Phosphate solubilization potential of PSB: An advance approach to enhance phosphorous availability for phytostimulation

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Phosphate solubilization potential of PSB: An advance approach to enhance phosphorous availability for phytostimulation

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Abstract

Rhizosphere engineering approach is considered a quantum leap in plant sciences. The current study focused on investigating rhizobacterial efficiency to mobilize bioavailable phosphate from insoluble-phosphate source. Phosphate-solubilization potential of four efficient phosphate solubilizing bacterial strains i.e., *Pseudomonas songnenensis* (GR3), *Stutzerimonas stutzeri* (HH2), *Bacillus bingmayongensis* (KH3) and *Achromobacter aegrifaciens* (MH1) was analyzed. The bacterial strain GR3 was observed as most efficient phosphate solubilizer. Interactions between various physiological parameters and phosphate solubilization efficiency of isolates was evaluated using surface response methodology. *In-vitro* experiments revealed that glucose significantly facilitated phosphorus solubilization at 37 ℃, with media having pH 7 and 0.5% phosphorous. Additionally, positive correlation among P-solubilization potential, acids produced and pH variations was observed. Plant microbe-interaction analysis was performed to evaluate the efficiency of these bacterial strains on various morpho-physiological responses of *Zea mays* L. For this purpose, various concentrations of tricalcium phosphate were applied to plants in the presence and absence of bacterial strains. The results showed that, lower phosphate levels trigger shoot development, improve plant weight and leaf formation whereas higher phosphate concentrations stimulated the development of longer root system. The bacterial strains GR3 and HH2 were observed as efficient phosphate-solubilizing bacteria (PSB) that positively stimulated plant morphological responses by triggering various biochemical attributes such as plant protein content, phytohormone homeostasis, macromolecule content, solute content and pigment content. Hence, the current study reviled that the use of these phosphate solubilizing PGPR are efficient phytostimulators used for crop production in replacement of chemical fertilizers which are carcinogenic and deteriorating our eco-system.

Keywords: Indole-3-acetic acid, phytostimulation, phosphate solubilization, PGPR, PSB, organic acids

Introduction

Phosphate (P) is an essential macronutrient required for various plant metabolic and physiological processes occurring in plant. It is associated with capturing, storage and transformation of bioavailable energy for the synthesis of various significant biomolecules (Borden et al. 2021). Phosphorous uptake by plants depends on the existence of bioavailable phosphate in root vicinity (Bargaz et al. 2021). Despite excessive presence of phosphate in agricultural soils, it is one of the most common crops limiting factor as its significant fraction is locked into various in soluble organic and inorganic forms (Adebayo et al. 2023). Plant available forms are quite low in soils i.e., 0.1 to 10 µM which is quit below the plant optimum phosphate demand. Phosphate in its bioavailable forms exist in the form of orthophosphate ions (H_2PO^{4-} , HPO_4^{2-}) in soil solutions. Therefore, increasing the bioavailability of phosphates in agricultural soils is one of the primary goals of sustainable management strategy (Moradali and Rehm, 2020). Application of synthetic phosphate fertilizers is common agricultural practice. However, only small portion of P-fertilizer is taken by plants as being highly reactive P-ions, they get immediately immobilized by binding with either by soil particles or by other mineral cations to form complexes (Kaur et al. 2021). Moreover, some fertilizers contain enough calcium to precipitate its own phosphate. Chemical phosphate fertilizers are considered ineffective due to unacceptable contaminations. The synthetic phosphate fertilizers are notorious for the existence of heavy and radioactive metals. The use of these chemicals pollutes environment, become part of food chain and ultimately assimilate in living organisms. Thereby, use of organic fertilizers is considered best possible solution for their cheap and environmentally friendly nature (Ait-Ouakrim et al. 2023). In this scenario, phosphate solubilizing bacteria (PSB) are the significant biotechnological tool that can mobilize the insoluble phosphate into bioavailable forms in root-soil interface (Wu and Wan, 2023). Low soil phosphorous

content is always a limiting factor in traditional agricultural system. It is one of the most common plants limiting nutrient, therefore, its acquisition is always considered a supreme problem throughout the world in agricultural sector. Rhizospheric soil phosphate solubilizing bacteria are the active agents that stimulate the presence of available phosphate in root vicinity and ensure its continuous supply to plants (Tariq and Ahmed, 2022 a). Due to their various advantages such as low cost, environment friendly nature, high biological efficiency and strong phytostimulatory potential, these microbes are the focus of interest for various agricultural researchers around the world. Plants treated with these microbes have shown great efficiency in various growth parameters (Samal and Sukla, 2022). These microbes have ability to produce various organic acids that trigger the conversion of bounded phosphate into bioavailable form. Moreover, their role in maintenance and regularizing soil phosphate by P-cycling mechanisms make them appropriate candidate to maintain bioavailability of phosphorous in soils (Pathania et al. 2020). In this scenario, the current study was designed to evaluate the phosphate solubilizing potential of bacterial strains and their impact on various morphological and biochemical attributes of maize plants under various levels of insoluble phosphate source.

Materials and methods

Screening of bacterial isolates

Already isolated twenty-five bacterial strains were used to screen bacterial isolates for the current study. The bacterial isolates having auxin producing ability with phosphate solubilization ability were used in the current study. For the present study, all bacterial strains were grown in Luria-Bertani media at 37°C for 24 hours of incubation.

Auxin production ability

The bacterial isolates were evaluated for their auxin production potential followed by Ahmed and Hasnain (2020) via colorimetric method. Briefly, the bacterial cultures were cultivated in a growth medium containing 1% tryptophan, underwent a centrifugation process. Following centrifugation, the supernatant was mixed with Salkowski reagent at a ratio of 1:2. This mixture resulted in the development of a pink colour, indicative of indole-3-acetic acid (IAA) production. To quantify the IAA concentration, absorbance was taken at a wavelength of 535 nm. The quantification was achieved by comparing the absorbance values obtained from the samples to a standard curve of known IAA concentration. Only auxin producing bacteria were further selected for the evaluation of phosphate solubilization potential.

Phosphate solubilization ability

Phosphate solubilization potential of PGPR was evaluated both qualitatively and quantitatively using Pikovskaya medium.

Qualitative analysis

The phosphate solubilizing index and efficiency of bacterial strains were analyzed using Pikovskaya (PVK) phosphate growth medium following Sanchez-Gonzalez et al. (2022). To assess the solubilization of insoluble phosphorus, pure bacterial colonies from each strain were spot-inoculated onto plates and then incubated at 37°C for a period of seven days. The degree of solubilization was quantified using the Phosphate Solubilization Index (PSI) and Phosphate Solubilization Efficiency (PSE) which was calculated using the following formula:

$$PSI = (\text{Colony diameter} + \text{halo zone diameter}) / \text{colony diameter}$$

$$PSE = (\text{Diameter of halo zone} / \text{colony diameter}) \times 100$$

Quantitative analysis

The bacterial strains were subjected to a quantitative assessment by measuring the soluble phosphorus content. To achieve this, the strains were cultured in liquid PVK growth medium in test tubes and then incubated at 37°C for a duration of seven days. After the incubation period, centrifugation was carried out at 6000 rpm for ten minutes and the absorbance of the culture supernatants was recorded at 882 nm using a spectrophotometer. Phosphate levels in the culture supernatant were determined through a colorimetric method, following the protocol outlined by Murphy and Riley (1962), which involves the molybdenum blue assay. In this process, molybdenum blue reagent was introduced into the supernatant and maintained at 20°C. After 24 hours, the optical density was measured using a spectrophotometer at 882 nm. The quantity of soluble phosphorus was calculated using a standard curve generated with dipotassium hydrogen phosphate.

Physiological profiling for phosphate solubilizing activity

Phosphate solubilization potential of the selected bacterial isolates were evaluated using various physiological parameters i.e., temperature, pH, phosphorous concentration and carbon sources. For this purpose, bacterial isolates were allowed to grow on phosphate media under three different temperature ranges (i.e., 25, 37, 45 °C), three different pH ranges (i.e., 5, 7 and 9) and three different levels of insoluble phosphate concentrations (i.e., 0.3, 0.5 and 0.9 %). Similarly, for the evaluation of the impact of carbon source the P-solubilizing activity three different carbon sources i.e., glucose, sucrose and lactose were used individually in phosphate media and quantitatively analyzed. Phosphate was estimated using colorimetric method via molybdenum blue assay following Murphy and Riley (1962).

Tailoring physiological variables for efficient phosphate solubilization

The bacterial strain *Pseudomonas songnenensis* (GR3) was selected for the tailoring physiological parameters using surface response methodology as this bacterial isolate showed great efficiency of solubilizing insoluble phosphate. Mutual interaction among temperature, pH, concentrations of phosphate used and phosphate solubilization was assessed. In this study, a central composite rotatable design (CCRD) consisting of two variables, five central points and 13 runs was employed for this purpose by using Design Expert-13 software. It provides a foundational design comprising both higher and lower values, in addition to central points. Three-dimensional response surface graphs were generated following Suleman et al. (2018).

Acid production analysis

In the context of the phosphate solubilization process, the production of acids in the culture medium was monitored by tracking changes in the pH of the broth culture. To analyze these acids, High-Performance Liquid Chromatography (HPLC) was employed. The procedure involved filtering the supernatant using a 0.2 µL Millipore nylon filter and subjecting it to analysis via an HPLC system equipped with a C-18 column. The mobile phase consisted of methanol and water in a ratio of 30:70 (v/v), and it was pumped through the column at a flow rate of 0.6 mL/min. Detection of signals was accomplished at a wavelength of 210 nm. To identify the specific acids and retention time of the chromatographic peaks were compared with those of standard organic acids. This was done to assess the relationship between the phosphate-solubilizing activity of these bacterial isolates, the variations in pH and the production of organic acids. This analysis aimed to establish correlations among these three parameters. To quantify these correlations, Pearson correlation analysis was conducted. This statistical analysis allowed for an examination of the relationships between pH levels, the organic acids produced by the bacterial isolates, and the phosphate-solubilization potential exhibited by bacterial isolates *Pseudomonas songnenensis* (GR3) and *Stutzerimonas stutzeri* (HH2).

Soil profiling

Various soil physiological parameters including electrical conductivity, pH, organic matter content, available phosphate, soil saturation, organic matter (%) and texture were analyzed before and after experiment.

