Contents lists available at ScienceDirect

# Heliyon



journal homepage: www.cell.com/heliyon

# Plant growth-promoting bacterial consortia improved the physiology and growth of maize by regulating osmolytes and antioxidants balance under salt-affected field conditions

Ali Afzal<sup>a</sup>, Muhammad Yahya Khan<sup>b,\*</sup>, Zahir Ahmad Zahir<sup>c,\*\*</sup>, Hafiz Naeem Asghar<sup>c</sup>, Atif Muhmood<sup>a</sup>, Muhammad Rashid<sup>a</sup>, Zeeshan Aslam<sup>a</sup>, Syed Ayyaz Javed<sup>d</sup>, Sajid Mahmood Nadeem<sup>b</sup>

<sup>a</sup> Institute of Soil Chemistry & Environmental Sciences, AARI, Faisalabad, Pakistan

<sup>b</sup> Sub-Campus Burewala, University of Agriculture, Faisalabad, Pakistan

<sup>c</sup> Institute of Soil & Environmental Sciences, University of Agriculture, Faisalabad, Pakistan

<sup>d</sup> Department of Soil & Environmental Sciences, College of Agriculture, University of Sargodha, Sargodha, Pakistan

# ARTICLE INFO

Keywords: Microbial consortia Rhizosphere Aggregation Phytohormones Endophytes

CelPress

#### ABSTRACT

This study was undertaken to see how microbial consortia influenced maize development and yield under salt-affected conditions. The efficacy of the pre-isolated bacterial strains *Burkholderia phytofirmans, Bacillus subtilis, Enterobacter aerogenes,* and *Pseudomonas syringae and Pseudomonas fluorescens* to decrease the detrimental effects of salt on maize was tested in four distinct combinations using Randomized Complete Block Design with three replicates. The results revealed that these strains were compatible and collaborated synergistically, with an 80% co-aggregation percentage under salt-affected conditions. Following that, these strains were tested for their ability to increase maize growth and yield under salt-affected field conditions. The photosynthetic rate (11–50%), relative water content (10–34%), and grain yield (13–21%) of maize were all increased by these various combinations. However, when *Burkholderia phytofirmans, Enterobacter aerogenes* and *Pseudomonas fluorescens* were combined, the greatest increase was seen above the un-inoculated control. Furthermore, as compared to the un-inoculated control, the same combination. These findings showed that a multi-strain consortium might boost maize's total yield response as a result of better growth under salt stress.

# 1. Introduction

Sodicity and salinity alone deteriorated 1128 million hectares of land throughout the world [1]. It is expected that 30% of arable areas would be impacted by salt in the next 25 years, and around 50% by the end of 2050 [2]. It has been reported that 10% annual increase in salt-affected soil around the globe [3]. Global trends indicate that to meet the food demands of the growing population, we must cultivate marginal land (salt-affected soils, for example) [4]. Salinity can be reduced by a variety of biotic and abiotic methods,

\* Corresponding author.

\*\* Corresponding author. E-mail addresses: yahya.khan@uaf.edu.pk (M.Y. Khan), zazahir@yahoo.com (Z.A. Zahir).

https://doi.org/10.1016/j.heliyon.2023.e17816

Received 11 January 2023; Received in revised form 25 June 2023; Accepted 28 June 2023 Available online 4 July 2023

2405-8440/© 2023 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

including the use of chemical additives (Gypsum), genetic engineering, and the breeding of salt-resistant cultivars [5]. The use of biological methods is the most promising method for removing salt stress [6] like plant growth-promoting bacteria (PGPB), mycorrhizal fungi, and nitrogen fixers are the most common bio-inoculants [7]. These microorganisms' tools might include nitrogen fixation in the atmosphere [8], nutrient solubilization [9], phytohormone synthesis [10], and release of metabolites and enzymes [11]. These activities improve nutrient uptake, control ethylene production, and cause biochemical changes (buildup of betaines, proline, and antioxidants). PGPB may settle efficiently at the roots of many plant species, improving plant mineral-nutrient adsorption and soil physical conditions, and therefore increasing output [12]. In addition to this mechanism, it is well known that ACC deaminase reduces stress-induced ethylene levels in plant species, resulting in tolerance to salt in a variety of crops. Through ACC deaminase activity, the plant growth promoting rhizo-bacteria, *Pseudomonas* spp., and *Enterobacter* spp. greatly increased maize biomass under varying salinity levels [13].

Due to their incapacity to fight pathogen assaults, limited survival capacity, and low colonization percentage, solitary stains find it challenging to interact with native soil populations under a variety of soil and environmental conditions [14]. Salinity resilience was generated by a single strain along the PGPR, either rhizobium or fungi. Under saline stress, for example, an Arbuscular mycorrhiza *Fugus, Glomus intraradices,* or *mosseae,* in combination with PGPR had greater growth and yield than when grown alone [15]. Several PGPR associations, such as *Herbaspirillum* and *Bacillus* [16], *Klebsiella* [17], *Rhizobium* [18], and *Burkholderia* [18], have shown salt stress mitigation in maize [19].

Microbial consortia, a biological method, have the potential to alleviate the consequences of salt stress. The five PGPR strains *Pseudomonas syringae* (S5), *Enterobacter aerogenes* (S14), *Pseudomonas fluorescens* (S20), *Burkholderia phytofirmans* (PsJN), and *Bacillus subtilis* (Y16) have been evaluated in laboratory and field experiments and have proven to be promising techniques for reducing salinity stress. Gaseous exchange, biochemical, ionic, and growth, and yield characteristics were used to evaluate the multi-strain consortia's potential for generating salt tolerance in field conditions on maize.

