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Ammar Ibnyasser [a,*,1], Rym Saidi [a], Wissal Elhaissoufi [a], Said Khourchi [a], Meryem Haddine [a], Rachid Ghani [a], Abdellatif Elghali [b], Abdallah Oukarroum [a], Abdellatif Barakat [c], Adnane Bargaz [a,*,2]

[a] AgroBiosciences Program, College of Agriculture and Environmental Sciences, Mohammed 6 Polytechnic University, Lot 660, Ben Guerir 43150, Morocco
[b] Geology & Sustainable Mining Institute, Mohammed 6 Polytechnic University, Lot 660, Ben Guerir 43150, Morocco
[c] IATE, University of Montpellier, INRAE, Agro Institute, Montpellier 34060, France

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ABSTRACT

Microbial bioremediation has emerged promisingly to improve crop tolerance to cadmium (Cd). Moreover, Cd tolerance and phosphate acquisition in plants positively correlated under P solubilizing bacteria inoculation, yet there is no evidence on specific mechanisms influencing Cd tolerance and plant P acquisition. The present study evaluates Cd tolerance in rock P-amended durum wheat in response to inoculation with P solubilizing bacteria (PSB) [three individual isolates *Bacillus siamensis*, *Rahnella aceris*, *Bacillus cereus* and their consortium (PSB_{Cs})] and consequently reveals key rhizosphere mechanisms involved in both Cd tolerance and P use efficiency. Results show that inoculation overall improved plant growth, rhizosphere parameters and nutrient uptake (P, N, K) under increasing Cd concentrations [8 (Cd_8) and 16 (Cd_{16}) ppm Cd^{2+}]. Under Cd_{16}, *Rahnella aceris* induced the most significant plant responses in terms of biomass [shoots (31 %), roots (40 %), and spikes (92 %)], rhizosphere available P (234 %) and root inorganic P (109 %) compared to uninoculated plant. Microbial biomass P (MBP) and root acid phosphatases (APase) were 33-and 13-times higher, respectively, than in uninoculated plants. In addition, inoculation (particularly using PSB_{Cs}) significantly decreased Cd translocation factor (TF) (Cd_8: −17 % and Cd_{16}: −22 %) and Cd bioaccumulation factor (BAF) (Cd_8: −6 % and Cd_{16}: −40 %) concomitantly to enhanced root morphological traits and P contents in shoots and spikes. Furthermore, PSB inoculation under Cd constraint increased (rhizosphere available P / MBP) and (Root APase / Rhizosphere Apase) ratios that significantly ($p < 0.05$) correlate with plant P uptake in shoots and spikes. Increase in both ratios was concomitant to a significant decrease in TF and BAF of Cd exemplified by negatively significant correlations (r^2=0.70 and r^2=0.57, $p < 0.05$). This finding elucidates the key role of bacterial inoculation that presumably triggered Cd tolerance and aboveground P owing to increased (rhizosphere available P / MBP) and (Root / Rhizosphere APase) ratios in PSB-inoculated wheat.

1. Introduction

The exploitation of mines, the by-products recycling, and wastewater irrigation contribute to contamination of soils with variable amounts of heavy metals, particularly cadmium (Cd) (Zoffoli et al., 2013). This element is a non-essential trace element for plants, toxic at low concentrations, known as one of the most mobile heavy metals and the most harmful for plants and for their associated microbes (Hart et al., 1998; Maria et al., 2007). The phytotoxicity of Cd is the result of its rapid uptake and translocation into sensitive plant tissues (Wen et al., 2020), which makes it a critical environmental problem impairing nutrient uptake, severe oxidative damage, retarding plant development and reducing crop productivity worldwide (Ahmad et al., 2014; Haider et al., 2021). This oxidative damage at the cellular level manifests via oxidation of nucleic acids, proteins and chlorophyll, excessively inducing reactive oxygen species (ROS) production, thus altering cell membrane structural integrity alongside multiple cellular dysfunctions (Ahmad et al., 2010; Nadeem et al., 2010; Al-Dhaibani et al., 2013).

Moreover, increased Cd concentrations negatively affect the morphology and physiology of the plant belowground components such as growth and architecture of roots and overall rhizosphere functioning including the microbial community structure and reduces biodiversity (Dimkpa et al., 2009; Seshadri et al., 2015). Consequently, this has negative impact on nutrient acquisition with a decrease in crop yields (Khan et al., 2016; Peng et al., 2017). To cope with Cd deleterious effects, plants implemented several tolerance strategies including increased synthesis and accumulation of organic solutes to maintain cell water content (Yan et al., 2013), simultaneous activation of antioxidants [superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), malonaldehyde (MDA) and glutathione (GSH)] to sequester ROS and mitigating oxidative stress (Yan et al., 2013; El Rasafi et al., 2022). For instance, organic acids and metallothioneins produced by plants and soil microorganisms are phytochelatins compounds exhibiting high affinity to Cd (Isaure et al., 2006). The sequestration of Cd through phytochelatins was demonstrated in Indian mustard roots owing to several phytochelatin-Cd complexes (e.g. Cd-malate, Cd-citrate or Cd-cysteine and Cd-glutathione) (Salt et al., 1997). However, this Cd sequestration depends on the endogenous content of phytochelatins and their relative abundance in different parts of the plant (Brunetti et al., 2011).

Furthermore, soil and plant-associated microorganisms, particularly the commonly known plant growth-promoting rhizobacteria (PGPR) have developed several bioremediation strategies to reduce Cd toxicity, including physical or chemical sorption, bio-sorption, precipitation, chelation, ion efflux/afflux, intracellular accumulation and biomineralization (Singh et al., 2019; Yin et al., 2024). These strategies involved releasing chelating agents (exo-polysaccharides and siderophores), acidification (protons and organic acids), phosphate (P) solubilization, and redox changes, which affect Cd mobility and availability in the rhizosphere (Jing et al., 2007; Tiwari et al., 2008; Khourchi et al., 2023a). For example, siderophore-producing microorganisms have been reported to reduce ROS synthesis and mitigate metal stress by forming siderophore-metal complexes (Dimkpa et al., 2009). In addition, extracellular adsorption (via functional group modification) and intracellular sequestration (by carbonic anhydrase secretion) were reported in Cd-resistant *Pseudomonas* sp. B7 that was able to immobilize a significant Cd concentration Wu et al. (2022). Inoculation of *Miscanthus floridulus* with *Klebsiella michiganensis,* identified as a PGPR producing indole-3-acetic acid (IAA), siderophores and 1-aminocyclopropane-1-carboxylate deaminase (ACC deaminase), stimulated plant growth and reduced total Cd in rhizosphere soil by 49 % (Liu et al., 2021). For example, Wang et al. (2022) showed that inoculation with *Enterobacter bugandensis* reduced the Cd content of green vegetables (water spinach) by 51 %, through chelation by polyamines and precipitation from cell walls.

Phosphate solubilizing bacteria (PSB) are well known for being a key rhizosphere driving force enabling higher P use efficiency and crop productivity (Aallam et al., 2022, 2021; Khourchi et al., 2023a; Benmrid et al., 2024a, 2023; Elhaissoufi et al., 2024, 2022; Nkir et al., 2024). This agronomic advantage due to PSB can be extended to Cd tolerance (Jeong et al., 2013, 2012; Yuan et al., 2017; Zhang et al., 2022) while preserving positive consequences on root P absorption (Ibnyasser et al., 2024). Wang et al. (2020) reported that *Bacillus subtilis* (PSB) enhanced Cd immobilization and consequently, reduced its availability and uptake by lettuce plants. This is taken to mean that bacterial P solubilization and Cd tolerance can be interconnected rhizosphere processes triggered by inoculation that induced elongation of root hairs and likely the rigidity of the root cell wall to reduce Cd entrance while increasing P acquisition (Ibnyasser et al., 2024). As per current knowledge, PSB have been employed as bioremediators with the capability to reduce Cd concentrations in the rhizosphere (Park et al., 2011; Jeong et al., 2013, 2012; Yuan et al., 2017; Teng et al., 2019; Zhang et al., 2022). This application underscores their potential role in mitigating the adverse effects of Cd pollution and paves the way to develop best management practices for plant tolerance in Cd-contaminated soils. However, understanding mechanisms governing relationships between Cd translocation and nutrient uptake (notably P) remains poorly understood, particularly at the root-rhizosphere-microbe interface with increased intertwined biotic interactions. The present study focuses on wheat root-PSB physiological and rhizosphere responses under both Cd stress and low P availability, examining new rhizosphere mechanisms implicated in the relationship between Cd translocation/bioaccumulation and P use and translocation into aboveground part of wheat plants. These functional mechanisms between enhanced P uptake and Cd tolerance were evaluated under inoculation using PSB [*Bacillus siamensis*, *Rahnella aceris* and *Bacillus cereus* and their consortium (PSB$_{Cs}$)] exhibiting, contrasting abilities (high-medium-low) to solubilize rock phosphate (RP) and tolerate increasing concentrations of Cd. The present study aims to provide valuable information on the existential interconnection between belowground parameters and improved Cd tolerance in response to P deficiency and Cd stress under RP fertilization.

2. Material and methods

The present study was carried out to evaluate the effect of PSB and RP application on the improvement of Cd tolerance and P acquisition efficiency in durum wheat [*Triticum turgidum subsp. durum, variety Karim*] under increasing Cd concentrations [0 (Cd$_0$), 8 (Cd$_8$) and 16 (Cd$_{16}$) ppm Cd^{2+}]. The three PSB used individually and in a consortium (PSB$_{Cs}$) were identified as *Bacillus siamensis* (*B. siam*), *Rahnella aceris* (*R. acer*), *Bacillus cereus* (*B. cer*) (Khourchi et al., 2022; Elhaissoufi et al., 2023). These strains exhibited contrasting P solubilizing capacities under increasing Cd^{2+} concentration (33 ppm – 201 ppm Cd^{2+}) according to the recent study by Ibnyasser et al. (2024). In addition to their high Cd bioaccumulation capacity, these strains significantly improved Cd tolerance of wheat seedlings by improving root morphophysiological root traits along with other plant growth promoting traits [e.g., production of IAA, exopolysaccharide (EPS), ammonium, ACC deaminase, siderophores] (Ibnyasser et al., 2024).

