



Growth improvement of *Vicia faba* planted in salt-affected soil by novel endophyte co-cultures

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Abstract

Salinity is a major problem that affects agricultural productivity. Plant growth-promoting bacteria (PGPB) may form a symbiotic relationship with their hosts to promote salt tolerance. Given the challenges of soil salinity in agriculture, this study explored the potential of halo-tolerant endophytic bacteria (halo-endophytes) as a bio-approach to promote plant growth under salinity stress. Five unique halo-endophyte strains (*Providencia rettgeri* DW3, *Bacillus licheniformis* DW4, *Salinicoccus sesuvii* DW5, *Bacillus amyloliquefaciens* DW6, and *Paenaltcaligenes suwonensis* DW7), were used for the first time as bacteria promoting the growth of *Vicia faba* and soil fertility as well. Various bacterial combinations were investigated in a controlled greenhouse experiment. The unique combination of the five strains proved the most effective in alleviating the salinity's harm to plant growth (growth percentages, photosynthetic rate, vegetative growth traits, and nutrient content) as well as proline content, which enhanced plant tolerance to salinity. Chemical and biological health features of the soil, measured by nutrient availability, microbial diversity and enzyme activity, improved by 110%, 753%, and 262%, respectively. This study demonstrates the effectiveness of co-inoculation with a bacterial mixture (*P. rettgeri* DW3 + *B. licheniformis* DW4 + *S. sesuvii* DW5 + *B. amyloliquefaciens* DW6 + *P. suwonensis* DW7) as a novel and innovative bio-based approach to sustainable agriculture in saline environments.

Keywords Salinity stress · Plant Growth-Promoting bacteria · Salt-Affected soil · Halo-Endophytic bacteria · Seed priming · *Vicia Faba*

Introduction

Vicia faba holds significant strategic importance in Egyptian agriculture. It serves as a valuable source of plant-based proteins for soil, humans, and animals (Boye et al. 2010; Sharan et al. 2021). Moreover, it contributes to human nutrition and animal feed. However, domestic production currently meets only 40% of the national demand for *Vicia faba*, necessitating the import of 60% to fulfill domestic consumption (Elbatrawy et al. 2023). Because it is extremely sensitive to salinity (Cordovilla et al. 1994; Shahzad et al. 2013) and its presence in this land is impossible as it competes with other strategic crops for agricultural land. Planting *Vicia faba* on salt-affected soil and/or increasing faba bean production is a key objective to fulfill the growing domestic demand and reduce reliance on faba bean imports (Zeidan 2003).

A staggering 5.2 billion hectares of arable land, representing approximately 50% of the global total, are impacted by salinity (Numan et al. 2018). Generally, if the EC_e (Electrical Conductivity of the Extract) of a wet soil sample is

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higher than 4 dS/m (deciSiemens per meter), the soil is considered saline (Rath and Rousk 2015). Salt-affected soils have high salt concentrations, low soil organic carbon (SOC), reduced fertility, and limited water, hindering crop production (Mandour 2016). Salt stress, often exacerbated by irrigation practices, threatens sustainable agriculture and food security. Soil salinization occurs when too much salt builds up in the soil, making it hard for plants to grow. This can hurt farms, the environment, and even the economy (Rengasamy 2006).

Salinity-induced abiotic stress remains a nightmare, causing serious environmental problems and diminished agricultural output (Yang et al. 2012). The rise of soil salinity stress poses an imminent threat to major crops globally (Munns and Tester 2008). Salinity harms crops in several ways. First, it hinders their ability to absorb nutrients, especially phosphate. (Grattan and Grieve 1998). Second, high salt concentrations create a double threat: reduced water uptake (osmotic stress) and cellular stress from toxic ions (ionic stress) (Kohler et al. 2009). Finally, the buildup of salts like sodium chloride disrupts many essential plant functions (Lee et al. 2008), decreases productivity, and causes plant death (Munns and Tester 2008; Argente-Martínez et al. 2024). Additionally, salt stress triggers Reactive oxygen species (ROS) generation. These ROS wreak havoc on critical cell constituents such as DNA, proteins, and lipids. They also disrupt how enzymes work and can even break down the cell membrane, the protective barrier around the cell (Demiral and Türkan 2005). PGPB isolated from halo-phytes offer a sustainable and eco-friendly soil desalinization. These PGPB can contribute to natural desalinization processes while offering an affordable approach to enhancing soil health and plant yield.

Endophytic bacteria colonize all internal plant parts, such as leaves, seeds, stems, and roots, without generating detrimental impacts (Verma et al. 2007; Mitter et al. 2021). Scientists worldwide have been studying how plants respond to being treated with beneficial bacteria (PGPB) for many years, conducting experiments in both fields and greenhouses. These PGPB can actually help plants grow better throughout their developmental stages. For example, *Bacillus subtilis* and *Enterobacter hormaechei* were isolated from the rhizosphere and used in the Promoting *Vicia faba* growth (Abdelkader et al. 2022). *Pseudomonas yamanorum*, *Rahnella aquatilis*, and *Pseudomonas fluorescens* were isolated from *Vicia faba* and used in growth promotion, and Tunisian cultivars of faba bean (Bahroun et al. 2018), *Serratia plymuthica* were isolated from the soil and used for faba bean growth promotion (Borgi et al. 2020) and *Azorhizobium caulinodans* were isolated from root exudates, were applied to enhance wheat growth (Wenli et al. 2021).

The occurrence of endophytes can significantly enhance and hasten the plant's stress response. PGPB can give plants a significant boost at various stages. They can improve seed germination rates, seedling health, emergence from the soil, root and stem development, overall plant size, and seed weight, and even encourage earlier flowering and higher fruit and seed yields (Panke-Buisse et al. 2015). These organisms are recognized as significant producers of bioactive compounds, including natural antibiotics and compounds that augment salt stress tolerance (Waller et al. 2005). The observed beneficial effects can be likely mediated by the production of a broad spectrum of phytohormones (Nabti et al. 2015), as well as the synthesis of secondary metabolites, including exopolysaccharides (Abbas et al. 2019) and osmolytes such as proline (Upadhyaya and Panda 2019).

The goal of this study is to assess the efficacy of novel mixtures of specific halo-endophytic bacteria to enhance the growth of *Vicia faba* L. and to mitigate the detrimental impacts of the salt-affected soil on plant growth parameters.

Materials and methods

Materials

The halo-endophytic bacteria *Bacillus licheniformis* DW4, *Bacillus amyloliquefaciens* DW6, *Salinicoccus sesuvii* DW5, *Paenaltcaligenes suwonensis* DW7, and *Providencia rettgeri* DW3 were earlier isolated from different halophytic plants (*Tamarix nilotica*, *Suaeda pruinosa*, and *Arthrocnemum macrostachyum*), identified through molecularly and were deposited in the gene bank, as described previously (Wael et al. 2024). All isolates displayed various plant growth-promoting (PGP) traits, such as exopolysaccharides (EPS), indole acetic acid (IAA), siderophores, gibberellic acid (GA-3), ammonia, and hydrogen cyanide (HCN) production. They also demonstrated the capability to solubilize phosphate and assimilate atmospheric nitrogen. Additionally, the potential antagonistic effects between the isolates were previously studied by Wael et al. (2024) and there was no antagonism between all isolates, and also they exhibited diverse enzymatic activities, producing cellulase, protease, lipase, amylase, and catalase enzymes. Healthy seeds of *Vicia faba* L. Mariout-2 were sourced from the Agricultural Research Centre, Giza, Egypt. Salt-affected soil samples originated from the Mediterranean Deltaic coast (31°24'28.4" N, 31°22'30.7" E).

Methods

Bacterial growth conditions and inoculum Preparation

Individual bacterial seed cultures were prepared by inoculating 20 mL of LB broth media with cells from pure colonies of *P. rettgeri* DW3 (OR083403.1), *B. licheniformis* DW4 (OR083404.1), *S. sesuvii* DW5 (OR083408.1), *B. amyloliquefaciens* DW6 (OR083409.1), and *P. suwonensis* DW7 (OR147937.1). Cultures were incubated with shaking at 150 rpm at 28 °C for 48 h, then adjusted to OD₆₀₀ = 0.8 (approximately 8×10^8 cells/mL). Based on preliminary observations indicating higher efficacy of combined treatments over individual strains (S1), five bacterial mixtures were prepared using equal volumes of the seed cultures: B mix(1) – *P. rettgeri* + *P. suwonensis*; B mix(2) – *S. sesuvii* + *P. suwonensis*; B mix(3) – *P. rettgeri* + *B. amyloliquefaciens*; B mix(4) – *P. rettgeri*, *B. licheniformis*, *S. sesuvii*, and *B. amyloliquefaciens*; and B mix(5).

For the preparation of each 5% (v/v) bacterial mixture, 17.5 mL of the mixed seed cultures (OD₆₀₀ = 0.8, equal volumes per strain) were added to a total volume of 350 mL of LB broth and incubated under the same conditions (28 °C, 150 rpm, 48 h). Following incubation, centrifugation of the cultures occurred at 6000 rpm for 15 min to remove the supernatant. The resulting bacterial pellets were resuspended in a pre-prepared 1% soil solution to produce the final bacterial suspensions used for experimental applications.

Seed biopriming and planting of *Vicia faba* in the greenhouse

This study took place in a temperature-controlled greenhouse (25–30 °C), size (50 × 20 × 3 m³), relative humidity level (between 65 and 80%), 10/14 day/night photo- period and light intensity 20–25 mol · m⁻² · d⁻¹ at the Faculty of Agriculture, Mansoura University, Egypt, commencing on May 25, 2023. The primary objective of this experiment was to explore the potential of PGPB to alleviate salt stress and enhance the growth of *Vicia faba* via seed priming with bacterial suspensions mixtures.