Plant bacterization assay

Certified seeds of *Zea mays* L. (var. DK-6714) were procured from Punjab Seed Corporation, Lahore, Pakistan. The inoculation experiment was conducted in triplicates. Seeds were surface sterilized with 0.1% mercuric chloride solution before inoculating with bacterial strains. The seeds were inoculated with bacterial cultures with ~ 10^6 –10^7 CFU/mL for one hour. Experiment was conducted in triplicates with six seeds sowed per pot containing 165 g of sterilized soil. The experiments were conducted in a complete block design (CBD) using four sets of treatments (i) control sets- plants without bacterial treatments and external phosphate supply (C_0), (ii) experimental pots having phosphate only- plants with only insoluble tricalcium phosphate of variable concentrations i.e., 10, 20, 30, 40 and 50 mM/g of soil (C_{10}, C_{20}, C_{30}, C_{40}, C_{50}), (iii) experimental sets having bacterial strains only- bacterially treated plants without insoluble phosphate (GR3, HH2, KH3, MH1) and (iv) experimental sets having phosphate and bacterial strains- bacterially treated plants with insoluble phosphate of variable concentrations ($GR3_{10}$, $GR3_{20}$, $GR3_{30}$, $GR3_{40}$, $GR3_{50}$; $HH2_{10}$, $HH2_{20}$, $HH2_{30}$, $HH2_{40}$, $HH2_{50}$; $KH3_{10}$, $KH3_{20}$, $KH3_{30}$, $KH3_{40}$, $KH3_{50}$; $MH1_{10}$, $MH1_{20}$, $MH1_{30}$, $MH1_{40}$, $MH1_{50}$).

Post-harvest analysis

Plant morphological characters such as length of shoot and root, fresh weight of plant and number of leaves per plant were measured. For plant biochemical profiling, analysis of various parameters such as protein content, analysis of macromolecules and cell solutes, phytohormone and pigment content were carried out. Plant protein content was determined following Lowry et al. (1951). For the analysis of various macromolecules nitrogen and phosphate content was evaluated via kjeldahl and ammonium molybdate method following Bradstreet (1954) and Murphy and Riley (1962) respectively. For phytohormones analysis, plant auxin content was estimated following Mahadevan (1984). For the determination of cell solutes, plant proline and total soluble carbohydrate content were determined following Habib and Ahmed (2022) and DuBois et al. (1956) respectively whereas plant pigment content i.e., chlorophyll 'a', chlorophyll 'b', carotenoids and total chlorophyll content were determined following Lichtenthaler and Wellburn (1983).

Statistical analysis

The data obtained were statistically analyzed by applying Duncan's Multiple Range test using "agricolae" package to determine significant differences and Pearson correlation analysis was studied via package "PerformanceAnalytics" through RStudio ver. 4.2.2. (P=0.05). Surface response and regression analysis was carried out using Design Expert software (State Ease, USA).

Results

Screening for bacterial isolates

Out of twenty-five bacterial strains nine bacteria with auxin producing ability were further evaluated for their phosphate solubilizing potential and only four bacterial strains with efficient auxin production and phosphate solubilization ability i.e., *Pseudomonas songnenensis* (GR3) (OP256752), *Stutzerimonas stutzeri* (HH2) (OP271490), *Bacillus bingmayongensis* (KH3) (OP274119) and *Achromobacter aegrifaciens* (MH1) (OP279752) were selected for further studies.

Auxin production ability

The maximum auxin production ability was recorded for the bacterial strains *Achromobacter aegrifaciens* (MH1) i.e., 145.1 µg/ml followed by the bacterial isolates *Bacillus bingmayongensis* (KH3), *Pseudomonas songnenensis* (GR3) and *Stutzerimonas stutzeri* (HH2) with auxin production ability of 85.7, 67.7 and 62.2 µg/ml respectively (Fig. 1).

Phosphate solubilization ability

Qualitative analysis

For qualitative analysis the phosphate solubilization index (PSI) and efficiency (PSE) was measured and it was observed that bacterial isolate *Pseudomonas songnenensis* (GR3) showed maximum PSI of 1.84 and PSE of 84.4% followed by the bacterial isolated *Bacillus bingmayongensis* (KH3), *Stutzerimonas stutzeri* (HH2) and *Achromobacter aegrifaciens* (MH1) with PSI of 1.82, 1.79 and 1.66 respectively and PSE of 82.7, 79.3 and 66.8% respectively (Fig. 1).

Quantitative analysis

Quantitative analysis revealed that maximum phosphate was solubilized by bacterial strain *Pseudomonas songnenensis* (GR3) is 73 µg/ml after 24 hours of incubation followed by *Stutzerimonas stutzeri* (HH2) with 71 µg/ml after 48 hours of incubation. Similarly, bacterial strains *Bacillus bingmayongensis* (KH3) have shown solubilization of 62.3 µg/ml after 24 hours of incubation and *Achromobacter aegrifaciens* (MH1) showed 56.5 µg/ml of solubilized phosphate after 72 hours of incubation. pH variations of inoculated media were regularly observed and decline from 7 to 4.7, 5.1, 4.5 and 4.7 by the bacterial isolates GR3, HH2, KH3 and MH1 was observed respectively (Fig. 1).

Physiological profiling for phosphate solubilizing activity

Phosphate solubilization potential of the selected bacterial isolates were evaluated using various physiological parameters were evaluated to observe the impact of physiological conditions on the P-solubilization efficiency of the bacterial isolates. The data revealed that bacterial strains efficiently solubilize phosphate under different conditions suitable for rhizobacteria. For the carbon source glucose was observed to be preferred carbon source for the bacterial strains to achieve highest tendency to solubilize phosphate. The trend of phosphate solubilization for all the bacterial isolates were observed as 25 ℃ > 45℃ > 37℃. For pH it was observed as pH 9 > pH 5> pH 7. In case of variable carbon sources of media, it was observed as lactose > sucrose > glucose. Similarly, for the phosphate concentrations of the incubation media, it was observed as 0.9 % > 0.3 %> 0.5 %. To give an overview of phosphate solubilization efficiency heat map study and hierarchical clustering of the phosphate solubilization efficiency indicated the phosphate solubilization potential of bacterial isolates at various physiological parameters was performed (Fig. 2)

Tailoring physiological variables for efficient phosphate solubilization

In our efforts to optimize the physiological conditions conducive to efficient phosphate solubilization, we conducted a comprehensive analysis of various parameters associated with the proficient bacterial strain *Pseudomonas songnenensis* (GR3). This analysis involved the use of surface methodology response analysis, which allowed us to discern the mutual interactions among key physiological factors, i.e., pH, temperature and phosphate concentrations and their impact on the phosphate solubilizing efficiency of this bacterial isolate. The results of our study revealed that an increase in temperature, pH and phosphate concentrations positively influences the efficiency of bacterial phosphate solubilization, up to a certain threshold point. Beyond this threshold, further increments in these parameters actually diminish the bacterial efficiency in solubilizing phosphate. Consequently, based on our findings, we have determined that the optimum conditions for efficient phosphate solubilization in our current study are pH 7, a phosphate concentration of 0.5%, and a temperature of 37°C (Fig. 3).

Acid production analysis

In our current study, High-Performance Liquid Chromatography (HPLC) analysis of the organic acids generated by the bacterial strains unveiled the presence of several lower molecular weight organic acids. Specifically, the bacterial isolate *Pseudomonas songnenensis* (GR3) was found to produce citric acid and malic acid, while the bacterial strain *Stutzerimonas stutzeri* (HH2) was observed to produce gluconic acid, malic acid, and oxalic acid.

The Pearson correlation analysis conducted on pH, organic acids, and phosphate solubilizing potential of the efficient bacterial isolates, specifically *Pseudomonas songnenensis* (GR3) and *Stutzerimonas stutzeri* (HH2), revealed noteworthy findings. It demonstrated that there is a significant positive correlation among pH, phosphate solubilization efficiency, and acid production capability of these bacterial strains. However, it's important to note that in the case of the bacterial isolate HH2, phosphate solubilization does not exhibit a direct correlation with pH changes. While a slight change in pH was observed, the more pronounced correlation exists between pH changes and the production of acids (Fig. 4)

Soil profiling

The soil used in current study was loamy. Before conducting experiment, the soil has electrical conductivity of 0.9 $mScm^{-1}$, 7.3 pH, 0.7% organic matter, 7.2 mg/Kg of available phosphate with 42 % saturation while after harvesting the electrical conductivity was 1.1 $mScm^{-1}$, 7.4 pH, 0.49 % organic matter, 6.4 mg/Kg of available phosphate with 42 % saturation.

Post-harvest analysis

Growth profiling

The current study revealed that soil phosphate content has variably affected plant morphophysiological and biochemical parameters, however, presence of bacterial strains efficiently triggered various plant developmental processes.

i. Shoot length

Bacterially treated plants have shown prominent increase in shoot length without insoluble phosphate application with increase of 119.4, 118.2, 84.4 and 74.4 % showed by bacterial strain HH2, GR3, KH3 and MH1respectively, as compared to control plant (C). In case of 10 mM of insoluble phosphate, maximum increase of 77.4 % was observed for the plants treated with bacterial strain $HH2_{10}$ when compared to C_{10}. Similarly, increase of 73.8, 68.7 and 33.5 % was observed in case of bacterial strains $MH1_{10}$, $GR3_{10}$ and $KH3_{10}$ respectively, when compared to C_{10}. In case of 20 mM of insoluble phosphate, increase of 78.7, 75.1, 72.8 and 71.7 % in the plants treated with bacterial strain $KH3_{20}$, $MH1_{20}$, $HH2_{20}$ and $GR3_{20}$ respectively, when compared with C_{20}. In case of 30 mM of insoluble phosphate, increase of 71.5, 66.4, 64.2 and 20 % was observed in plants treated with bacterial strain $GR3_{30}$, $KH3_{30}$, $MH1_{30}$ and $HH2_{30}$ respectively, when compared to C_{30}. In case of 40 mM of insoluble phosphate, maximum increase of 78, 76.2, 74.2 and 71.9% was observed in case of $KH3_{40}$, $HH2_{40}$, $GR3_{40}$ and $MH1_{40}$ respectively, when compared to C_{40}. In case of 50 mM of insoluble phosphate, increase of 75.5, 67.2, 60.1 and 58 % was observed in plants treated with bacterial strain $GR3_{50}$, $KH3_{50}$, $HH2_{50}$ and $MH1_{50}$ respectively as compared to C_{50}. On an average, the efficiency of bacterial strains was observed in order as GR3> MH1> KH3 > HH2 with insoluble phosphate applications (Fig. 5).

ii. Root length

Increase of 173.7, 149.8, 133.9 and 90.5 % in root length of plants treated with bacterial strain GR3, HH2, KH3 and MH1respectively, was observed as compared to non-inoculated plants. In case of 10 mM of insoluble phosphate, increase of 112.3, 95.7, 81.2 and 51.5 % was observed in plants treated with bacterial strains $GR3_{10}$, $HH2_{10}$, $MH1_{10}$ and $KH3_{10}$ respectively, as compared to C_{10}. In case of 20 mM of insoluble phosphate, increase of 45, 35, 28.9 and 26.6 % was observed in plants treated with $KH3_{20}$, $MH1_{20}$, $GR3_{20}$ and $HH2_{20}$ respectively, as compared to C_{20}. In case of 30 mM of insoluble phosphate, increase of 42, 34.9, 32.3 and 0.5% was observed in plants treated with $GR3_{30}$, $KH3_{30}$, $MH1_{30}$ and $HH2_{30}$ respectively, as compared to C_{30}. In case of 40 mM of insoluble phosphate, increase of 53, 43.9, 37.9 and 37.8 % in root length of plants treated with bacterial strain $KH3_{40}$, $MH1_{40}$, $HH2_{40}$ and $GR3_{40}$ respectively, have been observed as compared to C_{40}. In case of 50 mM of insoluble phosphate, increase of 44.1, 25.8, 25.7 and 14.9 % in root length of plants treated with bacterial strain

