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RESEARCH ARTICLE

Bacillus megaterium compensates for growth inhibition from phosphorus deficiency by improving photosynthetic capacity, changing antioxidant potential, and regulating non-structural carbohydrate concentrations in *Glycyrrhiza uralensis*

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ABSTRACT

This study assessed the effect of *Bacillus megaterium* on seedling growth of *Glycyrrhiza uralensis* Fisch. under control and phosphorus (P) deficiency conditions. The results showed that P deficiency improved 1) *G. uralensis* root growth, 2) superoxide dismutase, catalase, and peroxidase activities, 3) inorganic P, starch, and soluble sugar contents in roots, 4) dissipated energy flux per reaction center, trapped energy flux per reaction center, and absorption flux per reaction center, and 5) net photosynthetic rate, stomatal conductance, transpiration rate, maximum fluorescence intensity after dark adaptation, and variable fluorescence. However, P deficiency significantly decreased chlorophyll and carotenoid contents in *G. uralensis*, but enhanced chlorophyll and carotenoid contents in *B. megaterium*. Our findings on the regulatory mechanisms of *B. megaterium* in response to P starvation hold promise for improving the success of *G. uralensis* cultivation.

Keywords: *Bacillus megaterium*; *Glycyrrhiza uralensis* Fisch; Phosphorus deficiency; Chlorophyll fluorescence; Antioxidant enzymes superoxide; Non-structural carbohydrate

INTRODUCTION

As a crucial structural and regulatory macro-element for plant development (Zhong et al., 2023), phosphorus (P) not only contributes to the synthesis of plant components but also engages in signal transduction in photosynthesis, respiration, and other physiological processes (Rubio et al., 2001; Wang et al., 2010; Cheng et al., 2011). Although P is generally rich in soil, it is rapidly immobilized in soil organic and inorganic components, making it a constraining factor for plant development in numerous natural and agricultural ecosystems (Hinsinger et al., 2011; Richardson et al. 2009; Rouached et al., 2010). P deficiency has the potential to decrease photosynthetic rate at multiple levels. For instance, inorganic phosphate (Pi) serves as a substrate or activity modulator of various enzymes affecting photosynthesis and carbohydrate metabolism. While P deficiency can reduce electron transport rates and light-use efficiency (Zhang et al., 2019), certain studies have indicated that the photosynthetic rate per unit leaf area might remain unaffected (Balemi et al., 2011).

Under P deficiency, plants may generate cellular reactive oxygen species (ROS), activating superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) (Liu et al., 2021). Plants may also employ adaptive strategies to

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deal with P deficiency, such as altering root architecture, enhancing P-related gene expression, and adjusting starch-to-sucrose ratios (Ghannoum and Conroy, 2007). Soluble sugars, particularly those interacting with membranes, are effective ROS scavengers in plants (Keunen et al., 2013). Moreover, studies have indicated that soluble sugars could be transported to non-photosynthetic tissues to regulate the balance of non-structural carbohydrates (NSC, which consist of soluble sugars and starch) in the entire plant under stress (Li et al., 2023). However, these mechanisms have little effect on plant growth under conditions of extreme P deficiency. To overcome this negative impact on crop productivity caused by low P, farmers often use excessive P fertilizer, resulting in heavy metal contamination. Therefore, it becomes imperative to increase P absorption using an environmentally friendly biological or physicochemical approach.

Soil microbial organisms, particularly plant growth-promoting rhizobacteria (PGPR), are the driving force for nutrient mobility and transformation in terrestrial ecosystems (Gyaneshwar et al., 2002). Their metabolic activities also directly or indirectly affect soil physicochemical properties. Among PGPR, *Bacillus* and *Pseudomonas* have been found to be important genera (Radhakrishnan et al., 2017). *Bacillus* species are ubiquitous in nature and are widely studied to generate bioactive metabolites and nutrients for promoting plant development (Shi et al., 2022). In addition, they are the most efficient P-solubilizing microorganisms in the rhizospheres of legumes (Wani et al., 2007) and are utilized as microbial inoculants to promote plant yield (Rebi et al., 2022). Studies have demonstrated that *Bacillus megaterium* strains KAP5 and KAP6, which concurrently possess high P-solubilizing capabilities and 1-aminocyclopropane-1-carboxylate deaminase activities, boosted wheat development compared to other *B. megaterium* strains with only one of these traits (Baig et al., 2012). Moreover, *Bacillus safensis*, an efficient PGPR with P-solubilizing abilities, could promote *Mentha arvensis* L. growth and oil content (Prakash and Arora, 2019). Furthermore, *B. megaterium* is superior to *B. safensis* as a P-solubilizing agent (Mukhtar et al., 2017). Thus, using PGPR may be better for the environment and plant growth than fertilizer applications.

Legume crops, including licorice (*Glycyrrhiza uralensis* Fisch.), a perennial medicinal plant primarily grown in the arid loess plateau where P is usually a limiting macronutrient, require more P for nitrogen (N)-fixation (Zhong et al., 2023). In particular, licorice demands high P in the early growth stages (Xie et al., 2022). However, there is limited understanding regarding the potential of *Bacillus megaterium* to induce resistance to low P in licorice. This investigation aimed to evaluate the influences of (i) P nutrition and (ii) applying *B. megaterium* on photosynthetic efficiency, antioxidant activity, and inorganic P, starch, soluble sugars, chlorophyll, and carotenoid contents in licorice under normal conditions and P deficiency.