Prior to the germination test, all equipment and seedlings were thoroughly sterilized. Surface sterilization of seeds involved initial washing under running tap water, a 2-minute immersion in 70% ethanol, three rinses in sterile distilled water, a 5-minute treatment in 3% sodium hypochlorite solution, and finally, three more rinses in sterile distilled water (Fatima and Jabeen 2011; Elsayed et al. 2022).

Surface-sterilized *faba* seeds were treated with fifty mL of 0.8 OD soil solution containing bacteria at 600 nm for 24 h. Control seeds were saturated with Nile water for an equivalent duration. Following 24 h of incubation, the seeds

inoculated with bacterial suspensions were planted at a temperature of 25–30 °C in a 25 cm diameter agricultural plastic bag filled with 10 kg of soil. For each mixture, replica of twenty primed seeds were planted in two agricultural plastic bags at a temperature between 25 and 30 degrees Celsius (S2).

All seeds were planted in the greenhouse with a controllable temperature system at 25–30 °C and irrigated with suitable enough Nile water (Damietta branch) every three days for 50 days. Seven replica samples were taken after 33 days and 50 days of agriculture, and the growth parameters, height of plant, leaf count, length, fresh and dry biomass of root and shoot, and other criteria on plants and soil were then calculated at about 60% of water holding capacity of the soil.

Germination percentage and plant survival analysis

Throughout the experiment, germination counts were taken until all of the seeds had either germinated or died. Data were collected on the number of daily germinated seeds, germination percentage, pre-emergence damping off, and post-emergence damping off. The following equation was employed to calculate Germination Percentage (G%): (Keshavarzi et al. 2011):

$$G\% = \frac{\text{Number of germinated seeds}}{\text{Number of total seeds}} \times 100 \quad (1)$$

Pre-emergence damping-off was assessed by counting the number of dead seeds (%) before emergence in each treatment, and Post-emergence damping-off was analyzed through the evaluation of seedling damage following plant emergence from the soil in each treatment (Machón et al. 2006).

LCi-SD leaf chamber/soil respiration analysis system

A special device (LCi, ADC BioScientific Ltd., Hoddesdon, UK) (portable gas exchange equipment) was used to measure how well the plants were exchanging gases in their natural environment (in situ) around midday. The device uses an open-flow system, equipped with a 6.25 cm² leaf chamber. Measurements were taken under controlled conditions: 1500 mmol m⁻² s⁻¹ PPFD, 25 °C leaf temperature, and 400 μmol CO₂ mol⁻¹. We focused on 50-day-old leaves, making sure they were facing the sunlight directly. During the measurements, the photosynthetically active radiation (PAR) averaged 700 μmol m⁻² sec⁻¹, the temperature of 28 °C, and the carbon dioxide concentration in the air (ambient CO₂) was 360 μmol mol⁻¹ within the sample chamber. Some leaf gas exchange characteristics might be directly measured,

including photosynthetic rate (A), transpiration rate (E), stomatal conductance (gs), and intercellular CO₂ concentration (Ci). In addition, further gas exchange parameters were estimated from the directly measured ones using the following equations (Biesaga-Kościelniak et al. 2014), (Yin et al. 2006), (Kubota and Hamid 1992), (Agnihotri et al. 2009):

$$\text{Photosynthetic water use efficiency (pWUE)} = A/E \quad (2)$$

$$\text{Stomatal limitation (Ls)} = 1 - (Ci/Ca) \quad (3)$$

$$\text{Mesophyll conductance (gm)} = A/Ci \quad (4)$$

$$\text{Mesophyll efficiency (ME)} = Ci/gs \quad (5)$$

Measurements of vegetative growth parameters

Thirty-three (vegetative stage) and fifty-day-old (flowering stage) (Duan et al. 2021) bean plants ($n=5$) were removed, and vegetative growth parameters recorded included: height of plant, length of root and shoot, leaf count, and fresh and dry biomass determinations for both root and shoot biomass.

Mechanical, physical, and chemical soil analysis

Rhizospheric soil was collected by a sterilized spatula after planting from each treatment and analyzed with soil samples before planting (row soil) and conducted in the Agricultural Research Centre, Giza, Egypt. Physical, chemical, and mechanical analyses were conducted. For the mechanical analysis (soil texture) of the soil sample, to determine the particle size distribution of the sample, including gravel content, a sieve analysis was performed using a set of sieves with mesh sizes of: 2 millimeters (fine gravel), 0.2 millimeters (coarse sand), 0.02 millimeters (fine sand), and 0.002 millimeters (silt). Particles smaller than 0.002 millimeters (clay) passed through the sieve base and were captured in the pan.” The Mechanical analysis of salt-affected soil executed at the Agricultural Research Centre, Giza, Egypt, revealed that the mechanical analysis of the salt-affected soil used in the greenhouse experiment revealed sand (75.5%), silt (22%), and clay (2.5%). The soil texture was classified as loamy sand.

Physical analysis of salt-affected soil is pH, EC (electric conductivity) of soil solution, and SP (water saturation percentage of soil) analysis, while chemical analysis of salt-affected soil includes calcium carbonate, organic matter, anions like; HCO³⁻, Cl⁻ and SO₄²⁻, cations like; Ca²⁺, Mg²⁺, Na⁺ and K⁺, and macro and microelements analysis like; Nitrogen, Potassium, Phosphorus, Copper, Iron, Manganese and Zinc.

A pH and conductivity meter (Mettler Toledo, Switzerland) was used to measure the EC and pH of the soil after it had been mixed with water in a 1:2.5 ratio. Silver nitrate (AgNO₃) and potassium chromate (K₂CrO₄) solutions were used as indicators to measure the chloride ions (Cl⁻). The exchangeable (K⁺, Ca²⁺, and Na⁺) cations were assessed using ammonium acetate buffered at pH 7. With an ICP-OES (ICP-OES stands for Inductively Coupled Plasma Optical Emission Spectrometry), P, K⁺, Ca²⁺, and Na⁺ were measured following the extraction procedures. The soils' available iron (Fe), manganese (Mn), zinc (Zn), and copper (Cu) were determined using extraction techniques including diethylene triamine penta acetic acid (DTPA). The Smith-Weldon method, a chemical technique, was employed to quantify the organic matter content of the soil; 1:2 extracts were utilized to assess soil pH; and Page (1982) was followed to determine the calcium carbonate concentrations.

Soil biological properties

Estimation of organic matter percentage and nitrogen concentration The Smith-Weldon method, a chemical technique, was used to quantify rhizospheric soil's organic matter level; 1:2 extracts were used to determine the calcium carbonate concentrations (Soltanpour 1991). The Micro-Kjeldahl method was used to determine the total nitrogen concentration in rhizospheric soil (Juarez Rosete et al. 2014).

Estimation of total bacterial count Colony forming unit (CFU) or total bacterial count was estimated for soil sample before planting (row soil) and after planting control and soil samples after treatment after 50 days of planting by preparing soil solution of each soil sample by suspending 10 g of soil sample in 100 milliliters sterilized dist.H₂O, mixed them well for 5 min, when soil particle settled down, 10 µL of upper soil solution were pipetted on LB agar medium and were dispersed using a sterile bent glass rod on the agar surface of a 9-cm plate, and the dish was incubated for 48 h at 28 °C. Colony enumeration was performed.

Estimation of dehydrogenase activity Soil dehydrogenase activity was measured both pre-planting and following 50 days of plant growth and treatment, following the methodology described by Mierzwa-Hersztek et al. (2020). A glass rod was used to thoroughly mix one gram of sieved soil with one milliliter of a 3% aqueous solution (w/v) of 2,3,5-triphenyl tetrazolium chloride (TTC). Following the 96-hour incubation at 27 °C, 10 milliliters of ethanol were added to each test tube, vortexed for 0.5 min, and left to settle for

60 min in order to allow the suspended soil to settle. A test tube containing five milliliters of the filtrate was used to quantify the amount of dehydrogenases, which caused TTC to change into triphenyl-formazan (TPF). At a wavelength of 485 nm, the formazan's absorbance was measured spectrophotometrically. Dehydrogenase activity is measured in units of $\mu\text{g TPF g}^{-1}$ dry soil min^{-1} (Moussa et al. 2023).

Plant analysis

Assessment of nitrogen, potassium, and phosphorus According to Juarez Rosete et al. (2014), the Micro-Kjeldahl method was employed to quantify total nitrogen concentration. Through acid digestion, the minerals P and K were extracted. The ammonium molybdate method was used to quantify P concentration (Juarez Rosete et al. 2014). and a HACH spectrometer set to 470 nm in wavelength. The flamometry approach was applied for K in accordance to the methodology described by Chapman and Pratt (1962).

Assessment of total amino acids and proline Ten grams of fresh plant material was taken and blended with a chilled methanol solution (70% methanol, 30% water). This mixture sat overnight at 4 °C to allow the hormones to be extracted. The mixture was filtered to remove plant debris, then the methanol was evaporated using a vacuum. This left a concentrated extract containing the hormones and other compounds. The extract's acidity level (pH) was adjusted to slightly alkaline (pH 8.5), and then the hormones were separated into different groups using two organic solvents: ethyl acetate and diethyl ether. Each solvent attracts specific types of hormones. The separation process was repeated three times for each solvent. Any remaining water was removed from the final hormone extract using a drying agent (anhydrous sodium sulfate). Finally, the remaining solvent (diethyl ether) was evaporated using a vacuum, resulting in a dry residue containing the isolated plant hormones. This residue was then dissolved in a minimal amount of methanol (2.0 ml) and then stored at 4 degrees Celsius for further analytical procedures (Durley and Simpson 1982).

Statistical analysis

A randomized block design was employed for data organization. One-way ANOVA was utilized to assess treatment effects, followed by Tukey's HSD post-hoc test at a significance level of 0.05 to determine significant differences between treatment means. Statistical analyses were conducted using CoStat software (version 6.450, CoHort

Software, Birmingham, UK). To investigate the relationships between variables, Pearson correlation coefficients were calculated, and correlation matrices were generated using the Data Analysis ToolPak within Microsoft Excel 2016.