#### 2. Material and methods

The efficacy of an endophytic bacteria *Burkholderiaphytofirmans* in combination with salt-tolerant strains like *Bacillus subtilis*, *Enterobacter aerogenes*, and *Pseudomonasfluorescens* on maize growth and yield under salt stress conditions (EC 8.23 dS  $m^{-1}$ ) was tested in the field at the Postgraduate Agriculture Research Station, University of Agriculture, Faisalabad.

# 2.1. Compatibility test

These bacterial strains were investigated for their compatibility of growth as described by Raja et al., [20]. Each isolate was cultured in Luria-Bertani broth (50 mL) separately at  $28 \pm 1$  °C on shaker at 100 rpm for 48-72 h and all strains were cross streaked on same L.B agar plate. This step was repeated three times and these plates were incubated at  $28 \pm 1$  °C for 48-72 h and was examined for the inhibition zones around the colonies.

# 2.2. Synergism/antagonism tests

The synergistic/antagonistic activities of bacterial isolates were screened between these PGPR strains *Burkholderia phytofirmans, Bacillus subtilis, Pseudomonas fluoroscenes* and *syringae*, and *Enterobacter aerogenes*. For antibacterial assays, the bacterial isolates were cultivated in Luria-Bertani (LB) broth at  $28 \pm 1$  °C for 24 h. The bacterial isolates were spot-inoculated (10 µl aliquots) on LB plates preseded with 100 µl tested strains. The plates were incubated at 28 °C for 48 h and clear zones of inhibition were recorded [19].

#### 2.3. Co-aggregation and auto-aggregation tests

For co-aggregation assay, bacteria were grown in high C/N fructose minimal medium as described above and the cells were harvested by centrifugation at 5000g for 15 min, washed twice and re-suspended in phosphate buffered saline (0.1 mol L<sup>-1</sup>, pH 6.8) to give viable counts of  $10^8$  CFU mL<sup>-1</sup>. Equal volumes (2 mL) of each bacterial strain's cell suspension were mixed together in pairs by vortexing for 10s. Control tubes were maintained with 4 ml of bacterial suspension for each individual strains. The absorbance (A) at 600 nm of the suspensions was measured after mixing the strains and after 24 h of incubation at a temperature of  $28 \pm 2$  °C. The percentage of co-aggregation was calculated using the equation of Joe et al., [21].

$$Co-aggregation(\%) = \frac{(Ax + Ay + Az)/3 - A(x + y + z)}{Ax + Ay + Az/3} \times 100$$

where Ax, Ay and Az represent the absorbance of the three strains in the control tubes and

A (x + y + z) the absorbance of the mixture of the three strains after a period of 24 h.

Auto-aggregation assays were performed according to Joe et al. [21], with certain modifications to compare auto-aggregation potential of the strains with their co-aggregation efficiency. Cell suspensions (4 mL) were mixed by vortexing for 10 s and auto-aggregation was determined during 24 h of incubation at room temperature. After 24 h, 0.1 mL of the upper suspension was transferred to another tube with 3–9 mL of PBS buffer and the absorbance (A) was measured at 600 nm. The auto-aggregation percentage is expressed as

Auto – aggregation (%) =  $1 - At/A0 \times 100$ 

where At represents the absorbance at 24 h and A0 the absorbance at t = 0.

# 2.4. Inoculum preparation

The PGPR strains *Pseudomonas syringae* S5, *Enterobacter aerogenes* S14, *Pseudomonas fluorescens* S20, *Burkholderia phytofirmans* PsJN, and *Bacillus subtilis* Y16 were grown separately in LB medium for 24 h at  $28 \pm 1$  °C with shaking at 280 rev/min. After achieving the required population  $(10^7-10^8)$  CFU mL<sup>-1</sup> of the multi-strainconsortia, the cell suspensions of all three bacterial strain were prepared, mixed in equal proportions and incubated for 24 h at 28 °C with germ-free peat. After that, sterile maize seeds were coated by the help of 10% sugar solution. For the uninoculated control, the autoclaved broth was utilized to treat the seeds [22]. Following RCBD, five treatments C0 = Un-inoculated Control, C1 = (PsJN + S5+Y16), C2 = (PsJN + S14 + Y16), C3 = (PsJN + S14 + S20) and C4 = (PsJN + S20 + Y16) were duplicated thrice.

2.5. Determination of malondial dehyde contents, membrane permeability index, proline contents, relative water contents,  $K^+/Na^+$  ratio, chlorophyll contents, and gas exchange measurements

Jambu Nathan [23], method was used to calculate the leaf MDA concentration with the help of Beer and Lambert's equation at 600 and 532 nm. The membrane permeability index was computed using the formula for percent leakage of ions from leaves, as described by Jambu Nathan [23].

$$\% \text{ MPI} = \frac{\text{EC1} - \text{EC0}}{\text{EC2} - \text{EC0}} \times 100$$

At an absorbance of 520 nm, the free proline content in maize was determined using the technique described by Bates et al., [24]. Relative water content (RWC) was determined by following the procedure as explained by Mayak et al., [25]. A portable chlorophyll meter was used to determine the chlorophyll concentration [26]. CIRAS-III was used to measure the gaseousexchange measurements (MA, USA, PP System).