MATERIALS AND METHODS

Materials and treatment

Licorice (*G. uralensis* Fisch.) seeds were obtained from Xinjiang Uyghur Autonomous Region. Healthy and full seeds were selected, disinfected for 30 min with 30% H_2O_2, and germinated in a climatic chamber at 25 °C. Fifteen seedlings with uniform radicles of 1 cm were transplanted into hydroponic boxes loaded with 100 g of perlite containing 0.2% $Ca_3(PO_4)_2$ in a greenhouse with a 12 h light period, 60%–80% relative humidity, and 3000 lx light intensity at 26/20°C day/night. The seedlings were treated with Hoagland's complete nutrient solution (CK) or Hoagland's nutrient solution without P (-P). Plants in both CK and -P groups were further assigned into two subgroups and watered with 100 mL distilled water with or with *B. megaterium* (2.5×10^5 cfu·mL^{-1}). *B. megaterium* was purchased from Beihai Yishengwang Biotechnology Co., Ltd., Guangxi, China. Each treatment had nine biological replicates.

Biomass production

After 35 d of treatment, seedlings were collected and quickly rinsed with distilled water. After the removal of surface water using absorbent paper, they were dehydrated at 105 °C for 15 min and at 80 °C to reach a consistent weight. Leaf, stem, and root biomasses were individually determined. The plant's height and the main root length were determined using a ruler with an accuracy of 0.1 cm.

Antioxidant enzyme extraction and determination

Enzyme extraction

Licorice seedlings (0.5 g) were pulverized into powder in liquid nitrogen and subsequently homogenized in 10 mL of 50 mM phosphate buffered saline, pH 7.8, with 1% polyvinylpolypyrrolidone. After centrifugal separation at 4°C for 20 min at 12000 rpm, the supernatant was collected as crude enzyme for subsequent analysis. All absorbance values were measured using a double-beam UV-VIS spectrophotometer (Shimadzu UV 2600, Shimadzu Co., Kyoto, Japan).

Determination of SOD, POD, and CAT activities

SOD activity was assessed using the nitroblue tetrazolium (NBT) photoreduction method (Spitz and Oberley, 1989) in a 3.3 mL solution consisting of 1.5 mL of 50 mM phosphate buffer (pH 7.8), 300 μL of 100 μM EDTA-Na_2, 300 μL of 130 mM methionine (Met), 300 μL of 750 μM NBT, 300 μL of 60 μM riboflavin, 100 μL of crude

enzyme, and 500 μL of distilled water. After reacting for 15 min in the dark, the absorbance at 560 nm was measured.

POD activity was measured using the guaiacol oxidation method (Hemeda and Klein, 1990) in a 3 mL mixture consisting of 1.0 mL of 100 mM phosphate buffer (pH 6.0), 0.5 mL of 8 mM guaiacol, 0.5 mL of 2.75 mM H_2O_2, 100 μL of crude enzyme, and 0.9 mL of water. The increase in absorbance at 470 nm due to the formation of tetraguaiacol was measured at 3 min.

CAT activity was examined in a 3 mL solution consisting of 1 mL of 50 mM phosphate buffer (pH 7.0), 100 μL of crude enzyme, 200 μL of 200 mM H_2O_2, and 1.7 mL of distilled water. The decrease in absorbance at 240 nm was measured at 3 min (Bhuiyan et al., 2019).

Chlorophyll concentration determination

0.2 g of leaf disks were homogenized and soaked in 25 mL of 96% ethanol in the dark for 24 h. After centrifugation, the supernatant was collected, and the absorbances at 665 nm, 649 nm, and 470 nm were measured. Leaf chlorophyll content was calculated as previously reported using the following equations (Knudson et al., 1977):

$$\text{Chlorophyll } a \text{ (Chl } a\text{)} = 13.95\times A_{665\text{ nm}}\text{-}6.88\times A_{649\text{ nm}} \quad (1)$$

$$\text{Chlorophyll } b \text{ (Chl } b\text{)} = 24.96\times A_{649\text{ nm}}\text{-}7.32\times A_{665\text{ nm}} \quad (2)$$

$$\text{Carotenoid} = (1000\times A_{470\text{ nm}}\text{-}2.05\times\text{Chl } a\text{-}114.8\times\text{Chl } b)/245 \quad (3)$$

$$\text{Chlorophyll } a\text{+}b \text{ (Chl } a\text{+}b\text{)} = \text{Chl } a\text{+Chl } b \quad (4)$$

Inorganic phosphorus concentration determination

Inorganic phosphorus concentration was assessed by determining the absorbance at 660 nm with KH_2PO_4 as the standard, as previously reported (Veronica et al., 2017). In brief, 0.2 g of homogenized leaf and root samples were incubated with 5 mL of a triacid mixture (HNO_3+$HClO_4$+H_2SO_4, 10:4:1) until a milky color was observed. The solution was filtered into a 50 mL volumetric flask through a Whatman grade 42 filter paper and diluted to 50 mL. Subsequently, 5 mL of diluted samples were transferred into a 25 mL volumetric flask, mixed with 5 mL of Barton's reagent, and diluted to 25 mL. After 15–30 min, the absorbance at 660 nm was determined.