Results

Effect of seeds biopriming in halo-endophytic bacteria on germination percentage and survival of *Vicia faba*

Five halo-endophytic bacteria (*P. rettgeri*, *B. licheniformis*, *S. sesuvii*, *B. amyloliquefaciens*, *P. suwonensis*) were used in mixtures in the seed priming experiment (Fig. 1). The multi-comparison analysis showed that, seeds treated with B mix(5) achieved the highest germination rate, reaching 100% whereas B mix(4) bacteria showed percentage of germination lower than B mix(5) recorded as $98.33 \pm 2.88\%$, but both B mix(4) and B mix(5) showed percentage of germination higher than control of $85 \pm 2.00\%$ ($p \leq 0.05$). Seeds treated with halo-endophytic bacteria B mix(5) and B mix(4) showed the lowest pre and post-emergency damping off compared to control that was $18 \pm 2.64\%$, and $26.67 \pm 2.88\%$ ($p \leq 0.05$) and the best result were in seeds treated with halo-endophytic bacteria B mix(5) was zero in pre and post-emergency damping off (Table 1).

Effect of *Vicia faba* seeds biopriming on growth parameters

The effect of inoculating *Vicia faba* planted in salt-affected soil with different mixtures of halo-endophytic bacteria was assessed by evaluating changes in faba plant height, length of root and shoot, leaf count, and fresh and dry biomass of both roots and shoots for each mixture of halo-endophytic bacteria (Table 2).

Overall, the multi-statistical analysis showed that all five mixtures of halo-endophytic bacteria showed higher rates of all growth parameters in contrast to the control ($p \leq 0.05$), which displayed reduced growth parameters after 33 and 50 days, but B mix(4) and B mix(5) showed the highest results in all growth parameters. The highest percentages of increase recording values of 440% and 390% were in root fresh weight and found in seeds treated with halo-endophytic bacteria B mix(5) and B mix(4) respectively Table 2; Fig. 2c and Fig. 4, conversely, seeds treated with B mix(1) and B mix(2) showed a decrease in growth parameters after 50 days not 33 days compared to control as shown in Table 2, and Figs. 2 and 3, and 4. The correlation between different tested criteria is introduced in Table 3, all

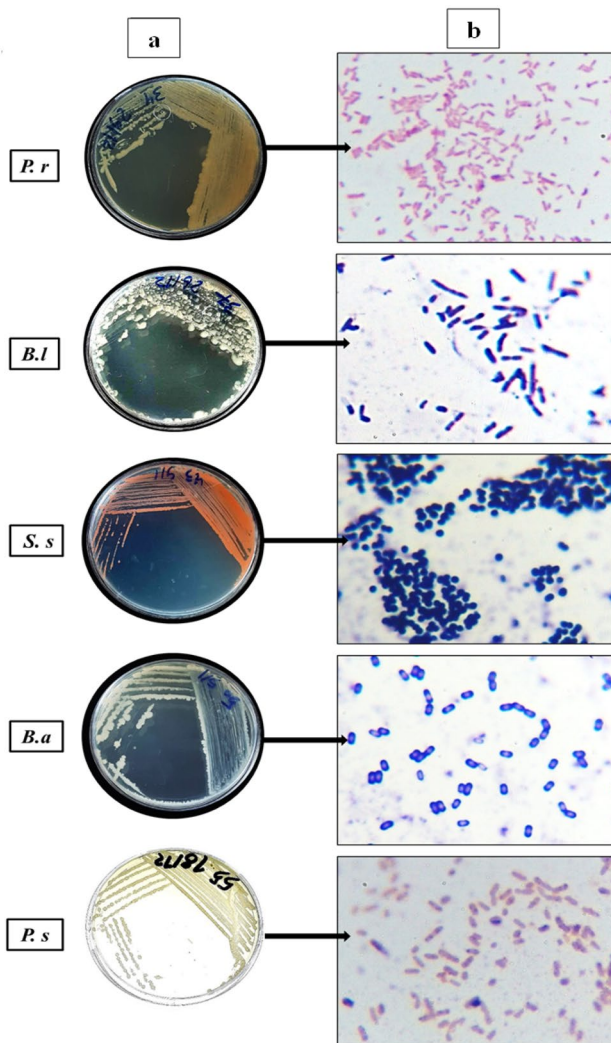


Fig. 1 Halo-endophytic bacteria used in the seed priming experiment of *Vicia faba* Mariout-2. a: halo-endophytic bacteria on L.B agar plate, b: Microscopic examination of Gram stain.; P.r: *P. rettgeri*, B.l: *B. licheniformis*, S.s: *S. sesuvii*, B.a: *B. amyloliquefaciens* and P.s: *P. suwonensis*

with each other.

Measurements of vegetative growth traits of *Vicia faba* in situ

Based on the results of the seeds bioprimering experiment, B mix(4) and B mix(5) were chosen for vegetative growth traits measurements. The statistical analysis showed that the maximum photosynthetic rate, internal carbon dioxide, mesophyll conductance, and mesophyll efficiency were found in seeds of *V. faba* treated with halo-endophytic bacteria B mix(5), while the minimum was found in the control ($p \leq 0.05$). The changes in transpiration rate, photosynthetic water use efficiency, and stomatal conductance were non-significant. The minimum stomatal limitation was -0.122 and found in seeds of *V. faba* treated with halo-endophytic bacteria B mix(5), and the maximum was 0.172 ± 0.005 and found in the control (Table 4).

Table 5 shows a positive correlation between the photosynthesis rate on one side and all tested criteria, except transpiration rate, stomatal conductance, and stomatal limitation, on the other side. A negative correlation was observed between transpiration rate from one side and all tested criteria except stomatal conductance and stomatal limitation from the other side, while a positive correlation was found between both photosynthetic water use efficiency, Internal CO_2 concentration, and all tested criteria except stomatal conductance and stomatal limitation from the other side. A negative correlation was observed between stomatal conductance from one side and all tested criteria except stomatal limitation from the other side, a positive correlation coefficient was observed between mesophyll conductance from one side and mesophyll efficiency from the other side, and a negative correlation with stomatal limitation from the other side. A negative correlation coefficient was observed between stomatal limitation on one side and mesophyll efficiency on the other side.

growth parameters of *Vicia faba* were positively correlated

Table 1 Germination percentage, pre-emergence damping off, and post-emergence damping off of *Vicia Faba* L. Mariout-2 using mixtures of halo-endophytic bacteria

Treatments	Bacterial type	G %	Pre-emergence damping off %	Post-emergence damping off %
Control	—	85 ± 2.00^d	18 ± 2.64^a	26.67 ± 2.88^a
B mix(1)	<i>P.r</i> + <i>P.s</i>	90.33 ± 1.52^{cd}	9.67 ± 1.52^b	15 ± 2.00^b
B mix(2)	<i>S.s</i> + <i>P.s</i>	92.33 ± 2.08^{bc}	8.67 ± 1.52^b	10 ± 1.00^{bc}
B mix(3)	<i>P.r</i> + <i>B.a</i>	92.67 ± 2.51^{bc}	9 ± 1.00^b	10 ± 2.00^{bc}
B mix(4)	<i>P.r</i> + <i>B.l</i> + <i>S.s</i> + <i>B.a</i>	98.33 ± 2.88^{ab}	8.33 ± 2.88^b	6.67 ± 2.88^c
B mix(5)	<i>P.r</i> + <i>B.l</i> + <i>S.s</i> + <i>B.a</i> + <i>P.s</i>	100 ± 0.00^a	0.0 ± 0.00^c	0 ± 0.00^d

Value: mean $\pm$ s.d. Numbers shared the same letter(s) are not significantly different, Tukey test ($p \leq 0.05$), $n=7$. P.r: *P. rettgeri*, B.l: *B. licheniformis*, S.s: *S. sesuvii*, B.a: *B. amyloliquefaciens* and P.s: *P. suwonensis*. Control (freshwater)

Table 2 Root and shoot length, leaf count, and fresh weight, dry weight of root and shoot of *V. faba* supplemented with mixtures of halo-endophytic bacterial isolates; B mix(1, 2, 3, 4, 5) and cultivated in salt-affected soil

