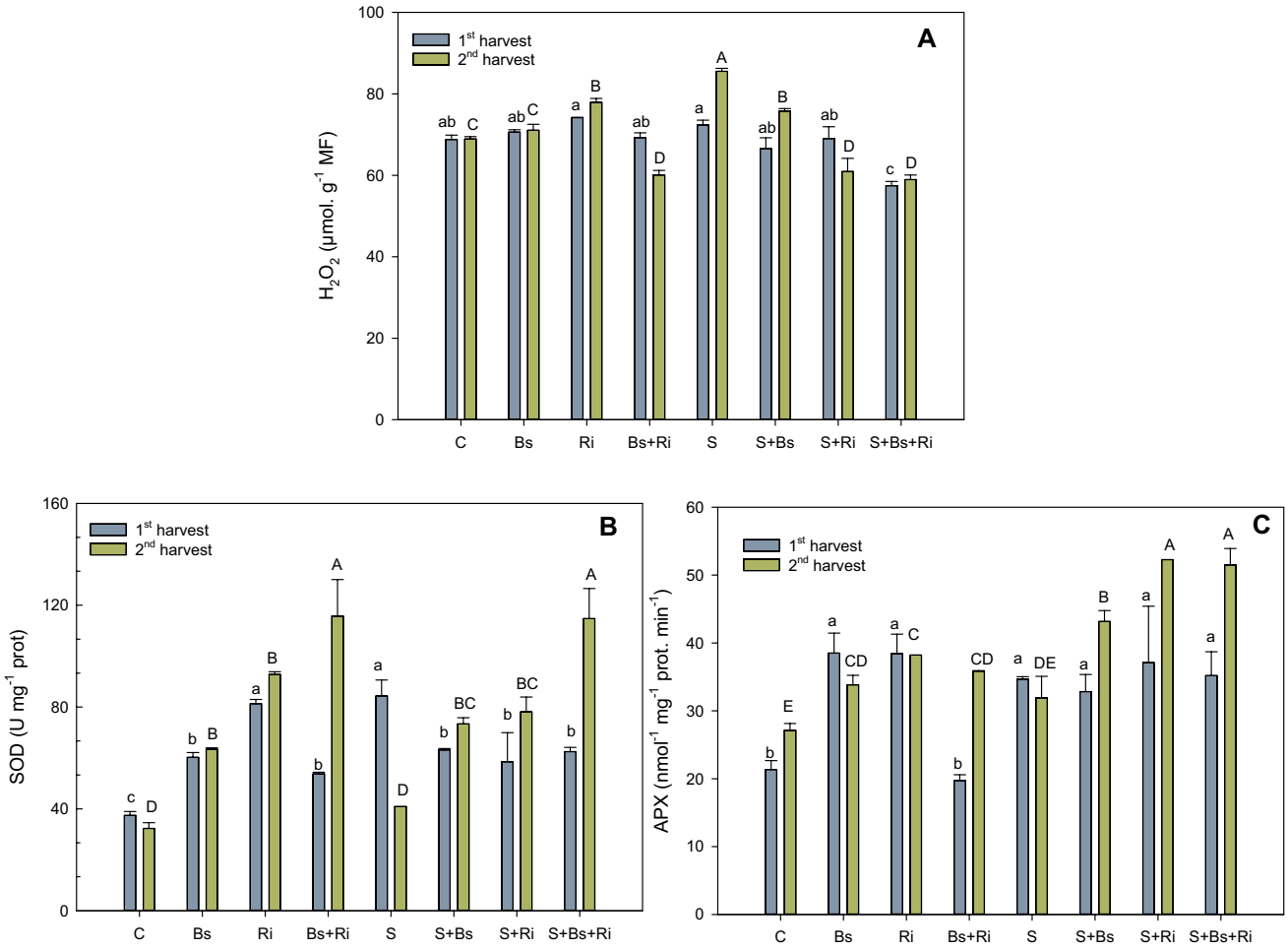


Fig. 4 Effect of inoculation with *R. intraradices* and/or *B. subtilis* on shoot H_2O_2 content (**A**), SOD activity (**B**), and APX activity (**C**) of eight-week-old *S. carnosa* plants challenged or not during 2 weeks with moderate salinity (100 mM NaCl) and during 2 other weeks with high salinity (200 mM NaCl). Data represent the mean ± SD of eight replicates and different letters indicate a significant difference ($p \leq 0.05$) using Duncan's test

NCED1 gene expression was shown to be inhibited in plants AMF inoculated with no significant differences were observed between plants inoculated with *B. subtilis* alone or in combination with *R. intraradices* and non-inoculated plants (Fig. 6b). Under salt stress condition, a significant induction of gene expression was observed in stressed inoculated plants with *B. subtilis* alone or combined with *R. intraradices* compared to non-inoculated plants, however, dual inoculation performed highest (Fig. 6b).

