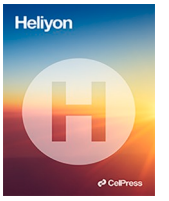
Heliyon 10 (2024) e38653

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Halotolerant phosphate solubilizing bacteria isolated from arid area in Tunisia improve P status and photosynthetic activity of cultivated barley under P shortage

Saber Kouas [a,b,*], Salem Djedidi [c], Imen Ben Slimene Debez [d], Imed Sbissi [e], Nouf M. Alyami [f], Ann M. Hirsch [g]

[a] Laboratory of Plant Physiology and Functional Genomics, Institute of Biotechnology, University of Sfax, BP "1175", 3038, Sfax, Tunisia
[b] Faculty of Sciences of Gabes, University of Gabes, Zrig, 6072, Gabes, Tunisia
[c] Institute of Agriculture, Tokyo University of Agriculture and Technology, Saiwai-cho, Fuchushi, Tokyo, 183–8509, Japan
[d] Laboratory of Bioactive Substances, Center of Biotechnology of Borj Cedria (CBBC), BP 901, Hammam-Lif, 2050, Tunisia
[e] Pastoral Ecosystems and Valorization of Spontaneous Plants and Associated Microorganisms Laboratory, Arid Regions Institute, University of Gabes, 4100, Medenine, Tunisia
[f] Department of Zoology, College of Science, King Saud University, PO Box-2455, Riyadh, 11451, Saudi Arabia
[g] Department of Molecular, Cell, and Developmental Biology and Molecular Biology Institute, University of California, Los Angeles, CA, USA, 90095, USA

ARTICLE INFO

Keywords:
Phosphorus-solubilizing bacteria
PAE
PUE
Auxin
Barley
Photosynthetic activity

ABSTRACT

Forty-seven (47) bacterial strains were isolated from soil of Gabes (an arid region in southern Tunisia) and were screened for their ability to produce Indole-3-Acetic Acid (IAA) and to solubilize phosphate (P). The characterization and molecular identification of the most successful P-solubilizing bacteria (PSB) were then carried out. When grown on suitable artificial media, the most salt-tolerant strains also showed the highest P solubilization capacity (up to 126.8 μg ml^{-1} of released phosphorus after 7 day incubation) and the strongest ability to produce IAA (up to 101.86 μg ml^{-1} after 3 day incubation). Overall, bacterial isolates displayed a different tolerance to varying pH, temperatures, and salinity. The molecular identification revealed that 11 strains belonged to three genera: *Bacillus*, *Pseudomonas* and *Mesorhizobium*. Inoculation of barley with P-solubilizing bacteria under tricalcium phosphate-induced P shortage significantly improved plant growth (biomass, shoot height, and root length) together with increasing total chlorophyll contents and photosynthetic activity. This was concomitant with (i) higher P uptake and translocation and (ii) increased phosphorus absorption and utilization efficiencies (PAE and PUE), which is indicative of a better plant P nutrition under P scarcity. Taken together, we provide strong arguments showing that bacteria native to extreme environments display PSB potential making them promising candidates to mitigate low Pi availability for crop plants.

* Corresponding author. Laboratory of Plant Physiology and Functional Genomics, Institute of Biotechnology, University of Sfax, BP "1175", 3038, Sfax, Tunisia.
E-mail address: kouas_saber@yahoo.fr (S. Kouas).

https://doi.org/10.1016/j.heliyon.2024.e38653
Received 21 May 2024; Received in revised form 20 September 2024; Accepted 26 September 2024
Available online 27 September 2024

1. Introduction

Phosphorus (P) represents about 0.2 % of plant dry weight and it is highly required for growth and development. Given its crucial significance for several metabolic activities such as DNA synthesis, cell division (growth and development), photosynthesis, and nutrient transport [1], P scarcity severely restricts crop yield [2]. Despite total P amount in soils is frequently high (between 400 and 1200 mg kg^{-1} soil), its bioavailability to plants is less than 1 % [3] because P is mainly found as organic form which represents between 20 % and 80 % of soil P pool [4]. Major compounds of organic P include inositol phosphates, nucleic acids and phospholipids [5]. Actually, low Pi bioavailability is a global issue that limits crop production in more than 60 % of the world's arable land [6]. The free inorganic P (Pi) available for plants absorption is limited because of its low mobility and strong adsorption in soils [7]. It is also known that P immobilization in P-insoluble complexes is a phenomenon that strongly depends on soil pH. P deficiency is more critical in highly withered soils of tropics and subtropics, as well as in calcareous/alkaline soils of the Mediterranean basin [8].

In order to enable crops to better cope with P deficiency, affordable and sustainable biological alternatives are needed to accelerate P transformation into bioavailable forms through multiple chemical reactions and biological interactions [3]. This includes using useful microorganisms like plant-growth-promoting rhizobacteria (PGPR) which are eco-friendly and highly efficient to increase agricultural productivity to face the environmental challenges posed by climate change and the concomitant land degradation [9–12]. PGPR application has proven to be an efficient strategy for facilitating plant nutrient uptake, conferring tolerance to abiotic stress, suppressing disease, and thus promoting plant growth [13]. Some PGPR strains such as phosphobacteria, are able to solubilize or mineralize soil unavailable P [14]. Bacteria with PGP traits also increase availability of essential nutrients, such as the macro-elements N, P and K and the microelement iron. Under nutrient shortage conditions, inoculation of plants with certain PGPR strains enhances the uptake of important nutrients and stimulates plant growth. With regard to phosphorus, P-solubilizing bacteria (PSB) are of major interest since they are capable of mineralizing insoluble P, which leads to an enhancement of P uptake by plants. The mechanisms of microbial P solubilization rely principally of the release of protons and organic acids that acidify the soil and also the mineralization of organic P through enzymes such as phytases and phosphatases [15]. PSB belong to different bacterial groups including, *Pseudomonas*, *Bacillus*, *Burkholderia*, *Agrobacterium*, *Rhizobium*, *Bradyrhizobium* are well-known and characterized bacteria that enhance P availability in soil [10,16,17].

Regardless of their lifestyle, bacteria with plant growth promoting (PGP) traits are known to exert their effects on plants through several mechanisms, both direct and indirect [18,19]. The most important direct actions are: (i) providing plants with nutrients through nitrogen fixation, phosphate and potassium solubilization, sequestration of iron via siderophores and (ii) synthesizing plant hormones such as indole acetic acid (IAA), cytokinins, gibberellins and others [20]. Among the auxins, indole-3-acetic acid (IAA) is the most important and well-known plant hormone. Bacteria from many genera have been described as IAA producers, including *Bacillus*, *Azospirillum*, *Rhizobium*, *Azotobacter* [21]. Bacterial IAA has significant effects on root growth and architecture thereby improving plant growth [22]. Additionally, some PGPB produce other phytohormones such as the cytokinins and gibberellins although some of the mechanisms are incompletely understood [22]. In fact, these microbes influence plant growth via numerous indirect mechanisms as well as directly.

There is a need to isolate P-solubilizing bacteria from the rhizosphere of several plants to have diverse applications in crop fields to maintain pH and to enhance phosphorus availability. The aims of this study were to (i) isolate phosphate-solubilizing bacteria from an

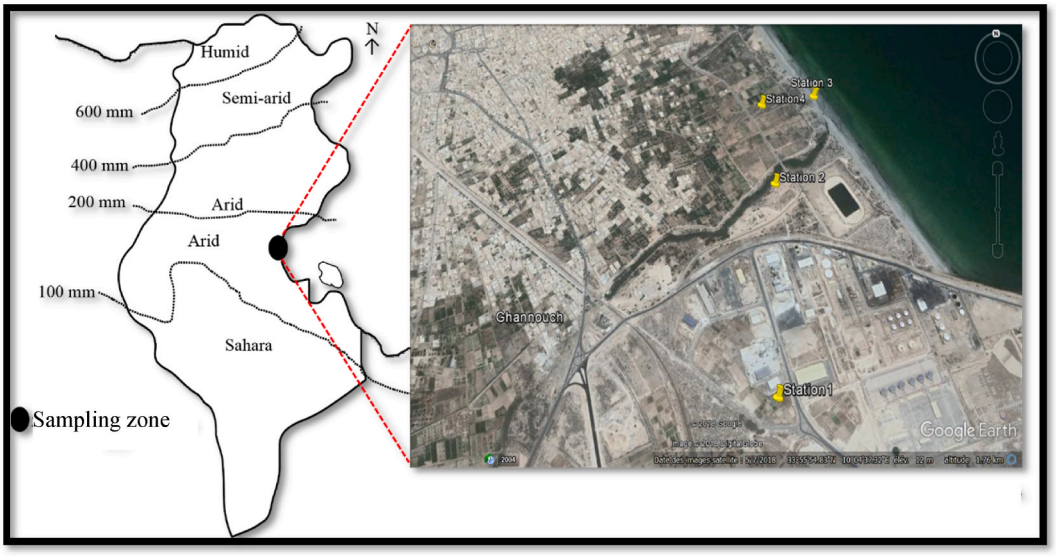


Fig. 1. Geographical localization (GPS coordinates) of the four soil sampling sites (stations) located in the region of Gabes. Station 1 (N33°55′41.63″, E10° 4′44.89″), Station 2 (N33°56′2.34″, E10° 4′47.50″), Station 3 (N33°56′12.05″, E10° 4′48.86″), and Station 4 (N33°56′10.58″, E10° 4′45.31″).

arid soil, (ii) perform physiological characterizations and molecular identifications of the most successful phosphorus solubilizing bacteria (PSB), and (iii) assess the efficacy of microbial inoculation on barley growth compared to the use of tricalcium phosphorus (an insoluble form of P that is inaccessible to plants thus remains in the groundwater).

2. Materials and methods

2.1. Soil sampling

The soils studied (3 replicates each) were sampled from four different stations in the region of Gabes, southern Tunisia (Fig. 1). Station 1 is a cultivated soil in the vicinity of the Tunisian chemical group whereas Station 2 is located on abandoned land, and station 3 is in a coastal area with the goal to isolate salinity-tolerant bacteria. Station 4 is located in a farm parcel close to the sea where *Medicago sativa* is cultivated. The physicochemical properties of the soil for the four stations are provided in Table 1.

2.2. Isolation of phosphate solubilizing bacteria (PSB)

The selective isolation of bacteria to assess their aptitude to solubilize phosphate is generally performed using a selective culture on Pikovskaya medium [23], which only allows the growth of bacteria that solubilize phosphate (referred to as phosphate-solubilizing bacteria, PSB). The isolation of phosphate-solubilizing bacteria from the soils of the four sites considered in this study was carried out using the serial dilution method, during which 10 g of soil from each sample was mixed with 90 ml of sterile distilled water before being vigorously stirred to obtain soil suspensions. Then, 1 ml was removed from each suspension and was added to a tube containing 9 ml sterile distilled water to obtain a dilution of 10^{-1}, after shaking. For each suspension corresponding to a soil sample, a dilution series was prepared until the 10^{-7} dilution was obtained. For the PSB isolation, 0.1 ml of the 10^{-5}, 10^{-6} and 10^{-7} dilutions of each suspension were spread on Petri dishes containing 20 ml of Pikovskaya's agar (PVK) medium containing glucose (10 g), $Ca_3(PO_4)_2$ (5 g), $(NH_4)_2SO_4$ (0.5 g), NaCl (0.2 g), KCl (0.2 g), $MgSO_4{\cdot}7H_2O$ (0.1 g), yeast extract (0.5 g), $MnSO_4$ (0.002 g), $FeSO_4$ (0.002 g), agar (15 g), and distilled water (1000 mL). The pH of this medium was previously adjusted to 7 before its sterilization by autoclaving. The plates were incubated at 28 ± 2 °C in the dark for 10 d. From each plate, one colony representing one morphological type and forming a transparent halo around the bacterial colony (phosphate solubilization zone) was picked and streaked for purity on LB agar plates.

2.3. Evaluation of bacterial isolates PGP activities

2.3.1. Phosphate solubilization

The phosphate solubilization capacity of each bacterial isolate was determined in liquid Pikovskaya medium containing 0.5 % $Ca_3(PO_4)_2$. Erlenmeyer flasks containing 20 ml of the above-mentioned medium were inoculated at an initial concentration of 10^5 CFU for each bacterial isolate and incubated at 28 ± 2 °C with shaking at 140 rpm for 7 days. After incubation, all bacterial cultures were centrifuged at 8000 rpm and 4 °C for 10 min. Soluble phosphorus in the supernatant of each isolate was then estimated by the nitro-vanado molybdate yellow colorimetric method [24]. One mL of supernatant was added to a mixture nitro-vanado molybdate water1 (1:3 vol) and the absorbance of the solution was read at 420 nm. The concentration of solubilized phosphates in the liquid medium was calculated using the equation of KH_2PO_4 obtained from the calibration curve.