$GR3_{50}$, $MH1_{50}$, $KH3_{50}$ and $HH2_{50}$ respectively, have been observed as compared to C_{50}. Similar trend was observed for root length of bacterially treated plants with insoluble phosphate application as for shoot length (Fig. 5).

iii. Plant fresh weight

Bacterial treatments greatly influence fresh weight of plants. Increase of 110, 75, 64 and 50 % was recorded in plants treated with bacterial strains KH3, GR3, MH1and HH2 respectively, as compared to non-treated plants. In case of 10 mM of insoluble phosphate, increase of 83.4, 81.6, 77.7, and 36.2 % in fresh weight of plants treated with bacterial strains $GR3_{10}$, $HH2_{10}$, $MH1_{10}$ and $KH3_{10}$ respectively, was observed as compared to C_{10}. In case of 20 mM of insoluble phosphate, increase of 64.3, 53.5, 45.4 and 40.2 % in fresh weight of plants treated with bacterial strains $MH1_{20}$, $HH2_{20}$, $KH3_{20}$ and $GR3_{20}$ respectively, was observed as compared to C_{20}. In case of 30 mM of insoluble phosphate, increase of 56.9, 37.6, 11.9 and 5.7 % in fresh weight of plants treated with bacterial strains $GR3_{30}$, $KH3_{30}$, $MH1_{30}$ and $HH2_{30}$ respectively, was observed as compared to C_{30}. In case of 40 mM of insoluble phosphate, increase of 35.7, 35.5, 27 and 19.3 % in fresh weight of plants treated with bacterial strains $MH1_{40}$, $GR3_{40}$, $KH3_{40}$ and $HH2_{40}$ respectively, was observed as compared to C_{40}. In case of 50 mM of insoluble phosphate, increase of 49.6, 45.6, 40 and 29.2 % in fresh weight of plants treated with bacterial strains $HH2_{50}$, $MH1_{50}$, $GR3_{50}$ and $KH3_{50}$ respectively, was observed as compared to C_{50}. On an average, bacterial strain MH1 efficiently improved fresh weight of plants at various phosphate concentrations followed by bacterial strains GR3, HH2 and KH3. On an average, the efficiency of bacterial strains was observed in order as MH1> GR3 > HH2> KH3 with insoluble phosphate applications (Fig. 5).

iv. Leaf count per plant

Increase of 88.5, 77.4, 75.6 and 68.4 % was observed in number of leaves of plants inoculated with bacterial strains MH1, KH3, HH2 and GR3 respectively, as compared to non-inoculated plants. In case of 10 mM of insoluble phosphate, increase of 128.3, 127.4, 127.4 and 87.7 % in number of leaves of plants treated with bacterial strains $HH2_{10}$, $GR3_{10}$, $MH1_{10}$ and $KH3_{10}$ respectively, was observed as compared to C_{10}. In case of 20 mM of insoluble phosphate, increase of 53.6. 52.9, 48.9 and 47.7 % in number of leaves of plants treated with bacterial strains $GR3_{20}$, $HH2_{20}$, $KH3_{20}$ and $MH1_{20}$ respectively, was observed as compared to C_{20}. In case of 30 mM of insoluble phosphate, increase of 61.9, 52.6, 51.1 and 9.8% in number of leaves of plants treated with bacterial strains $GR3_{30}$, $MH1_{30}$, $KH3_{30}$ and $HH2_{30}$ respectively, was observed as compared to C_{30}. In case of 40 mM of insoluble phosphate, increase of 73.1, 68.6, 58.1 and 9 % in fresh weight of plants treated with bacterial strains $HH2_{40}$, $KH3_{40}$, $GR3_{40}$ and $MH1_{40}$ respectively, was observed as compared to C_{40}. In case of 50 mM of insoluble phosphate, increase of 91.6, 80, 75, 69.2 % in fresh weight of plants treated with bacterial strains $GR3_{50}$, $MH1_{50}$, $KH3_{50}$ and $HH2_{50}$ respectively, was observed as compared to C_{50}. On an average, the efficiency of bacterial strains was observed in order as GR3 > KH3 > MH1> HH2 with insoluble phosphate applications (Fig. 5).

Biochemical profiling

i. Protein content

Increase of 69.2, 51.5, 49.7 and 42.7 % was observed in plants treated with bacterial strains KH3, HH2, GR3 and MH1 respectively as compared to control. In case of 10 mM of insoluble phosphate, increase of 76.7, 48.2, 43.5 and 37.6 % in protein content of plants treated with bacterial strains $HH2_{10}$, $GR3_{10}$, $KH3_{10}$ and $MH1_{10}$ respectively, was observed as compared to C_{10}. In case of 20 mM of insoluble phosphate, increase of 67.5, 66.9, 54.2 and 20.3 % in protein content of plants treated with bacterial strains $HH2_{20}$, $GR3_{20}$, $KH3_{20}$ and $MH1_{20}$ respectively, was observed as compared to C_{20}. In case of 30 mM of insoluble phosphate, increase of 91.6, 51.6, 35.3 and 27.7 % in protein content of plants treated with bacterial strains $KH3_{30}$, $GR3_{30}$, $HH2_{30}$ and $MH1_{30}$ respectively, was recorded as compared to C_{30}. In case of 40 mM of insoluble phosphate, increase of 70.5, 70.1, 48.1 and 2.1 % in protein content of plants treated with bacterial strains $HH2_{40}$, $KH3_{40}$, $GR3_{40}$ and $MH1_{40}$ respectively, was recorded as compared to C_{40}. In case of 50 mM of insoluble phosphate, increase of 54.2, 50.5, 48.2 and 21.8 % in protein content of plants treated with bacterial strains $HH2_{50}$, $GR3_{50}$, $KH3_{50}$ and $MH1_{50}$ respectively, was observed as

compared to C_{50}. On an average, the efficiency of bacterial strains was observed in order as KH3> HH2 > GR3> MH1 with insoluble phosphate applications (Fig. 6).

ii. Auxin analysis

Bacterial strains significantly increased auxin content by 181.5, 117.3, 107 and 76 % in plants treated with HH2, GR3, MH1and KH3 respectively, as compared to non-treated plants. In case of 10 mM of phosphate, increase of 173.8, 141.6, 107.1 and 104.5 % in auxin content of plants treated with bacterial strain $HH2_{10}$, $MH1_{10}$, $GR3_{10}$ and $KH3_{10}$ respectively, was recorded as compared to C_{10}. In case of 20 mM of insoluble phosphate, increase of 217, 149, 135 and 126 % in auxin content of plants treated with bacterial strains $HH2_{20}$, $GR3_{20}$, $MH1_{20}$ and $KH3_{20}$ respectively, was observed as compared to C_{20}. In case of 30 mM of insoluble phosphate, increase of 147, 103, 88 and 79.8 % in auxin content of plants treated with bacterial strains $HH2_{30}$, $GR3_{30}$, $MH1_{30}$ and $KH3_{30}$ respectively, was observed as compared to C_{30}. In case of 40 mM of insoluble phosphate, increase of 131.6, 90, 81.6 and 76.3 % in auxin content of plants treated with bacterial strains $HH2_{40}$, $GR3_{40}$, $KH3_{40}$ and $MH1_{40}$ respectively, was observed as compared to C_{40}. In case of 50 mM of insoluble phosphate, increase of 147.7, 96.1, 90.4 and 78.3 % in auxin content of plants treated with bacterial strains $HH2_{50}$, $MH1_{50}$, $GR3_{50}$ and $KH3_{50}$ respectively, was observed as compared to C_{50}. Similar trend was observed for auxin content of bacterially treated plants with insoluble phosphate application as for plant protein content (Fig. 6).

iii. Total soluble carbohydrate content

Increase of 131.6, 117.4, 58.1 and 56.6 % was observed in total soluble content of plants treated with KH3, GR3, HH2 and MH1respectively, as compared to control. In case of 10 mM of phosphate, increase of 188.8, 1169, 72 and 69.2 % in total soluble carbohydrate content of plants treated with bacterial strains $KH3_{10}$, $GR3_{10}$, $HH2_{10}$ and $MH1_{10}$ respectively, was recorded as compared to C_{10}. In case of 20 mM of insoluble phosphate, increase of 144.2, 121.8, 64.1 and 42.2 % in total soluble carbohydrate content of plants treated with bacterial strains $KH3_{20}$, $GR3_{20}$, $MH1_{20}$ and $HH2_{20}$, respectively, was recorded as compared to C_{20}. In case of 30 mM of insoluble phosphate, increase of 154.8, 109.2, 61.5 and 36.8 % in total soluble carbohydrate content of plants treated with bacterial strains $KH3_{30}$, $GR3_{30}$, $MH1_{30}$ and $HH2_{30}$ respectively, was observed as compared to C_{30}. In case of 40 mM of insoluble phosphate, increase of 114.5, 90.3, 59.3 and 40.5 % in total soluble carbohydrate content of plants treated with bacterial strains $KH3_{40}$, $GR3_{40}$, $MH1_{40}$ and $HH2_{40}$ respectively, was recorded as compared to C_{40}. In case of 50 mM of insoluble phosphate, increase of 123.1, 119.8, 53.7 and 47.8 % in total soluble carbohydrate content of plants treated with bacterial strains $GR3_{50}$, $KH3_{50}$, $MH1_{50}$ and $HH2_{50}$ respectively, was observed as compared to C_{50}. On an average, the efficiency of bacterial strains was observed in order as KH3> GR3> MH1> HH2 with insoluble phosphate applications (Fig. 7).