Correlation Analysis

In order to provide a comprehensive analysis of the traits characterizing *S. carnosa* plants subjected to salt stress and microbial inoculation, we conducted a correlation analysis at the second harvest time (Tables 2, 3 and 4). Correlation analysis clearly showed that shoot ABA content was positively correlated with SDW($r = 0.78$), *NCED1* gene expression ($r = 0.70$), SOD activity ($r = 0.85$) and negatively correlated with EL ($r = -0.93$) and H_2O_2 content ($r = -0.59$) under salt stress condition. Also root ABA content was positively correlated with APX activity ($r = 0.82$). The stomatal conductance was positively correlated to SDW ($r = 0.75$) and WPDW ($r = 0.64$) under salt-free condition. H_2O_2 content was negatively correlated with SOD and APX activities (r (SOD) = − 0.80; r (APX) = − 0.88) and positively correlated with to EL ($r = 0.69$) under salt stress condition. SOD showed a positive correlation with its gene expression ($r = 0.58$) under salt-free condition.

Salt treatment was negatively correlated with all growth traits (r (S DW) = − 0.55, r (R DW) = − 0.72, and r (WP DW) = − 0.7), and SOD and APX activities (r (SOD) = − 0.74 and r (APX) = − 0.84), indicating that salt stress without microbial inoculation led to a decrease in all these parameters. However, this treatment showed positive correlations for electrolyte leakage and H_2O_2 content (r