Determination of starch and soluble sugar contents

Total soluble sugar was assessed as previously reported (Turakainen et al., 2004). The anthrone solution was formulated by dissolving 2 g of anthrone in 1 L of 72% sulfuric acid. To extract soluble sugars, 250 mg of freeze-dried plant samples were subjected to three extractions with 3 mL of 80% ethanol each. After being spun down for 10 min at 3500 g, the supernatant was heated at 80 °C for 30 min to inactivate invertase. Subsequently, 0.25 mL of the supernatant was combined with 1.25 mL of ice-cold anthrone solution and boiled for 11 min. Once cooling down on ice, the absorbance at 540 nm was read, and the starch concentration was determined spectrophotometrically (Yemm and Willis, 1954).

Measurement of photosynthetic parameters

Net photosynthetic rate (P_n), stomatal conductance (G_s), transpiration rate (T_r), and intercellular CO_2 concentration (C_i) of the second fully expanded leaves were recorded manually using a CIRAS-3 portable photosynthesis system (PP Systems, United States) after 35 d of treatment. All measurements were executed randomly during the late morning (09:00–11:00 am) at 25 °C in a leaf chamber with light intensity of 800 μmol m^{-2} s^{-1} on the adaxial leaf surface provided with an LED source and ambient CO_2 concentration of 400 μmol·mol^{-1}.

Determination of chlorophyll fluorescence parameters

Chlorophyll fluorescence parameters were assessed using a Handy PEA$^+$ advanced continuous excitation chlorophyll fluorimeter (Hansatech Instruments Ltd., Kings Lynn, UK) on the same leaves at the same time. Leaves were clipped 20 min prior to measurements to adapt to the darkness. During the experiment, a light source with pulse intensity of 3500 μmol m^{-2} s^{-1} was applied to the middle leaf blade, avoiding the main leaf vein, for 1 s to determine the maximum fluorescence intensity after dark adaptation (F_m), variable fluorescence (F_v), minimal fluorescence (F_0), maximal PSII photochemical efficiency (F_v/F_m), maximum primary yield of photochemistry (F_v/F_0), non-photochemical quenching (NPQ), absorption flux per reaction center (ABS/RC), dissipated energy flux per cross section, t=t_m (DI_0/CS_m), trapped energy flux per reaction center (TR_0/RC), dissipated energy flux per reaction center (DI_0/RC), and electron transport flux per reaction center (ET_0/RC).

Statistical analysis

All measurements were processed using SPSS 26.0 software (SPSS Inc., Chicago, IL, United States) and presented as averages and standard errors (SE) of four replicates. Significant differences were assessed using the least significant difference (LSD) test at $P<0.05$.

RESULTS

Response of physiological indicators to different treatments

P controls most physiological processes in legume crops, such as nucleic acid and protein synthesis, respiration, and

photosynthesis, thereby directly affecting plant growth. We assessed the influences of various P treatments on licorice seedling growth. As depicted in Table 1, compared to the CK, *B. megaterium* augmented plant height (39.0%, P<0.05), fresh stem weight (86.6%, P<0.05), and fresh leaf weight (84.6%, P<0.05). *B. megaterium* also significantly augmented the dry weight of roots (49.0%, P<0.05), stems (96.8%, P<0.05), and leaves (90.5%, P<0.05), along with the total biomass (86.1%, P<0.05) under control conditions. Moreover, *B. megaterium* increased fresh weight (54.4%, P<0.05) and dry weight (55.2%, P<0.05) of roots and the total biomass (50.0%, P<0.05) under -P stress. Application of *B. megaterium* decreased the root-to-shoot ratio (22.3%, P<0.05) under control conditions. In addition, compared with the control conditions, P deficiency increased root length (38.0%, P<0.05) and decreased the root-to-shoot ratio (13.7%, P<0.05), fresh (35.9%, P<0.05) and dry (43.14%, P<0.05) weights of roots, and the total biomass (36.0%, P<0.05).

Response of antioxidant enzyme activities to different treatments

Antioxidant enzyme activities in response to different treatments were investigated to elucidate the impact of P deficiency on licorice seedlings. The analysis of SOD, POD, and CAT activities revealed that P deficiency increased root SOD (97.5%), POD (40.1%), and CAT (74.3%) activities and leaf SOD (40.0%), POD (17.1%), and CAT (238.3%) activities (P<0.05 for all) compared to the CK (Fig. 1). Conversely, *B. megaterium* reduced SOD, POD, and CAT activities by 41.6%, 27.8%, and 41.4% in roots and by 27.4%, 7.9%, and 36.2% in leaves, respectively, under -P stress (P<0.05 for all). Moreover, *B. megaterium* decreased SOD, POD, and CAT activities in roots by 64.0%, 34.3%, and 13.8%, respectively, under control conditions (P<0.05 for all).

Contents of inorganic P, starch, and soluble sugars under different treatments

Contents of inorganic P, starch, and soluble sugars are shown in Fig. 2. Compared to CK, P deficiency significantly increased starch content by 717.4% (P<0.05) and soluble sugar content by 1661.2% (P<0.05) in roots, but the root starch and soluble sugar contents remained virtually unchanged after *B. megaterium* treatment (P>0.05). Further analysis showed that -P stress increased the content of inorganic P by 34.7% (P<0.05) in roots but decreased inorganic P in leaves (21.2%, P<0.05) compared with the control conditions. Moreover, *B. megaterium* application decreased the inorganic P of roots and leaves by 36.3% and 20.9% (P<0.05 for both), respectively, under -P stress.