Treatments	Days old	Plant height (cm) (%variation)	Root			Shoot			Leaf count (%variation)
			Root length (cm) (%variation)	Fresh weight of root (g) (%variation)	Dry weight of root (g) (%variation)	Shoot length (cm) (%variation)	Fresh weight of shoot (g) (%variation)	Dry weight of shoot (g) (%variation)	
Control	33	29.8±1.64 ^f	6.8±0.83 ^c	0.2±0.02 ^c	0.07±0.008 ^{bc}	23±1.41 ^c	2.78±0.29 ^c	0.20±0.005 ^d	4.8±0.45 ^b
	50	55.2±3.34 ^c	10.4±1.94 ^d	0.44±0.06 ^d	0.09±0.01 ^b	44.8±1.78 ^c	3.52±0.32 ^d	0.29±0.07 ^c	6.2±0.45 ^c
B mix(1)	33	34.2±1.30 ^e (15%)	7.2±0.44 ^c (6%)	0.52±0.04 ^{bc} (160%)	0.07±0.008 ^{bc} (0%)	27±1.22 ^d (17%)	2.8±0.00 ^c (1%)	0.25±0.02 ^c (23%)	6.4±0.55 ^b (33%)
	50	44.4±0.82 ^e (−20%)	9.4±0.54 ^d (−10%)	0.67±0.04 ^c (51%)	0.09±0.005 ^b (4%)	35±0.35 ^d (−22%)	3.66±0.15 ^d (4%)	0.33±0.04 ^c (14%)	8.8±0.45 ^b (42%)
B mix(2)	33	38.6±2.51 ^d (30%)	10±1.41 ^b (47%)	0.5±0.03 ^c (150%)	0.05±0.004 ^c (−28%)	28.6±1.81 ^d (24%)	3.18±0.20 ^{bc} (14%)	0.28±0.02 ^c (36%)	6.6±0.55 ^b (37.5%)
	50	50.4±0.54 ^d (−9%)	13.2±0.44 ^{bc} (27%)	0.66±0.03 ^c (48%)	0.07±0.007 ^b (−26%)	37.2±0.83 ^d (−17%)	4.14±0.11 ^c (18%)	0.37±0.04 ^{bc} (27%)	9.2±0.45 ^{ab} (48%)
B mix(3)	33	48.4±1.67 ^b (62%)	8.8±0.83 ^{bc} (29%)	0.52±0.02 ^{bc} (160%)	0.07±0.005 ^{bc} (3%)	39.6±1.14 ^b (72%)	3.68±0.56 ^{ab} (32%)	0.34±0.04 ^b (67%)	7.8±0.45 ^a (62.5%)
	50	63±1.58 ^b (14%)	11.4±1.14 ^{cd} (9%)	0.68±0.02 ^c (53%)	0.09±0.004 ^b (7%)	51.6±1.14 ^b (15%)	4.76±0.11 ^b (35%)	0.45±0.03 ^b (55%)	8.8±0.45 ^b (42%)
B mix(4)	33	43.6±1.52 ^c (46%)	10±0.45 ^b (47%)	0.98±0.04 ^a (390%)	0.1±0.00 ^b (39%)	33.6±1.14 ^c (46%)	3.00±0.07 ^c (8%)	0.29±0.008 ^c (43%)	8.2±0.45 ^a (72%)
	50	80.8±1.30 ^a (46%)	14.4±1.51 ^{ab} (38%)	1.29±0.07 ^b (194%)	0.2±0.05 ^a (122%)	66.4±1.34 ^a (48%)	4.02±0.04 ^c (14%)	0.576±0.032 ^a (100%)	9.6±0.547 ^{ab} (55%)
B mix(5)	33	61±2.35 ^a (105%)	14±1.41 ^a (106%)	1.08±0.10 ^b (440%)	0.15±0.04 ^a (106%)	47±1.22 ^a (104%)	4.02±0.08 ^a (45%)	0.416±0.02 ^a (101%)	8.4±0.55 ^a (75%)
	50	80.4±2.96 ^a (46%)	16±1.22 ^a (54%)	1.43±0.044 ^a (224%)	0.25±0.07 ^a (175%)	64.4±1.81 ^a (44%)	5.14±0.11 ^a (46%)	0.67±0.07 ^a (133%)	10.2±0.84 ^a (65%)

Italics represents the highest percentage of increase compared to control. Value: mean±s.d, Number shared the same letter(s) are not significantly different, Tukey test ($p \leq 0.05$), $n = 7$. Numbers recorded between brackets represent the percentage of variation compared to the control. B mix(1) (*P. rettgeri* DW3 + *P. suwonensis* DW7), B mix(2) (*S. sesuvii* DW5 + *P. suwonensis* DW7), B mix(3) (*P. rettgeri* DW3 + *B. amyloliquefaciens* DW6), B mix(4) (*P. rettgeri* DW3 + *B. licheniformis* DW4 + *S. sesuvii* DW5 + *B. amyloliquefaciens* DW6) and B mix(5) (*P. rettgeri* DW3 + *B. licheniformis* DW4 + *S. sesuvii* DW5 + *B. amyloliquefaciens* DW6 + *P. suwonensis* DW7). Variation in parameter Estimation (%variation) signed by minus means less than the mean of the control

Physical and chemical analysis of the rhizosphere of salt-affected soil before and after planting

Physical analysis of salt-affected soil includes pH, EC, and SP analysis, while chemical analysis of salt-affected soil includes calcium carbonate, anions, cations and macro and microelements analysis of soil planted with seeds of *V. faba* inoculated with B mix(4) and B mix(5) showed the best results in all physical, chemical and biological analysis compared to control and soil sample before planting (row soil) which showed the worst results in all. pH analysis records non-significant changes in soil planted with seeds of *V. faba* inoculated with B mix(4) and B mix(5) compared to the control and soil samples before planting. The statistical analysis revealed that there are significant differences in EC of soil samples B mix(4) and B mix(5) compared to control and soil sample before planting; soil sample B mix(5) showed the lowest EC of 3.82 ± 0.03 then soil sample B mix(4) EC of 4.77 ± 0.015 , while the highest EC were found in soil sample before planting (row soil) and control

of 5.48 ± 0.01 and 5.02 ± 0.025 ($p \leq 0.05$), respectively as shown in Table 6.

Anions and cations concentrations decreased in post-treatment samples, especially soil samples B mix(4) and B mix(5) and the best was soil sample B mix(5) which showed Cl^- concentration of $20.9 \pm 0.1 \text{ mg.L}^{-1}$, Na^+ concentration of $16.2 \pm 0.25 \text{ mg.L}^{-1}$ and K^+ concentration of $0.79 \pm 0.02 \text{ mg.L}^{-1}$ compared to pretreatment sample (row soil) showed Cl^- , Na^+ and K^+ concentration of 32.5 ± 0.1 , 24.6 ± 0.3 and $1.15 \pm 0.05 \text{ mg.L}^{-1}$ respectively. Nitrogen and Iron concentrations increased in post-treatment samples, especially soil samples B mix(4) and B mix(5). The greatest concentration of nitrogen was $93 \pm 2 \text{ mg.kg}^{-1}$ soil, and iron was $12.38 \pm 0.02 \text{ mg.kg}^{-1}$ soil was found in soil samples B mix(5). Phosphorus concentration increased in post-treatment samples compared to pretreatment sample (row data), but soil samples B mix(5) showed the minimum phosphorus concentration of $7.38 \pm 0.02 \text{ mg.kg}^{-1}$ soil in post-treatment samples when compared with the control which showed $10.2 \pm 0.1 \text{ mg.kg}^{-1}$ soil ($p \leq 0.05$) (Table 6).

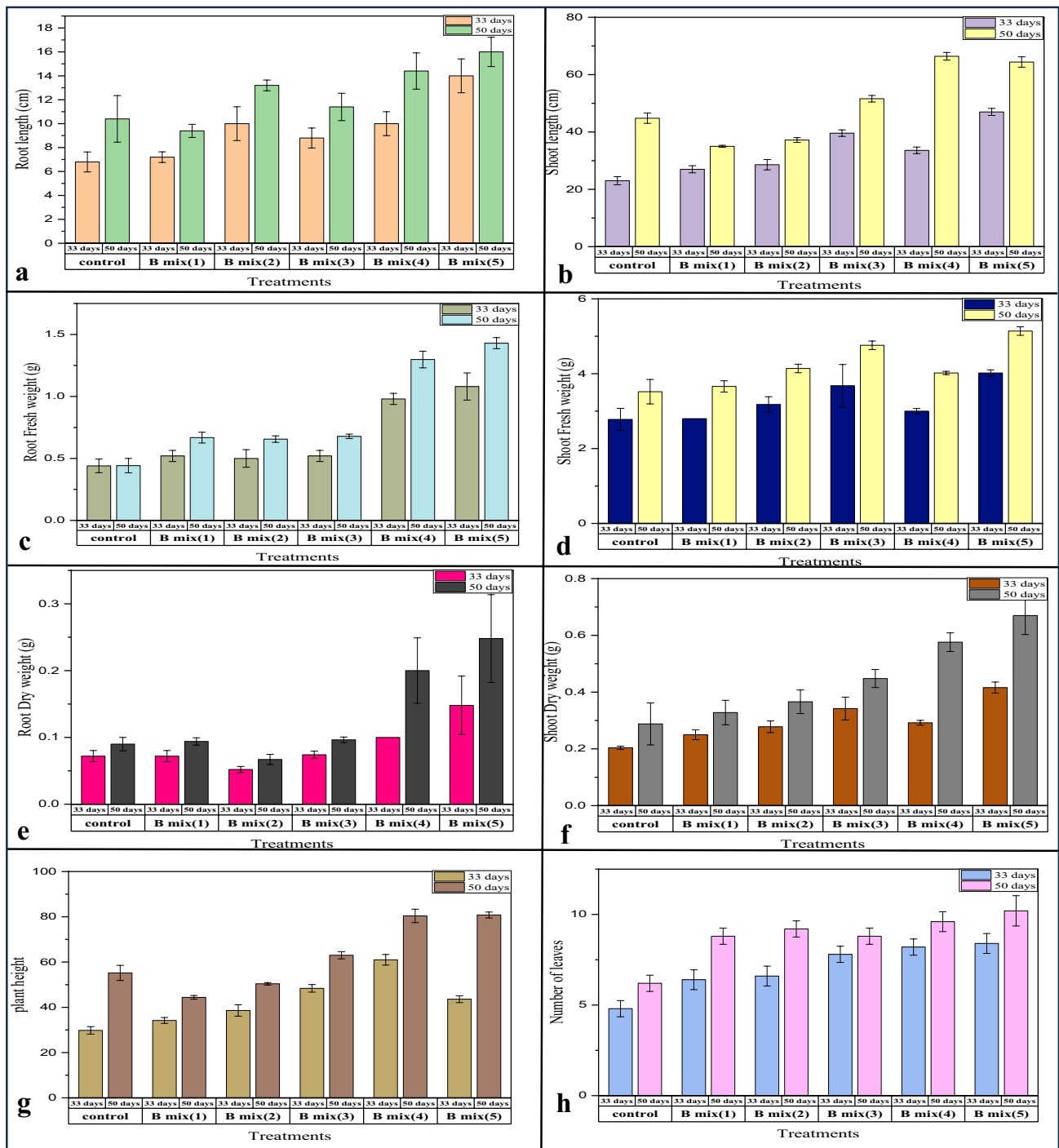


Fig. 2 Histogram of growth parameters of *V. faba* with and without halo-endophytic bacteria: a: length of root, b: length of shoot, c: fresh weight of root, d: fresh weight of shoot, e: dry weight of root, f: dry weight of shoot, g: plant height, and h: leaf count. B mix(1) (*P. rettgeri* DW3 + *P. suwonensis* DW7), B mix(2) (*S. sesuvii* DW5 + *P.*

suwonensis DW7), B mix(3) (*P. rettgeri* DW3 + *B. myloliuefaciens* DW6), B mix(4) (*P. rettgeri* DW3 + *B. licheniformis* DW4 + *S. sesuvii* DW5 + *B. myloliuefaciens* DW6) and B mix(5) (*P. rettgeri* DW3 + *B. licheniformis* DW4 + *S. sesuvii* DW5 + *B. amylioliquefaciens* DW6 + *P. suwonensis* DW7).