Fig. 1. Demonstrated results of antagonism/synergism assays where (A) positive control antagonistic interaction, (B) synergestic interaction *Burkholderia phytofirmans* (PsJN) versus *Bacillus subtilis* (Y16), (C) synergistic interaction of *Burkholderia phytofirmans* (PsJN) versus *Enterobacter aegogenes* (S14), (D) synergistic interaction of *Burkholderia phytofirmans* (PsJN) versus *Pseudomonas fluorescens* (S20).

#### 2.6. Enzymes assays

The role of Catalase and ascorbate peroxidase in maize leaves was studied using Qui et al. [27], method of fluctuating absorbance at 240 nm owing to hydrogen peroxide annihilation [28]. Similarly, the ability of ascorbate peroxidase was measured at 290 nm absorbance [29].

#### 2.7. Growth and yield attributes

At maturity, the growth and yield attributes including plant height, cob yield, total biomass, grain yield, straw yield, and 1000 grains weight were determined by standard methods.

#### 2.8. Minerals analysis

Macronutrients Nitrogen, Phosphorus, Potassium (NPK) and Sodium (Na) contents from plant samples were analyzed by using the method described by Ryan et al., [30].

# 2.9. Statistical analysis

Data was analyzed statistically under Randomized complete block design (RCBD) by using Statistics-8.1 (Statistics-8.1 Analytical Software, Tallahassee, USA).

# 3. Results and discussion

The findings of this study revealed that multi-strain combinations improved growth and yield qualities in a saline environment when compared to a linked control. First and foremost, the competence of selected strains was tested in the laboratory, where all strains demonstrated growth compatibility. Furthermore, all conceivable combinations of synergism/antagonism were investigated and shown to have favorable synergistic interactions with each other for activities relevant to plant development, as shown in Fig. 1 (A–D). All five strains that were eventually employed as multi-strain combinations were evaluated for auto-aggregation in the current investigation (Fig. 2). Auto-aggregation percentages ranged from 45 to 73%. All of the co-aggregates were bulky and quickly split, leaving a clear supernatant. The proportion of PsJN, S14, and S20 was determined to be 80% on average across replicates (Fig. 3).

#### 3.1. Gaseous exchange parameter

As shown in Table 1, the gas exchange characteristics improved considerably in all multi-strain combinations when compared to the un-inoculated control under saline field conditions. C3 had the best response in terms of photosynthetic apparatus regulation (50%), conductivity through stomata (49.16%), transpiration rate (18.85%), efficient use of water (26.50%), membrane permeability index (30.69%), and relative water content (27.26%) compared to the uninoculated control C0. However, when compared to each other, none of the combinations are statistically significant, but they are significant when compared to the control treatment. Due to soil salinity, the membrane permeability index and relative water content decreased, potentially preventing  $CO_2$  from reaching the plant through stomata closure. As a result, the plant's substomatal conductance increased, but other gas exchange metrics such as



Fig. 2. Auto-aggregation (%) of PGPB strains used in multi-strain combinations.



Fig. 3. Co-aggregation (%) of PGPB strains used in multi-strain combinations. PsJN = Burkholderia phytofirmans, S14 = Enterobacter aerogenes, S20 = Pseudomonas fluorescens, S5 = Pseudomonas syringae and Y16 = Bacillus subtilis.

 Table 1

 Effect of microbial consortia on gas exchange parameters under salt affected conditions.

Treat	PR	TR	WUE	SC	SSC	RWC	MPI
	$\mu mol \ CO_2 \ m^{-2} \ s^{-1}$	mmol $H_2O \ m^{-2} \ s^{-1}$	mmol $\rm CO_2 \ mol^{-1} \ H_2O$	mmol $H_2O \ m^{-2} \ s^{-1}$	$\mu mol \ CO_2 \ mol^{-1}$	%	%
C0 C1 C2 C3 C4 LSD	$\begin{array}{c} 23.2^c\pm 1.1\\ 25.8^{bc}\pm 2.3\\ 29.1^b\pm 2.3\\ 34.7^a\pm 1.3\\ 30.6^{ab}\pm 2.6\\ 5.60\end{array}$	$\begin{array}{l} 8.0^{b}\pm0.14\\ 8.2^{ab}\pm0.68\\ 8.8^{ab}\pm0.45\\ 9.5^{a}\pm0.44\\ 8.6^{ab}\pm0.22\\ 1.36\end{array}$	$\begin{array}{l} 2.9^{\rm b}\pm 0.12\\ 3.1^{\rm ab}\pm 0.37\\ 3.3^{\rm ab}\pm 0.21\\ 3.7^{\rm a}\pm 0.21\\ 3.5^{\rm ab}\pm 0.90\\ 0.72\end{array}$	$\begin{array}{c} 211^{c}\pm11\\ 225^{bc}\pm15\\ 300^{a}\pm14\\ 315^{a}\pm16\\ 268^{ab}\pm23\\ 48.3 \end{array}$	$\begin{array}{l} 221^{a}\pm 12\\ 196^{ab}\pm 9.0\\ 152^{bc}\pm 17\\ 140^{c}\pm 21\\ 170^{ac}\pm 8.0\\ 52.2 \end{array}$	$\begin{array}{l} 57.9^{\rm c}\pm2.8\\ 63.9^{\rm bc}\pm6.3\\ 70.9^{\rm ab}\pm4.4\\ 73.7^{\rm ab}\pm4.6\\ 77.7^{\rm a}\pm5.7\\ 12.4\end{array}$	$\begin{array}{c} 32.1^{a}\pm1.0\\ 28.3^{b}\pm3.1\\ 23.6^{cd}\pm0.4\\ 23.4^{d}\pm0.5\\ 25.4^{c}\pm0.6\\ 2.80\end{array}$