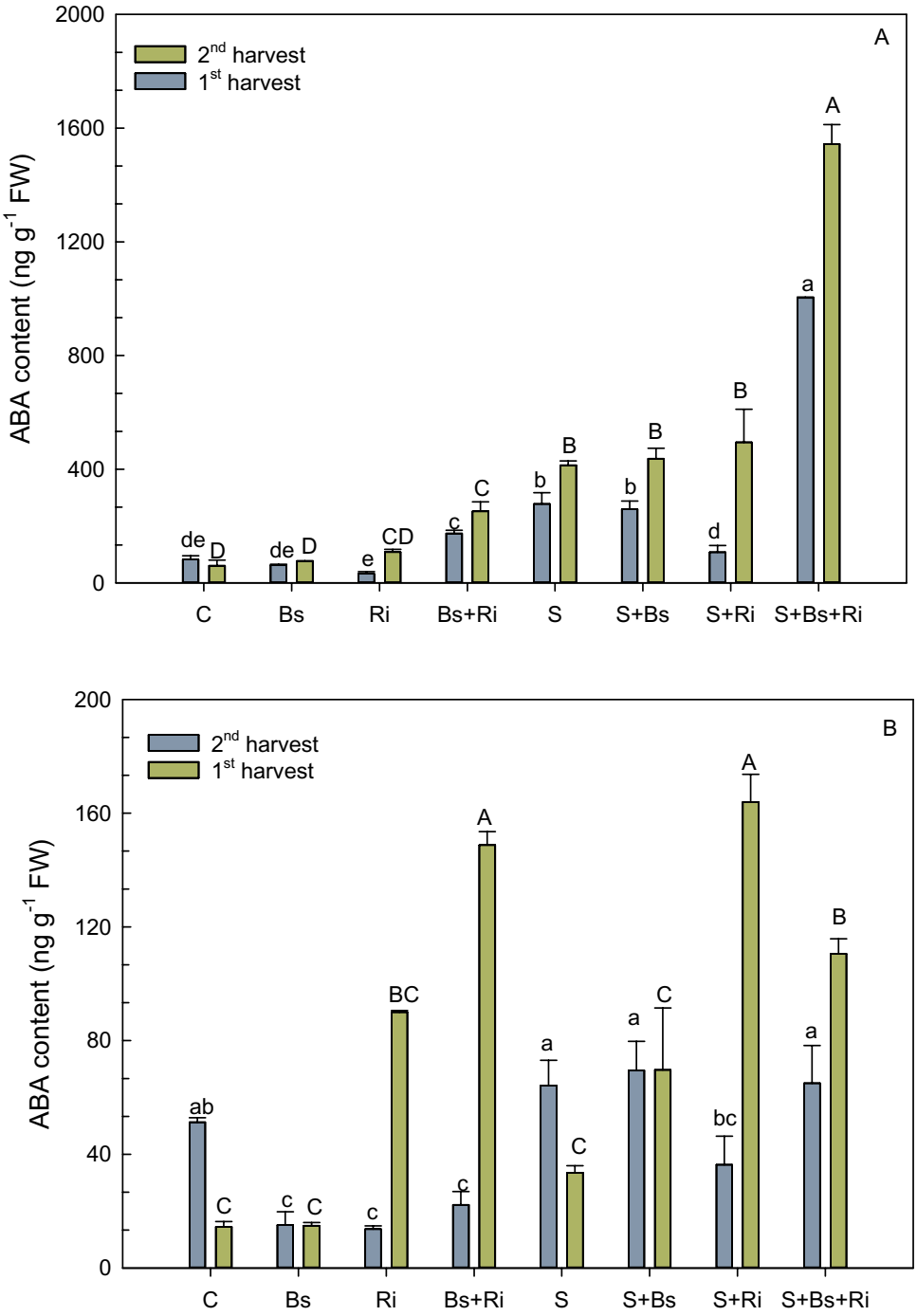


Fig. 5 Effect of inoculation with *R. intraradices* and/or *B. subtilis* on shoot ABA content (**A**) and root ABA content (**B**) of eight-week-old *S. carnosa* plants challenged or not during 2 weeks with moderate salinity (100 mM NaCl) and during 2 other weeks with high salinity (200 mM NaCl). Data represent the mean ± SD of eight replicates and different letters indicate a significant difference ($p \leq 0.05$) using Duncan's test

EL = 0.60) and *r* (H_2O_2 = 0.78)). Moreover, dual-inoculated plants with *B. subtilis* and *R. intraradices* showed positive correlations for shoot and whole plant dry weights (*r* (SDW) = 0.68 and *r* (WPD) = 0.64), shoot and root ABA content (*r* (shoot ABA) = 0.91 and *r* (root ABA) = 0.87), SOD activity (*r* = 0.69) and SOD gene expression (*r* = 0.9).

Remarkably, salt treatment with dual microbial inoculation was positively correlated with shoot ABA content (*r* = 0.98), SOD activity and its gene expression (*r* (SOD) = 0.79 and *r* (*SOD* gene) = 0.58) and *NCED1* gene expression (*r* (*NCED1* gene) = 0.76) and however, this treatment showed negative correlations for electrolyte leakage (*r* = − 0.90) and H_2O_2 content (*r* = − 0.58).

Discussion

Beneficial microbes provide additional support necessary for plant growth and survival under excess salinity stress through a range of direct and indirect mechanisms. It is