Chlorophyll content in *G. uralensis* seedlings under different treatments

B. megaterium increased chlorophyll *a*, chlorophyll *b*, carotenoid, and chlorophyll *a*+*b* contents by 1.9%, 3.8%, 3.9%, and 2.0% (P<0.05 for all), respectively, in *G. uralensis* (Fig. 3). Compared to CK, P deficiency significantly decreased chlorophyll *a*, chlorophyll *b*, carotenoid, and chlorophyll *a*+*b* contents by 9.8%, 8.1%, 8.8%, and 9.2% (P<0.05 for all), respectively. Furthermore, *B. megaterium* increased chlorophyll *a*, carotenoid, and chlorophyll *a*+*b* levels by 11.7%, 15.1%, and 9.9% (P<0.05 for the former two), respectively, under -P stress.

Leaf gas exchange parameters in *G. uralensis* under different treatments

To assess the influence of *B. megaterium* on photosynthesis and chlorophyll fluorescence of licorice under P deficiency, leaf gas exchange parameters in *G. uralensis* were determined after 35 d of cultivation under different treatments. Compared to CK, P_n, G_s, and T_r decreased by 17.6%, 51.5%, and 53.1% (P<0.05 for all), respectively, under -P treatment (Fig. 4). *B. megaterium* increased P_n, C_i, G_s, and T_r by 18.8%, 15.7%, 63.8%, and 83.5% (P<0.05 for all), respectively, in roots under -P treatment. Moreover, *B. megaterium* significantly increased P_n and T_r by 17.1% and 33.9% (P<0.05 for both), respectively, under the control conditions.

Chlorophyll fluorescence parameters in *G. uralensis* under different treatments

Chlorophyll fluorescence parameters of licorice seedlings are shown in Table 2. Compared with control conditions, P deficiency decreased F_v, F_m, F_v/F_m, and F_v/F_0 by 7.2%, 3.9%, 3.5% and 19.0%, respectively, while F_0, NPQ, DI_0/RC, TR_0/RC, and ABS/RC increased by 38.3%, 26.0%, 15.3%, 38.7%, and 78.1%, respectively, under the same conditions (P<0.05). *B. megaterium* increased F_m, F_v, and ET_0/RC by 8.9%, 8.0%, and 19.0%, respectively, under the control condition (P<0.05). Moreover, *B. megaterium* increased F_m, F_v, and ET_0/RC by 5.4%, 7.2%, and 19.1%, respectively, under -P stress (P<0.05). However, *B. megaterium* decreased F_0, NPQ, TR_0/RC, DI_0/RC, and ABS/RC by 21.1%, 19.0%, 20.6%, 46.9%, and 29.1%, respectively, under -P conditions (P<0.05).

DISCUSSION

In P-limiting environments, roots have high plasticity, including morphological and physiological changes, to enhance the capacity of P acquisition (Palonen, 1999). It has been shown that P deficiency stimulates root elongation in rice (Wissuwa, 2005) and promotes lateral root growth and secondary root branching in beans and *Arabidopsis* (Cao et al., 2013). Our study revealed P deficiency increased root length, likely due to increased contact between roots and soil. Previous studies showed that insufficient P can reduce shoot growth rates and increase carbohydrate