\*PR = photosynthesis rate, TR = transpiration rate, WUE = water use efficiency, SC = stomatal conductance, SSC = sub-stomatal conductance, RWC = relative water contents, MPI = membrane permeability index. Mean values followed by different letter(s) in the same column are statistically different ( $P \le 0.05$ ) C0 = Un-inoculated Control, C1 = (PsJN + S5 + Y16), C2 = (PsJN + S14 + Y16), C3 = (PsJN + S14 + S20) and C4 = (PsJN + S20 + Y16).

photosynthetic activity, transpiration rate, water usage efficiency, and stomatal conductance decreased [31]. The multi-strain combination significantly improved photosynthetic characteristics such as rate of transpiration, photosynthetic activity, water efficiency, and stomatal conductance in maize leaves. This might be attributed to an increase in root development as a result of ACC-deaminase activity, which reduces ethylene synthesis and allows roots to absorb more nutrients and water efficiently from the soil.

#### 3.2. Biochemical parameters

Table 2 displays the findings of biochemical factors such as proline, chlorophyll content, catalase content, ascorbate peroxidase content, and malondialdehyde content. In comparison to the un-inoculated control C0, the multi-strain combinations (C2, C3, C4) considerably enhanced the biochemical characteristics of the maize under salt impacted conditions. The most promising combination,

Table 2				
Effect of microbial consortia on biochemical	parameters	under	salt affected	conditions.

Treatments	Proline	Chlorophyll	MalondialdehaydeActivity	Ascorbate peroxidase Activity	Catalase Activity
	$\mu mol g^{-1}$	SPAD Value	$\mu mol \ min^{-1} \ mg \ protein^{-1}$		
C0 C1 C2 C3 C4	$\begin{array}{c} 2.34^{\rm c}\pm 0.04\\ 2.06^{\rm bc}\pm 0.23\\ 1.95^{\rm ab}\pm 0.04\\ 1.39^{\rm a}\pm 0.01\\ 1.56^{\rm ab}\pm 0.03\end{array}$	$\begin{array}{c} 36.0^{\rm c}\pm 0.92\\ 40.7^{\rm bc}\pm 0.83\\ 43.3^{\rm ab}\pm 1.78\\ 48.1^{\rm a}\pm 2.67\\ 45.8^{\rm a}\pm 0.95\end{array}$	$70.9^{a} \pm 2.16$ $64.8^{a} \pm 1.89$ $51.1^{b} \pm 1.11$ $41.4^{c} \pm 2.73$ $47.7^{b} \pm 5.02$	$9.73^{c} \pm 0.59$ $10.7^{c} \pm 1.10$ $11.9^{b} \pm 1.22$ $14.9^{a} \pm 0.68$ $13.4^{a} \pm 0.92$	$\begin{array}{l} 7.56^{\rm c}\pm 0.33\\ 8.45^{\rm bc}\pm 0.86\\ 9.95^{\rm a-c}\pm 0.80\\ 11.39^{\rm a}\pm 0.83\\ 10.47^{\rm ab}\pm 0.77\end{array}$
LSD	0.51	5.01	22.4	3.64	1.58

 $C0 = Un-inoculated Control, C1 = (PsJN + S5 + Y16), C2 = (PsJN + S14 + Y16), C3 = (PsJN + S14 + S20) and C4 = (PsJN + S20 + Y16) Mean values followed by different letter(s) in the same column are statistically different (P <math>\leq$  0.05).

C3 raised the levels of chlorophyll (33.68%), ascorbate peroxidase (53.31%), and catalase-peroxidase (5.66%) as compared to the uninoculated treatment C0, however, there was a reduction in malondialdehyde content (41.57%) and proline content (68.64%). Senescence in plants is caused by an increase in ethylene levels caused by stress [32]. Under salt-affected conditions, the current study found that PGPR multi-strain combinations C3, C4, and C2 enhanced chlorophyll content in maize plants compared to control C0. As a result, PGPR encourages the reduction of ethylene production to safeguard the plant's chlorophyll content from degradation. Similarly, limiting Na absorption and boosting Mg and N supply improves the chlorophyll synthesis [33]. Furthermore, multi-strains under salinity had a favorable effect on chlorophyll concentration [19,34,35].

Proline accumulation in the plant is an indication of stress tolerance since it acts as a protective osmolyte. It operates by regulating osmotic pressure, preventing dehydration of intercellular macromolecules, and scavenging hydroxyl radicals [31]. Under salt stress, the proline content of the multi-strain combination C3 was considerably reduced in this research.