2.3.2. Production of indole-3-acetic acid (IAA)

The ability of different bacteria to produce Indole-3-Acetic Acid was assessed in Tryptic Soy Broth (TSB) containing tryptophan (10 mg l^{-1}). Twenty ml of TSB containing tryptophan were inoculated with (0.02 McFarland equivalent of 10^5 CFU mL^{-1}) of bacterial suspension and they were incubated in incubator Shaker at 28 °C and 140 rpm/min for 72 h in dark. The bacterial cultures were centrifuged at 10,000 rpm and 4 °C for 10 min. The estimation of IAA produced was carried out by the colorimetric method [25]. Five hundred µl of the supernatant were then mixed with 500 µl of physiological water and 2 ml of Salkowski reagent (1 ml of 0.5 M $FeCl_3$ and 50 ml of 35 % $HClO_4$). The mixture was then incubated for 1 h at 37 °C in the dark. After 1 h in dark, the color change from pale yellow to pinkish red was the indication of IAA production for pink red color development, the optical density was measured at 530 nm using UV spectrophotometer. The amount of IAA produced was calculated by the standard graph of pure indole acetic acid. The concentration of IAA was determined using an IAA standard curve (0–100 µg ml^{-1} in Tripticase Soy-tryptophan medium). The test was performed in three replicates for each sample."

2.4. Tolerance test of strains to abiotic stresses

2.4.1. Salinity tolerance

This test is based on the culture of bacteria on Petri dishes containing a solid nutrient medium containing different concentrations of NaCl. In the present study, the effect of salinity was tested on a Trypticase Soy Agar medium containing (in g l^{-1}) Caseine peptone, 17; Soya peptone, 3; NaCl, 5; K_2HPO_4, 2.5; Glucose, 2.5 (pH 7.3), supplemented with increasing NaCl concentrations: 0 (control), 1, 2, 3, 4, 5, 7.5, and 10 %. On each dish, 10 µl of a culture of initial cell density of 10^5 CFU mL^{-1} of each strain was inoculated in three replicates and then incubated at 28 °C.

Table 1
Physicochemical properties of the soil sampled from the four chosen stations in the region of Gabes. Geographical localization is indicated in Fig. 1. Means of 9 replicates per each soil followed by the same letter are not significantly different at 5 % according to Fisher's LSD test.

Concentration (mg g^{-1} soil)											
	Na^{+}	N	P	K^{+}	Ca^{2+}	Mg^{++}	Fe^{2+}	Mn^{2+}	Electrical conductivity (ms cm^{-1})	pH	Organic matter (%)
Soil 1	0.16 ± 0.02 a	1.01 ± 0.05a	0.45 ± 0.025 b	0.038 ± 0.001 b	0.35 ± 0.01a	0.31 ± 0.08a	0.026 ± 0.005b	0.03 ± 0.004 b	2.52 ± 0.1b	8.3 ± 0.15bc	0.67 ± 0.019b
Soil 2	0.13 ± 0.018a	1.13 ± 0.08b	0.61 ± 0.03 c	0.049 ± 0.0011c	0.51 ± 0.02b	0.45 ± 0.05b	0.029 ± 0.003b	0.035 ± 0.001b	2.32 ± 0.3a	8.45 ± 0.14c	0.84 ± 0.028c
Soil 3	0.45 ± 0.015 c	0.76 ± 0.04c	0.33 ± 0.021a	0.015 ± 0.0014a	0.31 ± 0.012a	0.26 ± 0.063a	0.013 ± 0.001a	0.02 ± 0.002 a	6.24 ± 0.2d	8.1 ± 0.15a	0.36 ± 0.015a
Soil 4	0.28 ± 0.022 b	1.53 ± 0.6d	0.91 ± 0.051d	0.097 ± 0.0012d	0.78 ± 0.023c	0.67 ± 0.089c	0.062 ± 0.009c	0.05 ± 0.0016c	3.83 ± 0.1c	8.22 ± 0.12b	1.52 ± 0.26d

2.4.2. pH tolerance

Bacteria strains were cultivated on Petri dishes containing Trypticase Soy Agar medium whose pH was adjusted to different values. pH tolerance test was carried out by adjusting the pH of the medium to 5, 7 (control), 8, 9 and 10. On each dish, 10 μL of a culture of initial cell density of 10^5 CFU mL^{-1} of each strain was inoculated in three replicates and then incubated at 28 °C.

2.4.3. Temperature tolerance

The temperature response of selected bacterial isolates (10 μL of a culture with an initial cell density of 10^5 CFU mL^{-1}) was performed by inoculating them into Petri dishes containing Trypticase Soy Agar medium and incubating them at different temperatures: 10 °C; 28 °C (control), 37 °C, 45 °C and 50 °C.

2.5. Phylogenetic characterization of bacterial isolates

Amplification of the 16S rDNA gene was carried out using the universal primers S-D-Bact-0008-a-S-20 (5′- AGAGTTTGATCCTGGCTCAG-3′) and S-D-Bact-1495-a-S-20 (5′- CTACGGCTACCTTGTTACGA-3′) [26]. PCR reactions were carried out in a final volume of 25 μl. Each reaction volume contained 2.5 mM $MgCl_2$, 0.12 mM dNTPs, 0.3 mM of each primer, 1 U of Taq polymerase (Invitrogen) and 50 ng DNA. The amplification parameters were as follows: an initial denaturation of 10 min at 94 °C, 30 cycles of 1 min at 94 °C, 1 min at 55 °C, and 1 min at 72 °C, followed by the final step, an extension of 10 min at 72 °C. To perform their genetic identification, 13 strains were selected for sequencing of their 16S rDNA genes. The amplicons underwent a sequencing reaction using the same primers used for amplification. The sequences obtained were then read and checked using the SeqScanner software. Subsequently, the sequences obtained were compared with "reference" sequences from the GenBank database belonging to the NCBI portal (National Center for Biotechnology Information) to identify the selected strains. The comparison was performed using the "Nucleotide BLAST" tool (BLAST = Basic Local Alignment Search Tool) designated Blast available online in the NCBI portal.

2.6. Inoculation of barley by selected strains

Selected bacterial isolates were plated on Luria Broth (LB) agar plates and incubated at 28 °C in the dark for 24 h. Bacterial cells were then harvested from LB agar plates, transferred to Luria-Bertani liquid medium (LB medium) and cultured at 28 °C with shaking at 200 rpm. After centrifugation at 8000 rpm and 4 °C for 10 min, the obtained pellet of each isolate was washed twice with 50 mM phosphate saline buffer (PBS) and resuspended in 50 mL of sterilized distilled water at a concentration of 0.5 McF equivalent to 10^8 CFU mL^{-1}. Control uninoculated seeds were received 50 mL of sterilized distilled water [27].

Seeds of cultivated barley (Ardaoui variety: widely cultivated in Gabes were first sterilized and were sown in plastic pots (2 Kg) containing sterile sand (after autoclaving at 120 °C for 20 min) and cultivated for two months in a greenhouse under controlled conditions as follows: 16:8 h photoperiod (day:night), 20–25 °C temperature, and 65–75 % relative humidity. pH of the freshly prepared nutrient solution was 6.5. With regard to the final concentration of KOH used for pH 7.5 adjustment, it was 2 mM. For pH 7.5 fixation, Good's buffer 5 mM HEPES (pH 6.8–8.2; pKa = 7.55) was added to the nutrient solution. As for the stabilizer, to prevent ion precipitation, we used iron as 20 μM Fe-EDTA. EDTA (ethylenediaminetetraacetic acid) is known to have a high stability to prevent nutrient precipitation particularly phosphorus. Besides, the nutrient solution was renewed each 72 h.

The experimental units were arranged according to a completely randomized design (CRD). Six seeds of barley were sown in each pot and three seedlings per pot were maintained after germination. The experimental design contains different treatments (9 replicates per treatment: three pots containing three plants each) as follows:

Treatment 1 (T1): soluble phosphorus (KH_2PO_4) as the only source of P (control); Treatment 2 (T2): only soluble phosphorus (KH_2PO_4) and inoculation with one of the 8 strains that were selected based on their significant PGP activities (GS1c, GS2b, GS3b, GS3m, GS3g, GS3j, GS4d, GS4f); Treatment 3 (T3): 0.2 % tricalcium phosphate ($Ca_3(PO_4)_2$) as the only source of P; Treatment 4 (T4): 0.2 % tricalcium phosphate ($Ca_3(PO4)_2$) as the only source of P and inoculation with one of the 8 bacteria strains that were selected based on their significant PGP activities (GS1c, GS2b, GS3b, GS3m, GS3g, GS3j, GS4d, GS4f).

The Hewitt nutritive solution was used for growing plants in sand [28]. In T1 and T2 treatments, the plants were irrigated with the nutrient solution, whereas T3 and T4 plants were irrigated with a modified nutrient solution (without soluble P). For each treatment, irrigation frequency was 100 ml twice a week. Final harvest was performed on two month-old plants, showing a visible second node on stem. The following parameters were determined: shoot height (cm), root length (cm), root and shoot dry weights (respectively Root DW and shoot DW).

2.7. Phosphorus determination

Desiccated samples were ground to a fine powder using porcelain mortar and pestle, then digested in 4/1 (v/v) $HNO_3/HClO_4$. Phosphorus was assayed using the vanado-molybdate method [24].

Besides P content, P absorption efficiency (PAE) and P use efficiency (PUE) were calculated [29]. PAE (μmol P mg^{-1} root DW) reflects the capacity of roots to absorb P from the soil solution, i.e. PAE = Q/RDW, with Q and RDW, respectively, representing the quantity of P (μmol) taken up in each plant whereas the root dry weight (mg).

PUE reflects the dry weight increase per unit of P content (mg DW μmol^{-1} P). The value was calculated as the ratio between biomass production and P accumulation in plants.

2.8. Leaf gas exchanges and total chlorophyll content

Net CO_2 assimilation and stomatal conductance were recorded with a portable photosynthesis system (LCA4). Measurement conditions were as follows: 1200 μmol mm^{-2} s^{-1} photosynthetically active radiation (PAR), 378 μmol mol^{-1} ambient CO_2 concentration and 28 ± 2 °C leaf temperatures. Measurements were carried out in the morning between 10:00 and 12:00 (10 replicates per treatment). Data were automatically collected every minute after the photosynthesis rate had stabilized. For chlorophyll extraction, fresh leaf samples (100 mg) were incubated in 5 ml of 80 % acetone in obscurity at 4 °C for three days. After centrifugation at 5000 rpm for 5 min, the residue was then ground with 20 ml of 80 % acetone, centrifuged for 5min at 5000 rpm and the supernatant was transferred to the same beaker. These steps were repeated until having a colorless extract. The volume was adjusted to 100 ml with 80 % acetone. The absorbance of the extract solutions was read at 645 and 663 nm against the solvent (80 % acetone) blank. The total chlorophyll content was calculated as:

Total chlorophyll (as mg g^{-1} FW) = [20.2 (A645) + 8.02 (A663)] × V ÷ (1000 × W)

Here, A is the absorbance at specific wavelengths, Vis the final volume of chlorophyll extract in 80 % acetone (100 ml), and W is fresh weight (100 mg).

2.9. Statistical analysis

Analysis of variance (ANOVA), using StatisticaTM software was performed to detect differences between treatments. Mean separation procedures were carried out using the multiple range tests with Fisher's least significant difference (LSD) ($P < 0.05$).

3. Results

3.1. Physicochemical properties of the soil samples

The electrical conductivity ranged from 2.52 ms cm^{-1} for soil 1–6.24 ms cm^{-1} for soil 3 (Table 1). The pH values varied from 8.1 (soil 3) to 8.45 (soil 2), indicating that the soils analyzed are alkaline soils. Organic matter was the highest in soil 4 (1.52 %) whereas soil 3 displayed the lowest value (0.36 %).

With respect to mineral elements (Table 1), sodium content varied from 0.13 to 0.45 mg g^{-1} in soils 2 and 3, respectively. The highest value of nitrogen content was found in soil 4 (1.53 mg g^{-1}), very likely in relationship with the presence of *M. sativa* in this station, this legume known for contributing to enriching soil with nitrogen through the symbiotic nitrogen fixation. P content ranged from 0.33 mg g^{-1} in soil 3 to 0.91 mg g^{-1} in soil 4. Potassium concentration was found to vary between 0.015 (soil 3) and 0.097 mg g^{-1} (soil 4). Calcium concentration was the highest in soil 4 (0.78 mg g^{-1}), whereas soil 3 showed the lowest value. This was also true for magnesium (0.26 mg g^{-1} in soil 3 and 0.67 mg g^{-1} in soil 4). Iron content also varied between 0.013 mg g^{-1} and 0.062 mg g^{-1} for soils 3 and 4, respectively. Manganese was detected in the 4 sites considered with contents ranging from 0.02 to 0.05 mg g^{-1}, respectively in soils 1 and 4.

3.2. Isolation and purification of phosphate-solubilizing bacteria (PSB)

By using Pikovskaya medium containing tricalcium phosphate as the only source of P, we succeeded in isolating several bacteria, characterized by their variability in morphology (e.g. color, shape, size, and speed of growth). Forty-seven bacterial isolates showed a common yet variable ability to solubilize tricalcium phosphate, based on the appearance of a clear zone around the bacterial colony after a few days of incubation on Pikovskaya medium (Table 2). The selected colonies were re-streaked repeatedly to obtain pure colonies.

Table 2
Selected phosphorus solubilizing bacteria (BSP) isolated from soil in 4 stations.