iv. Proline content

Decrease of 6.8, 47.8 and 48.2 % in proline content of plants treated with bacterial strains MH1, KH3 and GR3 respectively, was observed as compared to control. In case of 10 mM of phosphate, increase of 43.8, % in proline content of plants treated with bacterial strain $HH2_{10}$ respectively, was observed as compared to C_{10}. However, decrease of 1.4, 25.1 and 34.2% in proline content of plants treated with bacterial strains $MH1_{10}$, $GR3_{10}$ and $KH3_{10}$ respectively, as compared to C_{10}. In case of 20 mM of insoluble phosphate, increase of 20.8 % in proline content of plants treated with bacterial strains $HH2_{20}$ as compared to C_{20}. While decrease of 13.5, 23.1 and 37.7 % in proline content of plants treated with bacterial strains $GR3_{20}$, $KH3_{20}$, and $MH1_{20}$ respectively, as compared to C_{20}. In case of 30 mM of insoluble phosphate, decrease of 25.1, 48.4, 55.8 and 64.2 % in proline content of plants treated with bacterial strains $HH2_{30}$, $MH1_{30}$, $KH3_{30}$ and $GR3_{30}$ respectively, was observed as compared to C_{30}. In case of 40 mM of insoluble phosphate, decrease of 35.4, 49.1, 59.4 and 65.6 % in proline content of plants treated with bacterial strains $HH2_{40}$, $MH1_{40}$, $KH3_{40}$ and $GR3_{40}$ respectively, was observed as compared to C_{40}. In case of 50 mM of insoluble phosphate, decrease of 37.3, 50.3, 62.5 and 64.8% in proline content of plants treated with bacterial strains $HH2_{50}$, $MH1_{50}$, $KH3_{50}$ and $GR3_{50}$ respectively, was observed as compared to C_{50}. On an average,

the efficiency of bacterial strains to decrease plant proline was observed in order as GR3> KH3> MH1> HH2 with insoluble phosphate applications (Fig. 7).

v. Pigment analysis

Chlorophyll 'a' content

Increase of 145.7, 143.2, 75.2 and 65.4 % in chlorophyll 'a' content of plants treated with MH1, KH3, HH2 and GR3 respectively, was observed as compared to untreated plants. In case of 10 mM of phosphate, increase of 59.4, 135.6, 77.9 and 8.7 % in chlorophyll 'a' content of plants treated with bacterial strain $HH2_{10}$, $KH3_{10}$, $GR3_{10}$ and $MH1_{10}$ respectively as compared to C_{10}. In case of 20 mM of insoluble phosphate, increase of 262.8, 151.4, 85.3 and 55.5 % in chlorophyll 'a' content of plants treated with bacterial strains $KH3_{20}$, $HH2_{20}$, $MH1_{20}$ and $GR3_{20}$ respectively, was observed as compared to C_{20}. In case of 30 mM of insoluble phosphate, increase of 174.6, 99.6, 89.6 and 82.7 % in chlorophyll 'a' content of plants treated with bacterial strains $HH2_{30}$, $KH3_{30}$, $GR3_{30}$ and $MH1_{30}$ respectively, was observed as compared to C_{30}. In case of 40 mM of insoluble phosphate, increase of 62.1 and 12.8 % in chlorophyll 'a' content of plants treated with bacterial strains $HH2_{40}$ and $MH1_{40}$ was observed, respectively, while no increase in plants treated with $KH3_{40}$, $GR3_{40}$ was observed respectively, when compared to C_{40}. In case of 50 mM of insoluble phosphate, increase of 70.4, 37.7 and 22.5 % in chlorophyll 'a' content of plants treated with bacterial strains $KH3_{30}$, $HH2_{50}$ and $MH1_{50}$ respectively, was recorded while no increase in case of $GR3_{50}$ was observed as compared to C_{50}. On an average, the efficiency of bacterial strains was observed in order as HH2> KH3> MH1> GR3 with insoluble phosphate applications (Fig. 8).

Chlorophyll 'b' content

Increase of 242, 208, 206 and 102 % in chlorophyll 'b' content of plants treated with KH3, MH1, HH2 and GR3 respectively, was observed as compared to untreated plants. In case of 10 mM of phosphate, increase of 162, 126, 89.2 and 50.7 % in chlorophyll 'b' content of plants treated with bacterial strain $KH3_{10}$, $HH2_{10}$, $MH1_{10}$ and $GR3_{10}$ was observed respectively, as compared to C_{10}. In case of 20 mM of insoluble phosphate, increase of 106.1, 101.6, 88.3 and 44.2 % in chlorophyll 'b' content of plants treated with bacterial strains $MH1_{20}$, $KH3_{20}$, $HH2_{20}$ and $GR3_{20}$ was observed respectively, as compared to C_{20}. In case of 30 mM of insoluble phosphate, increase of 173, 114.1, 64.7 and 34.6 % in chlorophyll 'b' content of plants treated with bacterial strains $KH3_{30}$, $MH1_{30}$, $HH2_{30}$ and $GR3_{30}$ was observed respectively, as compared to C_{30}. In case of 40 mM of insoluble phosphate, increase of 230, 189.9, 103.2 and 72.7 % in chlorophyll 'b' content of plants treated with bacterial strains $KH3_{40}$, $HH2_{40}$, $GR3_{40}$ and $MH1_{40}$ was observed, respectively, when compared to C_{40}. In case of 50 mM of insoluble phosphate, increase of 126.8, 110.5, 109.1 and 73.8 % in chlorophyll 'b' content of plants treated with bacterial strains $MH1_{50}$ $HH2_{50}$, $KH3_{50}$ and $GR3_{50}$ respectively, was recorded as compared to C_{50}. On an average, the efficiency of bacterial strains was observed in order as KH3> HH2> MH1> GR3 with insoluble phosphate applications (Fig. 8).

Carotenoid content

Increase of 112.2, 110.4, 96.3 and 52.2 % in carotenoid content of plants treated with MH1, HH2, KH3 and GR3 respectively, was observed as compared to untreated plants. In case of 10 mM of phosphate, increase of 97.4, 58.5, 47.6 and 20.9 % in carotenoid content of plants treated with bacterial strain $MH1_{10}$, $GR3_{10}$, $HH2_{10}$ and $KH3_{10}$ respectively as compared to C_{10}. In case of 20 mM of insoluble phosphate, increase of 55.9, 33.4 and 24.8 % in carotenoid content of plants treated with bacterial strains $HH2_{20}$, $KH3_{20}$ and $MH1_{20}$ was observed respectively, as compared to C_{20}. In case of 30 mM of insoluble phosphate, increase of 94.8, 78.8 and 67.8 % in carotenoid content of plants treated with bacterial strains $MH1_{30}$, $GR3_{30}$ and $HH2_{30}$ was observed respectively as compared to C_{30}. In case of 40 mM of insoluble phosphate, increase of % in carotenoid content of plants treated with bacterial strains $KH3_{40}$, $HH2_{40}$, $GR3_{40}$ and $MH1_{40}$ was observed, respectively, was observed when compared to C_{40}. In case of 50 mM of insoluble phosphate, increase of 396.6, 119.2, 116.2 and 68.4 % in carotenoid content of plants treated with bacterial strains $MH1_{50}$, $GR3_{50}$, $HH2_{50}$ and $KH3_{50}$ respectively, was recorded as compared to C_{50}. No increase in carotenoid content of plants treated with $GR3_{20}$, $KH3_{30}$ and $GR3_{50}$ was recorded compared with C_{20},

C_{30} and C_{50} respectively. On an average, the efficiency of bacterial strains was observed in order as MH1> HH2> GR3> KH3 with insoluble phosphate applications (Fig. 8).

Total chlorophyll content

Increase of 207, 182, 103 and 89.7 % in total chlorophyll content of plants treated with KH3, HH2, MH1and GR3 respectively, was observed as compared to non-treated plants. In case of 10 mM of phosphate, increase of 155.5, 134.5, 68.7 and 57.7 % in total chlorophyll content of plants treated with bacterial strain $KH3_{10}$, $HH2_{10}$, $MH1_{10}$ and $GR3_{10}$ respectively, was observed as compared to C_{10}. In case of 20 mM of insoluble phosphate, increase of 135, 101.7, 101.6 and 46.6 % in total chlorophyll content of plants treated with bacterial strains $KH3_{20}$, $MH1_{20}$, $HH2_{20}$ and $GR3_{20}$ respectively, was observed as compared to C_{20}. In case of 30 mM of insoluble phosphate, increase of 155, 106.3, 92 and 48.4 % in total chlorophyll content of plants treated with bacterial strains $KH3_{30}$, $MH1_{30}$, $HH2_{30}$ and $GR3_{30}$ respectively, was observed as compared to C_{30}. In case of 40 mM of insoluble phosphate, increase of 132.6, 128.4, 54.5 and 45.5 % in total chlorophyll content of plants treated with bacterial strains $HH2_{40}$, $KH3_{40}$, $GR3_{40}$ and $MH1_{40}$ respectively, was observed as compared to C_{40}. In case of 50 mM of insoluble phosphate, increase of 92.8, 84.7, 80.5 and 38.1 % in total chlorophyll content of plants treated with bacterial strains $KH3_{50}$, $MH1_{50}$, $HH2_{50}$ and $GR3_{50}$ respectively, was observed as compared to C_{50}. On an average, the efficiency of bacterial strains was observed in order as KH3> HH2> MH1> GR3 with insoluble phosphate applications (Fig. 8).

Macro-Analysis

i. Phosphate content

Increase of 51, 64.2, 38.7 and 21 % in phosphate content of plants treated with bacterial strains GR3, HH2, KH3 and MH1respectively, was observed as compared to non-treated plants. In case of 10 mM of phosphate, increase of 48.3, 40.8, 33.1 and 22.9 % in phosphate content of plants treated with bacterial strain $HH2_{10}$, $GR3_{10}$, $KH3_{10}$ and $MH1_{10}$ respectively, was recorded as compared to C_{10}. In case of 20 mM of insoluble phosphate, increase of 57.3, 37.3, 33.4 and 25.2 % in phosphate content of plants treated with bacterial strains $HH2_{20}$, $GR3_{20}$, $KH3_{20}$ and $MH1_{20}$, respectively, was observed as compared to C_{20}. In case of 30 mM of insoluble phosphate, increase of 45, 42.2, 26.6 and 24.3 % in phosphate content of plants treated with bacterial strains $HH2_{30}$, $GR3_{30}$, $KH3_{30}$ and $MH1_{30}$ respectively, was observed as compared to C_{30}. In case of 40 mM of insoluble phosphate, increase of 36.9, 28.9, 17.3 and 4.5 % in phosphate content of plants treated with bacterial strains $HH2_{40}$, $GR3_{40}$, $KH3_{40}$ and $MH1_{40}$ respectively, was observed as compared to C_{40}. In case of 50 mM of insoluble phosphate, increase of 47.2, 33.5, 24.3, 14.4 % in phosphate content of plants treated with bacterial strains $HH2_{50}$, $GR3_{50}$, $KH3_{50}$ and $MH1_{50}$ respectively, was observed as compared to C_{50}. On an average, the efficiency of bacterial strains was observed in order as HH2> GR3> KH3> MH1 with insoluble phosphate applications (Fig. 9).

ii. Nitrogen content

Inoculations with bacterial strains GR3, KH3, MH1and HH2 efficiently enhance nitrogen content of plants by 124.5, 97.4, 83.9 and 59.5 % respectively as compared to non-inoculated plants. In case of 10 mM of phosphate, increase of 99.6, 97, 58 and 55.5 % in nitrogen content of plants treated with bacterial strain $KH3_{10}$, $GR3_{10}$, $MH1_{10}$ and $HH2_{10}$ respectively, was observed as compared to C_{10}. In case of 20 mM of insoluble phosphate, increase of 79.7, 75.1, 49.7 and 42.8 % in nitrogen content of plants treated with bacterial strains $GR3_{20}$, $KH3_{20}$, $HH2_{20}$ and $MH1_{20}$, respectively, was observed as compared to C_{20}. In case of 30 mM of insoluble phosphate, increase of 116.4, 108.2, 81.2 and 67.6 % in nitrogen content of plants treated with bacterial strains $GR3_{30}$, $KH3_{30}$, $MH1_{30}$ and $HH2_{30}$ respectively, was observed as compared to C_{30}. In case of 40 mM of insoluble phosphate, increase of 93.8, 60.3, 48.3 and 38.7 % in nitrogen content of plants treated with bacterial strains $GR3_{40}$, $KH3_{40}$, $MH1_{40}$ and $HH2_{40}$ respectively, was observed as compared to C_{40}. In case of 50 mM of insoluble phosphate, increase of 125.1, 101.4, 80.6 and 54 % in nitrogen content of plants treated with bacterial strains $GR3_{50}$, $KH3_{50}$, $HH2_{50}$ and $MH1_{50}$

respectively, was observed as compared to C_{50}. On an average, the efficiency of bacterial strains was observed in order as GR3> KH3> HH2>MH1 with insoluble phosphate applications (Fig. 9).