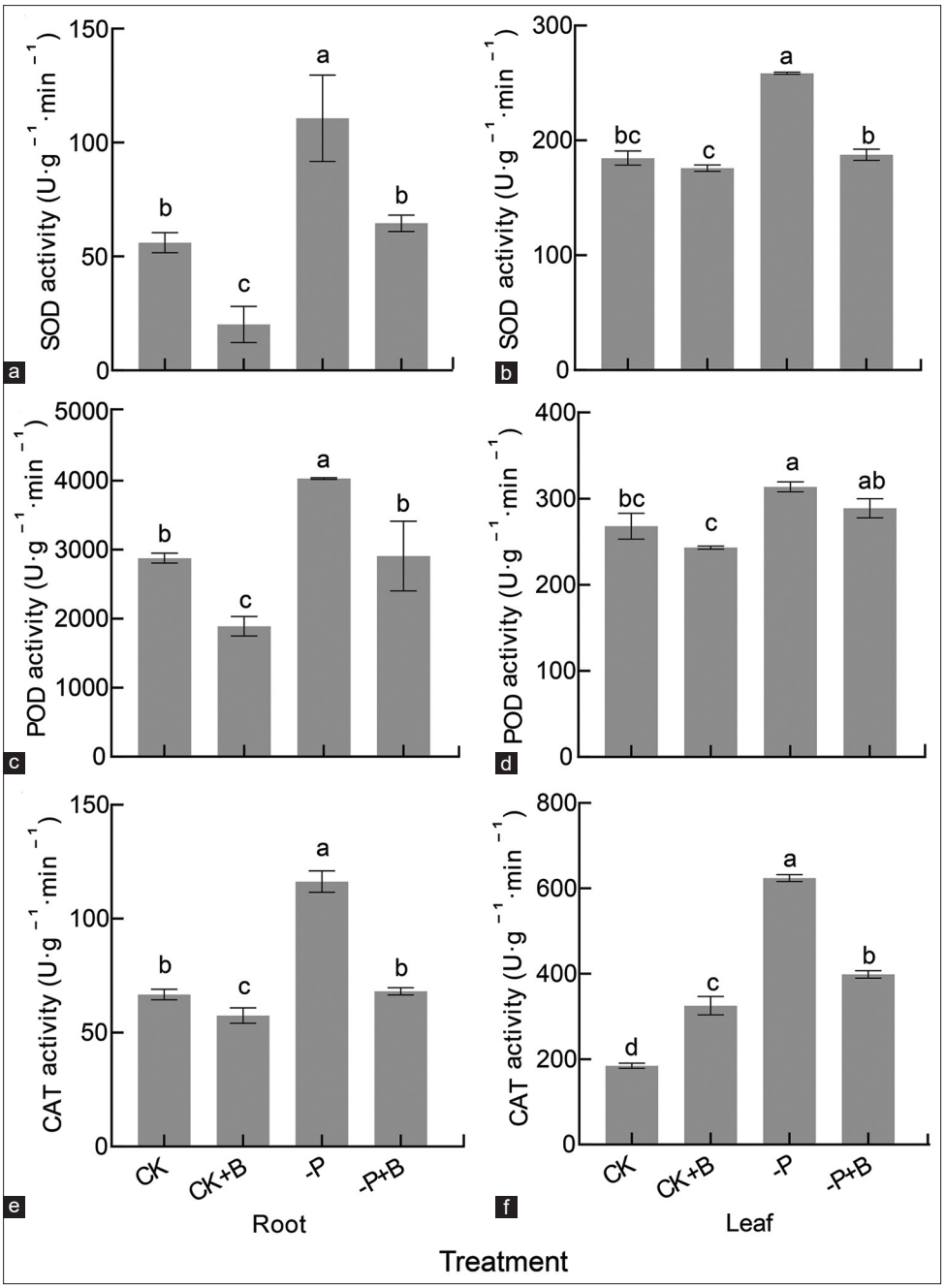

Fig 1. Changes in the activities of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) in roots (a, c, e) and leaves (b, d, f) of licorice after 35 d of treatment. CK indicates seedlings grown in Hoagland's complete nutrient solution. -P indicates seedlings grown in Hoagland's nutrient solution without P. +B represents *B. megaterium* (2.5×10^5 cfu·mL^{-1}) application. Data are presented as means ± standard errors (SE, n=9). Different lowercase letters indicate statistically significant differences at $P<0.05$ (least significant difference test).

concentrations in roots (Lynch and Brow, 2001). We observed that P deficiency significantly increased starch and soluble sugar concentrations in roots. High sugar content in the roots acts as a signal to trigger plant's responses to P deficiency (Hammond and White, 2008, 2011). P deficiency has been shown to increase root biomass, total root length, and root-to-shoot ratio, suggesting that the root system is pivotal for nutrient acquisition (Deng et al., 2020). However, our study indicated that P deficiency reduced root biomass and root-to-shoot ratio. This difference is possibly due to mild P deficiency augmented absolute root biomass, while more severe P deficiency reduced absolute root biomass.

In our study, *B. megaterium* increased biomass under both P deficiency and control treatments. As an effective P-solubilizing microorganism, *B. megaterium* colonizes soils and plant tissues, solubilizing phosphates via