Salinity stress induces the formation of reactive oxygen species (ROS) which causes the destruction of biomembranes and macromolecules [36]. Plants developed a variety of antioxidants to overcome these harmful effects. The increase in antioxidant activity in the plant can enhance the plant's tolerance to a variety of stressors. SOD, GR, APX, and CAT are examples of enzymes that operate as reactive oxygen species (ROS) scavengers [37,38]. Multi-strain consortia greatly increased antioxidant activity in the maize plant, as indicated in Table 2, causing increases in Catalase and ascorbate peroxidase activity. Reduced malondialdehyde levels, on the other hand, alleviated salt stress. In this investigation, it was discovered that all multi-strain combinations are statistically equivalent to each other except for C1, which is significant when compared to the inoculated control C0. This may be due to greater antioxidant activity in the presence of PGPR, which results in lower MDA levels and a lower membrane permeability index leading to improved plant salt stress resistance and biomass accumulation [39]. Moreover, it's possible that PGPR encouraged the buildup of antioxidant enzymes in the host plant to reduce salt stress by scavenging reactive oxygen species (ROS) [40].

# 3.3. Ionic parameter

When compared to the un-inoculated treatment C0, C3 showed the greatest improvement in ion parameters such as leaf  $K^+$  & Na $^+$ and macronutrient concentrations under salt stress. The multi-strain combination C3 increased K<sup>+</sup> uptake by 14.6% while decreasing  $Na^+$  uptake by up to 48.4% (Table 3). As a result, the  $K^+/Na^+$  Ratio was boosted by a factor of two (94.8%). It is self-evident that salinity promotes ion homeostasis while reducing nutrient supply in plants, resulting in stunted development [41]. It also upsets the balance of Na<sup>+</sup> and K<sup>+</sup> between the soil and the plant. As a result, the K<sup>+</sup>: Na<sup>+</sup> ratio is low [42]. The findings of this study show that inoculating maize with several strains reduces the absorption and accumulation of  $Na^+$  ions while increasing the demand for  $K^+$ , resulting in a higher K<sup>+</sup>: Na<sup>+</sup> ratio. It might be related to the PGPR's ACC-deaminase action causing the roots network to expand and investigate additional soil. As a result, the plant's growth has risen. In comparison to control CO, the multi-strains significantly improved ionic balance in maize by controlling Na<sup>+</sup> and K<sup>+</sup> ions. Several microbial strains have been shown to reduce salt toxicity by balancing the Na<sup>+</sup> and K<sup>+</sup> levels [15,43,44]. In the current work, inoculating maize straw and seeds with multi-strain combinations dramatically increased N, P, and K levels in a saline medium (Table 4). With multi-strain combination C4 as compared to control C0 treatment, the greatest increase in N straw and grains content was found to be 102% and 37% respectively. The multi-strain combinations C3 raised the supply of P and K concentration in straw by 52.4% and 19%, respectively, compared to the untreated control C0, but were statistically comparable to C4. In addition, C4 improved the P and K content of grain by up to 45.0% and 17%, respectively, as compared to the un-inoculated control. This enhanced supply of N, P, and K in the plant might be attributed to the inoculation of PGPR multi-stains, which boosted the root network, generation of growth-promoting hormones, and a variety of other unknown causes [8].

#### 3.4. Growth and yield parameters

With the C3 combination, the maximum increase in terms of growth and yield parameters was obtained (Table 5). However, the remaining three multi-strain consortia (C2, C4, and C1) increased maize growth and yield. Plant height, straw production, cob yield, 1000 grain weight, grains yield, and total biomass increased by 26%, 46.9%, 40.7%, 13.5%, 21.3%, and 49.5% respectively, when the C3 multi-strain combination was used. Multi-strain combinations offered a superior response to enhance yield and growth under saline stress in this experiment, and PGPR minimized the harmful effects of salinity stress on maize. This could be due to the increased actions of PGPR bacteria, such as biological nitrogen fixation and ACC-deaminase activity, which result in a decrease in ethylene production, nutrient solubilization, oxidative damage reduction, biochemical buildup (proline, betaine, antioxidants), and colonization with other beneficial soil microorganisms under saline conditions [8].

# 4. Conclusions

Based on the aforementioned data, it may be inferred that maize salt resistance was given by a multi-strain PGPR combination. Under salt-affected field conditions, it is clear that PGPR combinations enhance plant growth. Burkholderia phytofirmans, *Enterobacter aerogenes*, and *Pesudomonas florucens* were shown to be the optimum multi-strain combination (C3) for increasing growth and yield under salinity in this investigation. In comparison to other techniques, it is possible to say that PGPR in the form of multi-strain consortia is a successful tool for reducing salinity.

# Table 3

Effect of microbial consortia on ionic parameters in maize leaf under salt affected conditions.

	•		
Treatments	Na	К	K <sup>+</sup> /Na <sup>+</sup>
	%		
CO	$1.5^{\rm a}\pm 0.20$	$1.7^{ m c}\pm 0.05$	$2.9^{\text{c}}\pm0.23$
C1	$1.1^{\rm b}\pm0.10$	$1.8^{bc}\pm0.04$	$3.7^{bc}\pm0.17$
C2	$1.0^{ m bc}\pm 0.0$	$1.9^{\rm a}\pm 0.02$	$4.6^{ab}\pm0.34$
C3	$0.8^{ m c}\pm0.04$	$1.9^{\mathrm{a}}\pm0.01$	$5.7^{\rm a}\pm0.13$
C4	$0.9^{ m bc}\pm 0.0$	$1.9^{ab}\pm0.10$	$4.6^{ab}\pm0.04$
LSD	0.35	0.12	0.71

 $C0 = Un-inoculated Control, C1 = (PsJN + S5 + Y16), C2 = (PsJN + S14 + Y16), C3 = (PsJN + S14 + S20) and C4 = (PsJN + S20 + Y16) Mean values followed by different letter(s) in the same column are statistically different (p <math>\leq 0.05$ ).