	Soil 1	Soil 2	Soil 3	Soil 4
Strains	GS1a	GS2a	GS3a	GS4b
	GS1b	GS2b	GS3b	GS4c
	GS1c	GS2c	GS3c	GS4d
	GS1d	GS2d	GS3d	GS4e
	GS1f	GS2e	GS3e	GS4f
	GS1g	GS2f	GS3f	GS4g
	GS1h	GS2g	GS3g	GS4h
	GS1i	GS2h	GS3i	GS4i
	GS1j	GS2j	GS3J	GS4j
	GS1k	GS2k	GS3k	GS4k
	GS1l	GS2m	GS3l	
	GS1m	GS2n	GS3m	
			GS3n	

3.3. Characterization of bacterial isolates for plant growth-promoting (PGP) traits

3.3.1. Phosphorus quantitative solubilization and indole-3-acetic acid (IAA) production

Variability in P release from tricalcium phosphate was observed among the bacterial isolates (Fig. 2), with values of the levels of P released by the isolates ranging from 13.4 mg l^{-1} for strain GS2g to 126.8 μg ml^{-1} for strain GS4d, sampled from soils 2 and 4, respectively. Calculating the average concentration of released P of the isolates from each soil revealed that bacteria from soils 3 and 4 exhibited the highest P concentration (*ca.* 96 μg ml^{-1}), compared to isolates from soils 1 and 2, which had average values of 60.11 μg ml^{-1} and 44.12 μg ml^{-1}, respectively. Based on these data, 14 isolates native to soils 3 and 4 and representing 30 % of total isolates sampled, led to a concentration of released P higher than 100 μg ml^{-1}.

Indole-3-Acetic Acid (IAA) concentration released by the strains varied between 27.57 μg ml^{-1} for strain GS1a isolated from soil 1 and 101.86 μg ml^{-1} for strain GS3i isolated from soil 3 (Fig. 3). The average concentration of IAA released in each soil showed the influence of soil origin, with the highest mean value isolates from soil 3 (72.35 μg ml^{-1}) whereas isolates from soil 1 displayed the lowest concentration of IAA released (40.73 μg ml^{-1}).

The study of P solubilization and IAA production activities by the selected strains allowed us to classify them into several groups, independent of their soils of origin (Fig. 4); seven distinct groups were delineated. Group 1 and 2 strains showed high P solubilization activity but were low with regard to IAA production. The largest group was group 4 in which some isolates from both soil 3 and soil 4 were grouped together. These isolates demonstrated amounts of high IAA production as well as significant P solubilization levels. Group 5 included strains displaying lowest IAA production and P solubilization capacity. It comprised mostly strains from soils 1 and 2 (7 from 11 strains). Two isolates from soil 3 that were part of group 6 showed moderate P solubilization and IAA production. The last group, 7, contained strains with low IAA production and low P solubilization; these latter strains were collected from soils 1 and 2. Generally, this type of classification is used to detect the most effective or efficient bacterial strains. For our study, the group 3/4 strains were the most interesting. Based on our findings, strains from soils 3 and 4 were considered as strains with potential plant growth promoting traits.

3.3.2. Responses of selected bacterial isolates to some abiotic constraints

3.3.2.1. Responses to salinity. The salt tolerance aptitude of the selected isolates was studied in solid culture medium enriched with NaCl at increasing concentrations: (0, 1, 2, 3, 4, 5, 7.5, and 10 %), The salt tolerance index (STI), measured as the ratio between the bacterial colony diameter when incubated under a given NaCl salinity and the diameter of the colony from control conditions, was calculated. Globally, most of the isolates challenged with salinity (1 %–5 %) showed STI values higher or equal than 1, suggesting an improved or maintained growth under salinity (Table 3). However, the degree of salt tolerance was strain origin-dependent, as 75 % of isolates sampled from soils 3 and 4 had a STI $\geq$1, followed by isolates from soils 1 and 2 (68 % and 50 %, respectively). Interestingly, all strains were able to cope with up to 5 % NaCl salinity, despite a reduction in STI in some cases. At this salinity, the most tolerant strain was GS3m, whereas GS3e was the most sensitive strain (STI was respectively 1.67 and 0.58).

As mentioned in Table 3, the majority of strains showed a severe reduction in their growth at 7.5 % NaCl. At this concentration of NaCl (7.5 %), the selected strains GS3m, GS1c and GS4d were the most tolerant to salinity. Whereas at 10 % salinity, only GS1c, GS1l, GS3a, GS3k, GS3m, GS4d, GS4e and GS4f were able to grow at this concentration. At this high salinity, selected strain GS3m showed a maximum tolerance to 10 % NaCl.

3.3.2.2. Responses to pH variation. For each isolate, the pH tolerance index was calculated on the same basis as for STI. Response to pH variation in the culture medium was found to be strain origin-dependent (Table 4). At slightly acid pH (pH 5), several isolates from soil

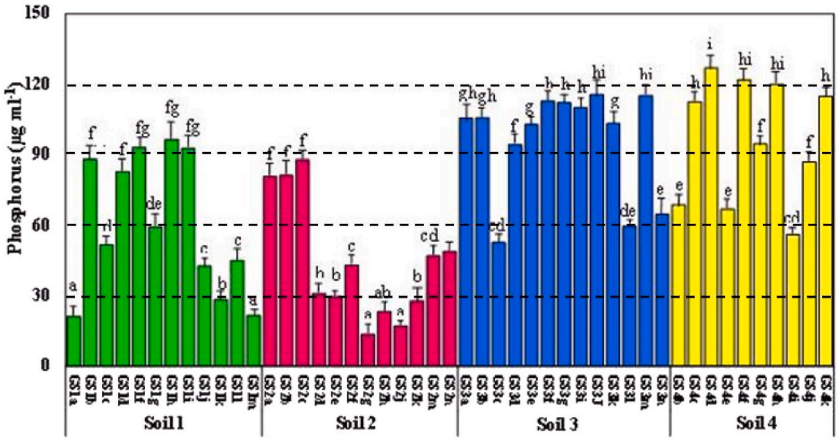


Fig. 2. Differential ability of the strains isolated from soils 1 to 4 in Gabes region to solubilize phosphorus, expressed as P released from tricalcium phosphate (means of 3 replicates per strain). Bars labelled with the same letter are not significantly different at 5 % according to Fisher's LSD test.

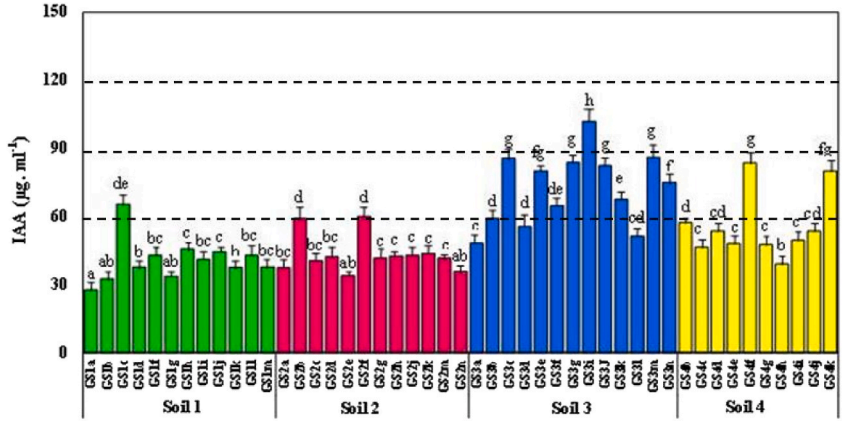


Fig. 3. Variability of IAA production among strains (means of 3 replicates per strain) isolated from soils 1 to 4 in Gabes region. Bars labelled with the same letter are not significantly different at 5 % according to Fisher's LSD test.

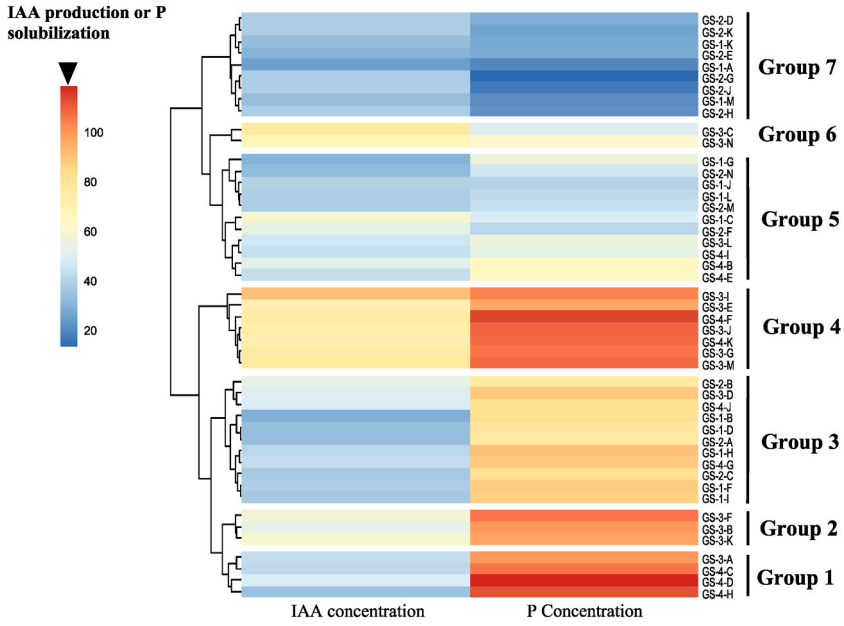


Fig. 4. Clustering of the isolates sampled from the different soils based on their capacity to produce IAA and solubilize P.

1 (especially GS1h and GS1c) showed values of pH tolerance index higher or equal than 1, whereas strains of other isolates appeared to be sensitive. Strain growth under alkaline pH (8–10) was impacted for isolates from soils 3 and 4, whereas isolates from soils 2 and 1 were much less sensitive, especially in the pH range 8–9 where most strains showed either no or improved growth (Table 3). At pH 10 and 5, the GS2b strain from soil 2 showed the highest tolerance index value (1.33). Interestingly, this strain also showed better growth in the pH range 8–9.

3.3.2.3. Responses to temperature. The results of the temperature tolerance tests showed that irrespective of their origin, the tested strains coped with high temperature (37°-45 °C), based pm their respective tolerance index values, which were generally greater than 1 at 45 °C (up to 1.82 in GS2a strain from soil 2) (Table 5). Yet, all the investigated isolates failed to grow when incubated at 50 °C (Table 5). In addition, only a limited number of isolates (2 from soil 1 and 7 from soil 3) grew at an incubation temperature of 10 °C (Table 4).

3.4. Phylogenetic characterization of some selected strains

Post-sequencing of the 16S rDNA genes, 11 strains were selected: GS1a and GS1c from soil 1; GS2b from soil 2; GS3e, GS3b, GS3g, GS3j, GS3k and GS3m from soil 3; and finally, GS4d and GS4f from soil 4. Comparing 16S rDNA sequences of the selected strains with

Table 3
Responses of selected bacterial isolates to increasing salinity in the medium (0–10 %) expressed as salinity tolerance index (means of 3 replicates per treatment and per strain).