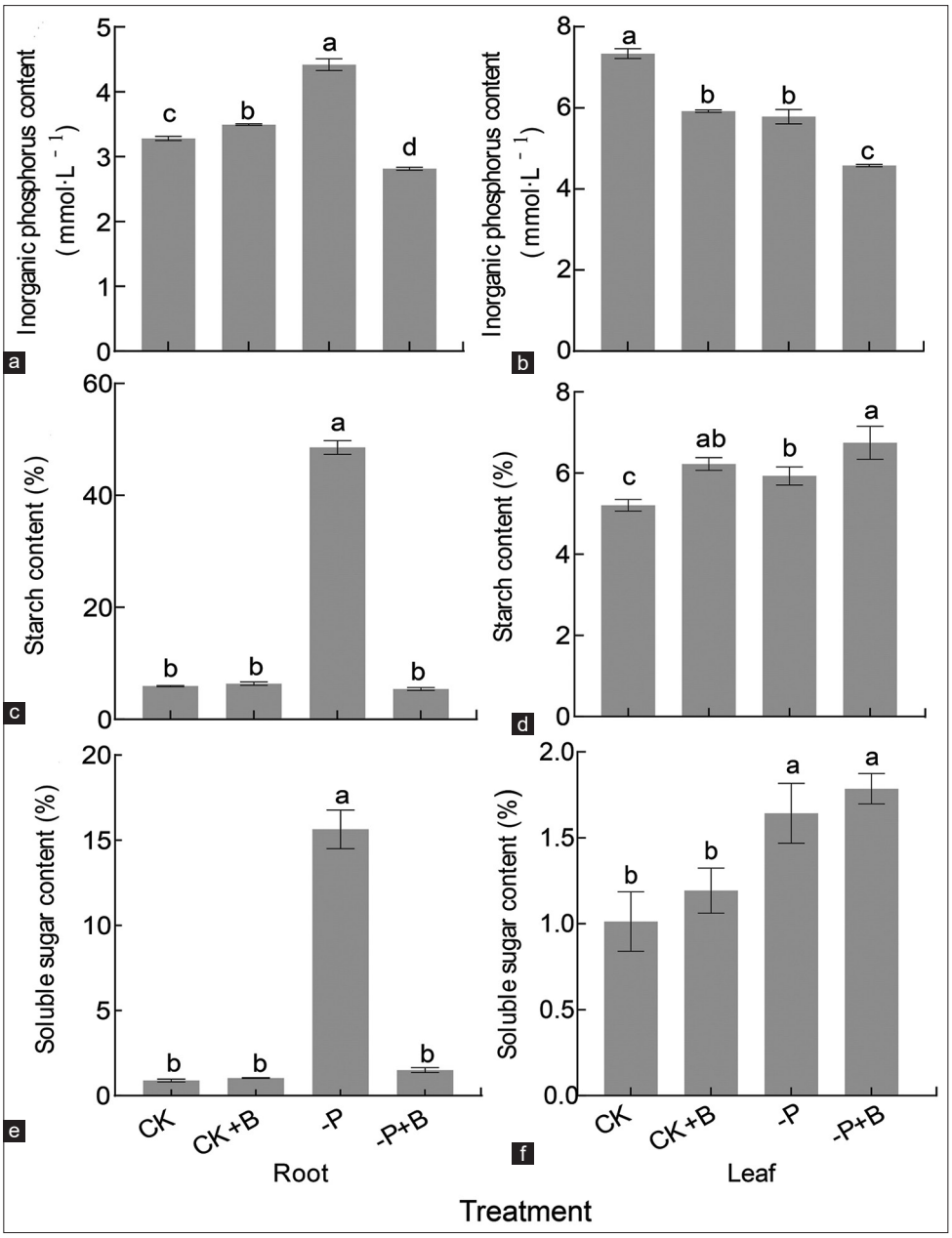

Fig 2. Inorganic P, starch, and soluble sugars concentrations of roots (a, c, e) and leaves (b, d, f) of licorice after 35 d of treatment. CK indicates seedlings grown in Hoagland's complete nutrient solution. -P indicates seedlings grown in Hoagland's nutrient solution without P. +B represents *B. megaterium* (2.5×10^5 cfu·mL^{-1}) application. Data are presented as means ± standard errors (SE, n=9). Different lowercase letters indicate statistically significant differences at P<0.05 (least significant difference test).

phosphatases and/or organic acids (Mukhtar et al., 2017). Studies have shown that *B. megaterium* enhances soil phosphate solubilization and crop growth rate in sugarcane (Shankaraiah et al., 2000) and increases tea plant growth and root and leaf phosphate content (Chakraborty et al., 2012). In addition, *B. megaterium* mj1212 acts as a phosphate biofertilizer in mustard plants, improving their growth by augmenting plant shoot and root length and fresh weight (Kang et al., 2014). Moreover, *B. megaterium* strains produce indole-3-acetic acid (an auxin), promoting root growth under salt stress (Abdel Motaleb et al., 2020). The findings imply that the mechanisms for enhancing plant growth under nutrient deficiency involve nutrient mobilization and the release of phytohormones by microorganisms (Chakraborty et al., 2012).

Plants produce peroxides and ROS under adverse conditions. PGPR produces metabolites to activate the defense system by elevating the activities of SOD, POD, CAT, and other ROS-scavenging enzymes, thereby mitigating ROS-induced damage (Rashid et al., 2022). SOD, as the first-line defense against ROS, converts O_2^- to O_2

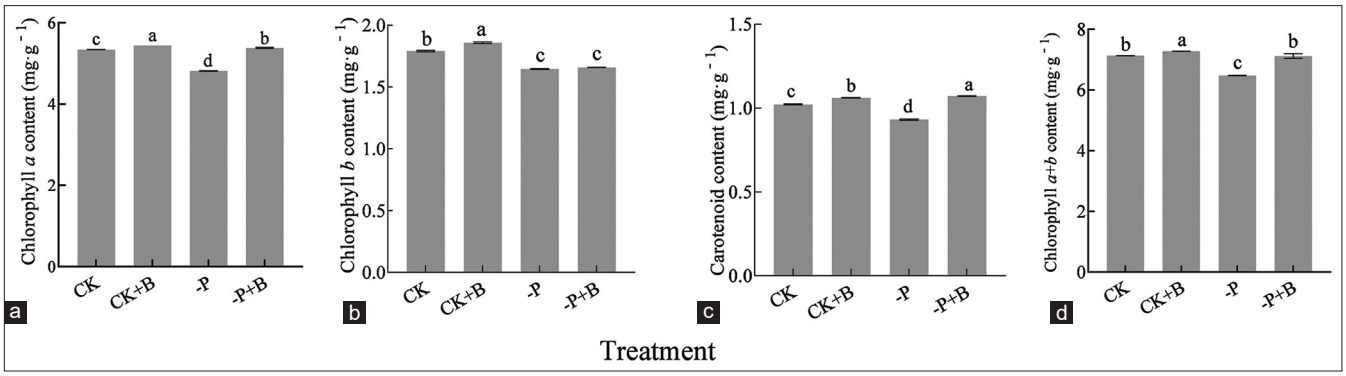

Fig 3. Chlorophyll *a* (a), chlorophyll *b* (b), carotenoid (c), and chlorophyll *a*+*b* (d) concentrations of licorice after 35 d of treatment. CK indicates seedlings grown in Hoagland's complete nutrient solution. -P indicates seedlings grown in Hoagland's nutrient solution without P. +B represents *B. megaterium* (2.5×10^5 cfu·mL^{-1}) application. Data are presented as means ± standard errors (SE, n=9). Different lowercase letters indicate statistically significant differences at P<0.05 (least significant difference test).