Discussion

The current study was designed to give insight into the implementation of phosphorous solubilizing PGPR for sustainable agriculture and for the evaluation of phosphate solubilization potential of PGPR to develop next generation agricultural tool for improving phosphorous use efficiency that will enhance plant responses in terms of agricultural yield. The present piece of work deals with evaluation of auxin producing- phosphate solubilizing bacteria for their potential to stimulate various plant morphophysiological and biochemical responses under different insoluble phosphorous levels in comparison with non-treated plants. The production of bacterial auxin is recognized as a highly impactful mechanism for promoting plant growth, particularly within the domain of plant growth-promoting rhizobacteria (PGPR). A large portion of bacteria associated with plants possesses the natural capacity to synthesize IAA. Consequently, the biosynthesis of auxin is considered a favorable attribute when developing biological inoculants (Pantoja-Guerra *et al*., 2023). Similarly, phosphorus is a crucial macronutrient for plants, playing a significant role as a structural and functional component in various co-enzymes and proteins, among other functions. To address phosphorus deficiency in soils and promote plant growth and crop production, the utilization of phosphate-solubilizing bacteria represents a valuable strategic approach applicable to the agricultural sector. These bacteria act as active agents, ensuring the presence of bioavailable phosphate in the root vicinity and maintaining a continuous supply to the plants. These microorganisms enhance the availability of soil phosphorus by converting inorganic phosphate into a soluble form and assimilating soluble phosphate, thus making it accessible for plant uptake (Lu et al., 2023). Rhizospheric bacteria having both auxin production potential and phosphate solubilization potential are the significant phytostimulatory agents under soil P-deficit conditions. To achieve this objective, efficient phosphate-solubilizing bacterial strains exhibiting the capability for auxin production were chosen for a thorough assessment of their impact on plant growth.

Firstly, the auxin production and phosphate solubilization efficiencies of the bacterial isolates were analyzed. The production of indole-3-acetic acid (IAA) by PGPR is a standout feature, embraced by over 80% of these rhizobacteria. The IAA produced by these bacteria closely resembles plant IAA both structurally and functionally, serving as a significant signaling molecule crucial for establishing and maintaining plant-microbe interactions (Iqbal *et al*., 2023; Tariq & Ahmed, 2023 b). In the current study bacterial isolate *Achromobacter aegrifaciens* (MH1) was observed as high auxin producing strain and *Pseudomonas songnenensis* (GR3) was observed as high phosphate solubilizing strain. Phosphate solubilization potential of selected bacterial strains were evaluated by determining phosphate solubility index and phosphate solubilizing efficiency (Blanco-Vargas et al. 2020). In a quantitative assay, these rhizobacteria efficiently solubilized an ample amount of phosphorus while causing a notable decrease in pH, dropping from 7 to as low as 4.5 in the liquid medium. Interestingly, a phosphate-solubilizing strain *Achromobacter aegrifaciens* (MH1) didn't exhibit a high solubilization index. However, it demonstrated significant phosphate solubilization in the liquid medium, indicating that the absence of a visible halo zone on agar medium cannot be relied upon as a definitive criterion for isolating PSB. This observation highlights that some rhizobacterial isolates, which do not exhibit halo zone formation on agar medium, are still capable of solubilizing insoluble phosphates in liquid culture medium. A similar finding was also documented by Suleman et al. (2018). To date various rhizobacterial genera have been evaluated for their phosphate solubilization potential such as *Rhizobium*, *Bacillus*, *Burkholderia, Pseudomonas* and *Enterobacter* (Adnan et al. 2022; Kirui et al. 2022). In current study, bacterial strains from four genera i.e., *Stutzerimonas*, *Bacillus*, *Pseudomonas* and *Achromobacter* were used. Different species of *Pseudomonas*, *Bacillus* and *Achromobacter* has already been reported for having phosphorous solubilizing efficiency (Mohamadpoor et al. 2022; Tan et al. 2022; Wu et al. 2023, Lu et al. 2023). However, to the best of our knowledge, the species used in current study have been discussing for the first time for their phosphate solubilization and phytostimulatory activity. The bacterial isolates adopted various mechanism to solubilize insoluble phosphate but the production of organic acids is the principal mechanism adopted by PSB for inorganic phosphate solubilization (Wu et al. 2019).

The potential for phosphate solubilization in bacterial strains varies under diverse physiological conditions due to the variations in the physiochemical properties of soil. Phosphate solubilization is subject to influence from a range of factors, including agronomic practices, soil composition, nutrient levels, ecological factors, and interactions with other microorganisms in the soil (Kirui et al., 2022). As a result, a comprehensive physiological profiling was conducted to evaluate various traits that impact phosphate solubilization. It's known that the level of phosphate solubilization by PSB can vary depending on the carbon source, temperature, pH and levels of insoluble phosphate used in the growth medium. The result indicated that the bacterial strains exhibited the highest phosphate solubilization potential at 37°C, under pH conditions of 7, and when glucose was used as the carbon source. These findings are consistent with the results reported by Suleman et al. (2018), which also indicated that bacterial strains exhibited the highest phosphate solubilization potential at 22°C, under pH conditions of 7, and when glucose was used as the carbon source. This alignment between the two studies underscores the significance of these specific conditions in promoting efficient phosphate solubilization by bacterial strains.

Consequently, the study examined the interaction of pH, temperature, and the concentration of insoluble phosphate in growth media and their combined effect on phosphate solubilization by *Pseudomonas songnenensis* (GR3) using Response Surface Methodology (RSM). The RSM plot revealed that phosphate solubilization increased with incubation temperature set at 37°C and a pH of 7, up to an optimal point. Beyond this optimum, further increases in temperature or pH could lead to a decrease in phosphate solubilization activity. Additionally, the concentration of the insoluble phosphate showed a positive correlation with phosphate solubilization, indicating that higher concentrations of insoluble phosphate were associated with increased phosphate solubilization efficiency until it reached to its threshold levels after which further increase in insoluble P-concentrations decrease the P-solubilization activity of the bacterial isolates.

In the present study, the HPLC analysis revealed the production of citric, malic, gluconic and oxalic acids by the bacterial isolates. Pearson correlation between pH, organic acids and phosphate solubilizing potential of efficient bacterial isolates i.e., *Pseudomonas songnenensis* (GR3) and *Stutzerimonas stutzeri* (HH2) indicated that pH, phosphate solubilization efficiency and acid production ability of the bacterial strains is significantly positively correlated. However, for the bacterial isolate HH2 phosphate solubilization is not directly corelated with pH changes as slight pH change was observed but the change in pH is positively corelated with acid production as noted by Flatian et al. (2021) some bacterial isolates did not significantly drop the pH of broth medium in spite of having high phosphate solubilizing ability this may be due to they have adopted different strategy to release bounded phosphate vis releasing proton for ion assimilation. Phosphate solubilization is positively correlated with its highest production of gluconic acid as reported by Hii et al. (2020).

Phytostimulation is conspicuous attribute for the evaluation of PSB activity. Before conduction plant-microbial analysis a brief analysis of soil was carried out which was used to conduct experiment. The soil used in current study was loamy with 7.3 pH which is near to the optimum pH required for P-availability to plants i.e., 5.5-7 (Etesami, 2020). The soil properties were analyzed before sowing and after harvesting and it was observed that the PSB present in soils modified soil physiological parameters that facilitate the solubilization of insoluble phosphate (Naureen et al. 2018). Different above and below ground vegetative characters of bacterially treated and non-treated plants were analyzed under different P-levels. *Pseudomonas songnenensis* (GR3) has shown significant results. It efficiently affected shoot length, root growth and number of leaves under various phosphate levels. Higher concentrations reduced bacterial phosphate solubilizing ability and it is completely inhibited at further increasing insoluble P-levels (Zeng et al. 2017). In current study, increasing soil phosphate negatively affected shoot length of non-inoculated plants with maximum increase observed with 20mM application of tricalcium phosphate. However, bacterially treated plants have shown prominent increase in shoot length without insoluble phosphate application. Moreover, longer and deeper root system was observed at lower P-levels however, increasing soil phosphate triggered shallow root growth near soil surface because increasing soil P-concentrations, decrease the growth of primary roots (Yadava et al. 2022). Plants roots tend to grow longer with increased root hair under lower P-concentrations (Gao et al. 2023). The stronger root morphological plasticity of bacterially treated plants under low P-levels is attributed towards the bacterially mediated upregulation of various P- transporter genes that improve P- absorbance capacity (Sun et al. 2022). However, higher shoot length was observed under higher P-levels. The increase in root to shoot ratio under lower P-levels is due to increased resource

allocations from shoots to roots that trigger root growth and reduce shoot growth (Liu, 2021). A variable effect on fresh weight and number of leaves in bacterially treated plants with various P-treatments have been recorded, however. Bacterial strains significantly enhance number of leaves of treated plants (Wang et al. 2022). Contrary to plants treated with MH1 and HH2, the plants treated with bacterial strains GR3 and KH3 showed negative impact on the fresh weight with phosphate applications when compared with bacterially treated plants without phosphate application. However, bacterial treatments showed significantly enhanced fresh weight compared to non-treated plants. The study of Khan et al. (2022) stated that PSB efficiently enhance fresh weight of plants however, variations in plant fresh weight was observed in the presence of insoluble phosphate treatments with PSB as compared to control (Khan et al. 2022). These improvements in various plant morphological traits with the addition of PSB are attributed to their ability of releasing P from insoluble sources to plants under influence of diverse actions adopted by these bacterial stains.