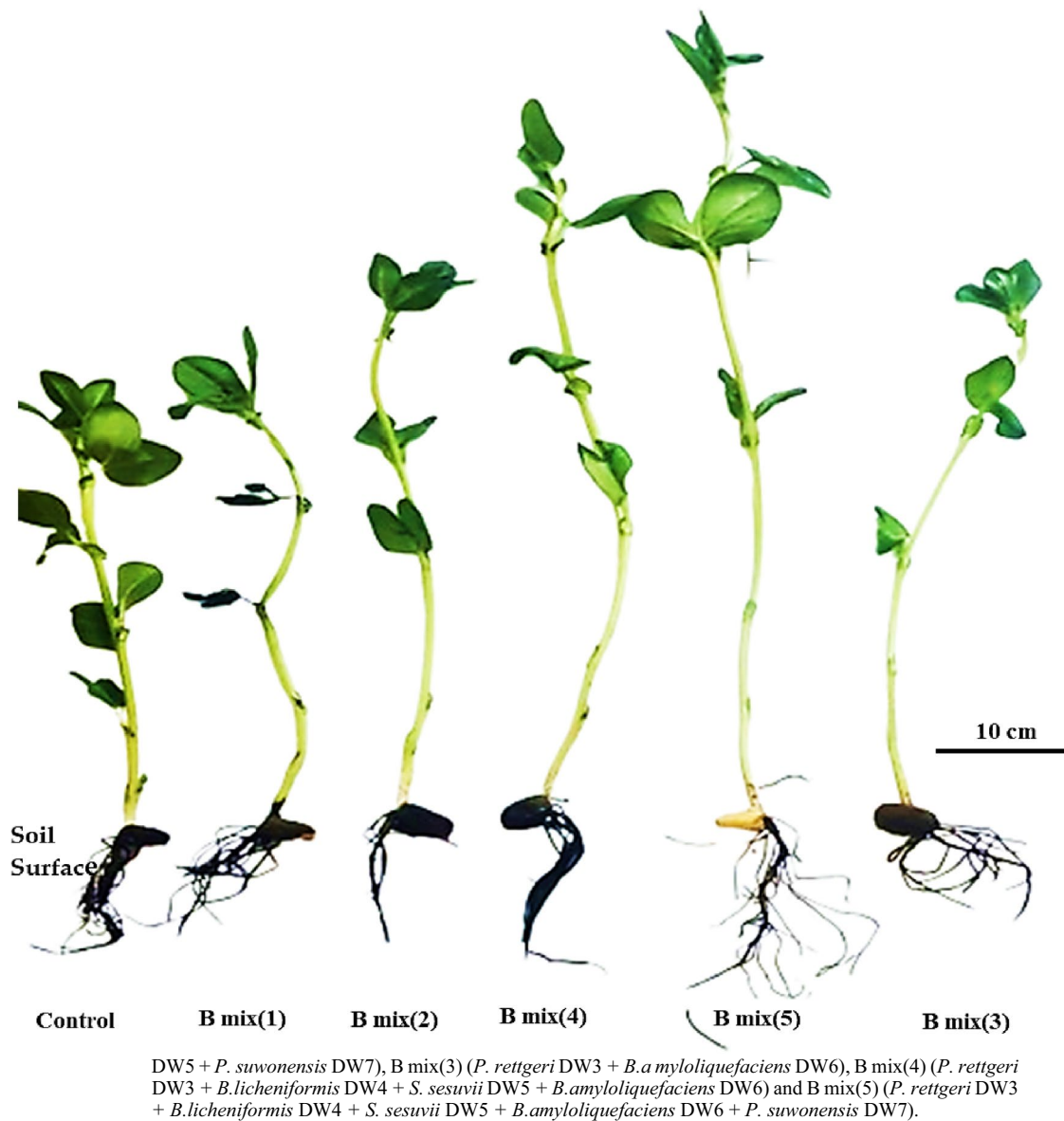


Fig. 3 Images of *V. faba* plants inoculated with various mixtures of halo-endophytic bacteria in comparison to the control and grown in salt-affected soil in the greenhouse after 55 days. B mix(1): *P. rettgeri* DW3 + *P. suwonensis* DW7; B mix(2): *S. sesuvii* DW5 + *P. suwonensis* DW7; B mix(4): *P. rettgeri* DW3 + *B. licheniformis* DW4 + *S. sesuvii*

DW5 + *B. amyloliquefaciens* DW6; Bmix(5): *P. rettgeri* DW3 + *B. licheniformis* DW4 + *S. sesuvii* DW5 + *B. amyloliquefaciens* DW6 + *P. suwonensis* DW7 and B mix(3): *P. rettgeri* DW3 + *B. amyloliquefaciens* DW6

Effect of halo-endophytic bacteria on soil biological activity

The statistical analysis showed that organic matter percentages increased in soil samples B mix(4) and B mix(5) compared to row soil, but the highest organic matter was $0.94 \pm 0.01\%$, found in soil sample B mix(5). Unlike control, control remained constant. Nitrogen concentrations increased in post-treatment samples, especially soil samples B mix(4) and B mix(5). The highest concentration of

nitrogen was $93 \pm 2 \text{ mg.kg}^{-1}$ ($p \leq 0.05$) soil was found in soil samples B mix(5) (Table 7).

The total bacterial count and dehydrogenase activity increased in post-treatment samples especially soil samples B mix(4) and B mix(5) and the maximum total bacterial count of $593 \times 10^3 \pm 1000 \text{ CFU.g}^{-1}$ and $0.962 \pm 0.01 \mu\text{g TPF g}^{-1} \text{ Dry Soil min}^{-1}$ soil was in soil sample B mix(5), on the other hand, pre-treatment sample (row soil) showed the minimum total bacterial count and dehydrogenase activity of $69.5 \times 10^3 \pm 1500 \text{ CFU.g}^{-1}$ soil and $0.266 \pm 0.0095 \mu\text{g}$

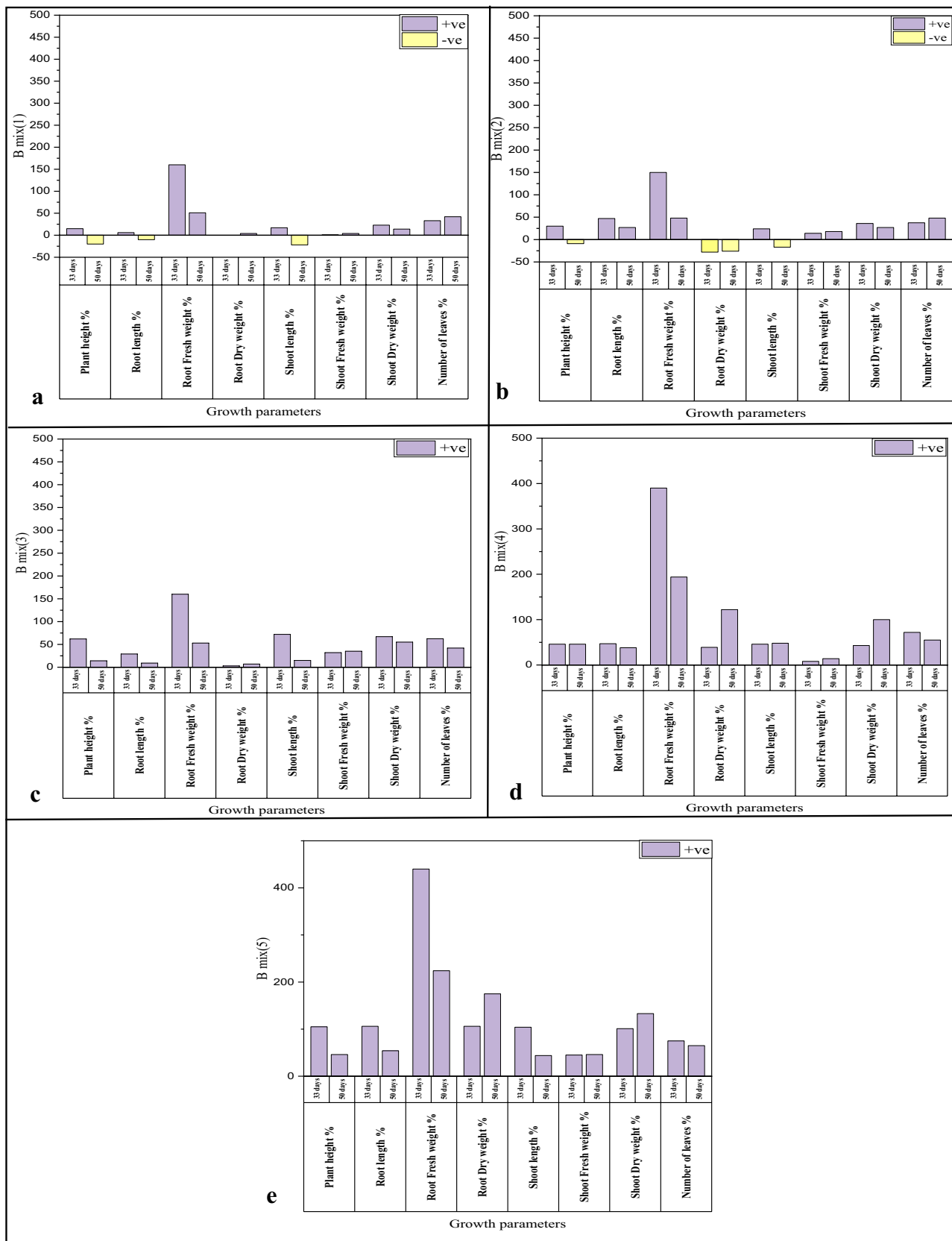


Fig. 4 The percentage of increase samples plant height, length of root, length of shoot, leaves count, root and shoot fresh biomass, and root and shoot dry biomass compared to the control. a: B mix(1) (*P. rettgeri* DW3 + *P. suwonensis* DW7), b: B mix(2) (*S. sesuvii* DW5 + *P. suwonensis* DW7), c: B mix(3) (*P. rettgeri* DW3 + *B. a. myloliuefaciens* DW6), d: B mix(4) (*P. rettgeri* DW3 + *B. licheniformis* DW4 + *S. sesuvii* DW5 + *B. amyloliquefaciens* DW6) and e: B mix(5) (*P. rettgeri* DW3 + *B. licheniformis* DW4 + *S. sesuvii* DW5 + *B. amyloliquefaciens* DW6 + *P. suwonensis* DW7). +ve represents a percentage value higher than the control, -ve represents a percentage value lower than the control