	Strains	Salinity tolerance index							
		0 %	1 %	2 %	3 %	4 %	5 %	7.5 %	10 %
Soil 1	**GS1a**	1.00	1.02 ± 0.035	1.00 ± 0.040	1.05 ± 0.047	1.00 ± 0.035	0.80 ± 0.032	0.49 ± 0.035	0.00
	GS1b	1.00	1.00 ± 0.041	1.18 ± 0.035	1.10 ± 0.052	0.91 ± 0.043	0.91 ± 0.041	0.57 ± 0.021	0.00
	GS1c	1.00	0.96 ± 0.05	1.00 ± 0.024	1.15 ± 0.058	1.50 ± 0.053	1.50 ± 0.062	1.02 ± 0.055	0.58 ± 0.035
	GS1d	1.00	1.04 ± 0.048	0.83 ± 0.039	1.05 ± 0.037	0.83 ± 0.041	0.67 ± 0.039	0.43 ± 0.039	0.00
	GS1f	1.00	0.87 ± 0.031	1.00 ± 0.051	1.00 ± 0.042	1.10 ± 0.036	1.00 ± 0.045	0.61 ± 0.041	0.00
	GS1g	1.00	1.06 ± 0.058	1.00 ± 0.027	1.07 ± 0.030	0.90 ± 0.047	1.00 ± 0.053	0.64 ± 0.034	0.00
	GS1h	1.00	0.85 ± 0.047	1.10 ± 0.053	0.95 ± 0.051	1.10 ± 0.052	0.90 ± 0.042	0.48 ± 0.056	0.00
	GS1i	1.00	1.00 ± 0.04	1.10 ± 0.065	0.99 ± 0.029	1.00 ± 0.049	1.10 ± 0.059	0.71 ± 0.048	0.00
	GS1j	1.00	1.00 ± 0.032	1.22 ± 0.073	1.22 ± 0.064	1.11 ± 0.039	1.11 ± 0.048	0.65 ± 0.053	0.00
	GS1k	1.00	1.10 ± 0.066	1.25 ± 0.061	1.17 ± 0.043	1.13 ± 0.062	1.00 ± 0.036	0.58 ± 0.035	0.00
	GS1l	1.00	0.85 ± 0.028	1.43 ± 0.083	1.00 ± 0.037	1.43 ± 0.068	1.14 ± 0.052	0.83 ± 0.049	0.39 ± 0.021
	GS1m	1.00	0.95 ± 0.043	1.25 ± 0.056	1.04 ± 0.025	1.25 ± 0.045	0.88 ± 0.032	0.52 ± 0.037	0.00
Soil 2	**GS2a**	1.00	1.00 ± 0.053	0.91 ± 0.037	0.91 ± 0.038	0.82 ± 0.043	0.80 ± 0.042	0.45 ± 0.041	0.00
	GS2b	1.00	1.10 ± 0.046	1.10 ± 0.043	1.20 ± 0.059	0.90 ± 0.049	0.80 ± 0.052	0.50 ± 0.032	0.00
	GS2c	1.00	1.20 ± 0.03	0.70 ± 0.028	1.00 ± 0.041	1.10 ± 0.055	0.90 ± 0.037	0.56 ± 0.028	0.00
	GS2d	1.00	0.91 ± 0.039	1.00 ± 0.045	1.00 ± 0.035	0.91 ± 0.043	0.73 ± 0.035	0.38 ± 0.024	0.00
	GS2e	1.00	0.91 ± 0.051	1.00 ± 0.048	1.00 ± 0.052	0.73 ± 0.037	0.73 ± 0.046	0.31 ± 0.035	0.00
	GS2f	1.00	1.10 ± 0.026	1.00 ± 0.053	1.00 ± 0.056	0.80 ± 0.042	0.89 ± 0.052	0.44 ± 0.027	0.00
	GS2g	1.00	1.00 ± 0.055	0.90 ± 0.032	1.00 ± 0.033	0.90 ± 0.056	0.80 ± 0.037	0.47 ± 0.037	0.00
	GS2h	1.00	1.05 ± 0.062	0.95 ± 0.041	1.05 ± 0.045	0.95 ± 0.035	0.84 ± 0.027	0.53 ± 0.041	0.00
	GS2j	1.00	1.00 ± 0.048	1.00 ± 0.038	1.00 ± 0.049	1.00 ± 0.053	1.00 ± 0.057	0.62 ± 0.059	0.00
	GS2k	1.00	1.00 ± 0.024	1.00 ± 0.025	1.00 ± 0.039	0.90 ± 0.040	0.80 ± 0.048	0.40 ± 0.031	0.00
	GS2m	1.00	0.82 ± 0.02	0.91 ± 0.052	1.09 ± 0.043	0.82 ± 0.029	0.73 ± 0.031	0.28 ± 0.036	0.00
	GS2n	1.00	1.00 ± 0.037	1.00 ± 0.057	1.00 ± 0.034	0.83 ± 0.051	0.83 ± 0.011	0.47 ± 0.031	0.00
Soil 3	**GS3a**	1.00	1.33 ± 0.072	1.33 ± 0.62	1.33 ± 0.059	1.33 ± 0.063	1.13 ± 0.062	0.85 ± 0.045	0.42 ± 0.037
	GS3b	1.00	1.00 ± 0.043	1.13 ± 0.045	1.25 ± 0.065	1.13 ± 0.040	1.00 ± 0.052	0.78 ± 0.053	0.00
	GS3c	1.00	1.11 ± 0.054	0.89 ± 0.035	1.22 ± 0.053	1.11 ± 0.039	0.89 ± 0.058	0.61 ± 0.041	0.00
	GS3d	1.00	1.00 ± 0.034	1.00 ± 0.047	1.11 ± 0.057	1.11 ± 0.048	1.11 ± 0.067	0.81 ± 0.023	0.00
	GS3e	1.00	0.92 ± 0.047	0.83 ± 0.038	0.75 ± 0.041	0.63 ± 0.035	0.58 ± 0.045	0.22 ± 0.020	0.00
	GS3f	1.00	1.25 ± 0.052	1.25 ± 0.027	1.25 ± 0.069	1.25 ± 0.046	1.00 ± 0.042	0.64 ± 0.035	0.00
	GS3g	1.00	1.18 ± 0.034	1.06 ± 0.053	1.18 ± 0.052	1.18 ± 0.035	0.82 ± 0.035	0.56 ± 0.041	0.00
	GS3i	1.00	1.11 ± 0.04	1.00 ± 0.042	1.11 ± 0.043	1.11 ± 0.041	1.00 ± 0.54	0.70 ± 0.050	0.00
	GS3J	1.00	1.25 ± 0.06	0.88 ± 0.031	1.25 ± 0.054	1.25 ± 0.038	0.88 ± 0.043	0.54 ± 0.046	0.00
	GS3k	1.00	1.00 ± 0.031	1.25 ± 0.037	1.38 ± 0.073	1.38 ± 0.059	1.25 ± 0.059	0.92 ± 0.051	0.57 ± 0.041
	GS3l	1.00	1.00 ± 0.028	0.90 ± 0.06	1.00 ± 0.046	0.80 ± 0.047	0.80 ± 0.034	0.45 ± 0.036	0.00
	GS3m	1.00	1.50 ± 0.063	1.67 ± 0.096	1.50 ± 0.061	2.50 ± 0.081	1.67 ± 0.071	1.23 ± 0.061	0.85 ± 0.059
	GS3n	1.00	1.00 ± 0.057	0.90 ± 0.028	0.80 ± 0.040	0.80 ± 0.035	0.80 ± 0.043	0.55 ± 0.039	0.00
Soil 4	**GS4b**	1.00	1.20 ± 0.038	1.00 ± 0.039	1.00 ± 0.036	0.90 ± 0.043	0.80 ± 0.045	0.53 ± 0.043	0.00
	GS4c	1.00	1.10 ± 0.042	1.10 ± 0.057	1.10 ± 0.043	1.00 ± 0.051	0.90 ± 0.051	0.57 ± 0.033	0.00
	GS4d	1.00	1.13 ± 0.051	1.13 ± 0.044	1.13 ± 0.057	1.25 ± 0.035	1.13 ± 0.061	0.89 ± 0.051	0.51 ± 0.049
	GS4e	1.00	1.00 ± 0.06	1.20 ± 0.03	1.10 ± 0.063	1.20 ± 0.062	1.20 ± 0.052	0.83 ± 0.034	0.43 ± 0.035
	GS4f	1.00	1.13 ± 0.071	1.13 ± 0.046	1.25 ± 0.062	1.00 ± 0.053	1.13 ± 0.068	0.85 ± 0.048	0.46 ± 0.042
	GS4g	1.00	1.00 ± 0.036	0.91 ± 0.025	0.91 ± 0.041	0.82 ± 0.046	0.82 ± 0.046	0.58 ± 0.039	0.00
	GS4h	1.00	1.00 ± 0.032	1.11 ± 0.067	0.89 ± 0.036	1.00 ± 0.051	0.89 ± 0.034	0.55 ± 0.041	0.00
	GS4i	1.00	1.10 ± 0.045	1.00 ± 0.04	1.00 ± 0.045	0.40 ± 0.032	0.90 ± 0.051	0.65 ± 0.051	0.00
	GS4j	1.00	1.10 ± 0.053	1.00 ± 0.036	1.20 ± 0.068	1.10 ± 0.066	1.10 ± 0.056	0.82 ± 0.037	0.00

those present in the GenBank database revealed that these strains were distributed over three bacterial genera (Fig. 5). The majority of selected isolates (GS1a, GS1c, GS2b, GS3e, GS3b, GS3j, GS3k and GS3m) were identified as member of the *Bacillus* genus. One strain (GS3g) and two from soil 4 (GS4d and GS4f) were identified as *Mesorhizobium* and *Pseudomonas* respectively.

Considering the length (in bp) of the sequences obtained and the strain identifications, the identities as well as the percentage of similarity between the various sequences were determined. Most were found to be *Bacillus*. In soils 1, 2 and 3, the following bacterial species could be distinguished: (i) GS1a showed 100 % sequence similarity with *Bacillus thuringiensis* MML5321 (MF687848), (ii) strains GS2b, GS3b and GS3j were 100 % identical to *Bacillus subtilis* V44 (MK229110), (iii) strains GS3k and GS3m exhibited 100 % sequence identity to *Bacillus cereus* EM10 (KJ612536) and *B.cereus* SVP4 (MF471326), respectively and (iv) the remaining two strains (GS1c and GS3e) displayed 100 % sequence similarity with *Bacillus* sp. ADMK50 (KU851006) and *Bacillus* sp. TS10 (MH057231), respectively. Strain GS3g sampled from soil 3 was 100 % identical to *Mesorhizobium* sp. A121 (HQ836191), and strains GS4d and GS4f isolated from soil 4 were 100 % identical to *Pseudomonas brassicacearum* Y21 (MH997640) and *Pseudomonas* sp. SA501 (MK294319), respectively.

Table 4
Responses of selected bacterial isolates to pH (5, 7, 8, 9 and 10) expressed as pH tolerance index (means of 3 replicates per treatment and per strain).

Soil	Strains	pH tolerance index				
		pH 5	pH 7	pH 8	pH 9	pH 10
Soil 1	**GS1a**	1.05 ± 0.06	1.00	1.75 ± 0.096	1.50 ± 0.076	1.08 ± 0.045
	GS1b	0.93 ± 0.045	1.00	1.57 ± 0.075	1.29 ± 0.045	1.14 ± 0.032
	GS1c	1.33 ± 0.075	1.00	1.83 ± 0.086	1.42 ± 0.056	1.17 ± 0.03
	GS1d	0.93 ± 0.081	1.00	1.57 ± 0.08	1.29 ± 0.05	1.00 ± 0.041
	GS1f	0.86 ± 0.035	1.00	1.43 ± 0.076	1.21 ± 0.039	1.00 ± 0.039
	GS1g	0.82 ± 0.039	1.00	1.18 ± 0.068	0.82 ± 0.032	0.94 ± 0.05
	GS1h	1.43 ± 0.089	1.00	1.43 ± 0.08	1.14 ± 0.043	1.00 ± 0.04
	GS1i	1.15 ± 0.071	1.00	1.54 ± 0.049	1.23 ± 0.04	1.08 ± 0.035
	GS1j	1.08 ± 0.053	1.00	1.46 ± 0.059	1.38 ± 0.056	1.15 ± 0.055
	GS1k	1.00 ± 0.048	1.00	1.36 ± 0.037	1.21 ± 0.047	1.14 ± 0.049
	GS1l	0.93 ± 0.061	1.00	1.27 ± 0.041	1.07 ± 0.032	1.00 ± 0.027
	GS1m	0.93 ± 0.052	1.00	1.13 ± 0.031	1.00 ± 0.027	0.93 ± 0.043
Soil 2	**GS2a**	1.18 ± 0.065	1.00	1.64 ± 0.071	1.55 ± 0.063	1.18 ± 0.031
	GS2b	1.17 ± 0.055	1.00	1.50 ± 0.081	1.33 ± 0.054	1.33 ± 0.058
	GS2c	1.00 ± 0.045	1.00	1.36 ± 0.054	1.14 ± 0.046	1.00 ± 0.039
	GS2d	0.81 ± 0.035	1.00	1.13 ± 0.042	1.06 ± 0.049	0.88 ± 0.023
	GS2e	0.63 ± 0.029	1.00	1.00 ± 0.048	1.00 ± 0.036	0.75 ± 0.041
	GS2f	0.67 ± 0.039	1.00	1.06 ± 0.032	0.83 ± 0.04	0.78 ± 0.03
	GS2g	0.75 ± 0.048	1.00	1.13 ± 0.058	1.06 ± 0.034	0.94 ± 0.05
	GS2h	0.88 ± 0.042	1.00	1.06 ± 0.039	1.00 ± 0.039	0.69 ± 0.02
	GS2j	0.93 ± 0.062	1.00	1.13 ± 0.041	1.00 ± 0.047	0.93 ± 0.048
	GS2k	0.88 ± 0.071	1.00	0.94 ± 0.057	1.13 ± 0.034	0.88 ± 0.036
	GS2m	0.75 ± 0.053	1.00	1.06 ± 0.032	1.06 ± 0.025	0.81 ± 0.05
	GS2n	0.88 ± 0.06	1.00	1.00 ± 0.038	1.00 ± 0.03	0.56 ± 0.028
Soil 3	**GS3a**	0.80 ± 0.046	1.00	1.07 ± 0.045	0.80 ± 0.045	0.80 ± 0.04
	GS3b	0.94 ± 0.052	1.00	0.94 ± 0.052	0.76 ± 0.037	0.59 ± 0.034
	GS3c	0.82 ± 0.038	1.00	0.88 ± 0.036	0.82 ± 0.03	0.71 ± 0.045
	GS3d	0.76 ± 0.041	1.00	0.94 ± 0.047	0.71 ± 0.045	0.76 ± 0.037
	GS3e	0.71 ± 0.034	1.00	0.82 ± 0.031	0.82 ± 0.052	0.82 ± 0.05
	GS3f	0.87 ± 0.028	1.00	1.07 ± 0.06	0.73 ± 0.028	0.87 ± 0.028
	GS3g	0.79 ± 0.054	1.00	0.93 ± 0.038	0.86 ± 0.051	0.71 ± 0.042
	GS3i	1.08 ± 0.063	1.00	1.15 ± 0.053	0.77 ± 0.041	0.85 ± 0.02
	GS3J	0.75 ± 0.041	1.00	0.88 ± 0.033	0.69 ± 0.026	0.75 ± 0.039
	GS3k	0.75 ± 0.036	1.00	0.75 ± 0.029	0.63 ± 0.039	0.63 ± 0.027
	GS3l	0.93 ± 0.068	1.00	0.86 ± 0.03	1.00 ± 0.03	0.86 ± 0.049
	GS3m	0.88 ± 0.051	1.00	0.88 ± 0.025	0.94 ± 0.046	0.81 ± 0.052
	GS3n	0.88 ± 0.039	1.00	1.31 ± 0.051	0.88 ± 0.035	0.81 ± 0.040
Soil 4	**GS4b**	1.08 ± 0.053	1.00	1.31 ± 0.06	1.00 ± 0.04	1.00 ± 0.06
	GS4c	1.00 ± 0.042	1.00	1.20 ± 0.048	1.07 ± 0.057	0.80 ± 0.051
	GS4d	0.81 ± 0.03	1.00	0.94 ± 0.027	0.94 ± 0.035	0.69 ± 0.023
	GS4e	0.88 ± 0.039	1.00	1.13 ± 0.035	0.94 ± 0.043	0.75 ± 0.036
	GS4f	0.93 ± 0.051	1.00	1.07 ± 0.04	1.00 ± 0.049	0.86 ± 0.045
	GS4g	1.06 ± 0.059	1.00	1.38 ± 0.052	0.81 ± 0.062	0.88 ± 0.030
	GS4h	0.76 ± 0.043	1.00	0.88 ± 0.036	0.71 ± 0.047	0.71 ± 0.029
	GS4i	0.93 ± 0.059	1.00	1.20 ± 0.068	0.93 ± 0.049	0.87 ± 0.056
	GS4j	0.94 ± 0.063	1.00	1.00 ± 0.03	0.81 ± 0.036	0.69 ± 0.039

3.5. Effect of PSB inoculation on barley grown under phosphorus scarcity

After performing the characterization and molecular identification of selected phosphorus solubilizing bacteria (BSP), the effect of inoculation by these strains (8 BSP) on growth and development of barley plants cultivated for 60 d in the presence of tricalcium phosphorus (an insoluble form of P that is inaccessible to plants) was determined. Plant growth (as root length, shoot height, shoot, and root dry weight), photosynthetic activity (as total chlorophyll content and leaf gas exchange parameters) and P status were measured.