Different levels of phosphate variably affect plant metabolic processes. Bacterially treated plants have adjusted various biochemical parameters for efficient nutrient acquisition under various P-levels. PSB strains have shown prominent increment in protein, auxin, carbohydrate, proline and total carbohydrate content of treated plants. Increased protein content of bacterially treated plants was observed. Auxin (IAA) content of bacterially treated plants were significantly higher than non-treated plants. Various species of *Bacillus*, *Pseudomonas*, *Achromobacter*, have been reported to produce IAA (Wagi and Ahmed, 2019; Santana et al. 2022; Jain et al. 2023). Production of bacterial IAA interacts with various plant metabolic pathways and maintain plant hormonal homeostasis with control overall plant development (Tariq and Ahmed, 2022 b). All the PSB have shown increment in plant morphophysiological traits depending upon bacterial nature and association with plants. Increase in total carbohydrate and protein content of bacterially treated plants has been observed in the present study. Increment in protein can be due to the accumulation of carbohydrates because higher number of polysaccharides and oligosaccharides in plants accelerate nitrogen incorporation and speed up the protein synthesis. Nitrogen is important constituent of chlorophyll besides various other enzymes. Increased in nitrogen content referees towards efficient nutrient uptake and chlorophyll activity (Wu et al. 2023). Increase in various biochemical attributes was observed under 10-20 mM concentrations of phosphate. Excessive application of phosphate reduces the enzymatic activity of bacterial strains for the release of bounded phosphate which reduces phosphate diffusion (Romero-Perdomo et al. 2021). The alterations in plant physiology might have occurred due to greater nutrient uptake and enhanced carbohydrates synthesis by photosynthesis (Pohan et al. 2019). Moreover, proline content of plants varies under various treatments. Increased proline content was observed under increasing phosphate concentrations. As indicated by Shirmohammadi et al. (2020), proline content in plants increased with increasing phosphate application. Proline is stress indicator, increased proline activity during unfavorable environmental conditions were mainly observed (Nacoon et al. 2022). However, bacterially treated plants showed decreased proline content of treated plants with tricalcium phosphate.

Various studies have explored the phosphate solubilization capabilities of rhizobacteria. However, to the best of our knowledge, only limited research has delved into their dual role, encompassing both phytostimulation and phosphate solubilization. Furthermore, while researchers have examined the phosphate solubilizing abilities of rhizobacteria across various concentrations of soluble phosphorus, there is a notable dearth of knowledge regarding their phytostimulatory potential in the presence of varying concentrations of insoluble phosphorus. Consequently, this study presents an evaluation of bacterial strains possessing both traits, i.e., auxin production and phosphate solubilization potential. Additionally, it offers a comprehensive investigation into the morphological and biochemical responses of plants in relation to these bacterial strains.

Conclusion

The current study revealed that the bacterial strains *Pseudomonas songnenensis* (GR3OP256752), *Stutzerimonas stutzeri* (HH2OP271490), *Bacillus bingmayongensis* (KH3OP274119) and *Achromobacter aegrifaciens* (MH1OP279752) are efficient phosphate solubilizing phytostimulators can be used as an alternative to chemical fertilizers to ameliorate the growth of *Zea mays* L. under phosphate deficit soils. The results accentuated that synergistic effect of phytohormone production and phosphate solubilization potential simulate plant growth as

well as soil fertility by release bounded phosphate into bioavailable forms for plant uptake. These agents significantly boost plant metabolic activities by significantly improving nutrient uptake that ultimately trigger the photosynthetic activity of plants. Hence, these findings suggested that manipulating these potential phytostimulators by commercialization will be promising bioengineering tool in sustainable agriculture.

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Author contributions

AA and AT contributed to conceptualization of study, AA contributed to the formal analysis, investigation, funding acquisition, resources and supervision. AT provided methodology and performed writing—original draft preparation, and writing—review and editing.

Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Code availability: Not applicable.

Declarations

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Consent for publication: The authors give their consent for the publication of all details to be published in this Journal.

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

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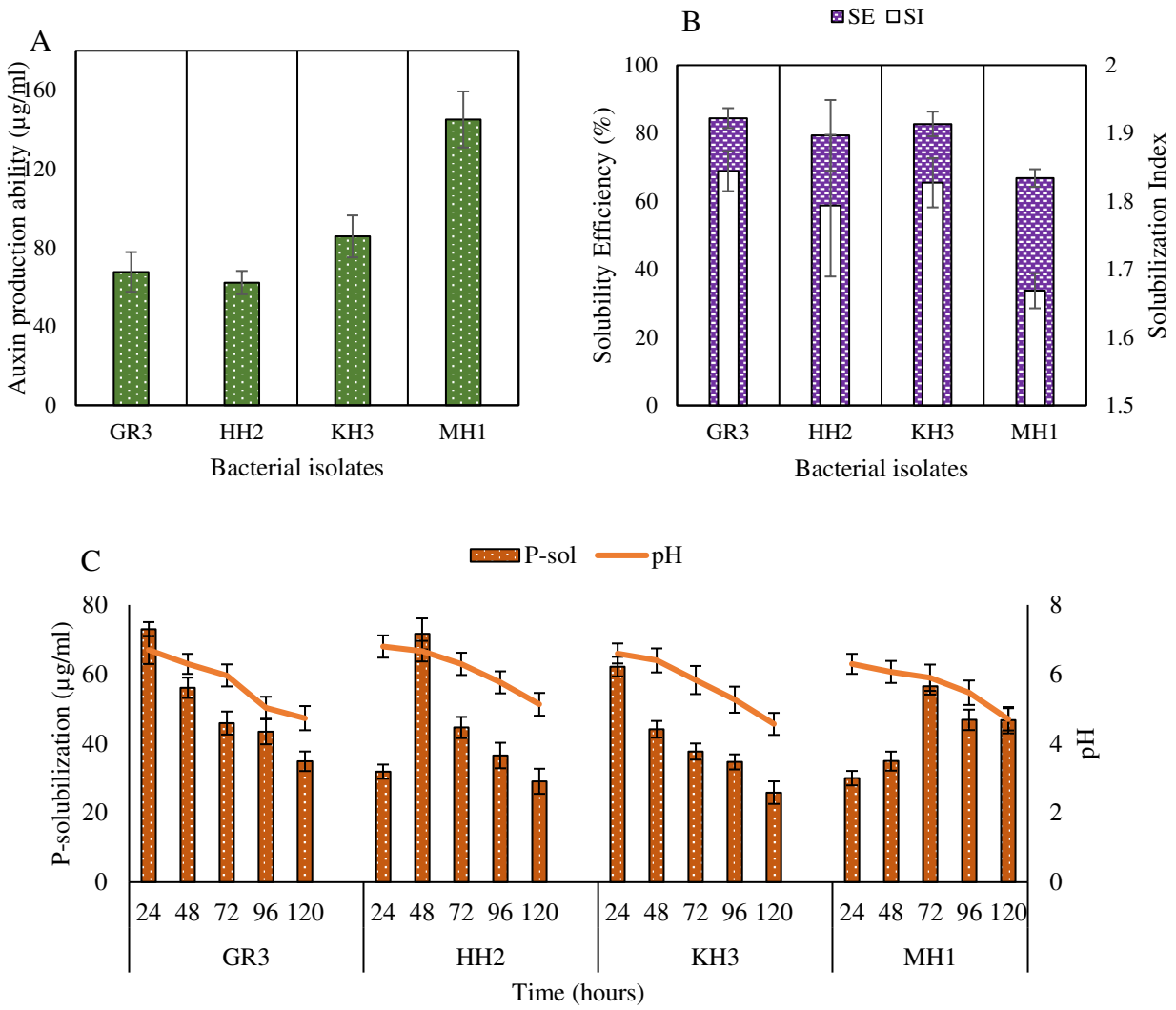

Figure 1. Auxin production potential (A) and qualitative (B) and quantitative (C) analysis of phosphate solubilization ability of the bacterial isolates (GR3 - *Pseudomonas songnenensis*, HH2 - *Stutzerimonas stutzeri*, KH3 - *Bacillus bingmayongensis*, MH1 - *Achromobacter aegrifaciens*)

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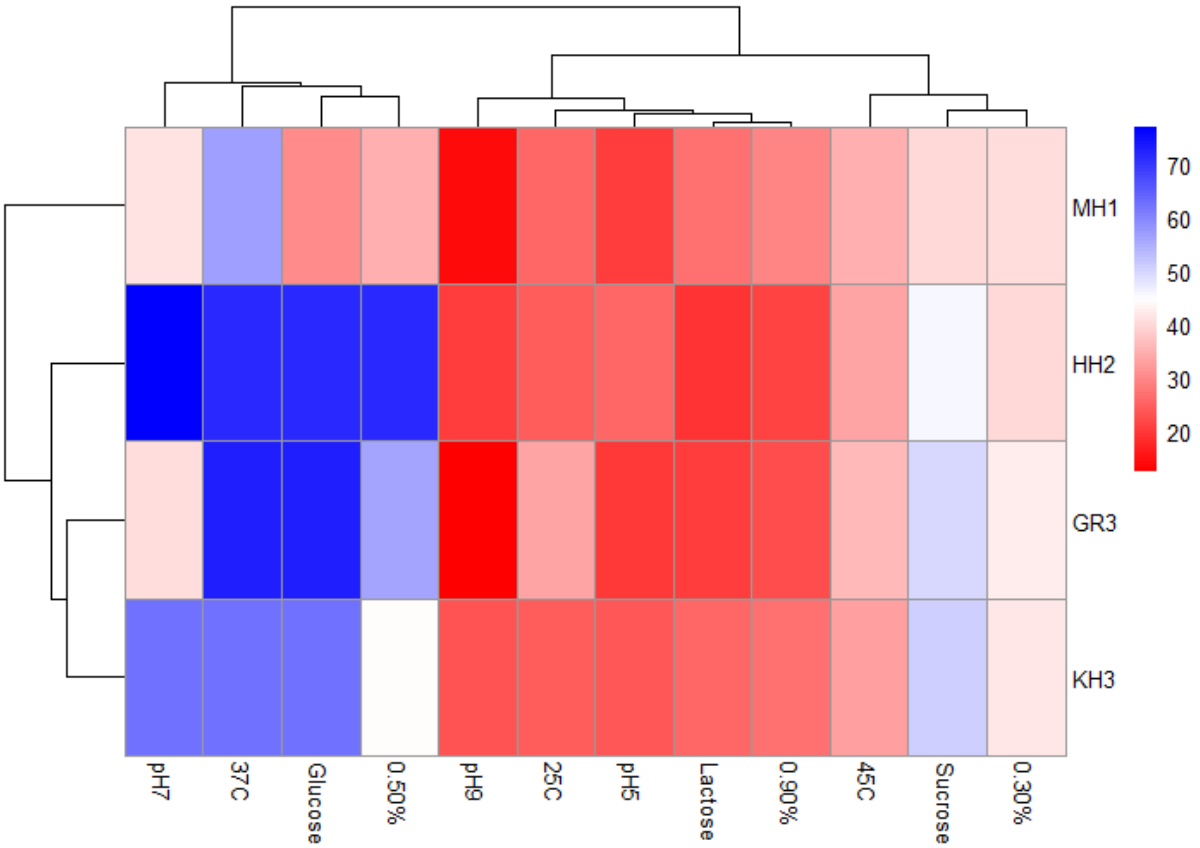