TPF g^{-1} Dry Soil min^{-1} , then control showed total bacterial count and dehydrogenase activity of $175 \times 10^3 \pm 1527$ CFU. g^{-1} soil and 0.394 ± 0.0051 μg TPF g^{-1} Dry Soil min^{-1} soil ($p \leq 0.05$) (Table 7).

Effect of halo-endophytic bacteria on *Vicia faba* phytochemicals

Following planting, analysis of *V. faba* revealed increased percentages of nitrogen, potassium, and phosphorus in plants treated with halo-endophytic bacteria, B mix(4) and B mix(5), relative to the control. Based on statistical analysis, the highest percentages of nitrogen, potassium and phosphorous were found in faba plants treated with halo-endophytic bacteria B mix(5) and recorded as $3.03 \pm 0.060\%$, $1.12 \pm 0.10\%$ and $0.68 \pm 0.01\%$ ($p \leq 0.05$) respectively, while the minimum percentage of nitrogen $2.7 \pm 0.051\%$, potassium was $0.95 \pm 0.025\%$ and phosphorus was $0.45 \pm 0.072\%$ ($p \leq 0.05$) and found in control (Table 8).

Total amino acids and proline percentages increased in faba plants treated with halo-endophytic bacteria, B mix(4) and B mix(5), in comparison to the control. The highest percentages of total amino acids and proline were $3.98 \pm 0.01\%$ and $5.87 \pm 0.02\%$ ($p \leq 0.05$) as found in faba plants treated with halo-endophytic bacteria B mix(5), alternatively, the minimum percentage of total amino acids and proline were $2.81 \pm 0.01\%$ and $2.61 \pm 0.015\%$ ($p \leq 0.05$) and found in control as shown in Table 8.

This finding was confirmed by the statistical analysis (Table 9), in which most of the relationships of the tested criteria showed a positive correlation between nitrogen percentage and proline percentage. So, the data recorded here is evidence for the efficiency of halo-endophytic bacteria B mix(5) in *V. faba* seeds biopriming under the salinity stress of salt-affected soil.

Discussion

Field crops irrigated with low-quality water in salt-affected soil produce more growth inhibitors and fewer growth promoters, which lowers soil quality and ultimately results in

cell death and low productivity. In this work, the combinations of the PGPB were utilized to increase soil quality and reduce damage to the faba bean from salt stress.

The symbiotic relationship between faba beans and N-fixing rhizobia makes them essential for nitrogen cycling in agronomy. Due to their frequent exposure to and adaptation to environmental conditions, rhizobial strains are known to have enzyme profiles that are necessary for enhancing abiotic stress tolerance (Alsenidi et al. 2020). The literature has ample evidence of the use of bacterial inoculation to encourage plant development (Adesemoye and Egamberdieva 2013). Apart from their capability to promote plant growth, *Paenaltcaligenes suwonensis*, *Bacillus licheniformis*, *Bacillus amyloliquefaciens*, *Salinicoccus sesuvii*, and *Providencia rettgeri* are also capable of withstanding salt stress, thus offering viable approaches for addressing salinity challenges in faba bean agriculture.

In the present study, the possibility that the use of PGPB like *P. rettgeri* DW3, *B. licheniformis* DW4, *S. sesuvii* DW5, *B. amyloliquefaciens* DW6, *P. suwonensis* DW7 in mixtures ameliorate the salinity effect in salt-affected soil and make agriculture possible in salt-affected soil and especially for the most important crop in Egypt, *Vicia faba*.

As far as we know, this study is the first to investigate the effects of these specific isolates as PGPB on *Vicia faba* under salinity stress. Biochemical characterization demonstrated that all five isolates exhibit a diverse array of plant growth-promoting traits, including the production of IAA, GA3, siderophores, exopolysaccharides (EPS), HCN, and ammonia. Moreover, they exhibit nitrogen-fixing and phosphate-solubilizing capabilities and produce various enzymes such as catalase, amylase, lipase, protease, and cellulase. All isolates demonstrated the capacity for growth in LB medium containing NaCl concentrations ranging from 170 mM (1%) to 3400 mM (20%) (Wael et al. 2024).

As there were no antagonism between isolates, the mixtures of bacterial isolates were used for PGP of *Vicia faba* to produce more PGP hormones than single isolates to help in *faba* growth and this corroborated by the findings of Wang et al. (2022) on IAA and EPS. The unique combination of these five isolates and the specific conditions used have not been previously reported in the literature. Moreover, the isolates *S. sesuvii* and *P. suwonensis* have not been studied as PGPB for any plant species, including *V. faba*. Isolate; *P. rettgeri* was used for the first time in the growth promotion of *V. faba*; on the other hand, *P. rettgeri* was used in the growth promotion of *Arabidopsis thaliana* (Mora-Ruiz et al. 2018). *B. licheniformis* was used for the first time in promoting *V. faba* growth; on the other hand, *B. licheniformis* was used in the growth promotion of mung beans (*Vigna radiata*) (Biswas et al. 2018) and common beans (*Phaseolus vulgaris*) (Chihaoui et al. 2015). *B. amyloliquefaciens* was

Table 3 Correlations between root and shoot length, leaf count and fresh weight, dry weight of root and shoot of *V. faba* during seed priming experiment

	Plant height (cm)	Root length (cm)	Shoot length (cm)	Leaf count	Fresh weight of root (g)	Fresh weight of shoot (g)	Dry weight of root (g)
Root length (cm)	0.89	1					
Shoot length (cm)	0.99	0.85	1				
Leaf count	0.77	0.81	0.75	1			
Fresh weight of root (g)	0.83	0.85	0.80	0.82	1		
Fresh weight of shoot (g)	0.84	0.82	0.82	0.80	0.63	1	
Dry weight of root (g)	0.86	0.79	0.86	0.66	0.90	0.65	1
Dry weight of shoot (g)	0.95	0.88	0.94	0.84	0.87	0.87	0.90

Table 4 *In situ* measurements of vegetative growth traits of *V. faba* treated with B mix(4) and B mix(5) using the LCi-SD Leaf Chamber/Soil Respiration Analysis System

Analysis	Control	B mix(4)	B mix(5)
Photo-synthesis rate (A)	1.263±0.015 ^b	1.346±0.050 ^{ab}	1.39±0.036 ^a
Transpiration rate (E)	0.943±0.110 ^a	0.873±0.113 ^a	0.913±0.023 ^a
Photo-synthetic water uses efficiency (pWUE)	1.35±0.146 ^a	1.55±0.138 ^a	1.52±0.048 ^a
Internal CO ₂ conc (C.I)	298±2 ^c	371±1.5 ^b	404±2 ^a
Stomatal conductance (G.S)	0.021±0.0017 ^a	0.018±0.0023 ^a	0.018±0.0023 ^a
Meso-phyll conductance (g _m)	0.0034±0.00011 ^b	0.0036±0.00015 ^b	0.0042±0.00006 ^a
Stomatal limitation (L _s)	0.172±0.005 ^a	−0.031 ^b	−0.122 ^c
Meso-phyll efficiency (ME)	14,256±1202 ^a	20,120±2764 ^a	21,883±2917 ^a

Value: mean±s.d. Numbers shared the same letter(s) are not significantly different, Tukey test ($p \leq 0.05$), $n=7$

used for the first time in the growth promotion of *V. faba*; on the other hand, *B. amyloliquefaciens* was used in the growth promotion of common beans (*Phaseolus vulgaris*) (Sendi et al. 2020), (Martins et al. 2015), and Tepary bean seedlings (*Phaseolus acutifolius*) (Prabhakaran et al. 2013).

Successful plant establishment hinges on germination and early growth stages. These initial phenophases (growth stages) are the most vulnerable, particularly when faced with poor soil conditions (Abo-Kassem 2007). It was revealed that the seeds of *V. faba* treated with halo-endophytic bacteria B mix(5) increased germination percentages under salinity stress of salt-affected soil, in comparison to non-inoculated seeds (control). This observation corroborates what has been described in previous studies by Piernik et al. (2017) on fodder beet and decreased pre- and post-emergence damping off. It was previously known from prior work (Wael et al. 2024) halo-endophytic bacteria were capable of producing Siderophores, which allows halo-endophytic bacteria to fight harmful pathogens in plant roots in two ways: by limiting the amount of available iron in the root zone (rhizosphere), essential for many harmful pathogens to grow and cause disease (Yu et al. 2011), producing various digestive enzymes, like amylase, cellulase, protease, and lipase. These enzymes possess the capacity to inhibit fungal pathogen growth by hydrolyzing the cell walls of fungi (Dukare and Paul 2018).