3.5.1. Plant growth

Generally, inoculation with PSB strains under optimal P levels resulted in a slight but significant improvement of shoot biomass (as DW) compared to the control (Fig. 6a). This was especially true for strains GS4d and GS4f. Phosphorus scarcity induced a drastic impairment of shoot DW (−46 %) in non-inoculated plants grown in the presence of tricalcium phosphate (TCP) as compared to plants grown under optimal phosphorus nutrition (Fig. 6a). Inoculated plants (especially the GS1c, GS3m, GS4d and GS4f isolates) exhibited significantly improved shoot DW compared to TCP treatment (up to +63 % in GS4f). This trend was also true for shoot height (Fig. 6b). Root DW and root length showed the same trend under P shortage and after inoculation (Fig. 6c and d), the most efficient isolates being GS1c, GS3m GS4d and GS4f (up to +96 % and +65 %, respectively, for root DW and root length as compared to TCP treatment).

Table 5
Responses of selected bacterial isolates to temperature (10, 28, 37, 45 and 50 °C) expressed as temperature tolerance index (means of 3 replicates per treatment and per strain).

	Stains	Temperature tolerance index				
		10 °C	28 °C	37 °C	45 °C	50 °C
Soil 1	**GS1a**	0.00	1.00	1.42 ± 0.061	1.33 ± 0.062	0.00
	GS1b	0.00	1.00	1.36 ± 0.051	1.14 ± 0.037	0.00
	GS1c	0.00	1.00	1.33 ± 0.045	1.25 ± 0.058	0.00
	GS1d	1.00	1.00	1.14 ± 0.054	1.07 ± 0.041	0.00
	GS1f	0.00	1.00	1.14 ± 0.043	1.21 ± 0.064	0.00
	GS1g	0.00	1.00	1.00 ± 0.051	1.12 ± 0.052	0.00
	GS1h	0.93	1.00	1.21 ± 0.039	1.43 ± 0.077	0.00
	GS1i	0.00	1.00	1.38 ± 0.075	1.23 ± 0.065	0.00
	GS1j	0.00	1.00	1.54 ± 0.059	1.38 ± 0.042	0.00
	GS1k	0.00	1.00	1.29 ± 0.053	1.14 ± 0.040	0.00
	GS1l	0.00	1.00	1.27 ± 0.037	1.27 ± 0.037	0.00
	GS1m	0.00	1.00	1.20 ± 0.058	1.20 ± 0.047	0.00
Soil 2	**GS2a**	0.00	1.00	1.82 ± 0.067	1.82 ± 0.086	0.00
	GS2b	0.00	1.00	1.33 ± 0.053	1.42 ± 0.052	0.00
	GS2c	0.00	1.00	1.29 ± 0.055	1.36 ± 0.057	0.00
	GS2d	0.00	1.00	1.00 ± 0.041	1.19 ± 0.041	0.00
	GS2e	0.00	1.00	1.06 ± 0.047	1.25 ± 0.034	0.00
	GS2f	0.00	1.00	0.94 ± 0.033	1.11 ± 0.039	0.00
	GS2g	0.00	1.00	1.25 ± 0.057	1.38 ± 0.051	0.00
	GS2h	0.00	1.00	1.25 ± 0.048	1.25 ± 0.043	0.00
	GS2j	0.00	1.00	1.33 ± 0.062	1.20 ± 0.054	0.00
	GS2k	0.00	1.00	1.19 ± 0.053	1.19 ± 0.034	0.00
	GS2m	0.00	1.00	1.13 ± 0.038	1.19 ± 0.048	0.00
	GS2n	0.00	1.00	1.19 ± 0.49	1.38 ± 0.062	0.00
Soil 3	**GS3a**	0.00	1.00	1.47 ± 0.073	1.33 ± 0.053	0.00
	GS3b	0.82±	1.00	1.00 ± 0.055	1.18 ± 0.041	0.00
	GS3c	0.82±	1.00	0.94 ± 0.035	1.06 ± 0.051	0.00
	GS3d	0.94±	1.00	1.12 ± 0.041	1.06 ± 0.032	0.00
	GS3e	0.00	1.00	1.06 ± 0.046	0.94 ± 0.039	0.00
	GS3f	0.93±	1.00	1.33 ± 0.059	1.33 ± 0.049	0.00
	GS3g	0.93±	1.00	1.14 ± 0.034	1.43 ± 0.068	0.00
	GS3i	0.92±	1.00	1.15 ± 0.048	1.38 ± 0.054	0.00
	GS3J	0.00	1.00	1.19 ± 0.053	1.25 ± 0.043	0.00
	GS3k	0.81±	1.00	1.00 ± 0.045	0.94 ± 0.037	0.00
	GS3l	0.00	1.00	1.29 ± 0.060	1.36 ± 0.059	0.00
	GS3m	0.00	1.00	1.19 ± 0.037	1.25 ± 0.046	0.00
	GS3n	0.00	1.00	1.19 ± 0.053	1.19 ± 0.058	0.00
Soil 4	**GS4b**	0.00	1.00	1.54 ± 0.057	1.38 ± 0.053	0.00
	GS4c	0.00	1.00	1.20 ± 0.036	1.20 ± 0.059	0.00
	GS4d	0.00	1.00	1.06 ± 0.041	1.25 ± 0.043	0.00
	GS4e	0.00	1.00	1.25 ± 0.039	1.25 ± 0.034	0.00
	GS4f	0.00	1.00	1.21 ± 0.051	1.14 ± 0.053	0.00
	GS4g	0.00	1.00	1.25 ± 0.061	1.19 ± 0.061	0.00
	GS4h	0.00	1.00	1.06 ± 0.049	1.06 ± 0.041	0.00
	GS4i	0.00	1.00	1.33 ± 0.054	1.27 ± 0.053	0.00
	GS4j	0.00	1.00	1.25 ± 0.031	1.19 ± 0.038	0.00

It is noteworthy that morphological observations confirmed the depressive effect of P shortage on shoot growth as compared to the control (Fig. 7a and b), which was significantly offset by the inoculation, notably with GS3m, GS4d and GS4f (Fig. 7c–e) strains.

3.5.2. Photosynthetic activity

A marked reduction in leaf chlorophyll content (−26 %) was observed in plants grown in the presence of tricalcium phosphate (TCP) compared to control plants (Fig. 8a). Inoculation of TCP plants, especially GS3b, GS4d and GS4f inoculants, resulted in significantly increased total chlorophyll content, reaching up to +100 % for GS4d and GS4F. Values of leaf gas exchange parameters (Fig. 8b) and net photosynthetic rate (P_N) were the highest in plants cultivated under optimal phosphorus supply. TCP-induced phosphorus deficiency strongly and significantly decreased this result (−65 % as to C), whereas PSB inoculation of plants led to a significant restoration of P_N compared to TCP. This was especially true for plants inoculated with GS1c, GS3m, GS4d and GS4f strains (2-fold–2.6-fold increase).

PSB inoculation mitigated the impact of P shortage on stomatal conductance (*gs*), shown by a significant increase of this parameter compared to TCP plants (Fig. 8c). To summarize, GS1c, GS3m, GS4d and GS4f were the most efficient strains in these tests. As for internal CO_2 concentration (*Ci*), it was highest in TCP plants, whereas plants with inoculated GS1c, GS3m, GS3b, GS4d and GS4f were

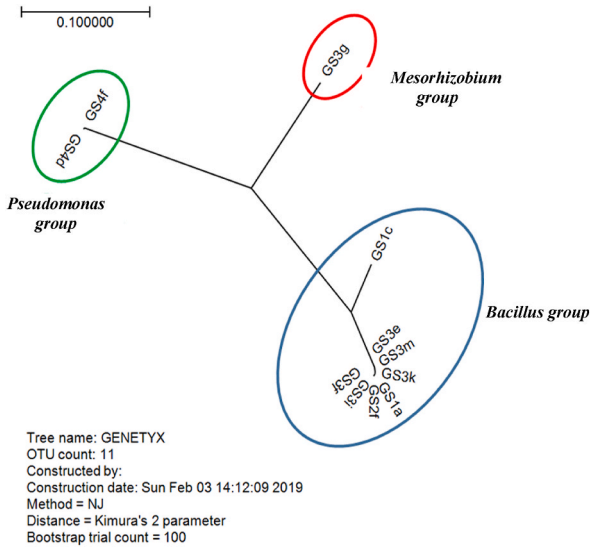


Fig. 5. Phylogenetic tree constructed using GENETYX software based on the sequences obtained from the 16S rDNA of the isolates. The method used is called Neighbors joining (NJ).

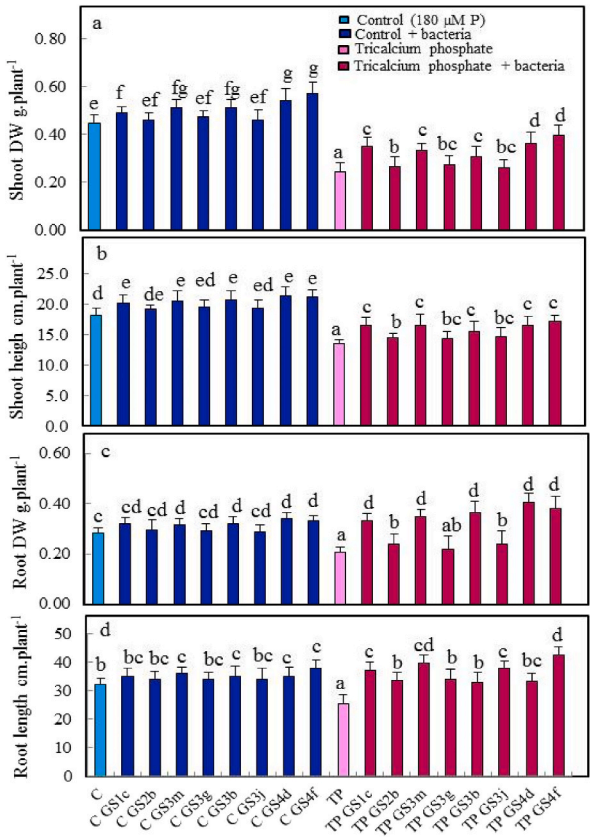


Fig. 6. Effect of the inoculation with the 8 most effective phosphorus solubilizing bacteria (BSP) strains selected from soils 1, 2, 3, and 4 on growth of two month-old barley plants (growth stage: second node of stem visible). Shoot and root biomass production (**a** and **c**, respectively), shoot height **(b),** and root length **(d**). GS1c, GS2b, GS3m, GS3g, GS3b, GS3j, GS4d and GS4f: inoculated plants; C: control *i.e.* non inoculated plants cultivated with KH_2PO_4 as unique source of P; TCP: non inoculated plants cultivated with tricalcium phosphate as unique source of P. Means of 9 replicates ± SD per treatment. Bars labelled with the same letter are not significantly different at 5 % according to Fisher's LSD test.