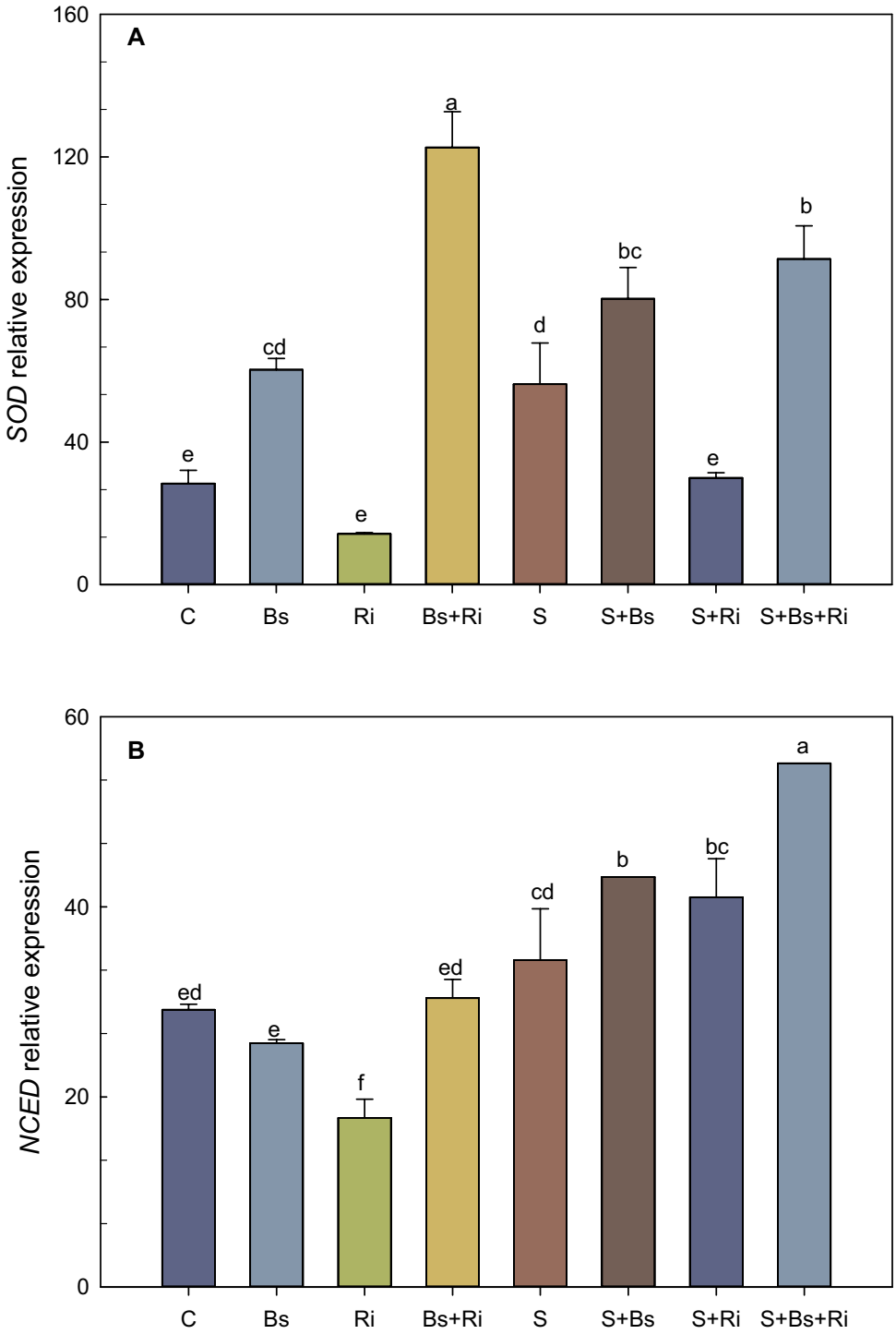


Fig. 6 Effect of inoculation with *R. intraradices* and/or *B. subtilis* on relative *SOD* gene expression (**A**) and relative *NCED1* gene expression (**B**) of eight-week-old *S. carnosa* plants challenged or not during 2 weeks with moderate salinity (100 mM NaCl) and during 2 other weeks with high salinity (200 mM NaCl). Data represent the mean ± SD of eight replicates and different letters indicate a significant difference ($p \leq 0.05$) using Duncan's test

crucial to recognize the implication of these microorganisms in the endogenous mechanisms regulating the various processes and mechanisms responsible for plant stress tolerance. The effect of inoculation with AMF and PGPR strain was studied on *S. carnosa* plants subjected to different concentrations of NaCl at different plant growth stages under greenhouse.

In the present study, increasing salinity to 200 mM (NaCl) led to a significant decrease in shoot and root dry biomass of *S. carnosa* plants in comparison to the control plants at the second harvest time. Salinization is recognized as the major threats to plant growth and results in impaired physiological processes, abnormal biochemical mechanisms, ion toxicity, nutrient (N, Ca, K, P, Fe, Zn) deficiency, abridged photosynthesis, inhibited enzymatic activities, loss of cell turgor and

Table 2 Correlation coefficients between biomass, physiological and biochemical parameters in *S. carnosa* plants under salt-free condition at the second harvest

Variables	SDW	RDW	WPDW	EL	SC	H_2O_2	Shoot ABA	Root ABA	SOD	APX	*SOD* gene	*NCED1* gene	
SDW	**1**	0.38	0.86***	− 0.44	0.75**	− 0.43	0.64*	0.63*	0.81**	0.63	0.75**	0.04	
RDW		**1**	0.59*	− 0.09	0.04	0.51	0.15	0.42	0.55	0.79**	− 0.18	− 0.77**	1
WPDW			**1**	− 0.13	0.64*	− 0.21	0.65*	0.74**	0.87***	0.77**	0.57	− 0.17	0.8
EL				**1**	− 0.41	− 0.08	0.30	0.30	− 0.08	− 0.18	− 0.06	0.25	0.6
SC					**1**	− 0.32	0.46	0.24	0.50	0.40	0.71**	0.25	0.4
H_2O_2						**1**	− 0.63*	− 0.48	− 0.24	0.23	− 0.85***	− 0.82**	0.2
Shoot ABA							**1**	0.88***	0.75**	0.43	0.83***	0.37	0
Root ABA								**1**	0.87***	0.64*	0.60*	0.03	− 0.2
SOD									**1**	0.79**	0.58*	− 0.18	− 0.4
APX										**1**	0.17	− 0.54	− 0.6
SOD gene											**1**	0.63*	− 0.8
NCED1 gene												**1**	− 1

SDW shoot dry weight, *RDW* root dry weight, *WPDW* whole plant dry weight, *EL* electrolyte leakage, *SC* stomatal conductance, H_2O_2 hydrogen peroxide, *ABA* abscisic acid, *SOD* superoxide dismutase, *APX* ascorbate peroxidase

*indicates a significant correlation at a significance level alpha ≤ 0.05

**indicates a significant correlation at a significance level alpha ≤ 0.01

***indicates a significant correlation at a significance level alpha ≤ 0.001

overproduction of ROS in plants, which causes impairment of redox homeostasis resulting in oxidative stress (Hidri et al. 2019; Arif et al. 2020; Atta et al. 2023). Nonetheless, the application of *B. subtilis* alone or in combination with *R. intraradices* consistently increased plant biomass compared to control plants in saline conditions at the second harvesting point. These beneficial microorganisms play key roles in adapting the host plant to saline environment.