Table 4	
Effect of microbial consortia on ionic parameters in maize straw and grain under salt affected conditions.	

Treatments	Straw N	Straw P	Straw K	Grain N	Grain P	Grain K
	%					
C0 C1 C2 C3 C4 LSD	$\begin{array}{c} 0.5^{c}\pm 0.07\\ 0.89^{b}\pm 0.05\\ 1.08^{ab}\pm 0.04act\\ 1.15^{a}\pm 0.02\\ 0.94^{ab}\pm 0.10\\ 0.20\end{array}$	$\begin{array}{c} 0.21^d \pm 0.0 \\ 0.24^{cd} \pm 0.01 \\ 0.29^{ab} \pm 0.01 \\ 0.32^a \pm 0.01 \\ 0.27^{bc} \pm 0.02 \\ 0.05 \end{array}$	$\begin{array}{c} 1.6^{\rm c}\pm 0.04\\ 1.7^{\rm c}\pm 0.05\\ 1.9^{\rm ab}\pm 0.0\\ 1.9^{\rm a}\pm 0.02\\ 1.8^{\rm bc}\pm 0.0\\ 0.12\end{array}$	$\begin{array}{c} 1.13^{c}\pm 0.15\\ 1.41^{ab}\pm 0.07\\ 1.55^{a}\pm 0.03\\ 1.67^{ab}\pm 0.02\\ 1.51^{ab}\pm 0.02\\ 0.40\end{array}$	$\begin{array}{c} 0.21^{c}\pm 0.0\\ 0.25^{bc}\pm 0.03\\ 0.29^{ab}\pm 0.02\\ 0.33^{a}\pm 0.03\\ 0.31^{ab}\pm 0.01\\ 0.06\end{array}$	$\begin{array}{c} 1.41^{\rm c}\pm 0.01\\ 1.48^{\rm bc}\pm 0.02\\ 1.53^{\rm b}\pm 0.03\\ 1.65^{\rm a}\pm 0.01\\ 1.51^{\rm b}\pm 0.02\\ 0.70\end{array}$

 $C0 = Un-inoculated Control, C1 = (PsJN + S5 + Y16), C2 = (PsJN + S14 + Y16), C3 = (PsJN + S14 + S20) and C4 = (PsJN + S20 + Y16) Mean values followed by different letter(s) in the same column are statistically different (P <math>\leq 0.05$ ).

# Table 5 Effect of microbial consortia on growth and yield parameters under salt affected conditions.

Treatments	Plant height	Total biomass	Straw yield	Cob yield	Grain yield	1000 Grain weight
	cm	t ha <sup>-1</sup>				grams
C0 C1 C2 C3 C4 LSD	$\begin{array}{c} 134^{c}\pm 5.14\\ 142^{bc}\pm 4.50\\ 169^{a}\pm 8.23\\ 170^{a}\pm 7.55\\ 160^{ab}\pm 5.30\\ 23.2 \end{array}$	$\begin{array}{c} 14.7^{c}\pm1.36\\ 16.1^{bc}\pm1.40\\ 20.3^{ab}\pm1.28\\ 22.0^{ab}\pm1.54\\ 18.2^{a\cdot c}\pm1.73\\ 4.52 \end{array}$	$\begin{array}{c} 2.23^c \pm 0.21 \\ 2.53^{bc} \pm 0.07 \\ 2.82^{ab} \pm 0.04 \\ 3.28^a \pm 0.37 \\ 3.09^{ab} \pm 0.04 \\ 0.56 \end{array}$	$\begin{array}{c} 10.3^{b}\pm 0.94\\ 11.8^{ab}\pm 0.71\\ 12.9^{ab}\pm 1.07\\ 14.5^{a}\pm 1.15\\ 13.9^{a}\pm 1.38\\ 2.80\end{array}$	$\begin{array}{l} 2.85^{\rm b}\pm 0.05\\ 3.21^{\rm ab}\pm 0.30\\ 3.38^{\rm ab}\pm 0.20\\ 3.46^{\rm a}\pm 0.11\\ 3.29^{\rm ab}\pm 0.09\\ 0.59\end{array}$	$\begin{array}{l} 208^{d}\pm3.03\\ 219^{c}\pm1.02\\ 236^{a}\pm1.20\\ 229^{ab}\pm2.27\\ 224^{bc}\pm2.38\\ 7.68 \end{array}$

 $C0 = Un-inoculated Control, C1 = (PsJN + S5 + Y16), C2 = (PsJN + S14 + Y16), C3 = (PsJN + S14 + S20) and C4 = (PsJN + S20 + Y16) Mean values followed by different letter(s) in the same column are statistically different (P <math>\leq$  0.05).

# Author contribution statement

Ali Afzal; Muhammad Yahya Khan, PhD: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Zahir Ahmad Zahir, PhD: Conceived and designed the experiments.

Hafiz Naeem Asghar; Atif Muhmood; Muhammad Rashid; Zeeshan Aslam; Syed Ayyaz Javed; Sajid Mahmood Nadeem: Contributed reagents, materials, analysis tools or data.

# Data availability statement

Data included in article/supp. material/referenced in article.

# Additional information

No additional information is available for this paper.

# Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### Acknowledgements

The authors are thankful to the Director, Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad, Pakistan for providing the required facilities for conducting the experimental work.