The inoculum of the different PSB was prepared in tryptone soy broth (TSB) and incubated on a rotary shaker (150 rpm) at 28°C for 24 hours. The bacterial pellet was collected by centrifugation and washed with sterilized physiological saline water (0.9 % NaCl). The PSB$_{Cs}$ inoculum was prepared by mixing equal proportions of the three PSB isolates. Of note, the three isolates did not present antagonistic interactions following a plate confrontation test. The cell density was then adjusted to 10^8 CFU.mL^{-1} for each inoculant before the inoculation was performed by soaking the disinfected wheat seeds in the corresponding inoculum for two hours under continuous agitation (90 rpm) at room temperature.

2.1. Experimental design and plant growth conditions

In the present study, a 75-day greenhouse experiment was conducted at the Agricultural Innovation and Technology Transfer Center (AITTC) of UM6P. Durum wheat was grown in a greenhouse in disinfected pot (14 cm diameter and 15 cm length) containing 2.5 Kg of sterilized P-deficient agricultural soil (3 ppm Olsen P), 2.5 ppm Cd (measured by inductively coupled plasma emission spectroscopy: ICP-OES), pH 8.24, and electrical conductivity 64 μs.cm^{-1} (measured by pH-meter Mettler Toledo). Wheat seeds were surface disinfected by successive immersion in ethanol (70 %, 1 min) and sodium hypochlorite (6 %, 5 min), followed by several washes with sterile distilled water. The disinfected wheat seeds were inoculated (soaked in the inoculum 2 hours / 90 rpm / room temperature) while the uninoculated seeds (used for control treatments) were soaked in sterile distilled water. The inoculated seeds were sown at a rate of six seeds per pot, with only three seedlings retained after germination. The RP (29 % total P, 2 ppm available P) was applied at the rate of 157 Kg.ha^{-1} according to Elhaissoufi et al. (2020). The amounts of N and K nutrients supplied were balanced for all treatments to a final rate of 180 and 80 Kg.ha^{-1}, respectively. The micronutrients were equally balanced for all treatments using Hoagland solution. Before planting, the soil was supplemented with Cd^{2+} at two concentrations of Cd$_8$ and Cd$_{16}$ ppm. This involved dissolving the specified amounts of Cd^{2+} in distilled water, and the treated soil was left two nights before sowing. Two Cd^{2+} concentrations were employed (Cd$_8$ and Cd$_{16}$), along with untreated control (Cd$_0$). Each concentration underwent five treatments: four with inoculation (utilizing *B. siam*, *R. acer*, *B. cer*, and PSB$_{Cs}$) and one as an uninoculated control. The experiment was conducted in a completely randomized design of six replicates per treatment, with two plants per replicate. Plants were regularly watered twice a week with distilled water to keep the soil moisture at approximately 60 % of water-holding capacity.

2.2. Measurements of above- and below-ground traits

At harvest (75-days of growth), spikes, shoots, roots, and rhizosphere soils were separately collected. Fresh samples of rhizosphere soil (obtained by carefully separating the roots from the adhering soil), shoots and roots were stored at −20°C for biochemical analysis [e.g., inorganic P (Pi) content, rhizosphere available P, microbial biomass P (MBP), acid phosphatase activity (APase), proline, and chlorophyll content]. Then, dry weights of shoots (SDW), roots (RDW) and spikes (SpDW) were determined at 80°C (for 72 h) before being ground into a fine powder for P and Cd analysis (Khourchi et al., 2023b).

2.3. Determination of Cd concentration in plant and soil

An aliquot of 0.5 g of each dried and ground shoot, root and rhizosphere sample was digested in a flask with 3 mL nitric acid (HNO$_3$, 70 %,) and 1 mL perchloric acid (concentrated HClO$_4$ 60 %) (v/v ratio 3:1). The samples were then heated at 350°C until dense white fumes appeared. The contents of the flasks were cooled, filtered for Cd determination using ICP-OES (Agilent Technologis, 5110). Translocation and bioaccumulation of Cd were calculated using the following formulas (Ahmad et al., 2014):

$$Translocation\ factor\ (TF) = \frac{Cd\ concentration\ in\ shoot}{Cd\ concentration\ in\ root} \quad (A1)$$

$$Bioaccumulation\ factor\ (BAF) = \frac{Cd\ concentration\ in\ shoot}{Cd\ concentration\ in\ soil} \quad (A2)$$

2.4. Determination of plant P content and rhizosphere P

2.4.1. Total P and Pi content in plant biomass

The determination of total P was carried out after mineralization of the plant biomass (shoot and spike) (Majeed et al., 2015). Samples were placed in the oven at 550°C for 6 h. The resulted ash was then added to 3 mL of HCl (6 N), evaporated on a hot plate and recovered with hot distilled water. The available P fraction was estimated spectrophotometrically using molybdenum blue method at 880 nm (Sun et al., 2018).

To determine root Pi content, samples were ground in cold sodium acetate buffer (0.2 M, pH 5.6). The mixture was centrifuged at 12,000 g for 10 min at 4°C and the resulting supernatant (50 μL) was used for Pi quantification as determined by Khourchi et al. (2022) and Benmrid et al. (2024b).

2.4.2. Rhizosphere available P

Dried and sieved samples of rhizosphere soil were used prior to determine P fraction. The rhizosphere available P was extracted with sodium bicarbonate (NaHCO$_3$) (pH 8.5) and measured colorimetrically at 880 nm. Briefly, 0.5 g of rhizosphere soil was mixed with 10 mL of NaHCO$_3$ (0.5 M) and shaked for 20 min at room temperature. After filtration, 1 mL of filtrate was used to measure the available P fraction in rhizosphere as described by Zheng et al. (2009).

2.4.3. Determination of soil microbial biomass P and calculation of P availability to MBP ratio in rhizosphere

Microbial biomass P (MBP) was determined using the chloroform (CHCl$_3$) fumigation-extraction method (Corstanje et al., 2007). Fresh rhizosphere samples (2 g) per treatment were fumigated with chloroform (ethanol-free) under 200 mbar vacuum pressure for 18 h. In parallel, non-fumigated rhizosphere samples in the same treatment were processed under similar conditions (200 mbar for 18 h), without adding chloroform. The MBP concentration was determined using the following formula:

$$MBP(ppm) = \frac{\Delta P}{K_{EP}} \quad (A3)$$

MBP (ppm): Microbial biomass P, ΔP: (NaHCO$_3$-extractable P in fumigated soil) – (NaHCO$_3$-extractable P in non-fumigated soil) and K$_{EP}$: The extraction efficiency factor (K$_{EP}$ = 0.40).

Since MBP is a pool contributing in enhancing rhizosphere P availability (Khourchi et al., 2023b), the ratio (A4) of rhizosphere available P to MBP was calculated to assess the interconnection between rhizosphere P availability and Cd mobility to the plant.

$$Ratio\ 1 = \frac{Rhizosphere\ available\ P}{Microbial\ biomass\ P} \quad (A4)$$

2.5. Acid phosphatases activities in rhizosphere and roots

The APase activities in rhizosphere and root were measured using the *para*-nitrophenyl phosphate (*p*-NPP) (Elhaissoufi et al., 2020; Khourchi et al., 2022). A mass of 0.125 g of fresh samples (soil and roots) were added to cold sodium acetate buffer (0.5 M, pH 5.6) and *p*-NPP (10 mM) as substrate. The homogenate was stirred for 30 s and incubated at 37°C for 1 h. After incubation, the enzymatic reaction was stopped by adding CaCl$_2$ (0.5 M) and NaOH (0.5 M). The APase activity (nmol *p*-NPP.g^{-1}.h^{-1}) was determined by amount of *para*-nitrophenol (*p*-NP) (reaction product) using the spectrophotometer FLUOstar Omega, UV/VIS absorbance spectra at 405 nm.

Since root and rhizosphere APase are significantly involved in P mineralization and internal P utilization efficiency processes in soils and roots, the ratio of root to rhizosphere APase was calculated following formula:

$$Ratio\ 2 = \frac{Root\ APase}{Rhizosphere\ APase} \quad (A5)$$

2.6. Proline and total chlorophyll content

The proline content was determined according to Bates et al. (1973).

An aliquot of 100 mg of fresh shoots was mixed with 2 mL of pure methanol (40 %) and centrifuged at 10,000 rpm for 15 min at 4°C. The supernatant (1 mL) was mixed with acidic ninhydrin (1 mL) and glacial acetic acid (1 mL), followed by an incubation period at 100°C for 1 hour. After incubation, toluene (2 mL) was added to the mixture, and upon agitation, phase separation occurred, yielding two distinct phases. The absorption of upper phase was subsequently measured at 520 nm. The proline content was determined by referencing a standard curve constructed with pure L-proline (99 %) and calculated using the following formula:

$$Proline \ (\mu mol. \ g^{-1}) \ FW = \frac{X \times \left(\frac{V \ toluene}{115.5}\right)}{FW} \tag{A6}$$

Where X: obtained through the standard curve, V: the volume of toluene and FW fresh weight.

Total chlorophyll (Chlt) content was measured according to Pérez-Patricio et al. (2018). Briefly, 100 mg of fresh leaf tissue was ground in 5 mL of acetone (80 %, v/v). Total chlorophyll was determined according to the following formula:

$$Chlt \ (mg.g^{-1}) \ = \ 8.2 \times (A663) + 20.20 \times (A645) \tag{A7}$$

where A663 and A645 are the absorbance measured from 663 nm and 645 nm, respectively.

2.7. Measurement of root morphological traits

The washed roots were used to measure the morphological root traits including root length (RL), root surface area (RSA) and root volume (RV). Roots were gently spread on a flat tray filled with a narrow layer of water and then placed into an Epson flatbed scanner with a resolution of 300 dpi. The morphological roots traits were automatically quantified using WinRhizo software (Regent Instruments Inc., Québec, QC, Canada) (Elhaissoufi et al., 2023, 2020; Khourchi et al., 2023b, 2022; Ibnyasser et al., 2024).