B. subtilis strain is one of the most widely studied plant growth-promoting rhizobacteria (Blake et al. 2021). It shows many direct and indirect mechanisms to promote plant growth, including improving nutrient availability, IAA and siderophore production, ACC deaminase activity, solubilization of the phosphorus, altering plant growth hormone homeostasis, and reducing the severity of abiotic stresses (Hidri et al. 2019; Yasmin et al. 2020; Blake et al. 2021). *B. subtilis* is well known for its ability to alleviate the damage effects of saline stress (Pinter et al. 2017; Wang et al. 2018; Metoui-Ben Mahmoud et al. 2024). *R. intraradices* is a regulator of stress by adjusting nutritional and hormonal balance, improving the antioxidant enzyme system and inducing systemic stress tolerance (Ruiz-Lozano et al. 2012; Hidri et al. 2019; Parihar et al. 2020; Yang et al. 2020). Several reports have also highlighted those beneficial microbes can assist their host plants in coping with salt stress (Niu et al. 2016; Pan et al. 2020a; Parihar et al. 2020; Hidri et al. 2023; Wang et al. 2024; Metoui-Ben Mahmoud et al. 2024). Diagne et al. (2020) have previously reported that inoculation with *P. agglomerans* and *Bacillus* sp. strains alone or in combination with *R. fasciculatus* can improve resistance to salinity of *Casuarina obesa* halophytic plants by increasing their growth parameters.

In the present study, the efficacy of co-inoculation with PGPR and AMF could be related to the ability of *B. subtilis* to solubilize phosphate and the effective absorption of solubilized P from soil through *R. intraradices* (Richardson et al. 2009; Hidri et al. 2019; Saia et al. 2020). Under salt-stressed conditions, stomatal conductance was impaired significantly at the second harvest point due to the closure of stomata to maintain the leaf water status. While some studies support the idea that chemical signals cause stomatal closure, others provide evidence that hydraulic signals do the same. The abscisic acid (ABA) plays a critical role in chemical signaling (Wilkinson and Davies 2002). The effect of microbial inoculation is enough to maintain higher stomatal conductance under salt stress conditions. ABA is the key factor controlling the stomatal closure in inoculated *S. carnosa* plants under saline conditions.

Excessive salt inhibits the electron transport chain causes excessive production of reactive oxygen species (ROS), creating oxidative stress in plants. ROS produced as a result of salinity stress could disrupt both the structure and functions of the plasma membrane at high concentrations (Pang and Wang 2008). Electrolyte leakage is involved in programmed cell death and metabolic adjustment of plants under salinity; thus, it can be used to estimate the cell membrane harm, and to obtain crucial information about the cell metabolic activities (Bajji et al. 2001; Demidchik et al. 2014). Increasing salinity levels in the soil caused severe electrolyte leakage, but inoculated plants, were less affected than non-inoculated

Table 3 Correlation coefficients between biomass, physiological and biochemical parameters in *S.carnosa* plants exposed to salt stress at the second harvest

Variables	SDW	RDW	WPDW	EL	SC	H2O2	Shoot ABA	Root ABA	SOD	APX	*SOD* gene	*NCED1* gene	
SDW	**1**	0.4	0.79**	− 0.86***	− 0.32	− 0.48	0.78**	0.09	0.65*	0.49	0.59*	0.65*	
RDW		**1**	0.71**	− 0.17	0.29	− 0.27	0.01	0.13	0.32	0.49	0.32	0.21	1
WPDW			**1**	− 0.72**	− 0.13	− 0.5	0.62*	0.21	0.67*	0.65*	0.61*	0.65*	0.8
EL				**1**	0.35	0.69*	− 0.93***	− 0.4	− 0.90***	− 0.61*	− 0.58*	− 0.76**	0.6
SC					**1**	− 0.27	− 0.51	0.33	− 0.18	0.27	− 0.49	− 0.07	0.4
H_2O_2						**1**	− 0.59*	− 0.79**	− 0.80**	− 0.88***	− 0.02	− 0.71*	0.2
Shoot ABA							**1**	0.24	0.85***	0.46	0.55	0.70*	0
Root ABA								**1**	0.51	0.82***	− 0.3	0.3	− 0.2
SOD									**1**	0.68*	0.42	0.74**	− 0.4
APX										**1**	− 0.02	0.48	− 0.6
SOD gene											**1**	0.44	− 0.8
NCED1 gene												**1**	− 1

SDW shoot dry weight, *RDW* root dry weight, *WPDW* whole plant dry weight, *EL* electrolyte leakage, *SC* stomatal conductance, H_2O_2 hydrogen peroxide, *ABA* abscisic acid, *SOD* superoxide dismutase, *APX* ascorbate peroxidase

*indicates a significant correlation at a significance level alpha ≤ 0.05

**indicates a significant correlation at a significance level alpha ≤ 0.01

***indicates a significant correlation at a significance level alpha ≤ 0.001

Table 4 Pearson's correlation matrix analyzing all different treatments and the different parameters studied at the second harvest