#### References

- S. Mandal, R. Raju, A. Kumar, P. Kumar, P.C. Sharma, Current status of research, technology response and policy needs of salt-affected soils in India a review, Ind. Soc. Coastal Agri. Res. 36 (2018) 40–53.
- [2] A. Azhar, Impact of subsurface drainage on soil salinity in Pakistan, J. Anim. Plant Sci. 20 (2010) 94–98.
- [3] S.A. Shahid, M. Zaman, L. Heng, Soil salinity: historical perspectives and a world overview of the problem, in: Guideline for Salinity Assessment, Mitigation and Adaptation Using Nuclear and Related Techniques, Springer, Cham, 2018, https://doi.org/10.1007/978-3-319-96190-3\_2.
- [4] H. Bouwer, Integrated water management: emerging issues and challenges, Agric. Water Manag. 45 (2000) 217–228.
- [5] A. Wahid, S. Gelani, M. Ashraf, M.R. Foolad, Heat tolerance in plants: an overview, Environ. Exp. Bot. 61 (2007) 199-223.
- [6] C. Dimkpa, T. Weinand, F. Ash, Plant-rhizobacteria interactions alleviate abiotic stress conditions, Plant Cell Environ. 32 (2009) 1682–1694.
- [7] E. Malusa, L. Sas-Paszt, J. Ciesielska, Technologies for beneficial microorganisms inocula used as biofertilizers, Sci. World J. 2012 (2012) 1–13.
- [8] M. Ahmad, M. Kibret, Mechanisms and applications of plant growth promoting rhizobacteria: current perspective, J. King Saud Univ. Sci. 26 (2014) 1–20.
   [9] A. Das, M. Kumar, R. Kumar, Plant growth promoting rhizobacteria: an alternative for chemical fertilizer for sustainable, environmental friendly agriculture, Res. J. Agric. For. Sci. 1 (2013) 21–23.
- [10] J.S. Singh, V.C. Pandey, D.P. Singh, Efficient soil microorganisms: a new dimension for sustainable agriculture and environmental development, Agric. Ecosyst. Environ. 140 (2011) 339–353.
- [11] B. Carmen, D. Roberto, Soil bacteria support and protect plants against abiotic stresses, ISBN: 978-953-307-394-1, in: A. Shanker (Ed.), Abiotic Stress in Plants-Mechanisms and Adaptations, InTech, 2011, pp. 143–170.
- [12] Z.S. Wu, Y.F. Zhao, I. Kaleem, C. Li, Preparation of calcium-alginate microcapsuled microbial fertilizer coating Klebsiella oxytoca Rs-5 and its performance under salinity stress, Eur. J. Soil Biol. 47 (2011) 152–159.
- [13] S.M. Nadeem, Z.A. Zahir, M. Naveed, S. Nawaz, Mitigation of salinity induced negative impact on the growth and yield of wheat by plant growth-promoting rhizobacteria in naturally saline conditions, Ann. Microbiol. 63 (1) (2013) 225–232.
- [14] E. Elkoca, M. Turan, M.F. Donmez, Effects of single, dual and triple inoculations with Bacillus subtilis, Bacillus megaterium and Rhizobium leguminosarumbv. Phaseoli on nodulation, nutrient uptake, yield and yield parameters of common bean, J. Plant Nutr. 33 (2010) 2104–2119.
- [15] J. Kohler, J.A. Hernandez, F. Caravaca, A. Roldan, Induction of antioxidant enzymes is involved in the greater effectiveness of a PGPR versus AM fungi with respect to increasing the tolerance of lettuce to severe salt stress, Environ. Exp. Bot. 65 (2009) 245–252.
- [16] M.K. Chelius, E.W. Triplett, Diazotrophic endophyte associated with maize, in: E.W. Triplett (Ed.), Prokaryotic Nitrogen Fixation: A Model System for the Analysis of a Biological Process, Horizon Scientific Press, Norfolk, UK, 2000, pp. 779–792.
- [17] Y. Dong, J.D. Glasner, F.R. Blattner, E.W. Triplett, Genomic interspecies microarray hybridization: rapid discovery of three thousand genes in the maize endophyte Klebsiella pneumonia 342, by microarray hybridation with Escherichia coli K12 open reading frames, Appl. Environ. Microbiol. 67 (2001) 1911–1921.
- [18] A. Montanez, A.R. Blanco, C. Barlocco, M. Beracochea, M. Sicardi, Characterization of cultivable putative endophytic plant growth promoting bacteria associated with maize cultivars (Zea mays L.) and their inoculation effects in vitro, Appl. Soil Ecol. 58 (2012) 21–28.
- [19] M. Naveed, M.B. Hussain, Z.A. Zahir, B. Mitter, A. Sessitsch, Drought stress amelioration in wheat through inoculation with Burkholderia phytofirmans strain PsJN, Plant Growth Regul. 73 (2014) 121–131.
- [20] P. Raja, S. Uma, H. Gopal, K. Govindarajan, Impact of bio inoculants consortium on rice root exudates, biological nitrogen fixation and plant growth, J. Biol. Sci. 6 (2006) 815–823.
- [21] M.M. Joe, V.S. Saravanan, T. Sa, Aggregation of selected plant growth promoting Methylobacterium strains: role of cell surface components and hydrophobicity, Arch. Microbiol. 195 (2013) 219–225.