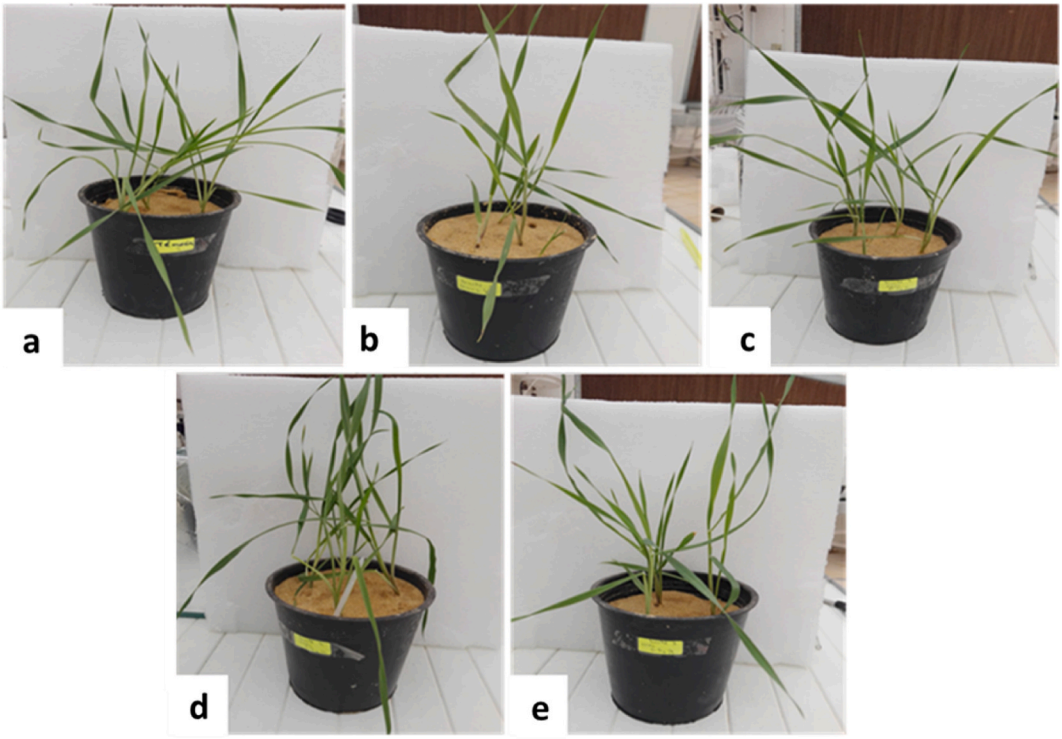


Fig. 7. Vegetative development of barley plants cultivated for two months under optimal P supply (control) or TCP-induced P deficiency. Control non inoculated plants (**a**), P-deficient non inoculated plants (**b**), P-deficient plants inoculated with either GS3m (**c**), GS4f (**d**), or GS4d (**e**).

very close to those recorded in control plants (Fig. 8d).

3.5.3. Plant phosphorus status

Tricalcium phosphate significantly decreased P concentration in both shoots and roots of barley \compared to the control (respectively −53 % and −40 %) (Table 5). PSB inoculation had a slight effect on this parameter under P optimal supply, whereas it significantly alleviated P shortage impact on P concentration in both organs (Table 6). The strongest increases were observed for plants inoculated with GS3m, GS3b, GS4d and GS4f (+55 % to +77 % in shoots and +57 % to +92 % in roots). P quantity in both roots and shoots was drastically reduced (−74 % on average) in plants cultivated solely with phosphate fertilizer (Table 5). The inoculation with PSB of P-deficient plants strongly increased this parameter in both organs (up to 3.8- and 2.8-fold as compared to TCP, respectively, in roots and shoots of inoculated plants).

4. Discussion

4.1. Tolerance of the bacterial strains studied to salinity pH and temperature

The ability of the selected isolates to tolerate salinity was studied in solid culture medium enriched with increasing NaCl salinity. Isolates were able to cope with salinity reaching up to 7.5 % NaCl despite some variability occurred, whereas viability of most strains was suppressed at 10 % NaCl. This diversity of salt tolerance was also documented [30]. When considering the salinity tolerance index, we noticed that several isolates showed an index equal or higher than 1, strongly indicating that their growth was improved or at least maintained despite exposure to high salinity. Several reports showed that bacteria isolated from arid or saline environments are able to survive at high saline concentrations compared to those isolated from non-saline habitats [31]. Habib et al. [32] revealed that bacteria such as *Enterobacter* sp. and *Bacillus cereus* isolated from saline soil selected for their PGPR activities showed salt tolerance traits. Shultana et al. [33] reported that plant growth-promoting rhizobacteria with exopolysaccharides (EPS) producing characters chelate different cations including Na^+. Besides, under salinity stress, bacteria can bind with the Na^+ ion through the secretion of EPS which consequently reduces sodium toxicity in the soil [34]. Therefore, a higher population of EPS producing bacteria in the rhizosphere zone is likely to reduce the concentration of available Na^+ for plants uptake and consequently alleviates salt stress effect on plants under saline environment [33].

Tolerance of the selected isolates to pH variation was studied in solid culture medium. In this test, the following pH values: 5, 7, 8, 9 and 10 were used. pH 7 served as a control treatment. According to Kumar et al. [35], bacteria mostly prefer near neutral pH value (*i.e.* 6.5–7.0) and are sensitive to pH change because this induces modification in bacterial protein and enzyme conformation and may

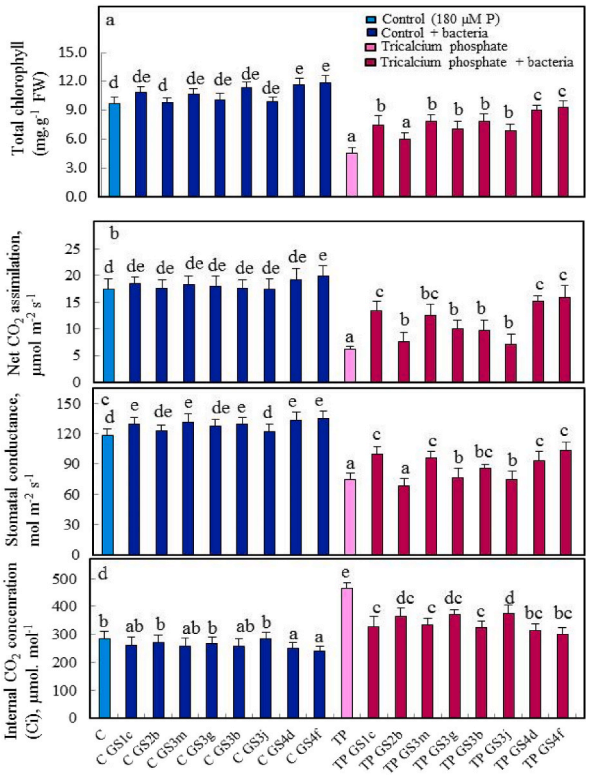


Fig. 8. Effect of the inoculation with the eight most effective phosphorus solubilizing bacteria (BSP) strains selected from soils 1, 2, 3, and 4 on leaf pigment content and photosynthetic gas exchanges of two-month-old barley plants (growth stage: second node of stem visible). Total chl. content (**a**), CO_2 net assimilation rate (**b**), stomatal conductance **(c),** and internal CO_2 concentration **(d)**. GS1c, GS2b, GS3m, GS3g, GS3b, GS3j, GS4d and GS4f: inoculated plants; C: control *i.e.* non inoculated plants cultivated with KH_2PO_4 as unique source of P; TCP: non inoculated plants cultivated with tricalcium phosphate as unique source of P. Means of 9 replicates ± SD per treatment. Bars labelled with the same letter are not significantly different at 5 % according to Fisher's LSD test.

Table 6
Effect of inoculation on phosphorus status in barley plants provided with KH_2PO_4 (control, C) or with tricalcium phosphate (TP). Means of 9 replicates per treatment followed by the same letter are not significantly different at 5 % according to Fisher's LSD test.

Treatment	P Root content (µmol g^{-1} DW)	P Root quantity (µmol $plant^{-1}$)	P Shoot content (µmol g^{-1} DW)	P Shoot quantity (µmol $plant^{-1}$)	PAE (µmol P mg^{-1} root DW)	PUE (mg DW $µmol^{-1}$)
C	135.1 ± 9.1a	38.5 ± 2.3a	190.2 ± 12.4a	84.9 ± 5.2a	0.43 ± 0.031 a	5.83 ± 0.28 a
C GS1C	136.4 ± 8.6a	43.5 ± 2.6b	195.6 ± 13.2a	90.7 ± 5.5b	0.44 ± 0.033 a	5.85 ± 0.25 a
C GS2b	134.5 ± 6.5a	39.7 ± 2.5a	194.3 ± 12.9 a	91.1 ± 4.8b	0.425 ± 0.036 a	5.77 ± 0.3 ab
C GS3m	138.5 ± 7.9ac	43.8 ± 3.1b	199.5 ± 13.56 b	92.7 ± 4.5b	0.445 ± 0.037 a	5.72 ± 0.34 b
C GS3g	135.7 ± 6.2a	39.8 ± 2.7 a	193.2 ± 11.9 a	87.8 ± 5.7 ab	0.443 ± 0.031a	5.79 ± 0.3 ab
C GS3b	137.8 ± 8.1ac	41.3 ± 3 ab	201.4 ± 12.8 b	95.2 ± 6.2bc	0.46 ± 0.035 ab	5.63 ± 0.26 c
C GS3j	135.6 ± 7.3a	38.9 ± 3.3 a	197.5 ± 12.6 b	90.46 ± 6.1b	0.45 ± 0.03 a	5.76 ± 0.29 ab
C GS4d	142.3 ± 12.1 bc	45.5 ± 4 bc	203.5 ± 13.6 b	94.47 ± 6bc	0.456 ± 0.034 ab	5.64 ± 0.35c
C GS4f	145.1 ± 11.8 b	46.2 ± 4.5c	197.5 ± 11.3 b	97.24 ± 5.9 c	0.46 ± 0.038 b	5.60 ± 0.23c
TP	52.4 ± 4.3d	10.7 ± 1.8d	90.3 ± 6.3 c	21.9 ± 2.1d	0.16 ± 0.01 c	6.9 ± 0.37d
TP GS1C	71.2 ± 5.1 ef	18.4 ± 2.4ef	115.8 ± 8.4 d	39.1 ± 3.4f	0.23 ± 0.014 d	8.5 ± 0.43 f
TP GS2b	65.4 ± 4.7f	15.7 ± 2.2 e	110.1 ± 7.9d	28.9 ± 2.5e	0.19 ± 0.012 c	7.5 ± 0.36 e
TP GS3m	85.6 ± 5.5g	23.6 ± 2.5 fg	130.2 ± 7.4 ef	43.1 ± 3.8 f	0.24 ± 0.023 d	8.8 ± 0.46 f
TP GS3g	70.2 ± 6.1 f	15.4 ± 1.9e	125.3 ± 6.5 e	40.28 ± 3.4f	0.25 ± 0.015d	7.8 ± 0.41 ef
TP GS3b	82 ± 6.4 g	25.7 ± 2.7 g	135.6 ± 8.7f	45.29 ± 4.3f	0.23 ± 0 .021d	8.9 ± 0.48 f
TP GS3j	74.2 ± 5.7 f	17.8 ± 1.7 e	120.2 ± 7.3 de	32.3 ± 3.2e	0.2 ± 0.012cd	7.7 ± 0.37ef
TP GS4d	100.2 ± 6.7 h	40.6 ± 3.2 h	160.2 ± 9.9 g	57.6 ± 4.9g	0.27 ± 0.026e	9.6 ± 0.46 g
TP GS4f	97.8 ± 6.3h	42.8 ± 3.7h	158.8 ± 9.4 g	62.4 ± 5.4g	0.3 ± 0.029e	10.2 ± 5.1g

hence lead to into dysfunctional form. Our data showed first that studied isolates are able to tolerate a slightly acidic pH (pH 5). Acidity tolerance by bacteria is correlated with the production of extracellular polysaccharides or polyamines and glutamate concentration in the cell. In this way, Muglia et al. [36] highlighted the role of glutathione (a tripeptide) in the growth of *R. tropici* under conditions

characterized by low pH. Watkin et al. [37] reported the ability of *R. leguminosarum* et *R. trifolii* to tolerate the acidity by accumulating more potassium and phosphorus as compared to acid-sensitive strains. In addition, in our study, all isolates were able to grow over a range of alkaline pH (from 8 to 10). Kumar et al. [35] reported that some isolates grow in a wide range of pH, from slightly acidic to highly alkaline conditions and hence, can be applied in slightly acidic to highly alkaline soils.

The capacity of the selected isolates to tolerate different temperatures was studied in solid culture medium incubated at increasing temperatures (10, 28, 37, 45 and 50 °C). Data showed that none of the isolates was able to grow on a culture medium incubated at 50 °C, but all investigated strains were able to survive at 45 °C. Bacteria tolerance to high temperature is a complex phenomenon because it involves proteins and lipopolysaccharides (LPS). During exposure of *Rhizobium* sp. resistant to heat (30 °C and 43 °C), modifications of the cell surface appeared, including exo polysaccharides (EPS), LPS and proteins [38]. Recently, Ali et al. [39] reported that the *Bacillus* sp. IHBT-705 could survive both under low and high temperatures. Besides heat-shock proteins, this behavior was associated with an over-expression of cspC (Cold shock proteins) under both low and high temperatures. Hence, presence of cspC gene makes *Bacillus* sp. IHBT-705 a valuable microbe from stress tolerance perspective. However, cspB and cspD exhibited a narrow range of functionality as both these genes were up-regulated only at low temperature.

Altogether, the bacterial isolates investigated in the present study showed a variable behavior to variation in pH, temperature, and salinity.