Variables	C	Bs	Ri	Bs + Ri	S	S + Bs	S + Ri	S + Bs + Ri	
SDW	− 0.83***	0.26	− 0.11	0.68*	− 0.55*	0.02	− 0.21	0.80**	
RDW	− 0.66*	− 0.07	0.76**	− 0.02	− 0.72**	0.70**	0.03	0.10	1
WPDW	− 0.76**	− 0.02	0.14	0.64*	− 0.70*	0.34	− 0.29	0.66*	0.8
EL	0.46	− 0.79**	0.13	0.18	0.60*	0.15	0.14	− 0.90***	0.6
SC	− 0.61*	0.60*	− 0.37	0.36	− 0.26	0.20	0.62*	− 0.51	0.4
H_2O_2	− 0.05	0.13	0.74**	− 0.83***	0.78**	0.28	− 0.48	− 0.58*	0.2
ABA shoot	− 0.45	− 0.34	− 0.11	0.91***	− 0.36	− 0.33	− 0.27	0.98***	0
ABA root	− 0.53	− 0.53	0.23	0.87***	− 0.68*	− 0.27	0.78**	0.18	− 0.2
SOD	− 0.76**	− 0.22	0.29	0.69*	− 0.74**	− 0.07	0.03	0.79**	− 0.4
APX	− 0.88***	0.01	0.60*	0.28	− 0.84***	− 0.10	0.49	0.45	− 0.6
SOD gene	− 0.38	0.05	− 0.57	0.90***	− 0.17	0.34	− 0.75**	0.58*	− 0.8
NCED1 gene	0.37	− 0.01	− 0.86***	0.50	− 0.58*	− 0.02	− 0.16	0.76**	− 1

The values in the table indicate the correlation coefficients (R) between the different variables studied. The different treatments were considered qualitative variables

*indicates a significant correlation at a significance level alpha ≤ 0.05

**indicates a significant correlation at a significance level alpha ≤ 0.01

***indicates a significant correlation at a significance level alpha ≤ 0.001

plants. The best results were obtained by dual microbial inoculation. This result indicated that both microorganisms help protect plants from cell membrane damage and enables them to adjust to salinity stress by regulating cell membrane function. Similarly, *Rhizophagus intraradices* and *Bacillus megaterium* were reported to improve salinity stress tolerance by decreasing electrolyte leakage in trigonella, bean and plants, respectively (Evelin et al. 2012; Motaleb et al. 2020). Therefore, *R. intraradices* and/or *Bacillus* sp. enhance tolerance to salinity stress by decreasing electrolyte leakage in leaves of *S. carnosa* plants.

To resist the stressful conditions, plants provide numerous antioxidant enzymes, which scavenge ROS in plant cells. So, antioxidant enzymes help to prevent or mitigate the detrimental effects of oxidative stress caused by salinity (Rasool et al. 2013).

It is known that SOD, one of the antioxidant enzymes, catalyzes the dismutation of superoxide to oxygen and hydrogen peroxide and CAT and APX appear to play an essential protective role in scavenging hydrogen peroxide process when coordinated with SOD, thereby providing protection, and enhance plant salt tolerance. In the present investigation, inoculation with *B. subtilis* and/or *R. intraradices* improved SOD and APX activities under salinity stress. Accordingly, *B. subtilis* and/or *R. intraradices* augments antioxidant enzyme activities and activation of the ROS scavenging process for enhancing tolerance to salinity stress in *S. carnosa* plants. Similar results were reported by Xun et al. (2015), where dual inoculation with *Rhizophagus intraradices* and *Acinetobacter* sp., significantly increased SOD, CAT, and POD activities of oat leaves grown in saline-alkali soil.

Several studies have shown that mycorrhizal plants had a higher accumulation of antioxidative enzymes and thus improve general plant growth under salt stress (Wang et al. 2022; Afshar and Abbaspour 2023; Zong et al. 2023). Our study showed that salinization induced oxidative damage through increased H_2O_2 level in *S. carnosa* plants. Yet, this parameter was significantly lower in *B. subtilis* and/or *R. intraradices* inoculated plants compared to that in plants inoculated with *Bacillus* sp. alone and non-inoculated plants in the second harvest, implying lower accumulation of ROS in mycorrhizal plans and lower membrane damage.

ABA is well known as the most important phytohormone mediating plant behavior under adverse environmental stimuli since the level of this hormone in plants usually increases during abiotic stress conditions, and elevated ABA can help plants to adapt and survive under stressful conditions (Sah et al. 2016). Under drought or salinity stress conditions, ABA is synthesized in roots and transported to leaves resulting in ABA accumulation in leaves; stimulating stomatal closure, change in gene expression, and adaptive physiological responses (Sah et al. 2016; Yoo et al. 2019). A typical effect of ABA on leaves is to minimize plants transpiration by increasing water influx into roots and controlling stomatal apertures which helps plants to adapt and survive stress conditions (Bharath et al. 2021). Furthermore, ABA has been demonstrated to regulates the vacuolar Na^+/H^+ antiporter gene linked to ion homeostasis in cells and stress-response genes, including RD29A, RAB18, and P5CS, when plants experience salinity stress (Shi and Zhu 2002; Saavedra et al. 2010).