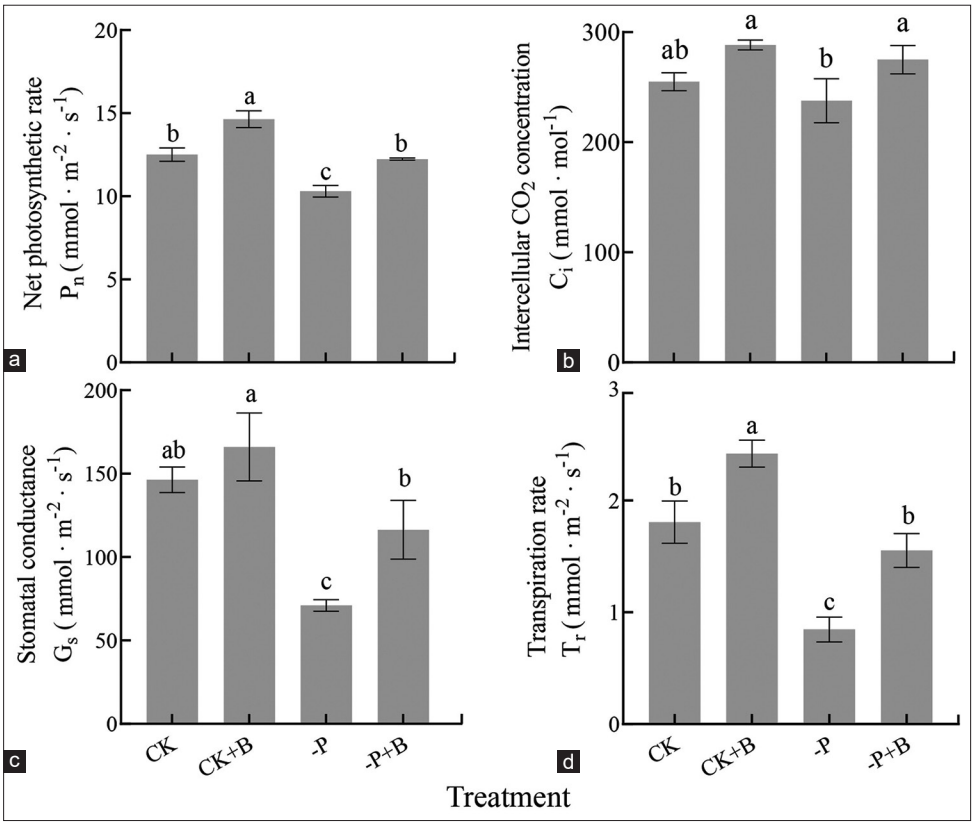

Fig 4. P_n (a), C_i (b), G_s (c) and T_r (d) in licorice after 35 d of treatment. P_n: net photosynthetic rate, G_s: stomatal conductance, T_r: transpiration rate, C_i: intercellular CO_2 concentration. Data are presented as means ± standard errors (SE, n=9). Different lowercase letters indicate statistically significant differences at P<0.05 (least significant difference test).

and H_2O_2 (Alscher et al., 2002). The latter may be further degraded to water by CAT and several other enzymes (Groß et al., 2013; Khan et al., 2017). Our findings revealed that P deficiency activated these enzymes in roots and leaves, consistent with the findings in maize (Kaya et al., 2020), rice (Fu et al., 2014), tomato (Zhang et al., 2019), *Arabidopsis* (Kandlbinder et al., 2004), *Phaseolus vulgaris* (Shirinpour et al., 2020), and tea (Meng et al., 2021). These findings indicate that the elevated enzyme activities protect *G. uralensis* against P deficiency-induced oxidative stress. Among antioxidant enzymes, the coordinated action of SOD and CAT is pivotal in alleviating oxidative stress (Rasool et al., 2013).

POD is known to catalyze the last steps in lignin and H_2O_2 biosynthesis in plants, playing a crucial role in detoxifying H_2O_2 (González-Gordo et al., 2023). In our study, *B. megaterium* did not affect POD activity in leaves.

However, inoculation with *B. megaterium* reduced SOD, CAT, and POD activities in roots under P limitation and control conditions. These findings suggest that PGPR assist host plants in alleviating oxidative stress not by directly scavenging ROS but rather by augmenting the activities of antioxidant enzymes. The main mechanisms deserve further elucidation.

B. megaterium significantly increased CAT activity in untreated leaves, indicating that root colonization by *B. megaterium* could induce CAT activity in licorice. A previous study on *Populus tremuloides* showed that inoculating *Laccaria bicolor* reduced POD and CAT activities compared to the control under P limitation (Chen et al., 2022). In addition, treatment with other PGPR strains, such as *B. subtilis* and *Arthrobacter*, reduced antioxidant enzyme activities in wheat (Arias Padró et al., 2021). However, inoculating *Bacillus* spp. increased the activities of antioxidant enzymes against *Pyricularia oryzae* (Shirinpour et al., 2020). The inconsistent results might stem from differences in plant species, treatment conditions, or other factors between experiments. These results highlight that *Bacillus* spp. may change the activity of antioxidant enzymes under P deficiency, with responses differing among plant species.