4.2. Impact of PSB on plant growth and phosphate status in cultivated barely grown under phosphorus scarcity

Microbiome of soils in arid ecosystems is diverse and rich in several extremophile microorganisms that could be exploited to improve plant response to environmental stressors, including phosphorus scarcity. To facilitate P uptake by cultivated plants, using soil-borne beneficial microorganisms, such as PSB as eco-friendly agents is gaining interest [11]. In this study, shoot growth (as biomass and height) of cultivated barley (Ardaoui variety), were significantly restricted in plants challenged with tricalcium phosphate (TCP), as compared to plants cultivated under optimal conditions. The TCP induced decrease in plant growth was concomitant with a significant reduction in total chlorophyll contents and gas exchange parameters (net CO_2 assimilation and stomatal conductance), hence confirming the assumption that the reduction in growth could partly result from a depressive effect of P deficiency on photosynthesis [40,41]. Being involved in several cellular processes (energy conservation, metabolic regulation, and signal transduction), P deficiency generally inhibits photosynthesis. This is ascribed to a feedback inhibition resulting from reduced leaf growth [40]. In our study, both P_N and stomatal conductance decreased under low P conditions, along with an increase in *Ci*, suggesting a clear dawn inhibition of photosynthesis, due to the lack of P carriers [41]. P deficiency might lower the stomatal opening, resulting in reduced recycling of ATP and NADPH, thus inhibiting plant photosynthetic capacity and growth [42].

Inoculation with PSB significantly improved plant growth grown despite the presence of tricalcium phosphate (TCP). This growth stimulation was mainly observed in plants inoculated with GS3m (*Bacillus cereus* EM10, KJ612536), GS4d (*Pseudomonas brassicacearum* Y21, MH997640) and GS4f (*Pseudomonas* sp SA501, MK294319) strains. Shoot height and root length were also enhanced with inoculation by the above mentioned strains. Some PSBs improve root length and growth due to the secretion of growth-stimulating phytohormones such as auxin, a major direct mechanism explaining PGPR action. In our study, the selection of the efficient strains was carried out according to their tolerance to environmental factors (e.g. salinity, temperature, and pH), along with their ability to solubilize P and to produce IAA. For the latter, the highest value was found in *Bacillus cereus* strain, followed by *Pseudomonas* sp. In order to assess the involvement of the bacteria auxin production in the root growth, the relationship between root length of plants challenged with tricalcium phosphate and bacteria IAA production was investigated (Fig. 9). The results showed a strong and significant correlation ($R = 0.83$) between root length and auxin production, which suggests a strong positive relationship between these two parameters.

Hence, inoculation with high IAA producing bacteria (e.g. GS1c, GS4f and Gs3m) was associated with the highest root length under P shortage. It has been documented that the elongation of plant root system for water and nutrient uptake needed for plant growth was enhanced by the production of auxin by plant growth-promoting rhizobacteria [43]. Under TCP treatment, bacterial strains GS1c (*Bacillus* sp ADMK50, KU851006), GS3m (*Bacillus cereus* EM10, KJ612536), GS4d (*Pseudomonas brassicacearum* Y21, MH997640) and GS4f (*Pseudomonas* sp SA501, MK294319) significantly stimulated root development of barley, further suggesting that PSB play a critical role in improving Pi acquisition under Pi-deficient conditions. It has been observed that many plant species develop long and abundant roots in response to Pi starvation [44].

Data obtained also indicated that bacterial strains GS1c (*Bacillus* sp ADMK50, KU851006), GS3m (*Bacillus cereus* EM10, KJ612536), GS4d (*Pseudomonas brassicacearum* Y21, MH997640) and GS4f (*Pseudomonas* sp SA501, MK294319) are high auxin producers. IAA synthesis by rhizobacteria contributes to promote plant growth under nutrient deficiency and is seen as a desirable PGP trait notably in programs aiming at selecting IAA-producing strains [45]. However, inconsistent efficiency and lack of reproducibility between controlled and natural conditions were documented [46]. Indeed, IAA bacterial production, endogenous auxin signaling in plants, and the soil auxin like effect, their interactions are influenced by genetic and environmental factors, which affect the efficacy of biofertilizers and biostimulants. Therefore, these authors proposed to consider IAA production in the plant-soil-bacteria framework. According to Pasternak et al. [47], auxin induced cell and tissue division and differentiation, and accelerates the production of xylem vessels and roots. During the process of lateral root formation, the activated endoderm/pericycle cells initiate several cell divisions, leading to the development of root primordia and ensuing differentiation into new lateral roots [48].

Some PSB also improved P status in barley under tricalcium phosphate (TCP), as evidenced by the strong increase of P content (as μmol g^{-1}DW) and quantity (as μmol $plant^{-1}$) in shoots and roots in plants inoculated by GS3m (*Bacillus cereus* EM10, KJ612536), GS4d (*Pseudomonas brassicacearum* Y21, MH997640) and GS4f (*Pseudomonas* sp SA501, MK294319). This finding is in agreement with recent

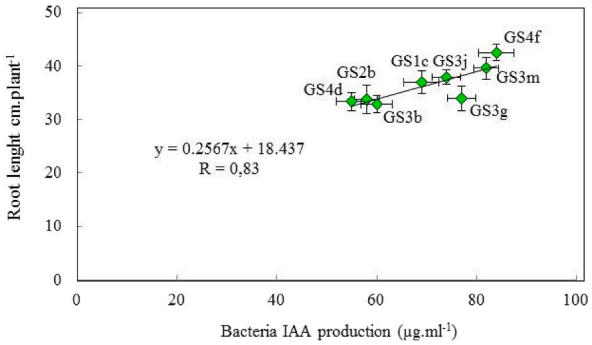


Fig. 9. Relationship between IAA production (as µg ml^{-1}) of the eight most effective phosphorus solubilizing bacteria (BSP) strains selected from soils 1, 2, 3, and 4 and root length of barley plants inoculated by eight BSP cultivated with Tricalcium phosphate as sole P source.

studies showing better P status in PSB inoculated plants under low P availability [15]. Furthermore, this result confirms that root length stimulation present an important mechanism in plant Pi acquisition under P scarcity [44]. To further ascertain the involvement of PSB in facilitating P nutrition in barley, the relationship between P content in roots and shoots and Bacteria P solubilization was assessed under P deficiency conditions (Fig. 10). Results showed a strong positive correlation ($R > 0.8$) between the two parameters in both roots (Fig. 10a) and shoots (Fig. 10b), indicating that the higher P contents in organs of barley exposed to P shortage were reached after inoculation with bacteria displaying the higher capacity for P solubilization.

The mechanisms involved in P solubilization and used by soil microorganisms include: (i) the release of metabolites for mineral dissolution of soil particles, e.g. organic acids, siderophores, protons, hydroxyl ions, and CO_2, (ii) and of extracellular enzymes, such as acid phosphatases and phytases that are generally responsible for biochemical mineralization of P and (iii) also the release of P following the degradation of an insoluble P-rich substrate [49,50]. In this work, bacterial strains including GS3m (*Bacillus cereus* EM10, KJ612536), GS4d (*Pseudomonas brassicacearum* Y21, MH997640) and GS4f (*Pseudomonas* sp SA501, MK294319) showed to be able to change insoluble form of phosphate and increase the availability of this nutrient.

In response to phosphorus deficiency barley plants adopted several mechanisms, phosphorus acquisition efficiency (PAE) is a vital adaptive trait for plants growing in low P conditions, since increased PAE might reflect a better capacity of the root system to explore larger volumes of the rhizosphere and hence improve plant capacity to take up available P. Data inferred from the present study

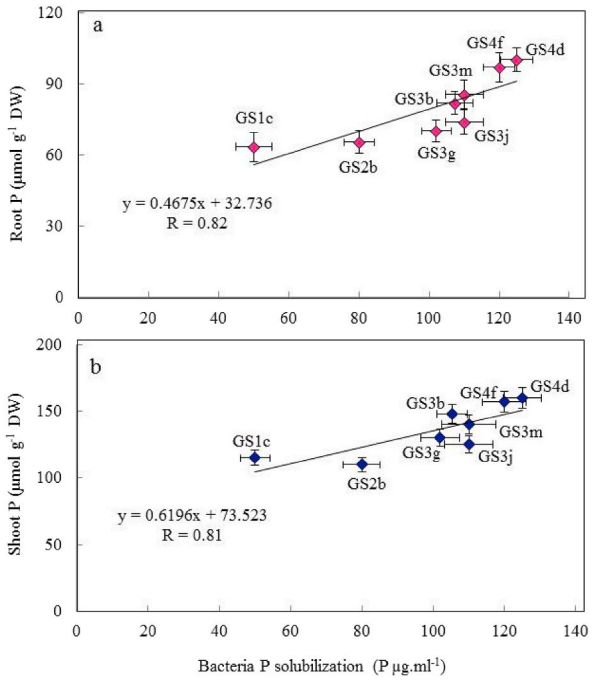


Fig. 10. Relationship between P solubilization capacity (as P mg l^{-1}) of the eight most effective phosphorus solubilizing bacteria (BSP) strains selected from soils 1, 2, 3, and 4 and P content (as µ mol g^{-1} DW) in roots (**a**) and shoots (**b**) of barley plants inoculated by eight BSP cultivated with Tricalcium phosphate as sole P source.

(Table 6) revealed that under P scarcity, inoculation with PSB (mainly GS3m (*Bacillus cereus* EM10, KJ612536), GS4d (*Pseudomonas brassicacearum* Y21, MH997640) and GS4f (*Pseudomonas* sp SA501, MK294319) increased PAE by up to two-fold compared to the un-inoculated plants (TP). This supports our hypothesis that *Bacillus cereus* EM10 (KJ612536), *Pseudomonas brassicacearum* Y21 (MH997640) and *Pseudomonas* sp SA501 (MK294319) stimulated root development under Pi-deficient conditions and further highlight their significance in enhancing Pi acquisition in barley. Plants also adapt to low Pi by optimizing the internal use of Pi and Pi-derived metabolites to maintain growth, *i.e.* increase the ratio of biomass or yield generated per molecule of acquired Pi (Pi use efficiency, PUE). Our findings on PUE (Table 6) highlight that barley plants inoculated with PSB, notably GS3m (*Bacillus cereus* EM10, KJ612536), GS4d (*Pseudomonas brassicacearum* Y21, MH997640) and GS4f (*Pseudomonas* sp SA501, MK294319) under tricalcium phosphate showed significantly higher PUE values and hence better growth, as compared to TCP plants. Finally, the efficient bacterial strains (GS3m (*Bacillus cereus* EM10, KJ612536), GS4d (*Pseudomonas brassicacearum* Y21, MH997640) and GS4f (*Pseudomonas* sp SA501, MK294319) improved phosphate nutrition; this nutrient plays crucial role in the maintain of higher photosynthetic activity and plant growth of cultivated barley grown under tricalcium phosphate.

5. Conclusion

As conclusion, it could be inferred from the present study that inoculation with PSB strains isolated from arid areas (generally seen as marginal zones) in Tunisia can be considered as a promising environmentally friendly technology to increase the availability of Pi from organic phosphorus form (tricalcium phosphate), resulting in an increase of barley growth under P limiting conditions. In this work, the selected phosphorus solubilizing bacteria are characterized as high salinity tolerant, high IAA producers and high phosphate solubilization ability as well as able to grow over a range of alkaline pH (from 8 to 10). Therefore, a more attention could be needed to investigate the ability of selected bacterial strains to solubilize and mobilize inorganic phosphorus in calcareous soils to enhance crop production of barley under natural condition.

Data availability statement

Data are available upon request to the corresponding author.

Competing interests

The authors declare no competing interests.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

CRediT authorship contribution statement

Saber Kouas: Writing – original draft, Validation, Data curation, Conceptualization. **Salem Djedidi:** Validation, Methodology, Data curation. **Imen Ben Slimene Debez:** Formal analysis. **Imed Sbissi:** Methodology. **Nouf M. Alyami:** Writing – review & editing, Funding acquisition. **Ann M. Hirsch:** Writing – review & editing, Validation, Supervision.

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

Authors gratefully acknowledge the Research Support Program (Project number RSP2024R177) from King Saud University, Riyadh, Saudi Arabia.

References

[1] Q. Li, L. Fu, Y. Wang, D. Zhou, B.E. Rittmann, Excessive phosphorus caused inhibition and cell damage during heterotrophic growth of *Chlorella regularis*, Bioresour, Technol. 268 (2018) 266–270, https://doi.org/10.1016/j.biortech.2018.07.148.
[2] L. Bononi, J.B. Chiaramonte, C.C. Pansa, M.A. Moitinho, I.S. Melo, Phosphorus-solubilizing Trichoderma spp. from Amazon soils improve soybean plant growth, Sci. Rep. 10 (2020) 2858, https://doi.org/10.1038/s41598-020-59793-8.
[3] J. Zhu, M. Li, M. Whelan, Phosphorus activators contribute to legacy phosphorus availability in agricultural soils: a review,, Sci. Total Environ. 612 (2018) 522–537, https://doi.org/10.1016/j.scitotenv.2017.08.095.
[4] R.C. Dalal, Long-term phosphorus trends in Vertisols under continuous cereal cropping, Soil Res. 35 (1997) 327–340, https://doi.org/10.1071/S96052.
[5] T.I. McLaren, R.J. Smernik, M.J. McLaughlin, A.L. Doolette, A.E. Richardson, E. Frossard, The chemical nature of soil organic phosphorus: a critical review and global compilation of quantitative data, Adv, Agron 160 (2020) 51–124, https://doi.org/10.1016/bs.agron.2019.10.001.
[6] D. Liu, Root developmental responses to phosphorus nutrition, J. Integr. Plant Biol. 63 (2021) 1065–1090, https://doi.org/10.1111/jipb.13090.