Modulating endogenous phytohormones levels such as ABA, with beneficial microorganisms can help engineer plant metabolism and reduce the harmful effects during stress (Wani and Kumar 2015; Dar et al. 2017). Recently, some PGPR such *Bacillus mesonae*, *Bacillus licheniformis*, *Azospirillum* sp., and *Pseudomonas* sp. were reported to induce salinity stress tolerance in plants by enhancement of ABA content in leaves (Naz and Bano 2015; Zhou et al. 2017; Yoo et al. 2019). Additionally, the influence of arbuscular mycorrhizal on ABA balance under salt stress conditions has been documented in many plant species like maize (*Zea mays)* lettuce (*Lactuca sativa*), tomato (*Solanum lycopersicum*), and olive (*Olea europaea*) (Danneberg et al. 1993; Aroca et al. 2013; Yoo et al. 2019; Ouledali et al. 2019). While the impact of single inoculation with PGPR or AMF on ABA content has been well documented in recent investigations, the effect of dual inoculation is not well studied.

In the present study, dual inoculation with *B. subtilis* and *R. intraradices* increased endogenous levels of ABA compared to that in non-inoculated plants at both harvesting points after the salinity stress challenge. This might mean that *B. subtilis and R. intraradices* together conferred induced tolerance to salinity stress by inducing ABA accumulation in *S. carnosa* plants.

Furthermore, relative expression level of the *NCED1* gene in plants treated with *B. subtilis* and *R. intraradices* was higher than that in non-inoculated plants under salinity stress at the second harvest point under salinity stress condition. In the ABA biosynthetic pathway, 9-cis-epoxy carotenoid dioxygenase (*NCED*) is the key rate-limiting enzyme (He et al. 2018); the *NCED* encoded gene induced by abiotic stresses, led to ABA endogenous accumulation; *Bacillus* sp associated to *R. intraradices* induces overexpression of the *NCED1* caused overproduction of ABA, and enhanced salt stress tolerance (Iuchi et al. 2000; Thompson et al. 2000). A significant positive correlation ($r=0.70$) linking shoot ABA content with *NCED1* gene expression was revealed by correlation analysis under salt stress condition at the second harvest point. Accordingly, *B. subtilis and R. intraradices* regulated ABA metabolism including ABA accumulation and expression of ABA-related genes, *NCED1* to tolerate salinity stress in *S. carnosa* plants. The higher concentration of ABA in leaves of *B. subtilis* and *R. intraradices* inoculated plants