2.8. Nutrients (N and K) contents in shoot and spike

Total N and K contents were measured in shoot and spike using dried and finely ground samples. The shoots and spikes powders were digested with nitric acid and analyzed for K content using ICP-OES (Agilent 5110 ICP-OES, USA). The total N content (shoots and spikes) was determined by the Kjeldahl method (KjelMaster K-375, Netherlands) (Khourchi et al., 2023b).

2.9. Statistical analysis

The statistical data analysis was carried out by IBM® SPSS® Statistics V. 25 software. Univariate and multivariate one and two-way ANOVA were used, followed by Tukey's post hoc test to determine the significant difference between the treatment means at the 0.05, 0.01, and 0.001 level of significance.

3. Results

3.1. Effects of PSB on Cd-mobility and rhizosphere P availability under combined Cd-stress and P deficiency

The application of PSB under RP fertilization significantly reduced Cd mobility from soil to plants. This combined application of PSB, individually or in consortium (PSB$_{Cs}$), with RP significantly decreases Cd uptake and accumulation in shoot and root of Cd-stressed wheat plants. This effect is observed under increasing Cd concentrations (Cd$_8$ and Cd$_{16}$) compared to uninoculated plants (Figs. 1A and B). Under RP-fertilization, the Cd BAF (Fig. 1A) and its TF (Fig. 1B) in wheat plants was significantly decreased in response to PSB inoculation compared to
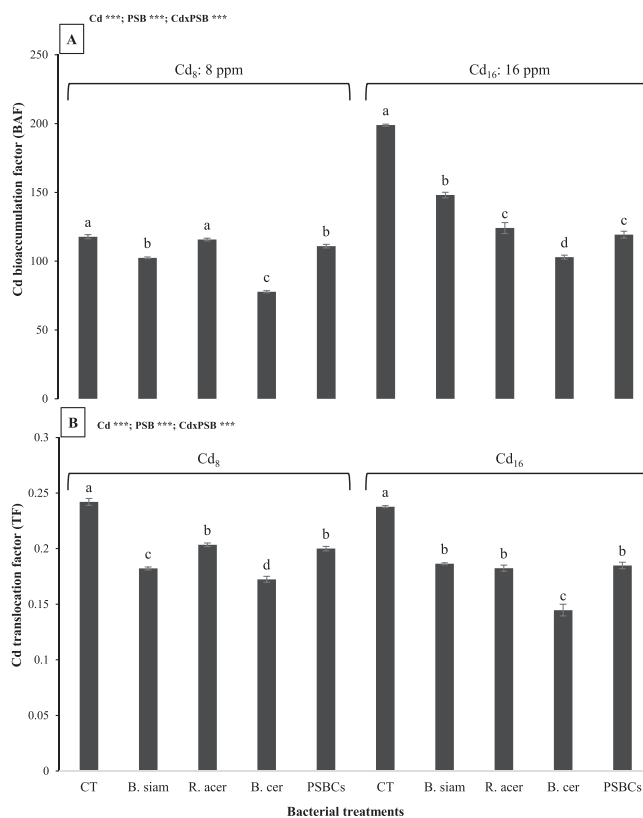

Fig. 1. Cd bioaccumulation (A) and translocation factor (B) in 75-day old wheat plant inoculated by PSB (*Bacillus siamensis: B. siam, Rahnella aceris: R. acer, Bacillus cereus: B. cer* and their consortium: PSB$_{Cs}$) under RP fertilized and increased Cd concentrations (Cd$_8$ and Cd$_{16}$ ppm Cd^{2+}). Each value represents an average of six replicates. Mean values labelled with the same superscript letter are not significantly different at $p < 0.05$ under the same Cd concentration. Asterisks indicate significant differences between Cd, PSB, and their interactions (Cd x PSB) (*** $p < 0.001$). CT: uninoculated plants, BAF: bioaccumulation factor, TF: translocation factor and RP: rock phosphate.

uninoculated and Cd-stressed plants. Notably, the inoculation with *B. cer* significantly decreased Cd TF to shoot by 29 % and 39 % and Cd BAF in plant by 34 % and 48 % under Cd$_8$ and Cd$_{16}$ concentrations, respectively compared to uninoculated and Cd-stressed plants. Following *B. Cer*, inoculation with *R. acer* and PSB$_{Cs}$ significantly reduced Cd TF to shoots and BAF in plants under Cd$_8$ and Cd$_{16}$ concentrations. This reduction was most pronounced under Cd$_{16}$, with BAF decreasing by 38 % and 40 % and TF decreasing by 23 % and 22 % in response to *R. acer* and PSB$_{Cs}$, respectively compared to uninoculated plants. The effect of Cd, PSB inoculation, and their interaction (Cd x PSB) were positively significant at $p < 0.001$.

Concerning the effect of PSB on P availability, the combined application of PSB inoculation and RP fertilization enhanced the concentrations of available P and MBP in the rhizosphere compared to uninoculated plants (Fig. 2A). Specifically, inoculation with PSB$_{Cs}$ increased rhizosphere available P, with improvements of up to 299 % under Cd$_8$ condition and 386 % under Cd$_{16}$ condition, respectively compared to the uninoculated plants. The increase of P availability was significantly more pronounced with the application of PSB$_{Cs}$, especially at elevated Cd$_{16}$ concentration. Comparing this effect to single inoculations, *R. acer* (18.99 ppm) and *B. siam* (15.85 ppm) exhibited the most substantial effects, particularly in soils without Cd addition. Regarding MBP, inoculation with PSB$_{Cs}$ (8.40 and 15.03 ppm) and *R. acer* (9.15 and 10.16 ppm) significantly enhanced MBP at Cd$_8$ and Cd$_{16}$, respectively. Without the application of Cd (under Cd$_0$ concentration), the MBP was highest in the rhizosphere of plants inoculated
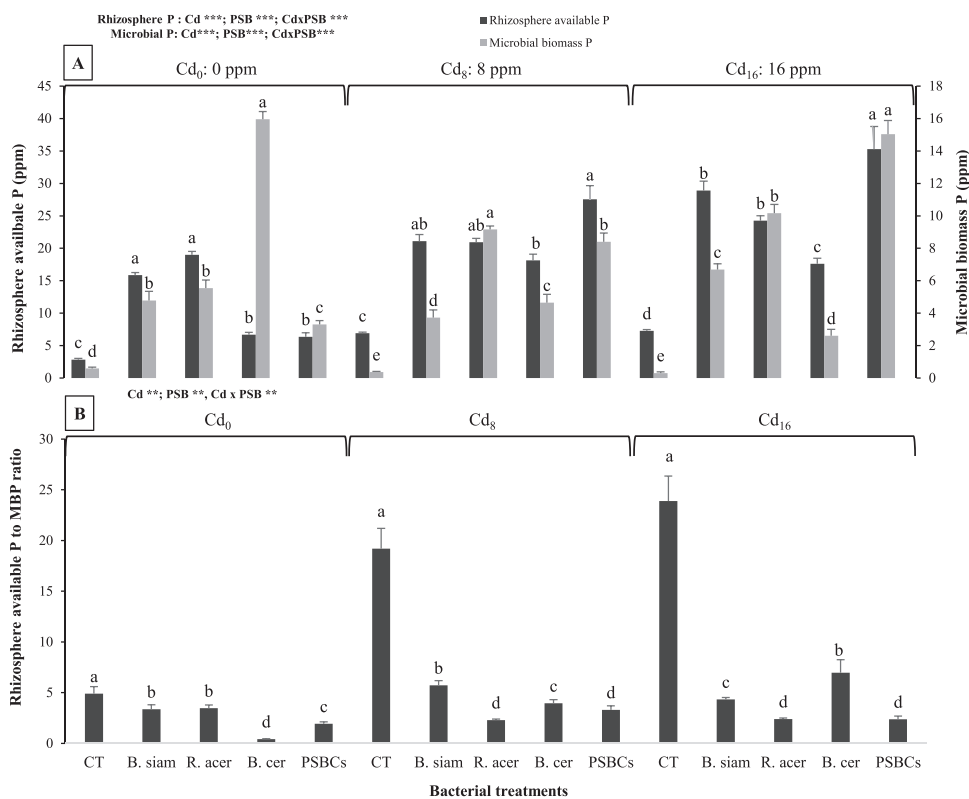
Fig. 2. Rhizosphere available P and MBP (A) and their ratio (B) in 75-day old wheat plant inoculated by PSB (*Bacillus siamensis: B. siam, Rahnella aceris: R. acer, Bacillus cereus: B. cer* and their consortium: PSB$_{Cs}$) under RP fertilized and increased Cd concentrations (Cd$_0$, Cd$_8$ and Cd$_{16}$ ppm Cd^{2+}). Each value represents an average of six replicates. Mean values labelled with the same superscript letter are not significantly different at $p < 0.05$ under the same Cd concentration. Asterisks indicate significant differences between Cd, PSB and their interactions (Cd x PSB) (*** $p < 0.001$; ** $p < 0.01$). CT: uninoculated plants; MBP: microbial biomass P and RP: rock phosphate.

with *B. cer* (15.96 ppm) followed by *R. acer* (5.54 ppm) and *B. siam* (4.78 ppm). The effects of Cd, PSB, and their interaction (Cd x PSB) significantly influenced available P and MBP in the rhizosphere soil ($p < 0.001$).

The contribution of PSB inoculation to balance the distribution of P between different P pools and ensure optimal P availability for plant uptake was assessed by measuring the ratio of rhizosphere available P to MBP (Fig. 2B). This ratio was significantly increased in the uninoculated plants under all increasing Cd concentrations compared to the inoculated treatments. However, the rhizosphere available P to MBP ratio was higher in plants inoculated with *B. cer* and *B. siam* compared to those inoculated with *R. acer* and PSB$_{Cs}$ under Cd-stressed conditions (Cd$_8$ and Cd$_{16}$). The effects of Cd, PSB, and their interaction (Cd x PSB) significantly ($p < 0.01$) influenced the rhizosphere available P to MBP ratio.