To evaluate the influence of bacterial isolates on faba growth, several physiological parameters were evaluated. Relative to the control, *V. faba* treated with B mix(5) exhibited significant improvements in plant height, length of root and shoot, leaf count, fresh and dry biomasses of both roots and shoots, and germination percentage. Furthermore, these bacterial treatments effectively reduced both pre- and post-emergence damping-off in *V. faba* under saline stress. These results are consistent with prior research on *V. faba* (Mahgoub et al. 2021) and Maize (Aslam and Ali 2018), which also demonstrated the positive impact of bacterial inoculation on plant growth parameters.

Plant development and yield are greatly influenced by salinity stress, which has a substantial impact on vital physiological functions like photosynthesis and energy metabolism (Feng et al. 2014). The key to maintaining high photosynthetic rates in salt-tolerant plants (halophytes) under stress lies in their ability to preserve the integrity

Table 5 Correlations between different vegetative growth traits of *V. faba in situ* using the LCi-SD Leaf Chamber/Soil Respiration Analysis System during the seed priming experiment

	Photosynthesis rate (A)	Transpiration rate (E)	Photosynthetic water uses efficiency (pWUE)	Internal CO ₂ conc (C.I)	Stomatal conductance (G.S)	Mesophyll conductance (gm)	Stomatal limitation (Ls)
Transpiration rate (E)	−0.58	1					
Photosynthetic water uses efficiency (pWUE)	0.88	−0.89	1				
Internal CO ₂ conc (C.I)	0.99	−0.61	0.90	1			
Stomatal conductance (G.S)	−0.94	0.82	−0.99	−0.952	1		
Mesophyll conductance (gm)	0.89	−0.16	0.59	0.88	−0.69	1	
Stomatal limitation (Ls)	−0.99	0.61	−0.90	−1	0.95	−0.87	1
Mesophyll efficiency (ME)	0.99	−0.67	0.93	0.99	−0.97	0.84	−0.99

Table 6 Effect of *V. faba* seeds biopriming by halo-endophytic bacteria, B mix(4) and B mix(5) on salt-affected soil before and after 50 days of planting.

Analysis	Before planting	After planting		
		Control	B mix(4)	B mix(5)
<i>Physical</i>				
pH	7.85±0.01 ^d	7.95±0.007 ^c	8.03±0.015 ^a	7.98±0.01 ^b
EC (dS/m)	5.48±0.01 ^a	5.02±0.025 ^b	4.77±0.015 ^c	3.82±0.03 ^d
SP	25±0.2 ^b	26±0.1 ^a	25±0.1 ^b	26±0.2 ^a
<i>Chemical</i>				
Cal- cium carbon- ate %	2.52±0.02 ^b	2.1±0.1 ^a	2.52±0.02 ^b	2.1±0.05 ^a
<i>Anions (mg/L)</i>				
HCO 3 [−]	1.5±0.1 ^a	1±0.2 ^b	1±0.1 ^b	1±0.104 ^b
Cl [−]	32.5±0.1 ^a	29.7±0.2 ^b	20.9±0.1 ^c	20.9±0.1 ^c
SO ₄ ^{2−}	19.75±0.02 ^a	18.19±0.01 ^b	14.91±0.01 ^c	13.77±0.005 ^d
Total anions	54.75	48.89	36.81	35.67
<i>Cations (mg/L)</i>				
Ca ²⁺	16.5±0.4 ^a	16.4±0.70 ^a	12.05±0.427 ^b	12.06±0.25 ^b
Mg ²⁺	12.5±0.2 ^a	7.7±0.22 ^b	7.7±0.11 ^b	7.69±0.02 ^b
Na ⁺	24.6±0.3 ^a	24.56±0.15 ^a	17.1±0.45 ^b	16.2±0.25 ^c
K ⁺	1.15±0.05 ^a	1.14±0.04 ^a	1.05±0.05 ^b	0.79±0.02 ^c
Total cations	54.75	49.8	37.9	36.74
<i>Macro and microelements (mg/kg soil)</i>				
Potas- sium	89±2 ^a	83±1 ^b	78±0.1 ^c	80±0.05 ^c
Phos- phorus	4.85±0.01 ^d	10.2±0.1 ^a	8.48±0.005 ^b	7.38±0.02 ^c
Copper	0.04±0.001 ^b	0.05±0.003 ^a	0.03±0.001 ^c	0.02±0.002 ^d
Iron	5.88±0.01 ^d	9.24±0.01 ^c	10.22±0.01 ^b	12.38±0.02 ^a
Man- ganese	1.23±0.02 ^a	0.79±0.01 ^b	0.64±0.03 ^c	0.51±0.021 ^d
Zinc	0.36±0.01 ^a	0.35±0.001 ^a	0.31±0.01 ^b	0.25±0.005 ^c

Value: mean±s.d, Numbers shared the same letter(s) are not significantly different, Tukey test ($p \leq 0.05$), $n = 7$

of their photosynthetic systems and maintain internal CO₂ levels (Sui and Han 2014). One of the main functions that salinity (Munns et al. 2006) affects is photosynthesis (Argente-Martínez et al. 2024). Direct consequences include the reduced CO₂ availability brought on by constraints in gas diffusion across stomatal pores and within the leaf mesophyll (Galmés et al. 2007).

The data analysis demonstrated a significant enhancement in the rate of photosynthesis (A) among plants treated with B mix(4) and B mix(5) in comparison to the control. This suggests that the B mixes positively influenced the plants' ability to harness and utilize light energy and convert it into chemical energy through photosynthesis as evidenced by increased the length of the plant and shoot, leaf number, shoot fresh and dry biomasses were the highest in plant treated with B mix(4) and B mix(5) and this is likely because of the presence of phytohormones such as IAA and GA-3 produced by PGPB. This finding is consistent with observations from previous studies, where the exogenous application of certain compounds enhanced photosynthetic activity in various plant species (Abdel Latef et al. 2021; Djebaili et al. 2021; Rossi et al. 2021).

Interestingly, the transpiration rate (E) demonstrated no statistically significant differences between treatments. Despite boosting photosynthesis, the B mixes didn't alter stomatal water loss or photosynthetic water use efficiency (pWUE). This suggests that even with enhanced carbon assimilation, plants might increase water usage to sustain ideal gas exchange rates, preventing an improvement in pWUE (Grassi et al. 2002; Sefton et al. 2002).

B mixes may have promoted the ability of plants grown in salt-affected soils to access water located further away by encouraging the development of longer roots. This is evident in the significantly greater root length and weight (both fresh and dry) observed in plants inoculated with B mixes compared to the control.

Internal CO₂ concentration (Ci) significantly increased in plants inoculated with B mixes in comparison with the control. This indicates that the B mixes likely induced greater

Table 7 Rhizosphere analysis: percentage of organic matter, concentration of nitrogen, dehydrogenase, and total bacterial count treated with halo-endophytic bacteria; B mix(4) and B mix(5) before and after 50 days of planting as an indicator of soil fertility

Analysis	Before planting	After planting		
	Row soil	Control	B mix(4)	B mix(5)
Organic matter %	0.44±0.02 ^c	0.44±0.005 ^c	0.5±0.01 ^a	0.94±0.01 ^b
Nitrogen (mg.kg ⁻¹ soil)	64±1 ^d	67±0.1 ^c	83±1 ^b	93±2 ^a
Total bacterial count (C.F.U.g ⁻¹ soil)	69.5×10 ³ ±1500 ^d	175×10 ³ ±1527 ^c	296×10 ³ ±1000 ^b	593×10 ³ ±1000 ^a
Dehydrogenase (μg TPF.g ⁻¹ Dry Soil.min ⁻¹)	0.266±0.0095 ^d	0.394±0.0051 ^c	0.725±0.01 ^b	0.962±0.01 ^a

Value: mean±s.d, Numbers shared the same letter(s) are not significantly different, Tukey test ($p\leq 0.05$), $n=7$

Table 8 Effect of inoculation with mixtures of halo-endophytic bacteria, B mix(4) and B mix(5), on phytochemicals of *V. faba* after 50 days of planting

Analysis	Control	B mix(4)	B mix(5)
Nitrogen %	2.7±0.051 ^b	3.02±0.072 ^a	3.03±0.060 ^a
Potassium %	0.95±0.025 ^b	1.026±0.064 ^{ab}	1.12±0.10 ^a
Phosphorus %	0.45±0.072 ^b	0.63±0.035 ^{ab}	0.68±0.01 ^a
Total amino acids %	2.81±0.01 ^c	3.63±0.015 ^b	3.98±0.01 ^a
Proline %	2.61±0.015 ^c	2.71±0.01 ^b	5.87±0.02 ^a

Value: mean±s.d, Numbers shared the same letter(s) are not significantly different, Tukey test ($p\leq 0.05$), $n=7$.

Table 9 Correlations among different phytochemicals of *V. faba* in the seed priming experiment after 50 days of planting.

	Nitrogen %	Potassium %	Phosphorus %	Total amino acids %
Potassium %	0.85	1		
Phosphorus %	0.98	0.93	1	
Total amino acids %	0.96	0.96	0.99	1
Proline %	0.55	0.91	0.68	0.75

stomatal opening, facilitating a reduction in C_i . Lower C_i allows for faster diffusion of CO_2 from the atmosphere into the chloroplasts, potentially explaining the observed increase in photosynthesis rate (A) with B mixes. This finding is consistent with prior research, where improved stomatal conductance due to environmental factors or specific treatments led to enhanced CO_2 assimilation rates (Morrison 1987; Panwar 1991; Elanchezhian and Panwar 1997; Bashan et al. 2006).

Mesophyll conductance (gm), a parameter indicative of CO_2 diffusion within the leaf mesophyll, showed significantly greater levels in B mix(5) than in both the control and B mix(4). This suggests that the B mix(5) treatment specifically improved the movement of CO_2 within the leaf mesophyll, facilitating its delivery to the chloroplast sites of photosynthesis. This enhanced diffusion likely contributed to the observed increase in photosynthetic activity in B mix(5) plants. Stomatal limitation (L_s), a measure of the extent to which stomatal closure limits CO_2 assimilation, exhibited negative values for B mix(4) and B mix(5). This

indicates that stomatal conductance was not a limiting factor for CO_2 assimilation in these treatments. In contrast, the positive L_s value in the control suggests that stomata might have been partially closed, potentially limiting CO_2 diffusion into the leaf. These findings suggest that B mixes, particularly B mix(5), improved CO_2 assimilation by promoting stomatal opening (reducing C_i) and enhancing mesophyll conductance for CO_2 diffusion.