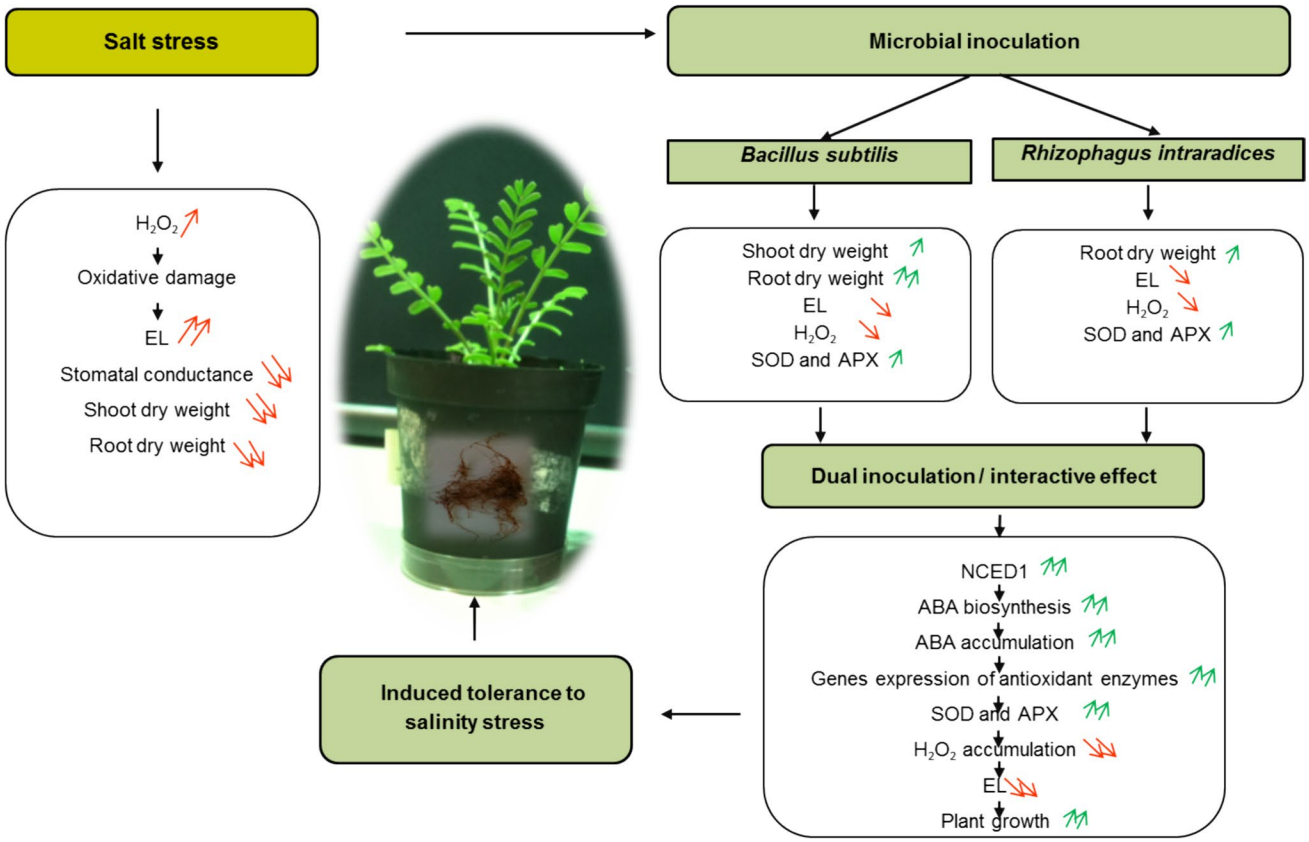


Fig. 7 Overview of mode of action of *B. subtilis* and *R. intraradices* for induced tolerance to salinity stress in *S. carnosa* plants. *B. subtilis* and *R. intraradices* improve plant growth and reduce electrolyte leakage and H_2O_2 in plant leaves. In addition, *B. subtilis* and *R. intraradices* lead to an increase of antioxidant enzyme activities including SOD and APX; ABA accumulation; upregulate *NCED*1 gene expression in *S. carnosa* plants

could be a specific mechanism of resilience to salt stress. Thus, beneficial microorganisms stimulate ABA accumulation that control biochemical and physiological mechanisms under salt stress condition. Similar results were reported, where beneficial microbes induced salinity tolerance by improving ABA levels in plants (Iuchi et al. 2000; Ouledali et al. 2019; Yoo et al. 2019).

Correlation analysis revealed a perfect match between the ABA content with the antioxidant enzymes activities resulting in significant positive relationship ($r = 0.85$ for shoot ABA content and SOD activity, and 0.82 for root ABA content and APX activity). Dual microbial inoculation increased endogenous ABA and SOD and APX activities and improved salt tolerance. Hence, there is a growing interest in leveraging plant hormones to improve antioxidant systems activities and enhance plant tolerance to oxidative stress induced by salinity. ABA improves the activities of enzymatic antioxidant defense systems. Zhou et al. (2017) found that the inoculation of Chrysanthemum plants with *Bacillus licheniformis* SA03 induced ABA to activate a series of adaptive responses in host plants under saline–alkaline stress, such as increased antioxidant enzymatic activities. Correlation analysis revealed no significant correlation between *B. subtilis* and/or *R. intraradices* treatments and root dry weight under salt stress conditions. Duan et al. (2013) and Fernando and Schroeder (2016) showed that endodermal cells activate ABA signaling and arrest growth so that lateral root do not elongate into highly saline environments, thus, in the presence of sodium ion ABA is a key signaling molecule in restriction lateral root growth.

Our findings also revealed that dual *B. subtilis and R. intraradices* improves plant fitness and ameliorate high salinity stress damage in plants by inducing ABA-dependent tolerance. Inoculation with beneficial microbes that stimulate genes responsible for the biosynthesis of plant hormones or phytohormones, such as abscisic acid (ABA), could be used to develop climate-resilient crops with improved yields under stressful conditions.

Conclusion

In this study, inoculation with PGPR and/or AMF showed to efficiently improve resistance to salinity of *S. carnosa* plants by increasing their growth parameters. In general, microbial inoculation influenced leaf and root ABA balance, antioxidant activities and stomatal functions under salt stress conditions. This positive effect of inoculation was more pronounced with *R. intraradices* and *B. subtilis*. In dual-inoculated plants, ABA concentration as well as the enzyme involved in ABA biosynthesis gene expression increased as salinity stress intensified. The plants which were inoculated with *R. intraradices* and *B. subtilis* also showed enhanced the activities of enzymatic antioxidant defense systems. Thus, dual inoculation with halotolerant PGPR and AMF could protect *S. carnosa* from severe salt stress by increasing ABA levels and improving the antioxidant enzyme activities (Fig. 7). Overall, dual inoculation with salt-tolerant PGPR and/or AMF could be a solution for the rehabilitation of salt-affected land in arid and semi-arid regions.

Author Contributions RH and RA conceived and designed the study, performed the experiments and analyzed data. RH wrote the manuscript. RA, WZ, AD, MBMO, MR, and CA revised the manuscript. All authors contributed to the article and approved the submitted version.

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Declarations

Competing Interests The authors declare that they have no competing interests.

Ethical Approval We confirm that all authors have consented to adhere to ethical standards.

Consent to Participate We confirm that all authors consent to participation.

Consent for Publication We confirm that all authors have given their consent to participate.

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