[7] J. Paz-Ares, M.I. Puga, M. Rojas-Triana, I. Martinez-Hevia, S. Diaz, C. Poza-Carríon, et al., Plant adaptation to low phosphorus avail-ability: core signaling, crosstalks and applied implications, Mol. Plant 15 (2022) 104–124, https://doi.org/10.1016/j.molp.2021.12.005.

[8] P. Hinsinger, C. Plassard, C. Tang, B. Jaillard, Origins of rootmediated pH changes in the rhizosphere and their environment constraints – a review, Plant Soil 248 (2003) 43–59, https://doi.org/10.1023/A:1022371130939.

[9] L.N. Castagno, A.I. Sannazzaro, M.E. Gonzalez, F.L. Pieckenstain, M.J. Estrella, Phosphobacteria as key actors to overcome phosphorus deficiency in plants, Ann. Appl. Biol. 178 (2021) 256–267, https://doi.org/10.1111/aab.12673.

[10] N.S. Raymond, B, Gacute;omez-Muñoz, F.J. van der Bom, O, L.S. Nybroe, D.S. Jensen, Müller-Stöver, et al., Phosphate-solubilising microorganisms for improved crop productivity: a critical assessment, New Phytol. 229 (2021) 1268–1277, https://doi.org/10.1111/nph.16924.

[11] J. Tian, F. Ge, D. Zhang, S. Deng, X. Liu, Roles of phosphate solubilizing microorganisms from managing soil phosphorus deficiency to mediating biogeochemical P cycle, Biology 10 (2021) 158, https://doi.org/10.3390/biology10020158.

[12] M. Pantoja-Guerra, M. Burkett-Cadena, J. Cadena, C.A. Dunlap, C.A. Ramírez, Lysinibacillus spp.: an IAA-producing endospore forming-bacteria that promotes plant growth, Antonie vn Leeuwenhoek (2023) 1–16.

[13] A. Chauhan, R. Saini, J.C. Sharma, Plant growth promoting rhizobacteria and their biological properties for soil enrichment and growth promotion, J. Plant Nutr. 45 (2021) 273–299, https://doi.org/10.1080/01904167.2021.1952221.

[14] P.J. Barra, S. Viscardi, M.A. Jorquera, P.A. Duran, A.J. Valentine, M. De la Luz Mora, Understanding the strategies to overcome phosphorus-deficiency and aluminum-toxicity by ryegrass endophytic and rhizosphere phosphobacteria, Front. Microbiol. 9 (2018) 1155, https://doi.org/10.3389/fmicb.2018.01155.

[15] Q. Zeng, X. Ding, J. Wang, X. Han, H. Iqbal, M. Bilal, Insight into soil nitrogen and phosphorus availability and agricultural sustainability by plant growth-promoting rhizobacteria, Environ. Sci. Pollut. Res. 29 (2022) 1–18, https://doi.org/10.1007/s11356-022-20399-4.

[16] E.T. Alori, B.R. Glick, O.O. Babalola, Microbial phosphorus solubilization and its potential for use in sustainable agriculture, Front. Microbiol. 8 (2017) 971, https://doi.org/10.3389/fmicb.2017.00971.

[17] G. Kalayu, Phosphate solubilizing microbes: promising approach as biofertilizers, Int. J. Agron 1 (2019) 1–7, https://doi.org/10.1155/2019/4917256.

[18] S. Kouas, N. Khan, A.M. Hirsch, The hologenome hypothesis and its application to plant-microbe interactions on an evolutionary scale, Advances in Environmental Microbiology, Microbes, The Foundation Stone of the Biosphere (2021) 425–440, https://doi.org/10.1007/978-3-030-63512-1_21.

[19] J.A.M. Moore, P.E. Abraham, J.K. Michener, W. Muchero, M.A. Cregger, Ecosystem consequences of introducing plant growth promoting rhizobacteria to managed systems and potential legacy effects, New Phytol. 234 (2022) 1914–1918, https://doi.org/10.1111/nph.18010.

[20] R.M. Makki, Sustainable farming using plant growth promoting, Appl. Ecol. Environ. Sci. 2 (2023) 2363–2382, https://doi.org/10.15666/aeer/2103_23632382.

[21] C.M.H. Ferreira, H.M.V.M. Soares, E.V. Soares, Promising bacterial genera for agricultural practices: an insight on plant growth-promoting properties and microbial safety aspects, Sci. Total Environ. 682 (2019) 779–799, https://doi.org/10.1016/j.scitotenv.2019.04.225.

[22] R. Backer, J.S. Rokem, G. Ilangumaran, J. Lamont, D. Praslickova, E. Ricci, et al., Plant growth-promoting rhizobacteria: context, mechanisms of action, and roadmap to commercialization of biostimulants for sustainable agriculture, front, Plant Sci. (2018), https://doi.org/10.3389/fpls.2018.01473.

[23] C.S. Nautiyal, An efficient microbiological growth medium for screening phosphate solubilizing microorganisms, FEMS, Microbiol. Lett. 170 (1999) 265–270, https://doi.org/10.1111/j.1574-6968.1999.tb13383.x.

[24] S. Kouas, A. Debez, T. Slatni, N. Labidi, J.J. Drevon, C. Abdelly, Root proliferation, proton efflux, and acid phosphatase activity in common bean (*Phaseolus vulgaris*) under phosphorus shortage, J. Plant Biol. 52 (2009) 395–402, https://doi.org/10.1007/s12374-009-9050-x.

[25] J.M. Brick, R.M. Bostock, S.E. Silverstone, Rapid in situ assay for indole acetic acid production by bacteria immobilized on nitrocellulose membrane, Appl. Environ. Microbiol. 57 (1991) 535–538, https://doi.org/10.1128/aem.57.2.535-538.1991.

[26] L.J. Harrell, G.L. Andersen, K.H. Wilson, Genetic variability of *Bacillus anthracis* and related species, J. Clin. Microbiol. 33 (1995) 1847–1850, https://doi.org/10.1128/jcm.33.7.1847-1850.1995.

[27] R.M. Mortensen, D.A. Conner, S. Chao, A. Geisterfer-Lowrance, J. Seidman, Production of homozygous mutant ES cells with a single targeting construct, Mol. Cell Biol. 12 (1992) 2391–2395, https://doi.org/10.1128/mcb.12.5.2391-2395.1992.

[28] E.J. Hewitt, Sand, Water Culture, Methods used in the study of plant nutrition, 2nd eds, Commonwealth Bureau of Horticulture Tech (1966).

[29] O.Z. Talbi, Z. Barhoumi, S. Kouas, M. Ghandour, I. Slama, C. Abdelly, Insights into the physiological responses of the facultative halophyte *Aeluropus littoralis* to the combined effects of salinity and phosphorus availability, J. Plant Physiol. 189 (2015) 1–10, https://doi.org/10.1016/j.jplph.2015.08.007.

[30] C. Zhang, R.J. Simpson, C.M. Kim, N. Warthmann, E. Delhaize, L. Dolan, et al., Do longer root hairs improve phosphorus uptake? Testing the hypothesis with transgenic *Brachypodium distachyon* lines overex-pressing endogenous RSL genes, New Phytol. 217 (2018) 1654–1666, https://doi.org/10.1139/cjm-2017-0571.

[31] R. Hayat, R. Khalid, M. Ehsan, I. Ahmed, A. Yokota, S. Ali, Molecular characterization of soil bacteria for improving crop yield in Pakistan, Pakistan J. Bot. 45 (2013) 1045–1055. https://www.researchgate.net/publication/236980604.

[32] S.H. Habib, H. Kausar, H.M. Saud, Plant growth-promoting rhizobacteria enhance salinity stress tolerance in Okra through ROS-scavenging enzymes, Biomed, Res. Intell. (2016), https://doi.org/10.1155/2016/6284547.

[33] R. Shultana, A.T.K. Zuan, M.R. Yusop, H.M. Saud, Characterization of salt-tolerant plant growth-promoting rhizobacteria and the effect on growth and yield of saline-affected rice, PLoS One 1–16 (2020), https://doi.org/10.1371/journal.pone.0238537.

[34] N.K. Arora, T. Fatima, J. Mishra, I. Mishra, S. Verma, R. Verma, et al., Halo-tolerant plant growth promoting rhizobacteria for improving productivity and remediation of saline soils, J. Adv. Res. 26 (2020) 69–82, https://doi.org/10.1016/j.jare.2020.07.003.

[35] A. Kumar, M. Kumari, P. Swarupa, M. Shireen, Characterization of pH dependent growth response of agriculturally important microbes for development of plant growth promoting bacterial consortium, J. Pure Appl. Microbiol. 13 (2019) 1053–1061, https://doi.org/10.22207/JPAM.13.2.43.

[36] C.I. Muglia, D.H. Grasso, O.M. Aguilar, *Rhizobium tropici* response to acidity involves activation of glutathione synthesis, Microbiol. 153 (2007) 1286–1296, https://doi.org/10.1099/mic.0.2006/003483-0.

[37] E.L.J. Watkin, G.W. O'Hara, A.R. Glenn, Physiological responses to acid stress of an acid-soil tolerant and an acid-soil sensitive strain of *Rhizobium leguminosarum* biovar trifolii, Soil Biol. Biochem. 35 (2003) 621–624, https://doi.org/10.1016/S0038-0717(03)00012-9.

[38] K. Nandal, A.R. Sehrawat, A.S. Yadav, R.K. Vashishat, K.S. Boora, High temperature-induced changes in exo-polysaccharides, lipopolysaccharides and protein profile of heat-resistant mutants of *Rhizobium* sp. (*Cajanus*), Microbiol. Res. 160 (2005) 367–373, https://doi.org/10.1016/j.micres.2005.02.011.

[39] N. Ali, M.K. Swarnkar, R. Veer, P. Kausha, A.M. Pati, Temperature-induced modulation of stress-tolerant PGP genes bioprospected from *Bacillus sp*, IHBT-705 associated with saffron (Crocus sativus) rhizosphere: A natural–treasure trove of microbial biostimulants, Front. Plant Sci (2023), https://doi.org/10.3389/fpls.2023.1141538.

[40] X. Meng, W.W. Chen, Y.Y. Wang, Z.R. Huang, X. Ye, L.S. Chen, L.T. Yang, Effects of phosphorus deficiency on the absorption of mineral nutrients, photosynthetic system performance and antioxidant metabolism in *Citrus grandis*, PLoS One 16 (2) (2021), https://doi.org/10.1371/journal.pone.0246944, 0246944.

[41] M. Kayoumu, A. Iqbal, N. Muhammad, X. Li, L. Li, X. Wang, et al., Phosphorus availability affects the photosynthesis and antioxidant system of contrasting low-P-tolerant cotton genotypes, Antioxidants 12(2) (2023) p 466, https://doi.org/10.3390/antiox12020466.

[42] D. Neocleous, D. Savvas, The effects of phosphorus supply limitation on photosynthesis, biomass production, nutritional quality, and mineral nutrition in lettuce grown in a recirculating nutrient solution,, Sci. Horticult 252 (2019) 379–387, https://doi.org/10.1016/j.scienta.2019.04.007.

[43] D. Egamberdieva, J. Stephan, S.J. Wirth, A. Abdulaziz, A.A. Alqarawi, E.F. AbdAllah, et al., Phytohormones and Beneficial Microbes: Essential Components for Plants to Balance Stress and Fitness, Front. Microbiol, 2017, https://doi.org/10.3389/fmicb.2017.02104, 8.

[44] S. Ying, E.B. Blancaflor, F. Liao, W.R. Scheible, A phosphorus-limitation induced, functionally conserved DUF506 protein is a repressor of root hair elongation in plants,, New Phytol. 233 (2022) 1153–1171, 10.1111/nph.17862.

[45] S. Park, A.L. Kim, Y.K. Hong, J.H. Shin, S.H. Joo, A highly efficient auxin producing bacterial strain and its effect on plant growth,, J. Genet. Eng. Biotechnol. 179 (2021) 1–9, https://doi.org/10.1186/s43141-021-00252-w.

[46] M. Pantoja-Guerra, N. Valero-Valero, C.A. Ramírez, Total auxin level in the soil–plant systemas a modulating factor for the effectivenessof PGPR inocula: a review, Chem. Biol. Technol, Agric. For. 10 (6) (2023), https://doi.org/10.1186/s40538-022-00370-8.

[47] T. Pasternak, K. Palme, J.M. P′erez-P′erez, Role of reactive oxygen species in the modulation of auxin flux and root development in *Arabidopsis thaliana*, Plant J. 114 (2023) 83–95, 10.1111/tpj.16118.
[48] H. Singh, Z. Singh, R. Kashyap, S.R. Yadav, Lateral root branching: evolutionary innovations and mechanistic divergence in land plants, New Phytol. 238 (4) (2023) 1379–1385, https://doi.org/10.1111/nph.18864.
[49] U.P. Dhuldhaj, N. Malik, Global perspective of phosphate solubilizing microbes and phosphatase for improvement of soil, food and human health, Cell. Mol. Biomed, Repura 2 (2022) 173–186, https://doi.org/10.55705/cmbr.2022.347523.1048.
[50] A.M. Dhole, H.N. Shelat, H.K. Patel, Y.K. Jhala, Evaluation of the Co-inoculation effect of Rhizobium and plant growth promoting non-rhizobial endophytes on *Vigna radiata*, Curr. Microbiol. 80 (2023) 167, https://doi.org/10.1007/s00284-023-03266-4.