The figure (Fig. 3) shows that combined application of PSB with RP fertilization significantly influences Cd translocation and bioaccumulation in wheat plants under Cd stress conditions. A strong negative correlation ($r^2 = 0.70$, $p < 0.05$) was observed between Cd TF and the rhizosphere available P to MBP ratio in inoculated plants (*B. Siam, R. Acer, B. Cer,* and PSB$_{Cs}$) confounded Cd stress (Cd$_8$-Cd$_{16}$) (Fig. 3A). However, uninoculated plants exhibited a not significant negative correlation ($r^2 = 0.24$, ns) (Fig. 3B) between Cd TF and the rhizosphere available P to MBP ratio. Similarly, a significant negative correlation ($r^2 = 0.57$, $p < 0.05$) was found between the Cd BAF and the rhizosphere available P to MBP ratio in inoculated plants with PSB (*B. Siam, R. Acer,* and *B. Cer*) and PSB$_{Cs}$ (Fig. 3C), while this correlation was not significant ($r^2 = 0.15$, ns) in uninoculated plants (Fig. 3D).

3.2. Effects of PSB on root and rhizosphere acid phosphatase activity

With increasing Cd-concentrations (Cd$_0$, Cd$_8$, and Cd$_{16}$), combined

application of RP with PSB as both single and consortium inoculation (*B. siam, R. acer, B. cer,* and PSB$_{Cs}$) induced a significant increase in APase activity in root and rhizosphere (Fig. 4A). Root APase activity of PSB$_{Cs}$-inoculating plants was increased (412.21, 469.72, and 480.10 nmol.g^{-1}.min^{-1}) compared to uninoculated plants (45.67, 35.56, and 27.03 nmol.g^{-1}.min^{-1}) under Cd$_0$, Cd$_8$, and Cd$_{16}$, respectively. This enhancement was followed by plants inoculated with *R. acer* and *B. siam* which exhibited significantly higher root APase activity across all Cd-concentration levels (Cd$_0$, Cd$_8$, and Cd$_{16}$). In addition, rhizosphere APase activity significantly enhanced by PSB inoculation, particularly in response to *B. siam* inoculation followed by PSB$_{Cs}$-inoculating plants that increased rhizosphere APase activity by 272.50 % and 218 % under Cd$_8$, and by 588 % and 246 % under Cd$_{16}$, respectively, compared to uninoculated plants. Overall, the PSB, Cd application and their interaction significantly ($p < 0.001$) influence APase activity in roots and rhizosphere. The evaluation of Root / rhizosphere APase ratio provided valuable insight into the efficiency of P-solubilization and uptake by plants under Cd-stress conditions. In response to inoculation with *R. acer* a high root-to-rhizosphere APase ratio was observed, showing increases of 213 %, 265 %, and 375 % under increasing Cd concentrations (Cd$_0$, Cd$_8$, and Cd$_{16}$, respectively), compared to uninoculated plants. Similarly, PSB$_{Cs}$ exhibited notable increases in the root-to-rhizosphere APase ratio, with enhancements of 260 %, 314 %, and 413 % under Cd$_0$, Cd$_8$, and Cd$_{16}$ conditions, respectively, compared to uninoculated plant (Fig. 4B).

Under Cd stress (Cd$_8$ and Cd$_{16}$), the results indicate a negative correlation ($r^2 = 0.20$, ns) between the Cd TF and root to rhizosphere APase ratio (Fig. 5A) in response to PSB inoculation. Similarly, a negative correlation ($r^2 = 0.42$, ns) between the Cd BAF and the root to rhizosphere APase ratio across inoculated treatments was noted under Cd stress (Cd$_8$-Cd$_{16}$) (Fig. 5C).
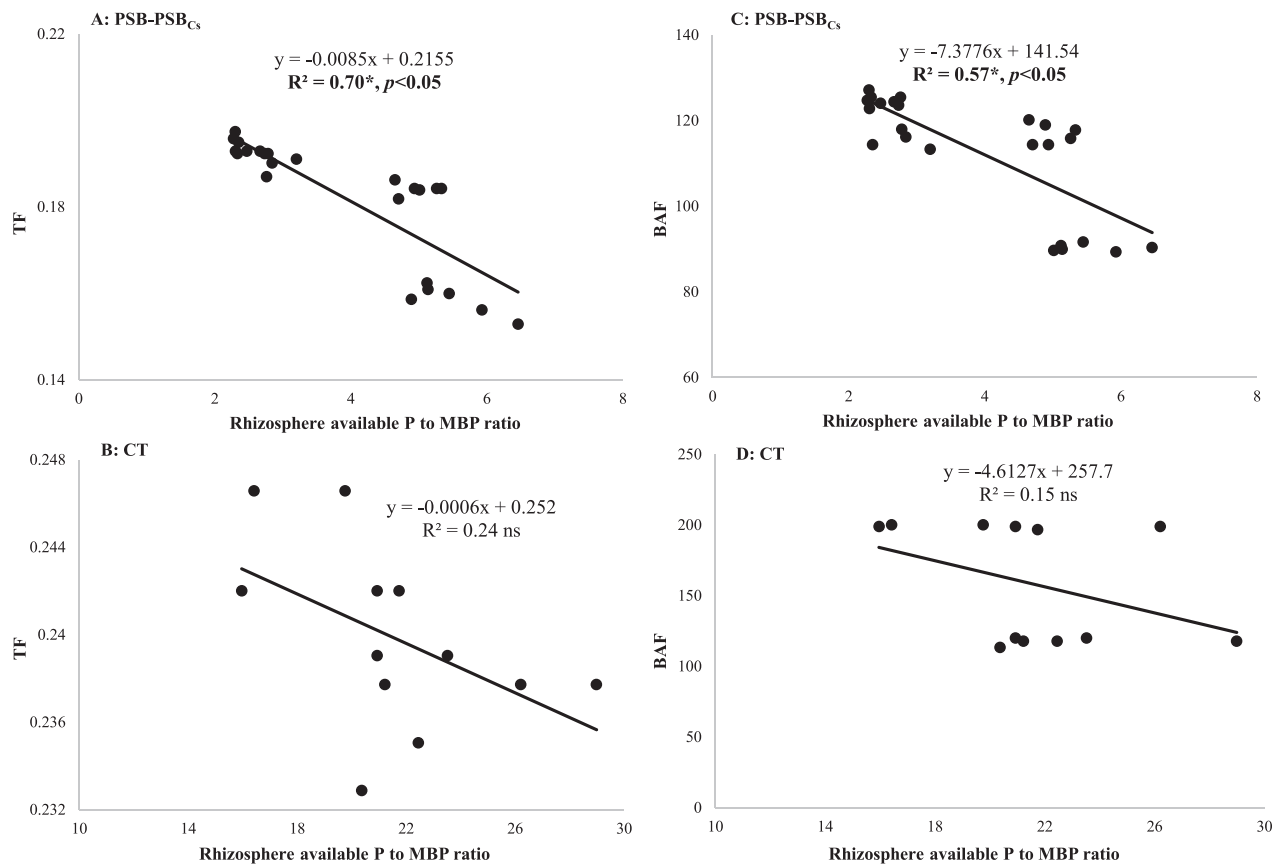
Fig. 3. Pearson correlations between rhizosphere available P to MBP ratio, TF and BAF, in 75-day old wheat plant inoculated by PSB (*Bacillus siamensis: B. siam, Rahnella aceris: R. acer, Bacillus cereus: B. cer* and their consortium: PSB$_{Cs}$) under RP fertilized and increased Cd concentrations (Cd$_0$, Cd$_8$ and Cd$_{16}$ ppm Cd^{2+}). Asterisks indicate significant differences between treatments (* $p < 0.05$; ns: not significant). The values used are averages replicates of all PSB inoculation (twenty-four replicates), compared to uninoculated control (CT: twelve replicates), under the combined Cd application of Cd$_8$-Cd$_{16}$. TF: translocation factor, BAF: bio-accumulation factor, MBP: microbial biomass P and RP: rock phosphate.

3.3. Impact of PSB on plant biomass and root morphological traits in Cd-stressed wheat

The table (Table 1) presents the effect of PSB inoculation both individually (*B. siam, R. acer, B. cer*) and in PSB$_{Cs}$ on SDW, RDW, and SpDW of RP fertilized wheat plants under increased Cd stress levels (Cd$_0$, Cd$_8$, and Cd$_{16}$). Combined application of PSB inoculation and RP fertilization showed a significant improvement in SDW, RDW, and SpDW of wheat plants compared to uninoculated and unfertilized plants, under Cd$_0$ condition. Especially, under Cd$_8$ concentration, *R. acer* inoculation significantly increased aboveground parts (SDW and SpDW) with 16 % and 3 % as compared to uninoculated plants. Under increased doses of Cd, SpDW was significantly improved up to 8 % and 35 % at both Cd concentrations (Cd$_8$ and Cd$_{16}$) in response to PSB$_{Cs}$ inoculation as compared to uninoculated plants. In addition, inoculation with *R. acer* and *B. cer* significantly increased SDW up to 30 % and 29.68 %, RDW up to 40 % and 9 %, and SpDW up to 92 % and 16 %, respectively under Cd$_{16}$ concentration compared to uninoculated plants (Table 1).

Regarding root growth, our results showed that all PSB treatments significantly enhance morphological traits (e.g., RL, RSA, and RV) at both Cd-stress concentrations (Cd$_0$, Cd$_8$, and Cd$_{16}$). The root images (supplementary file Fig. S4) confirmed that PSB inoculation significantly influenced root development under Cd stress (Table 2). Specifically, the RL was notably increased up to 29 % and 60 % in response to PSB$_{Cs}$ inoculation compared to uninoculated plants at both Cd$_8$ and Cd$_{16}$, respectively (Table 2). Additionally, inoculation with *R. Acer, B. Cer,* and PSB$_{Cs}$ showed the highest RSA under Cd$_8$ (14 %, 27 %, and 32 %, respectively), and RV under Cd$_{16}$ (50 %, 50 %, and 44 %, respectively),

compared to uninoculated plants.