Figure 2. Heatmap with hierarchical grouping describing physiological profiling of bacterial P-solubilizing activity (GR3 - *Pseudomonas songnenensis*, HH2 - *Stutzerimonas stutzeri*, KH3 - *Bacillus bingmayongensis*, MH1 - *Achromobacter aegrifaciens*)

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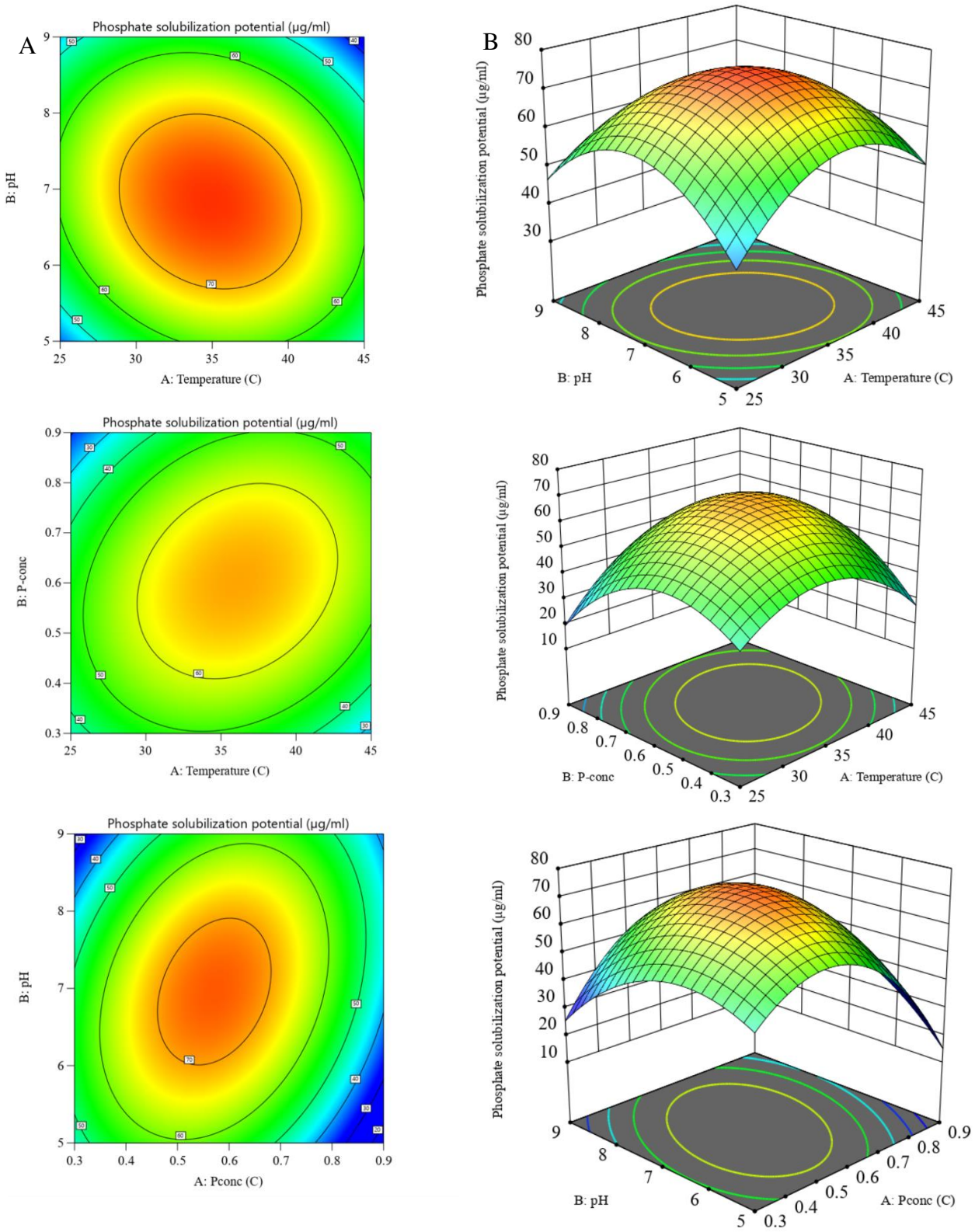

Fig. 3 Effect of mutual interaction among temperature, pH, P-solubilization potential and phosphate solubilization potential of bacterial isolate *Pseudomonas songnenensis* (GR3) using contour plot (A) and response surface plot (B).

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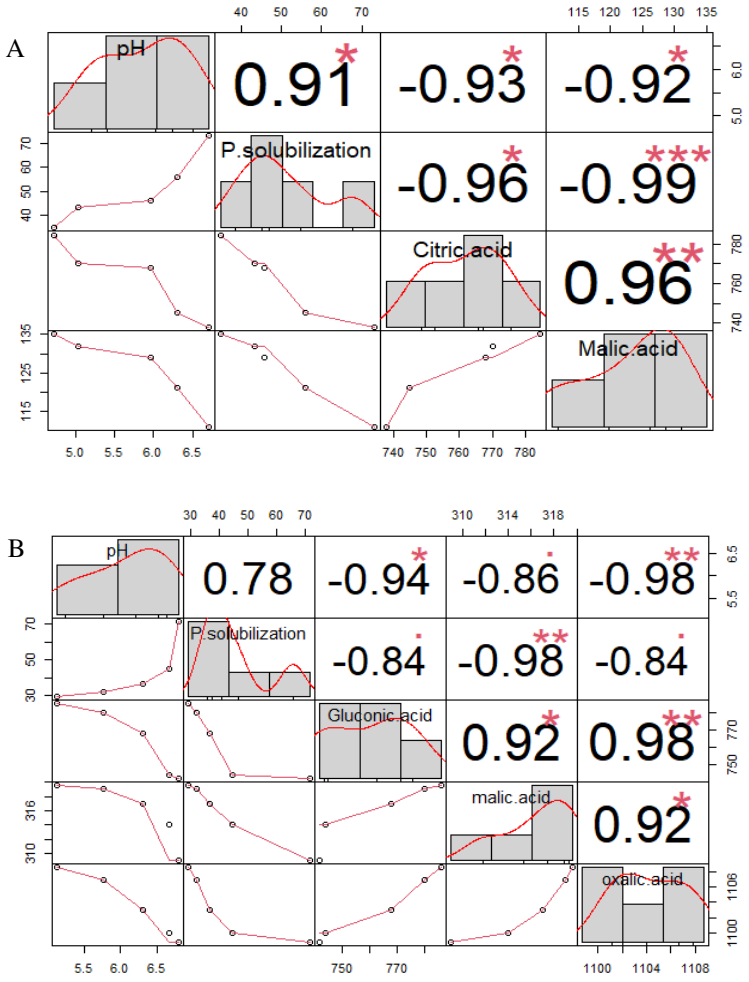

Fig. 4 Pearson's correlation coefficients matrix on phosphate solubilizing potential, pH and organic acids produced by *Pseudomonas songnenensis* (GR3) (a) and *Stutzerimonas stutzeri* (HH2) (b). Significance of correlations is indicated by the asterisk as *** for P < 0.001, ** for P < 0.01, and * for P < 0.05.

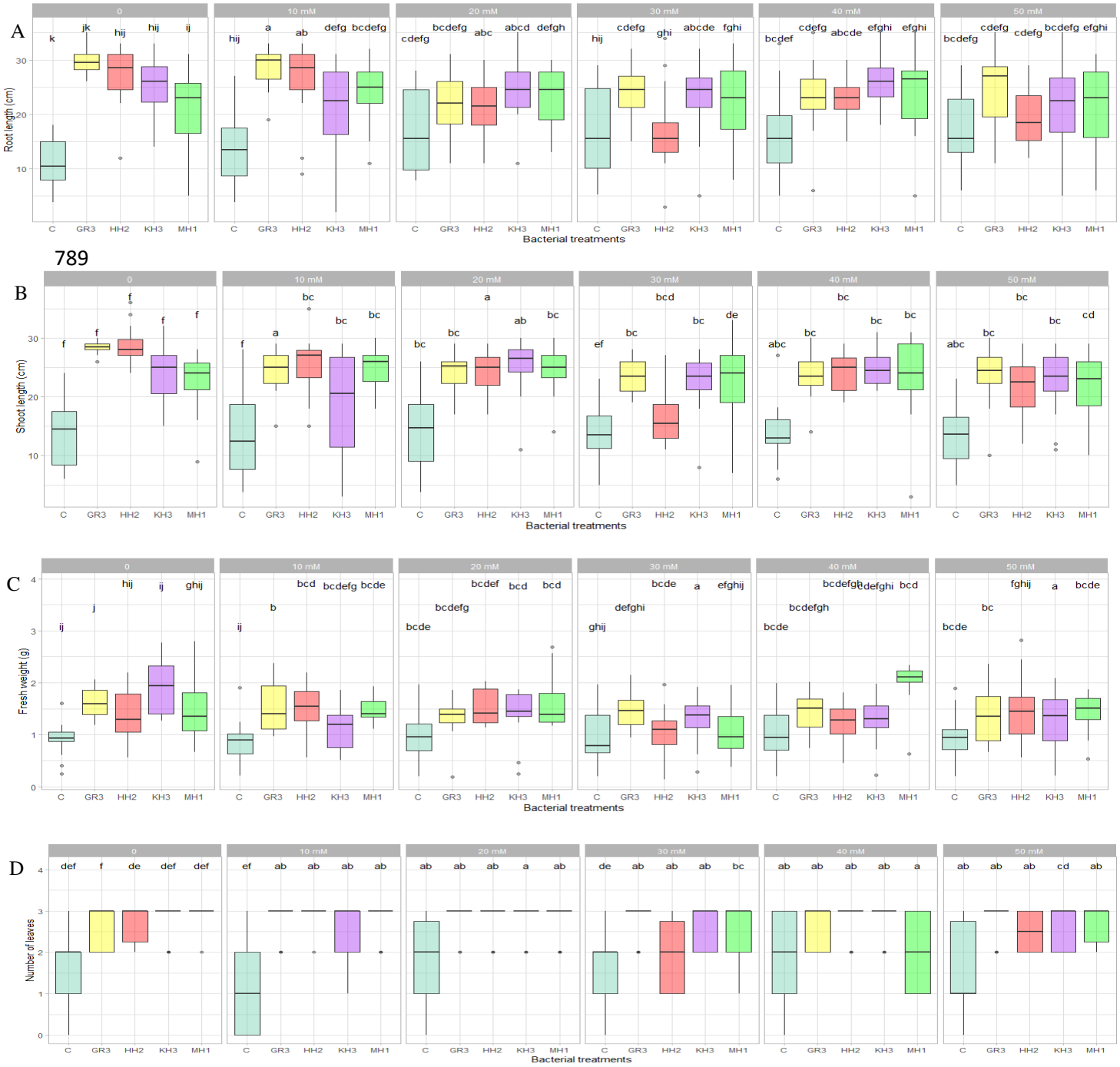

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Figure 5. Effects of bacterial treatments (GR3 - *Pseudomonas songnenensis*, HH2 - *Stutzerimonas stutzeri*, KH3 - *Bacillus bingmayongensis* MH1 - *Achromobacter aegrifaciens*) under various concentrations of phosphate (0, 10, 20, 30, 40 and 50 mM) on plant height (a), root length (b), fresh weight (c) and number of leaves (d) of *Zea mays* L.Different letters indicate significant differences between treatments using Duncan's multiple range test (P=0.05)