- [22] S. Iqbal, M.Y. Khan, H.N. Asghar, M.J. Akhtar, Combined use of phosphate solubilizing bacteria and poultry manure to enhance the growth and yield of mung bean in calcareous soil, Soil Environ. 35 (2) (2016).
- [23] N. Jambunathan, Determination and detection of reactive oxygen species (ROS), lipid peroxidation, and electrolyte leakage in plants, in: R. Sunkar (Ed.), Plant Stress Tolerance, Methods in Molecular Biology 639, Humana press, Springer New York Dordrecht Heidelberg London, 2010, pp. 291–297.
- [24] L. Bates, R. Waldren, I. Teare, Rapid determination of free proline for water-stress studies, Plant Soil 39 (1973) 205–207.
- [25] S. Mayak, T. Tirosh, B.R. Glick, Plant growth-promoting bacteria confer resistance in tomato plants to salt stress, Plant Physiol. Biochem. 42 (2004) 565–572.
- [26] W. Khan, B. Prithiviraj, D.L. Smith, Photosynthetic responses of corn and soybean to foliar application of salicylates, J. Plant Physiol. 60 (2003) 485–492.
- [27] Z.B. Qiu, X. Liu, X.J. Tian, M. Yue, Effects of CO2 laser pretreatment on drought stress resistance in wheat, J. Photochem. Photobiol. B Biol. 90 (1) (2007) 17–25.
   [28] I. Cakmak, H. Marschner, Magnesium deficiency and high light intensity enhance activities of superoxide dismutase, ascorbate peroxidase and glutathione reductase in bean leaves, Plant Physiol. 98 (1992) 1222–1227.
- [29] Y. Nakano, K. Asada, Hydrogen peroxide is scavenged by ascorbate- specific peroxide I spinach chloroplasts, Plant cell Physoil 22 (1981) 867-880.
- [30] J. Ryan, G. Estefan, A. Rashid, Solid and plant analysis laboratory manual, in: International Centre for Agriculture in Dry Areas (ICARDA). Syria, second ed., 2001.
- [30] N.B. Talaat, B.T. Shawky, Protective effects of arbuscular mycorrhizal fungi on wheat (Triticum aestivum L.) plants exposed to salinity, Environ. Exp. Bot. 98 (2014) 20–31.
- [32] M. Arshad, W.T. Frankenberger Jr., Ethylene: Agricultural Sources and Applications, Kluwer Academic/Plenum Publ., New York, 2002.
- [33] O. Alizadeh, S. Sharafzadeh, A.H. Firoozabadi, The effect of plant growth promoting rhizobacteria in saline condition, Asian J. Plant Sci. 11 (2012) 1-8.
- [34] V. Sandhya, S.Z. Ali, M. Grover, G. Reddy, B. Venkateswarlu, Effect of plant growth promoting Pseudomonas spp. on compatible solutes, antioxidant status and plant growth of maize under drought stress, Plant Growth Regul. 62 (2010) 21–30.
- [35] S. Vardharajula, S.Z. Ali, M. Grover, G. Reddy, V. Bandi, Drought tolerant plant growth promoting Bacillus spp.: effect on growth, osmolytes, and antioxidant status of maize under drought stress, J. Plant Interact. 6 (2011) 1–14.
- [36] R. Mittler, Oxidative stress, antioxidants and stress tolerance, Trends Plant Sci. 7 (2002) 405-410.
- [37] O. Fernandez, A. Theocharis, S. Bordiec, R. Feil, L. Jasquens, C. Clement, F. Fontaine, E. AitBarka, Burkholderia phytofirmansPsJN acclimates grapevine to cold by modulating carbohydrate metabolism, Mol. Plant Microbe Interact. 25 (2012) 496–504.
- [38] A. Theocharis, S. Bordiec, O. Fernandez, S. Paquis, S. Dhondt-Cordelier, F. Baillieul, C. Clement, E.A. Barka, Burkholderia phytofirmans PsJN primes
- Vitisvinifera L. and confers a better tolerance to low nonfreezing temperatures, Mol. Plant Microbe Interact. 25 (2012) 241–249.
- [39] M. Jain, G. Mathur, S. Koul, N.B. Sarin, Ameliorative effects of proline on salt stress-induced lipid peroxidation in cell line of groundnut (Arachishypgaea L.), Plant Cell Rep. 20 (2001) 463–468.
- [40] Y. Himabindu, T. Chakradhar, M.C. Reddy, A. Kanygin, K.E. Redding, T. Chandrasekhar, Salt-tolerant genes from halophytes are potential key players of salt tolerance in glycophytes, Environ. Exp. Bot. 124 (2016) 39–63.
- [41] H. Han, K. Lee, Plant growth promoting rhizobacteria effect on antioxidant status, photosynthesis, mineral uptake and growth of lettuce under soil salinity, Res. J. Agric. Biol. Sci. 1 (2005) 210–215.

- [42] N. Tank, M. Saraf, Salinity-resistant plant growth promoting rhizobacteria ameliorates sodium chloride stress on tomato plants, J. Plant Interact. 5 (2010) 51-58.
- [43] S. Nadeem, I. Hussain, M. Naveed, H. Asghar, Z. Zahir, M. Arshad, Performance of plant growth promoting rhizobacteria containing ACC-deaminase activity for improving growth of maize under salt-stressed conditions, Pakistan J. Agric. Sci. 43 (2006) 114–121.
  [44] A. Bano, M. Fatima, Salt tolerance in Zea mays (L.) following inoculation with Rhizobium and Pseudomonas, Biol. Fertil. Soils 45 (2009) 405–413.