3.4. Effects of PSB on wheat P content under Cd stress

The P content of shoot, spike and root significantly increased in response to all PSB inoculation under low P availability and in RP-fertilized plants without Cd addition (Cd$_0$). Under Cd$_{16}$, spike P content was significantly increased in plants inoculated with *B. siam* (45 %), *R. acer* up to (176 %), *B. cer* (62 %), and PSB$_{Cs}$ (38 %), compared to uninoculated plants (Table 3). A significant increase in shoot P content was observed in response to *B. siam* inoculation up to 71.5 % compared to uninoculated plants under Cd$_{16}$. In addition, root Pi content significantly increased in response to all PSB combinations studied, at different Cd concentrations (Cd$_8$ and Cd$_{16}$) compared to uninoculated and Cd-stressed plants (Table 3). Dual PSB x Cd application significantly affected ($p < 0.001$) wheat P content.

Fig. 6 shows the correlation between important parameters: 1/ rhizosphere available P / MBP ratio and plant P uptake, and 2/between root / rhizosphere APase ratio and plant P uptake. In response to PSB inoculation shoot ($r^2 = 0.59$, $p < 0.05$) and spike ($r^2 = 0.54$, $p < 0.05$) P content was positively correlated with rhizosphere available P / MBP ratio (Fig. 6A). Additionally, under increased Cd concentrations, PSB inoculation (both individual strains and PSB consortium) positively influenced plant P content in shoots and spikes. Moreover, root / rhizosphere APase ratio was positively correlated with shoot ($r^2 = 0.53$, $p < 0.05$) and spike P ($r^2 = 0.50$, $p < 0.05$) content of wheat plants inoculated with PSB (Fig. 6C).
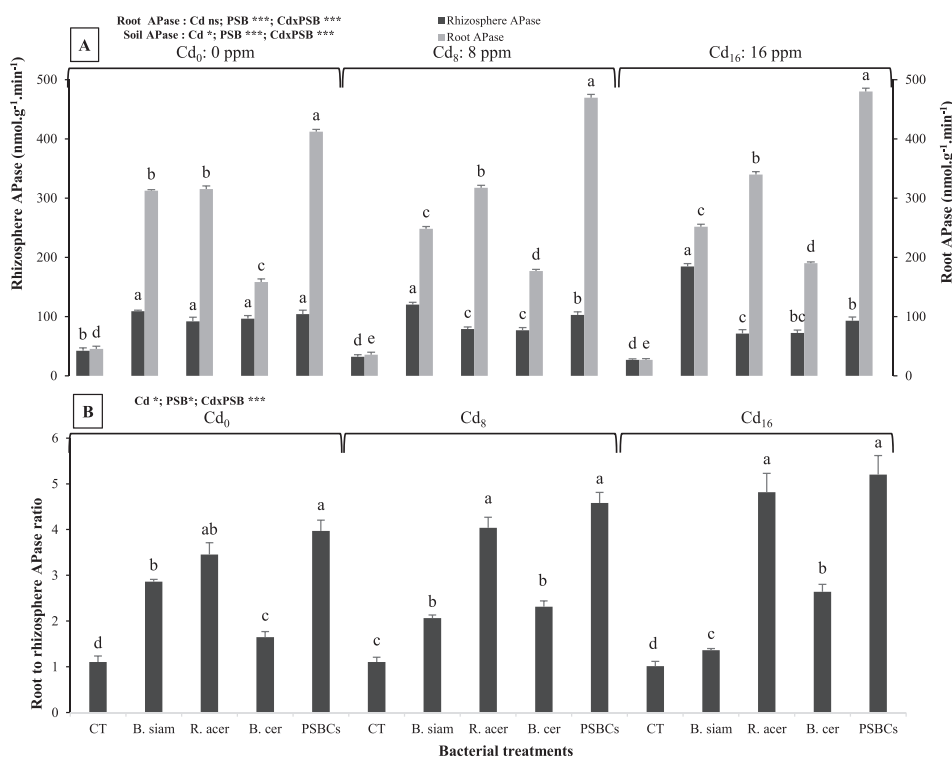
Fig. 4. Root and rhizosphere APase (A) and their ratio (B) in 75-day old wheat plant inoculated by PSB (*Bacillus siamensis: B. siam, Rahnella aceris: R. acer, Bacillus cereus: B. cer* and their consortium: PSB$_{Cs}$) under RP fertilized and increased Cd concentrations (Cd$_0$, Cd$_8$ and Cd$_{16}$ ppm Cd^{2+}). Each value represents an average of six replicates. Mean values labelled with the same superscript letter are not significantly different at $p < 0.05$ under the same Cd concentration. Asterisks indicate significant differences between Cd, PSB and their interactions (Cd x PSB) (*** $p < 0.001$; * $p < 0.05$; ns: not significant). CT: uninoculated plants, APase: acid phosphatase activity and RP: rock phosphate.

3.5. Effects of PSB on plant nutrient content (N and K) under Cd stress

Under Cd stress, wheat plants inoculated with PSB exhibited beneficial effects on shoot and spike N and K contents (Table 4). Under Cd$_{16}$, shoot N content showed enhanced up to 4 % in response to *B. siam* inoculation while *R. acer* significantly improved spike N content by 92 % compared to uninoculated plants. Although no significant differences were observed in shoot K content between inoculated and uninoculated plants, at all Cd concentrations. However, inoculation with *R. acer* notably increased spike K content by 73 % at Cd$_{16}$ compared to uninoculated plants.

3.6. Effects of PSB on proline, and chlorophyll content

Among all PSB studied, the inoculation with *B. cer* and PSB$_{Cs}$, significantly increased proline content under both Cd$_8$ (24 % and 83 %) and Cd$_{16}$ (90 % and 74 %) concentrations compared to uninoculated plants. Indeed, the proline content was higher following the inoculation with *B. siam* up to 111 % as compared to uninoculated plants specifically under Cd$_{16}$. The PSB, Cd and their interaction significantly influenced proline content at $p < 0.001$ (supplementary file Fig. S1).

In addition, PSB inoculation showed also positive effects on total chlorophyll content (supplementary file Fig. S2). The inoculation with *R. acer* significantly improved total chlorophyll content across all Cd concentrations (Cd$_0$, Cd$_8$, and Cd$_{16}$) by 12 %, 12 %, and 18 % respectively, compared to uninoculated plants. Likewise, under Cd$_{16}$ stress, the total chlorophyll content was higher up to 19 % in response to PSB$_{Cs}$ inoculation as compared to uninoculated plants (Fig. S2).

4. Discussion

This study enriches the existing knowledge regarding the ecological

advantages of bacterial inoculation using PSB on key rhizosphere processes such as P solubilization and Cd tolerance. The key findings demonstrate that application of PSB (either individually *B. siam*, *R. acer*, and *B. cer*, or PSB$_{Cs}$) can be exploited as a two-faceted strategy for enhancing stress tolerance of both Cd and low P availability. This approach improves not only the agronomic efficiency of RP, but also reduces Cd accumulation in roots and its translocation to aboveground parts. The study highlighted the impact of PSB on the reduction of Cd translocation/bioaccumulation and the improvement of P uptake through a favorable ratio between P availability in the rhizosphere (rhizosphere available P to MBP ratio) and between root to rhizosphere APase ratio. This is a novel addition to existing knowledge on soil bioremediation by providing clear evidence that activity at the root - rhizosphere interface, through mechanisms linked to P availability, is directly involved in reducing the transfer of Cd to the aerial part of durum wheat. These specific mechanisms can be explained by a positive interconnection between the morphological and physiological plant traits likely induced by PSB inoculation and manifested through the two ratios. However, the action of PSB at the root-rhizosphere interface must be maintained and optimized spatially and timely to reduce Cd concentrations to reasonable levels and increase nutrient availability as required.

4.1. Influence of Cd mobility on P availability and its impact on plant P uptake

Correlation between Cd translocation/bioaccumulation and rhizosphere P availability.

Inoculation with PSB, in particular *B. cer*, significantly reduced Cd mobility (at both Cd concentrations) from rhizosphere to plants through reducing both Cd bioaccumulation and translocation in plants (Figs. 1A, 1B). These results demonstrate that inoculation with PSB reduced the Cd
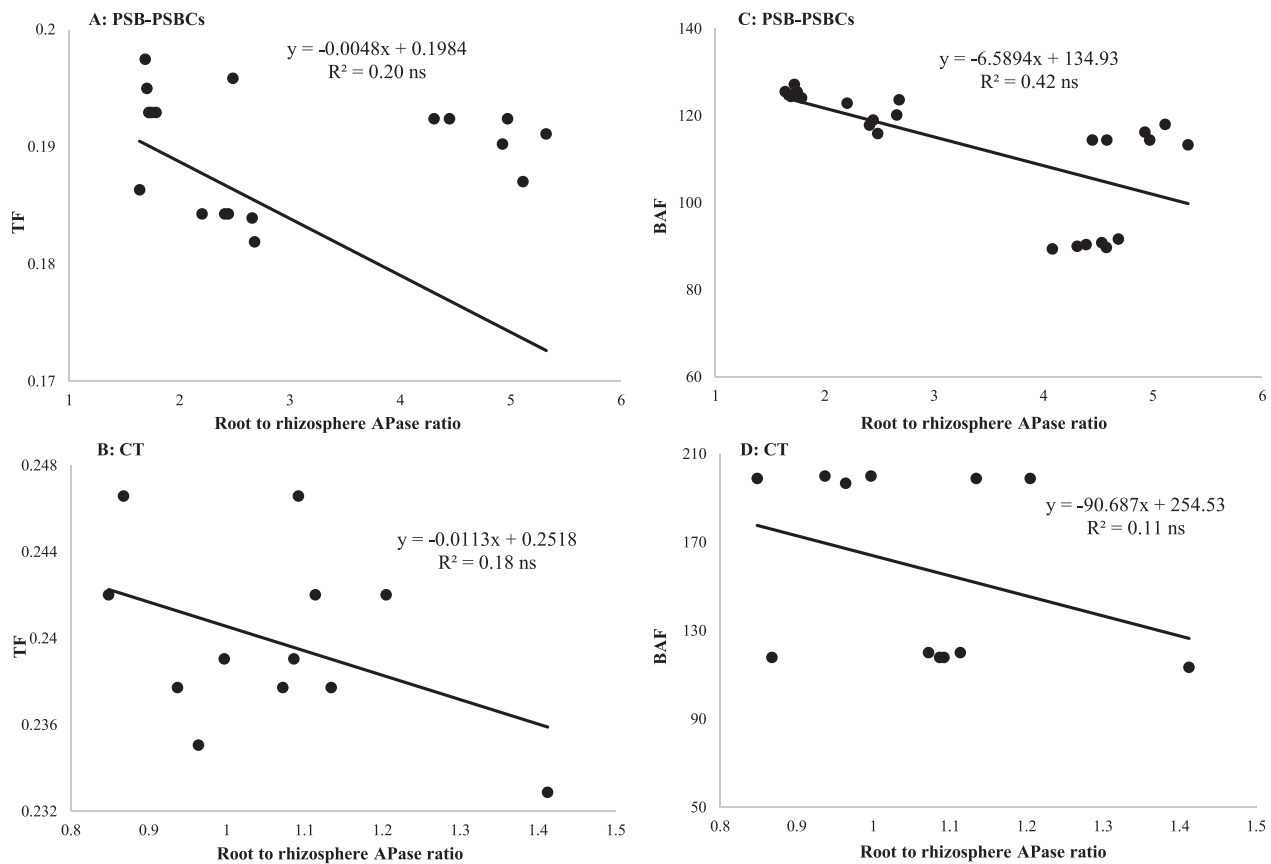
Fig. 5. Pearson correlations between root to rhizosphere APase ratio, TF and BAF, in 75-day old wheat plant inoculated by PSB (*Bacillus siamensis: B. siam, Rahnella aceris: R. acer, Bacillus cereus: B. cer* and their consortium: PSB_{Cs}) under RP fertilized and increased Cd concentrations (Cd_0, Cd_8 and Cd_{16} ppm Cd^{2+}). Asterisks indicate significant differences between treatments (* $p < 0.05$; ns: not significant). The values used are averages replicates of all PSB inoculation (twenty-four replicates), compared to uninoculated control (CT: twelve replicates), under the combined Cd application of Cd_8-Cd_{16}. TF: translocation factor, BAF: bioaccumulation factor, APase: acid phosphatase activity and RP: rock phosphate.