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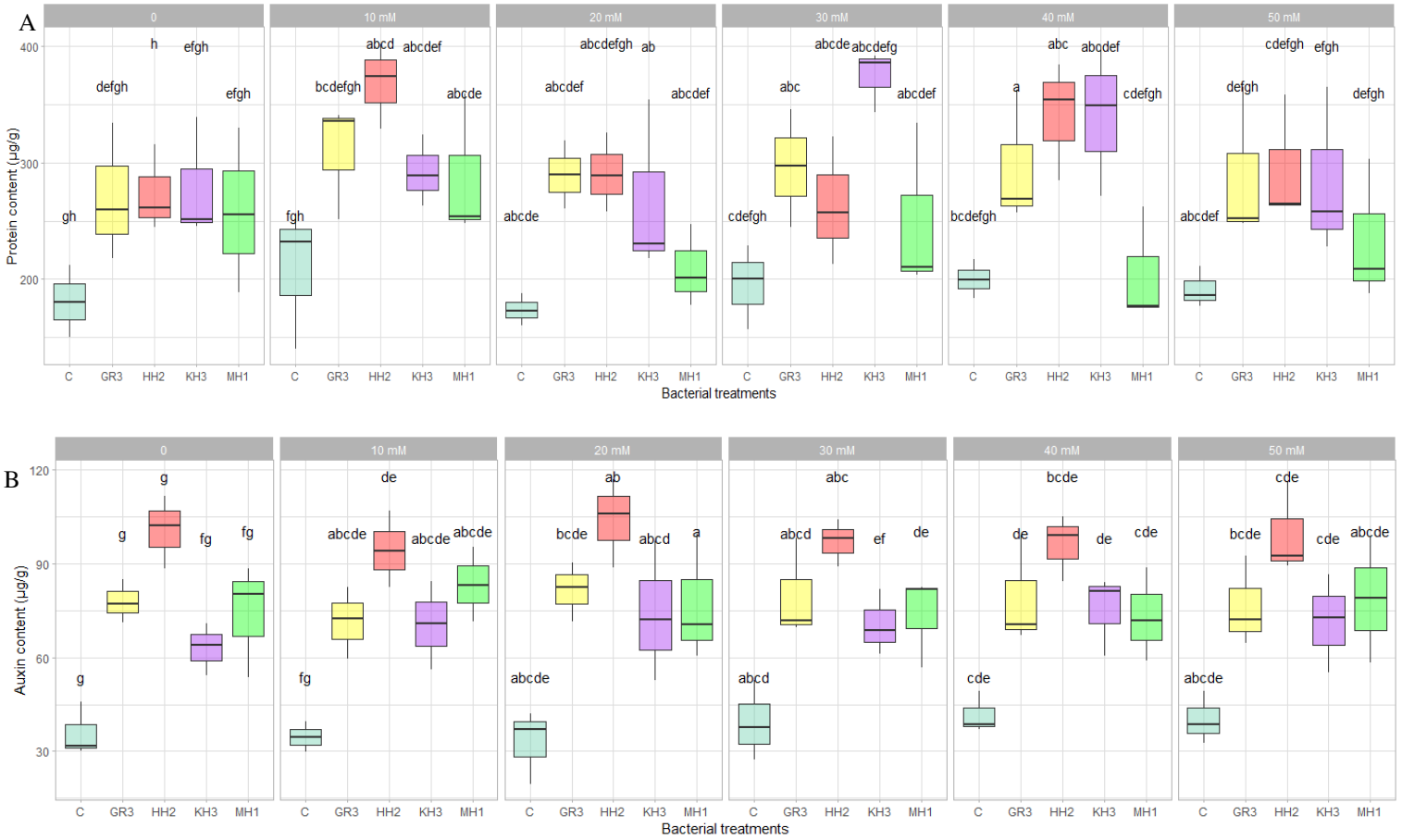

Figure 6. Effects of bacterial treatments (GR3 - *Pseudomonas songnenensis*, HH2 - *Stutzerimonas stutzeri*, KH3 - *Bacillus bingmayongensis*, MH1 - *Achromobacter aegrifaciens*) under various concentrations of phosphate (0, 10, 20, 30, 40 and 50 mM) on protein content (a) and auxin content (b) of *Zea mays* L.Different letters indicate significant differences between treatments using Duncan's multiple range test (P=0.05)

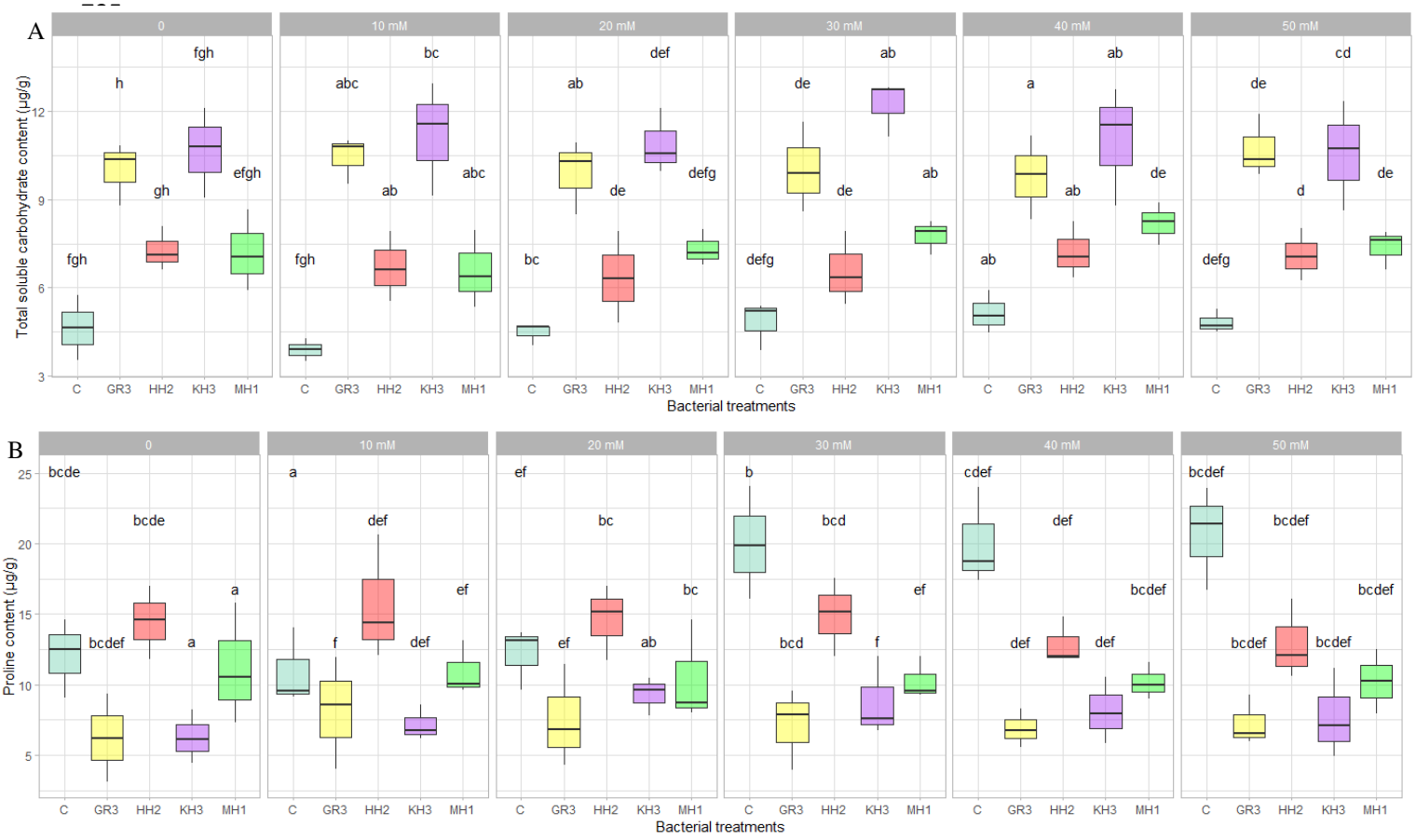

Figure 7. Effects of bacterial treatments (GR3 - *Pseudomonas songnenensis*, HH2 - *Stutzerimonas stutzeri*, KH3 - *Bacillus bingmayongensis,* MH1 - *Achromobacter aegrifaciens*) under various concentrations of phosphate (0, 10, 20, 30, 40 and 50 mM) on total soluble carbohydrate content (a) and proline content (b) of *Zea mays* L.Different letters indicate significant differences between treatments using Duncan's multiple range test (P=0.05)

Figure 8. Effects of bacterial treatments (GR3 - *Pseudomonas songnenensis*, HH2 - *Stutzerimonas stutzeri*, KH3 - *Bacillus bingmayongensis,* MH1 - *Achromobacter aegrifaciens*) under various concentrations of phosphate (0, 10, 20, 30, 40 and 50 mM) on total chlorophyll content (a), chlorophyl 'a' content (b), chlorophyl 'b' content (c) and carotenoids content (d) of *Zea mays* L.Different letters indicate significant differences between treatments using Duncan's multiple range test (P=0.05)

Figure 9. Effects of bacterial treatments (GR3 - *Pseudomonas songnenensis*, HH2 - *Stutzerimonas stutzeri*, KH3 - *Bacillus bingmayongensis,* MH1 - *Achromobacter aegrifaciens*) under various concentrations of phosphate (0, 10, 20, 30, 40 and 50 mM) on phosphate content (a) and nitrogen content (b) of *Zea mays* L.Different letters indicate significant differences between treatments using Duncan's multiple range test (P=0.05)