The photosynthetic rate (A), stomatal conductance (gs), and transpiration rate (E) were reduced under salinity stress, but B mix(5) increased photosynthetic rate, internal carbon dioxide, mesophyll conductance, and mesophyll efficiency of *Vicia faba* relative to the control. Other research in this area corroborates these results on Common Purslane (Hnilickova et al. 2021) and wheat (Sharma et al. 2005).

While the B mixes, particularly the B mix(5), showed promise in enhancing plant photosynthetic activity, to fully understand these effects, further research is necessary to identify the specific components involved. Unraveling the mechanisms of action of the B mixes on the photosynthetic machinery would be valuable for optimizing their formulation. Additionally, exploring strategies to improve water use efficiency alongside enhanced photosynthesis is crucial for sustainable plant growth, with a particular emphasis on water-scarce environments.

To assess the impact of bacterial isolates on seed germination and plant growth, several physiological parameters were measured. The mixtures of halo-endophytic bacteria, *P. suwonensis* DW7, *B. licheniformis* DW4, *B. amyloliquefaciens* DW6, *S. sesuvii* DW5, and *P. rettgeri* DW3 increased the height of the plant, length of root and shoot, leaf count, root and shoot fresh and dry biomasses of *Vicia faba* under salinity stress of salt-affected soil relative to the control. These results are consistent with the findings of other studies on related topics on wheat (Lastochkina et al. 2023), *Vicia faba* (Mahgoub et al. 2021; Wael et al. 2024), maize (Aslam and Ali 2018), and prostrate pigweed (Ikhajiagbe et al. 2021). the percentage of variation compared to the control was the highest in root fresh weight, as it was the most affected parameter by salt stress. May be the presence of high EC in salt-affected soil stimulated halo-endophytic bacteria to increase root fresh weight as a

response to salinity, corroborated by the findings of Yousefi et al. (2017).

EC, the quantity of Firmicutes rose in the hypersaline to saline soils, possibly as a result of the presence of numerous species of Firmicutes that, because of their Gram-positive cell walls and capacity to generate spores, can withstand severe environmental stresses (Kalwasińska et al. 2017).

Generally, EC concentrations decreased due to several reasons: watering with freshwater, exopolysaccharides production by halo-endophytic bacteria, and increasing soil organic matter concentration. Beneficial bacteria treatments can enhance plant tolerance to salty soils in two ways: by improving water retention within the plant (Wittenmayer and Merbach 2005; AbuQamar et al. 2024; Altynbay et al. 2024), create clumps in the soil that improve drainage and allow the soil to hold more water, creation of exopolysaccharide–cation–soil complex, neutralizing harmful sodium ions in the soil, making them less damaging to the plant, and this reduces the amount of sodium the plant takes up (Upadhyay et al. 2011).

Scientists have studied how sugary substances released by soil bacteria (exopolysaccharides or EPS) interact with various minerals in the soil, such as potassium, magnesium, sodium, and calcium. These interactions can form complexes (EPS–cation–soil complex) that help retain water in the soil (Morel et al. 1991). The effectiveness of this water retention depends on the specific chemical makeup of the EPS produced by the bacteria, which can influence how well they form stable clumps (aggregates) in the soil.

When land is irrigated (regularly watered), the salinity of the soil (electrical conductivity or EC) tends to go down over time (Cui et al. 2012; Rath and Rousk 2015). As the soil becomes less salty, the amount of soil organic carbon (SOC) also increases, this is because improving plant growth leads to more plant residues being left behind in the soil, increasing the organic carbon content and changing in the soil's physical, chemical, and biological properties as it becomes less salty may improve mechanisms that stabilize SOC (Wichern et al. 2006; Rath et al. 2019). Unveiling how microbes react to varying salinity and plant residue inputs is essential, as these factors significantly influence the creation of soil organic carbon (SOC) through microbial decomposition of plant matter. In this study, EC, anion, and cation concentrations decreased in post-treatment samples compared to the row soil sample and control, especially soil samples B mix(4) and B mix(5), and the best was soil sample B mix(5). These findings are supported by other studies on related topics on the planting of sunflower and its impacts on soil fertility (Urooj et al. 2022) and EC (Moula et al. 2021).

Halo-endophytic bacterial mixtures significantly influenced the levels of macro- and micro-elements. These act as biological fertilizer formulations that increase N and Fe

content in soil compared to the control, as they make nitrogen fixation and produce siderophores and enhance plant health by boosting iron intake, inhibiting other microbes by releasing antibiotic molecules that directly suppress their growth, and reducing iron availability, thereby hindering pathogen growth. These findings are consistent with the observations reported by Erdogan et al. (2018). Also, phosphorus and potassium contents with bacterial applications in *Vicia faba* were increased, whereas decreased in the soil as these bacteria helped faba beans in uptake of P and K due to the capability of these bacteria to make phosphate solubilization and convert insoluble phosphorus into soluble one and make it available for plant, these findings align with results from other investigations on related endophytic bacterial strains *Bacillus subtilis* by Oppenheimer-Shaanan et al. (2022). Halo-endophytic bacteria nutrient mining to increase absorption of soil nutrients such as N, P, K, and Fe by roots (White et al. 2019).

Previous studies have indicated that soil with more organic matter has a larger population of active microbes, especially in harsh environments like salty soils (Akhtar 2019; Zhang et al. 2024). This suggests that adding organic matter to soil could be a way to counteract salt stress. Our findings support this idea, as soil samples treated with halo-endophytic bacteria (which can increase organic matter) showed improvement. Similar results have been found in other studies on managing salty soils (Hammer et al. 2011). Salt-loving and salt-tolerant bacteria (halophilic and halo-tolerant) can build up certain organic compounds inside themselves to cope with salty environments. These compounds, like polyols, amino acids, sugars, and betaines, help them maintain a balance of water and salt within their cells (Brown 1976; Akhtar 2019; Linekha et al. 2024).

The total bacterial count increased in post-treatment samples, especially soil samples with halo-endophytic bacteria, as the total bacterial count had a positive correlation with soil organic carbon (C) and root exudates. The results were in agreement with the study of Barin et al. (2015), Radhakrishnan and Varadharajan (2016) and Shi et al. (2021). This study examined how applying a mixture of specific bacteria (halo-endophytic bacteria) affects the biological health of the soil around the roots of faba bean plants (*V. faba*). The activity of an enzyme called dehydrogenase increased in the soil treated with the bacteria. This enzyme can be considered a robust indicator of overall soil health, given that its activity directly reflects the extent of microbial activity within the soil ecosystem (Kumar et al. 2013; Moussa et al. 2023). Dehydrogenase is found only inside living microbes, so its presence indicates active microbes in the soil, and these microbes play a crucial role in breaking down the organic matter in the soil, which is vital for plant survival and growth (Wolińska and Stepniewska 2012). The

increase in dehydrogenase activity suggests that the bacteria mixture improved the microbial population and the overall health of the soil. (Ujjainiya et al. 2022). This aligns with previous research showing that specific bacteria used in this study (*B. licheniformis*, *P. rettgeri*, and *B. amyloliquefaciens*) can enhance dehydrogenase activity and improve soil properties (Gupta et al. 2021; Jeyasundar et al. 2021; Jiang and Li 2021), respectively.

When plants experience salt stress, microorganisms create special compounds to help them survive. These compounds, like proline and other amino acids, act like tiny sponges, soaking up excess salt and water to balance the pressure inside the microbes' cells. However, making these compounds takes a lot of energy, which the microbes get by breaking down soil organic matter (Idder et al. 2019). For plants, proline is also helpful. It protects them from salt stress by maintaining their water balance and getting rid of harmful molecules called ROS. This helps keep the plant's cell membranes intact and protects its enzymes and proteins (Ashraf and Foolad 2007). This study investigated how faba bean plants (*V. faba*) responded to salt stress when treated with mixtures of halo-endophytic bacteria, compared with untreated plants (control). The results demonstrated that the plants treated with bacteria accumulated more proline and total amino acids in their leaves than the control, and this was thanks to these bacteria as their ability to make nitrogen fixation and make nitrogen available to the plant, which is a substantial part of amino acids, essential for building proteins. This finding aligns with similar studies by Alsenidi et al. (2020) and Yang et al. (2022), suggesting that halo-endophytic bacteria may play a role in helping plants cope with salt stress.

5. Conclusions

This study successfully promoted *Vicia faba* growth under salinity stress using a novel co-inoculation of five halotolerant endophytic bacteria. This unique combination significantly improved growth parameters, proline content (indicating stress tolerance), and soil health compared to controls. These findings demonstrate the potential of co-inoculation with these distinct halo-endophytes as a sustainable and cost-effective bio-approach for *Vicia faba* cultivation in saline environments, particularly in arid and semi-arid regions. To further validate these promising results, future research should focus on: (1) assessing the efficacy of this co-inoculation strategy under diverse field settings, encompassing varying salinity levels and soil types; and (2) elucidating the specific mechanisms by which these halo-endophytes promote plant growth under salinity stress. Understanding these mechanisms will be crucial for

optimizing this bio-approach and facilitating its development into a commercially viable product. The co-inoculation strategy could be developed into a bio-fertilizer product tailored for crops grown in saline-affected regions, enhancing agricultural productivity in challenging environments. Additionally, it could be marketed as part of an integrated crop management system, offering sustainable solutions for farmers facing soil salinization issues. This innovation holds promise for improving food security by enabling the cultivation of salt-sensitive crops in previously unsuitable areas.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Competing interests The authors declare no competing interests.

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