Table 1
Biomass of shoot (SDW), root (RDW and spike (SpDW), in 75-day old wheat plant inoculated by PSB (*Bacillus siamensis: B. siam, Rahnella aceris: R. acer, Bacillus cereus: B. cer*, and their consortium: PSB_{Cs}) under RP fertilized and increased Cd concentrations (Cd_0, Cd_8 and Cd_{16} ppm Cd^{2+}). Each value represents an average of six replicates. Mean values labelled with the same superscript letter are not significantly different at $p < 0.05$ under the same Cd concentration. Asterisks indicate significant differences between Cd, PSB, and their interactions (Cd x PSB) (*** $p < 0.001$; ns: not significant). CT: uninoculated plants; SDW: shoot dry weight; RDW: root dry weight; SpDW: spike dry weight and RP: rock phosphate.

Bacterial treatments	SDW (g.pot⁻¹)			RDW (mg.pot⁻¹)			SpDW (mg.pot⁻¹)		
	Cd_0	Cd_8	Cd_{16}	Cd_0	Cd_8	Cd_{16}	Cd_0	Cd_8	Cd_{16^2}
CT	1.31[c]	1.81[bc]	1.54[b]	262.60[d]	450.00[b]	328.30[d]	533.30[e]	723.30[c]	525.00[d]
B. siam	2.34[a]	1.81[bc]	1.70[b]	478.30[a]	420.00[c]	407.60[b]	950.00[a]	625.00[d]	493.30[d]
R. acer	2.04[a]	2.10[a]	2.01[a]	433.30[b]	440.00[b]	460.00[a]	880.00[b]	743.30[b]	1007.60[a]
B. cer	1.36[bc]	1.62[c]	2.00[a]	490.00[a]	483.30[a]	357.60[c]	767.60[c]	720.00[c]	607.60[c]
PSB_Cs	1.66[b]	1.98[ab]	1.62[b]	355.00[c]	415.00[c]	327.60[d]	633.30[d]	783.30[a]	708.30[b]
Cd, PSB, Cd x PSB	***	***	***	***	***	***	ns	***	***

uptake, and thus reducing its toxicity. This could be presumably done through either Cd sequestration or inducing functional root traits limiting Cd absorption, which significantly reduce its translocation to root and shoot (Mitra et al., 2018a). These results are consistent with studies by Ahmad et al. (2014) and Gadd, (2004), who reported that rhizosphere bacteria exude metals-chelating agents (e.g., organic acids, EPS, biofilm, and siderophores) exhibiting, which significantly reduce their availability in the rhizosphere as well as their uptake by plants (Madhaiyan et al., 2007; Kuffner et al., 2008; Tiwari et al., 2008; Park et al., 2011; Dourado et al., 2013). The decreased Cd mobility along with increased rhizosphere P availability observed in this study (Fig. 2A) could be explained by a mutualization of the P release and Cd chelation

processes through organic acid production by PSB (Qin et al., 2023; Ibnyasser et al., 2024). This is confirmed by the significant interaction observed between Cd and PSB co-application on Cd translocation/bioaccumulation and P bioavailability in the rhizosphere, showing that inoculation simultaneously affected Cd mobility and P availability in the rhizosphere ($p < 0.001$).

Our results suggest that PSB significantly improve P availability and MBP under Cd stress (Fig. 2A). Previous results have reported that the application of Cd-tolerant PSB significantly increased the P available content in the rhizosphere via producing organic acids (Chen et al., 2006; Yuan et al., 2017; Tang et al., 2018; Afraz et al., 2021). However, the available literature failed to establish a comprehensive mechanism

Table 2

Root morphological traits (RL, RSA and RV), in 75-day old wheat plant inoculated by PSB (*Bacillus siamensis*: *B. siam*, *Rahnella aceris*: *R. acer*, *Bacillus cereus*: *B. cer*, and their consortium: PSB_{Cs}) under RP fertilized and increased Cd concentrations (Cd_0, Cd_8 and Cd_{16} ppm Cd^{2+}). Each value represents an average of six replicates. Mean values labelled with the same superscript letter are not significantly different at $p < 0.05$ under the same Cd concentration. Asterisks indicate significant differences between Cd, PSB and their interactions (Cd x PSB) (*** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; ns: not significant). CT: uninoculated plants; RL: root length; RSA: root surface area; RV: root volume and RP: rock phosphate.

Bacterial treatments	RL (cm)			RSA (cm^2)			RV (cm^3)		
	Cd_0	Cd_8	Cd_{16}	Cd_0	Cd_8	Cd_{16}	Cd_0	Cd_8	Cd_{16}
CT	393.36[c]	363.92[d]	285.17[c]	26.32[c]	26.85[c]	23.15[c]	0.14[b]	0.16[c]	0.16[c]
B. siam	478.70[ab]	407.22[c]	397.80[b]	34.95[a]	29.94[b]	32.30[b]	0.21[a]	0.19[b]	0.21[b]
R. acer	486.91[a]	430.34[b]	426.24[ab]	33.84[a]	32.85[ab]	42.40[a]	0.19[a]	0.20[b]	0.24[a]
B. cer	449.33[b]	431.42[b]	305.60[c]	35.56[a]	34.20[a]	32.62[b]	0.23[a]	0.23[a]	0.24[a]
PSB_{Cs}	488.10[a]	471.00[a]	455.70[a]	31.07[b]	35.42[a]	31.06[b]	0.19[a]	0.21[b]	0.23[a]
Cd, PSB, Cd x PSB	***	***	***	ns	***	***	**	*	ns

Table 3

Total P content of spike, shoot and root Pi, in 75-day old wheat plant inoculated by PSB (*Bacillus siamensis*: *B. siam*, *Rahnella aceris*: *R. acer*, *Bacillus cereus*: *B. cer*, and their consortium: PSB_{Cs}) under RP fertilized and increased Cd concentrations (Cd_0, Cd_8 and Cd_{16} ppm Cd^{2+}). Each value represents an average of six replicates. Mean values labelled with the same superscript letter are not significantly different at $p < 0.05$ under the same Cd concentration. Asterisks indicate significant differences between Cd, PSB and their interactions (Cd x PSB) (*** $p < 0.001$). CT: uninoculated plants, Pi: inorganic P and RP: rock phosphate.

Bacterial treatments	Spike P content (mg.pot^{-1})			Shoot P content (mg.pot^{-1})			Root Pi content (µg.g^{-1})		
	Cd_0	Cd_8	Cd_{16}	Cd_0	Cd_8	Cd_{16}	Cd_0	Cd_8	Cd_{16}
CT	0.77[d]	1.20[a]	0.68[c]	0.87[c]	1.27[a]	1.02[b]	17.88[c]	14.71[c]	13.96[d]
B. siam	1.61[a]	1.08[ab]	0.99[b]	1.56[a]	0.90[b]	1.75[a]	25.61[a]	27.09[ab]	30.63[a]
R. acer	1.64[a]	0.99[b]	1.88[a]	1.28[b]	1.11[a]	1.00[b]	22.02[ab]	28.57[a]	29.11[b]
B. cer	1.28[b]	1.09[b]	1.1[b]	0.81[c]	0.89[b]	1.00[b]	21.46[b]	21.94[b]	23.64[c]
PSB_{Cs}	1.05[c]	0.99[b]	0.94[b]	1.27[b]	0.85[b]	0.98[b]	21.42[b]	29.10[a]	30.30[a]
Cd, PSB, Cd x PSB	***	***	***	***	***	***	***	***	***

that likely link the increased P availability and Cd tolerance in response to inoculation with PSB. Hence, our study demonstrates that Cd-tolerant PSB, in addition to producing acids to help improve P availability (Ibnyasser et al., 2024), accumulate P as MBP providing a pool of P available in the short -medium term (Khourchi et al., 2023b). This MBP probably contributes to improving P availability and Cd chelation in the soil, which is supported by a significant negative correlation ($r^2 = 0.70$, $p < 0.05$) between the Cd translocation factor and the rhizosphere available P to MBP ratio (Fig. 3A).

To understand the mechanism involved in improving Cd tolerance, the present study suggests a ratio of rhizosphere-available P to MBP as an indicator of the ability of PSB inoculation to improve rhizosphere P availability, while minimizing Cd mobility (Fig. 2B). This important belowground trait can presumably explain the tight relationship between P availability and Cd mobility. Indeed, the translocation and bioaccumulation factors of Cd in plants were negatively correlated ($r^2 = 0.70$ and $r^2 = 0.57$ $p < 0.05$, respectively) with the rhizosphere available P to MBP ratio in response to PSB inoculation (Figs. 3A, 3C), reflecting that low MBP and high available P in the rhizosphere favors a drastic decrease in Cd mobility. This shows that uniform distribution of available P in the rhizosphere and MBP are the two key factors in both P incorporation in the MBP pool and reduced Cd mobility. This corroborates with previous studies carried out by Qin et al. (2023) and Zhang et al. (2023) suggesting that MBP may contribute to Cd immobilization via different mechanisms including 1) immobilization of Cd in cell walls, 2) induction of Cd precipitation in the presence of P soluble 3) binding of Cd with calcium or iron P leading to a reduction in available Cd content in rhizosphere soil. This was also demonstrated by Han et al. (2024) and Ma et al. (2022) that available P in the soil plays an important role in inhibiting Cd uptake by crops and its accumulation in wheat grains. Overall, our results suggest that the decrease in Cd translocation to the plant may be tightly associated with bio-solubilization mechanisms (especially organic acids), resulting in both enhanced P availability (through favorable ratio of available P to MBP) and Cd immobilization/precipitation (Figs. 3A, 3C).

Rhizosphere P availability affects plant P uptake under Cd stress.

Our findings showed that spike, shoot and root P contents were significantly increased in response to PSB inoculation, with the highest contents in response *B. siam* under Cd_{16} (spike P: 46 %; shoot P: 72 %; root Pi: 119 %) (Table 3). This increase can be attributed to the ability of PSB to produce organic acids, phosphatases (Zhang et al., 2023), and siderophores (Chen et al., 2018) under Cd stress, with double action enabling increased P availability and serving as Cd chelators allowing simultaneous increase of Cd detoxification, and P availability (Dixit et al., 2022; Qu et al., 2022). Additionally, improved P nutrition was accompanied by enhanced N and K contents in both spikes and shoots (Table 4). In this regard, previous studies have demonstrated that inoculation with individual PSB significantly improved mineral nutrition (P, N, and K) and reduced Cd uptake in watermelon plants (Zhang et al., 2022). Considered together, these results suggest that PSB can induced the efficient use of various nutrients by secreting relevant substances (e.g., organic acids and phosphatase enzymes) that are beneficial to plant physiological activities or by converting complex soil-substances into plant available forms (Ju et al., 2020; Naqqash et al., 2016). Considering that our PSB (individually and in consortium) reduced Cd mobility and improved P, N, and K uptake, a positive correlation (shoot: $r^2 = 0.59$ and spike: $r^2 = 0.54$, $p < 0.05$) was found between plant P uptake and the rhizosphere available P to MBP ratio (Fig. 6A). This sufficiently demonstrates the link between rhizosphere P availability, Cd mobility to the plant and plant P uptake. These results are coherent with above-mentioned findings highlighting the importance this rhizosphere ratio as relevant indicator for enhanced Cd tolerance and P acquisition under increasing Cd concentrations.

The PSB-induced phosphatases activities in root / rhizosphere mediated the increased P availability and Cd mobility.

In addition to organic acids production, the increased rhizosphere P availability in response to PSB is often attributed to increased phosphatase activity in the root-rhizosphere interface. Phosphatases participate in the mineralization of organic phosphates, thus enhancing soil P availability (Huang et al., 2024; Yu et al., 2020). Our findings showed PSB inoculation significantly induced an increase of APase activity in root and rhizosphere after inoculation (Fig. 4A), particularly PSB_{Cs},
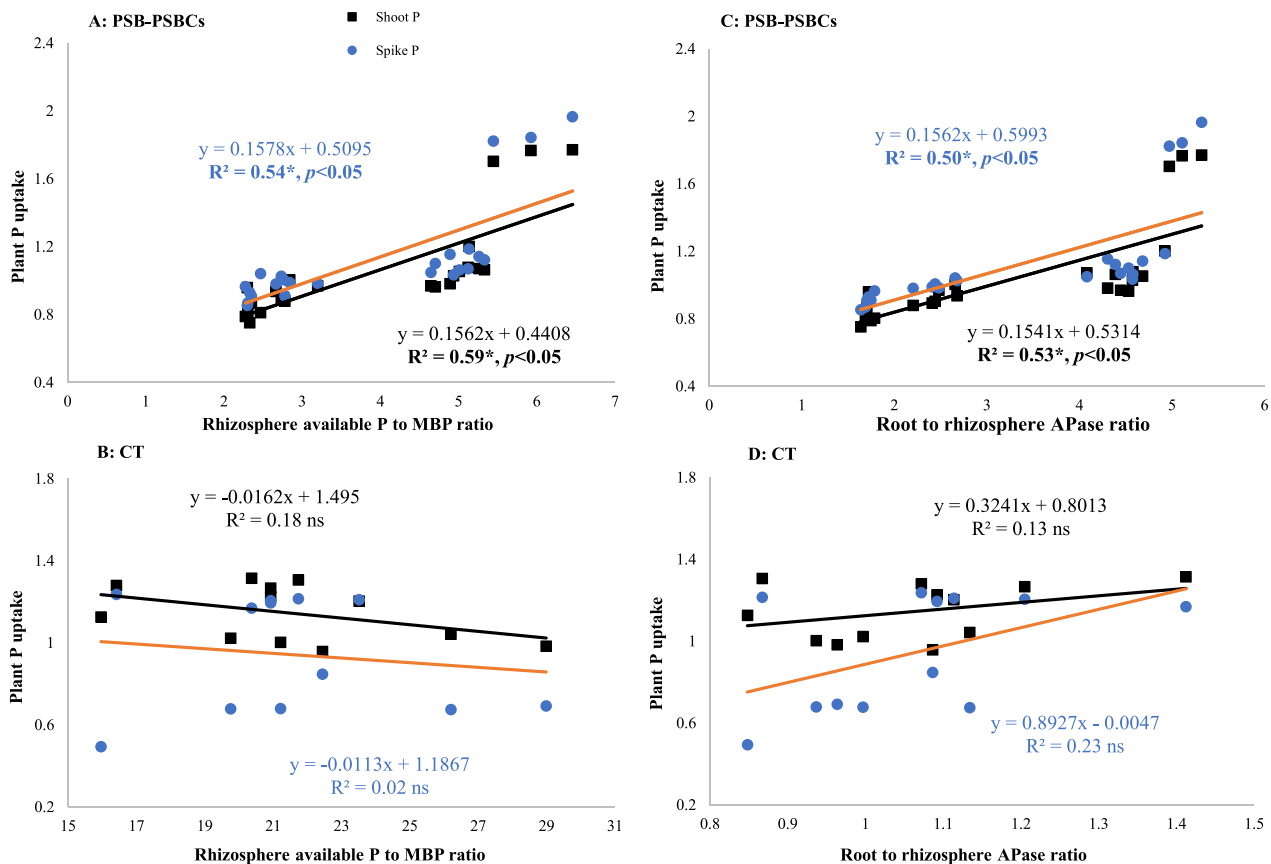
Fig. 6. Pearson correlations between rhizosphere available P / MBP ratio and plant P uptake (A: inoculated and B: uninoculated plant), and between root / rhizosphere APase ratio and plant P uptake (C: inoculated and D: uninoculated plant), in 75-day old wheat plant inoculated by PSB (*Bacillus siamensis: B. siam, Rahnella aceris: R. acer, Bacillus cereus: B. cer* and their consortium: PSB$_{Cs}$) under RP fertilized and increased Cd concentrations (Cd$_0$, Cd$_8$ and Cd$_{16}$ ppm Cd^{2+}). Bleu circles represent spike P content and black square represent shoot P content. Asterisks indicate significant differences between treatments (* $p < 0.05$; ns: not significant). The values used are averages replicates of all PSB inoculation (twenty-four replicates), compared to uninoculated control (CT: twelve replicates), under the combined Cd application of Cd$_8$-Cd$_{16}$. MBP: microbial biomass P, APase: acid phosphatase activity and RP: rock phosphate.

Table 4

Total N and K content of shoot and spike, in 75-day old wheat plant inoculated by PSB (*Bacillus siamensis: B. siam, Rahnella aceris: R. acer, Bacillus cereus: B. cer*, and their consortium: PSB$_{Cs}$) under RP fertilized and increased Cd concentrations (Cd$_0$, Cd$_8$ and Cd$_{16}$ ppm Cd^{2+}). Each value represents an average of six replicates. Mean values labelled with the same superscript letter are not significantly different at $p < 0.05$ under the same Cd concentration. Asterisks indicate significant differences between Cd, PSB and their interactions (Cd x PSB) (*** $p < 0.001$; * $p < 0.05$). CT: uninoculated plants and RP: rock phosphate.

Bacterial treatments	Shoot						Spike					
	N (mg.pot^{-1})			K (mg.pot^{-1})			N (mg.pot^{-1})			K (mg.pot^{-1})		
	Cd$_0$	Cd$_8$	Cd$_{16}$	Cd$_0$	Cd$_8$	Cd$_{16}$	Cd$_0$	Cd$_8$	Cd$_{16}$	Cd$_0$	Cd$_8$	Cd$_{16}$
CT	19.30[ab]	20.23[bc]	19.81[b]	18.10[bc]	26.36[a]	22.50[ab]	10.25[a]	14.40[a]	9.70[c]	4.80[d]	6.53[a]	4.33[b]
B. siam	20.94[a]	15.10[d]	20.62[a]	24.00[a]	17.90[b]	19.62[bc]	12.41[a]	9.70[b]	8.60[c]	7.53[a]	4.74[c]	4.64[b]
R. acer	16.93[ab]	22.04[ab]	18.50[b]	21.70[ab]	23.60[a]	24.20[a]	13.26[a]	11.02[b]	18.60[a]	6.80[ab]	5.23[bc]	7.51[a]
B. cer	15.50[b]	18.52[c]	19.30[b]	16.63[c]	19.51[b]	23.02[ab]	12.20[a]	12.15[ab]	10.60[bc]	6.34[bc]	5.51[bc]	5.30[b]
PSB$_{Cs}$	20.30[a]	24.40[a]	15.56[c]	21.13[ab]	23.34[a]	17.81[c]	10.73[a]	13.70[a]	11.86[b]	5.46[cd]	5.74[ab]	5.14[b]
Cd, PSB, Cd x PSB	*	***	***	***	***	***	***	***	***	***	***	***

which is consistent with findings by Prasad et al. (2012) that *Pseudomonas fluorescens* enhanced root APase of *Chrysanthemum indicum L*, and consequently facilitated plant P acquisition. Our study indicates for the first time that the root / rhizosphere APase ratio under both Cd$_8$ and Cd$_{16}$ significantly increased in response to inoculation with PSB$_{Cs}$ compared to uninoculated plants (Fig. 4B). Moreover, this root / rhizosphere APase ratio positively correlated (shoot: r^2=0.53 and spike: r^2= 0.50, $p < 0.05$) with plant P uptake (Fig. 6C). The increase in root to rhizosphere APase ratio, reflecting low rhizosphere APase and high root APase, presumably resulted in increased internal P utilization efficiency and shoot P allocation (Abd_Allah et al., 2015). This finding was

supported by Ma et al. (2022) stating that wheat roots must release more phosphatases to catalyze the hydrolysis of a portion of phosphate (organic) to meet its P demand when soil P content is low.

A non-significant negative correlation was found between the root / rhizosphere APase ratio and Cd translocation (TF) (r^2=0.20, ns) and bioaccumulation (BAF) (r^2=0.42, ns) in response to PSB inoculation (Figs. 5A, 5C). This suggests that a high root / rhizosphere APase ratio in the root-rhizosphere interface could contribute not only to P mobilization but also probably to Cd complexation (via substrate active site binding) in the rhizosphere and consequently reduce its efflux to the plant (Bellomo et al., 2016; Maret, 2013). Although the limited studies

investigating interactions between phosphatases enzymes and Cd immobilization and availability, Michel et al. (2000) reported that *Desulfovibrio desulfuricans* can enzymatically reduce uranium to uranite UO_2, which could be the case for Cd.

Inoculation with PSB improved above and belowground growth performance of wheat under Cd stress.

In this study, PSB inoculation significantly enhanced SDW, RDW and SpDW under increasing Cd concentration (Table 1), confirming the ability of these bacterial inoculants to enhance wheat tolerance to Cd. Our results are similar to the findings of Jeong et al. (2012), who demonstrated that inoculation of *Brassica juncea* with the PSB *Bacillus megatorium*, under Cd application, significantly improved total biomass from 0.087 to 0.448 g compared with the uninoculated control. Furthermore, Wang et al. (2016) demonstrated that inoculation with Cd-tolerant bacteria producing siderophores and IAA improved significantly plant biomass of Chinese cabbage and radish under Cd. Improved plant biomass could be partly explained by the fact that PSB can accumulate and immobilize significant amounts of Cd, reducing its translocation to the plant, which has already been reported as one of the bacterial mechanisms for mitigating the deleterious effects of Cd on plant growth (Ahmad et al., 2014; Huang et al., 2015; Lin et al., 2016).

Moreover, our findings demonstrated that PSB inoculation, particularly *R. acer* and PSB$_{Cs}$, improves wheat morphological root traits (RL, RSA, and RV) (Table 2 – supplementary file Fig. S4). These findings suggested that PSB induced adaptive morphological root traits enabling both Cd tolerance and enhanced P uptake. This was consistent with previous studies highlighting that morphological and anatomical root traits are significantly involved to enhancing Cd tolerance (Huang et al., 2015; Yang et al., 2019), as well as in essential functions such as water and nutrient uptake for plant growth (Barber, 1995; Ibnyasser et al., 2024). For instance, Zhang et al. (2022) reported wheat plants exposed to Cd stress developed thicker root and significant deposition of the apoplastic barrier in their roots as defensive strategy to reduce Cd uptake its translocation to shoot. More recently, Ibnyasser et al. (2024) reported that inoculation with Cd-tolerant PSB induced proliferation of root hairs in both piliferous zone and root cap under increasing Cd concentration, which enhanced the absorption of water and nutrient to the detriment of Cd. In addition, finding by Mitra et al. (2018a), (2018b) found that Cd-tolerant bacteria (*Klebsiella michiganensis* and *Enterobacter sp.*) significantly improved seedling vigor index, length and dry weigh of root and shoot, thus mitigating the negative effect of Cd on rice rooting system. All these root morphological changes (RL, RSA, RV) stimulated by direct or indirect mechanisms of PSB strongly contribute to the defensive adaptation to decrease Cd translocation and accumulation in plant tissues (Sabella et al., 2022) and consequently mitigate heavy metal stress (Huang et al., 2015, 2014; Jinadasa et al., 2016).

Proline is one of the most frequently accumulated non-enzymatic antioxidants involved in the internal detoxification of metal-induced phytotoxicity (Islam et al., 2016). Our results show that inoculation with PSB$_{Cs}$ significantly improved proline concentrations at Cd$_8$ and Cd$_{16}$ compared to Cd-stressed and uninoculated plants (supplementary file Fig. S1), suggesting that PSB may alleviate the negative effects of Cd on plant growth through inducing accumulation proline and thus detoxification. These results are supported by Jan et al. (2019), where inoculation with *Bacillus Cereus* increased proline content in rice plants compared to uninoculated plants under Cd stress. Proline is known to sequester free radicals, stabilize the proteins and cell membrane, and improve cellular redox buffering (Kamran et al., 2015). Under abiotic stress (Cd), the plant increases the synthesis and accumulation of osmolytes such as proline to maintain tissue water content and plant growth (Hashem et al., 2016).

Photosynthetic pigments such as chlorophyll a and b are the basis of photosynthesis. Inhibition of their synthesis and function under Cd stress could significantly reduce the photosynthetic activity (Somashekaraiah et al., 1992). Our findings showed that inoculation with *R. acer* significantly increased total chlorophyll content (supplementary file Fig. S2), under both Cd concentrations. These results confirm that Cd mitigating potential of PSB inoculation can be extended to improvement of photosynthesis efficiency and better physiological performance of wheat under Cd stress. Similar results were observed in *Plantago ovata* (Haneef et al., 2014) and rice seedlings (Pramanik et al., 2016) exposed to Cd stress.

5. Conclusions

This study shows that inoculation with Cd-tolerant PSB improves both P acquisition and Cd tolerance in durum wheat by favorably adjusting the balance between rhizosphere available P / MBP pools, influencing root to rhizosphere APase ratio, modulating morphophysiological root traits, and reducing the mobility of Cd from the rhizosphere to the plant. This adjustment involved a simultaneous improvement of both P availability and Cd tolerance, as both processes shared organic acids exudation (serving as chelators of Cd and increased the availability of RP) as the key mechanism responsible for enhanced P availability under Cd stress. Inoculation with PSB$_{Cs}$ induced a significant increase of APase activity in root and rhizosphere soil, which probably led to increased P availability and wheat P use efficiency. Bacterial inoculation limited the translocation of Cd to aboveground plants through strong immobilization of Cd in the soil through intense rhizosphere activity probably implicating exudations (organic acids and phosphatase enzymes), resulting in high P use by the PSB in terms of microbial biomass. In addition, the beneficial effects of PSB under combined Cd-stress and P-limited conditions on morpho-physiological characteristics such as plant biomass, nutrient's allocation to shoots and spikes and photosynthetic performance, suggest that PSB through their multifunctional activities (mainly P solubilization and Cd immobilization), may contribute to a coordinated action between the above and below-ground traits of wheat, resulting in improved rhizosphere P availability, P acquisition and reduced Cd mobility in the rhizosphere soil, which positively influence P use efficiency and wheat yield under Cd stress conditions. However, additional inoculation experiments under controlled and field conditions are needed to confirm the observed effects and to investigate the importance of microbial diversity and key plant gene expression plausibly responsible for enhanced wheat tolerance to Cd stress.

CRediT authorship contribution statement

Abdellatif Barakat: Writing – review & editing, Methodology. **Adnane Bargaz:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Conceptualization. **Meryem Haddine:** Methodology. **Rachid Ghani:** Methodology. **Abdellatif Elghali:** Methodology. **Abdallah Oukarroum:** Methodology, Investigation. **Ammar Ibnyasser:** Writing – review & editing, Writing – original draft, Software, Methodology, Investigation, Conceptualization. **Rym Saidi:** Methodology. **Wissal Elhaissoufi:** Methodology. **Said Khourchi:** Methodology.

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.

Appendix A

$$Translocation\ factor(TF) = \frac{Cd\ concentration\ in\ shoot}{Cd\ concentration\ in\ root} \tag{A1}$$

$$Bioaccumulation\ factor(BAF) = \frac{Cd\ concentration\ in\ shoot}{Cd\ concentration\ in\ soil} \tag{A2}$$

$$MBP(ppm) = \frac{\Delta P}{K_{EP}} \tag{A3}$$

$$Ratio\ 1 = \frac{Rhizosphere\ available\ P}{Microbial\ biomass\ P} \tag{A4}$$

$$Ratio\ 2 = \frac{Root\ APase}{Rhizosphere\ APase} \tag{A5}$$

$$Proline\ (\mu mol.\ g^{-1})\ FW = \frac{X \times (\frac{V\ toluene}{115.5})}{FW} \tag{A6}$$

$$Chlt\ (mg.g^{-1})\ =\ 8.2 \times (A663) + 20.20 \times (A645) \tag{A7}$$

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2024.117402.

Data Availability

Data will be made available on request.

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