Low P may energize thylakoid membrane, decrease electron flow, and limit ribulose 1,5-biphosphate regeneration in the photosynthetic carbon reduction cycle, ultimately decreasing the photosynthesis rate (Xu et al., 2007). Our findings indicate that the decline in photosynthesis under P deficiency in licorice may be attributed to several factors: (i) P deprivation decreases the total chlorophyll and carotenoid contents; (ii) decreased G_s restricts the uptake of CO_2 as a primary substrate for photosynthesis; (iii) induced damage to the photosynthetic apparatus and inactivation of PSII reaction centers; and (iv) low leaf inorganic phosphorus may limit P_n by constraining Rubisco or RuBP regeneration (Thomas et al., 2006). As a result, P deficiency reduced plant biomass. In addition, we showed that *B. megaterium* caused a significant improvement in P_n, G_s, C_i, T_r, and Chl and carotenoid contents under P deficiency. These results indicate that *B. megaterium* may increase plant tolerance to low P by changing photosynthetic pigments, elevating photosynthetic capacity, and preventing oxidative damage to the photosystems (Pan et al., 2019).

Chlorophyll fluorescence, a nonintrusive marker enabling the swift assessment of photosynthesis *in vivo* without leaf damage, serves as a potent tool to assess the capability of PSII to withstand abiotic stress (Yang et al., 2021). Therefore, measuring these parameters can offer valuable insights into the state of photon energy in photosynthesis, particularly under various stresses. According to our results, P deficiency decreased chlorophyll fluorescence parameters (F_m, F_v/F_m, F_v/F_0, and F_v) and increased F_0 and NPQ. F_v/F_0 measures the activity of PSII (Tripathi et al., 2014), and F_0 estimates the relative antenna pigment level in the PSII complex (Huang et al., 2004). High F_0 and low F_m indicate that P deficiency might damage to the photosynthetic apparatus, thereby affecting PSII activity (Tripathi et al., 2014; Pshenichnikova et al., 2019). Under P deficiency, increased NPQ values reflect light energy absorbed via fluorescence radiation energy in PSII, protecting seedlings from photodynamic damage (Tripathi et al., 2014). Although P deficiency reduced F_v/F_m, the value was still higher than 0.8, which is normally seen in healthy plants (Gao et al., 2001). Thus, F_v/F_m may be insensitive to P deficiency, aligning with the findings under nitrogen deficiency (Živčák et al., 2015). Furthermore, *B. megaterium* significantly decreased F_0 and NPQ and increased F_m, F_v/F_m, F_v/F_0, and F_v, which indicates that *B. megaterium* may alleviate the damage of the photosynthetic apparatus under P deficiency.

A fast chlorophyll fluorescence transition has been applied in numerous studies to assess environmental effects on plants. Significant increases in ABS/RC, DI_0/RC, and TR_0/RC were observed in the P deficiency treatment. The higher values of ABS/RC and TR_0/RC indicate that certain reaction centers were inactivated because of deactivation of the oxygen-evolving complex and the silencing of active reaction centers (Liang et al., 2019; Mihaljević et al., 2021; Sousaraei et al., 2021). Significant increases in DI_0/RC and decreases in ET_0/RC under P deficiency increased the energy dissipation rate of untrapped excitations, indicating that excess energy was dissipated as heat (Živčák et al., 2015). The decreases in ABS/RC, DI_0/RC, and TR_0/RC and increases in F_m, F_v, and ET_0/RC in *B. megaterium*-treated plants indicate that *B. megaterium* can relieve the inactivation of some PSII reaction centers caused by P deficiency to maintain high photosynthetic capacity.

CONCLUSION

This study demonstrated that P deficiency decreased biomass, growth, and photosynthetic capacity in licorice seedlings. For *G. uralensis*, tolerance to low P involved three major strategies: (1) elongating the roots to enlarge the roots-soil contact area; (2) enhancing starch and soluble sugars concentrations in roots as a response to P stress; and (3) increasing F_0 and NPQ to safeguard the photosynthetic system from harm. In addition, applying *B. megaterium* alleviated the damage caused by phosphorus stress: (1) *B. megaterium* increased P_n, G_s, C_i, T_r, chlorophyll and carotenoid contents, F_m, F_v/F_m, F_v/F_0, and F_v and decreased ABS/RC, DI_0/RC, TR_0/RC, F_0, and NPQ to alleviate the damage to the photosynthetic apparatus and maintain high photosynthetic capacity under P deficiency

stress; and (2) *B. megaterium* decreased POD, SOD, and CAT activities and starch and soluble sugars concentrations, illustrating that low phosphorus causes a stress response in *G. uralensis*. These results reveal that *B. megaterium* mitigates low P stress by relieving the inactivation of some PSII reaction centers to improve photosynthetic capacity and changing antioxidant enzymes' activity and starch and soluble sugars concentrations under P deficiency. Moreover, our study revealed the effects of phosphorus deficiency on *G. uralensis* and provided evidence for *B. megaterium* enhancing tolerance of *G. uralensis* to low phosphorus. These results have implications for agronomic management of *G. uralensis* in P-deficient soils.

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Author contributions

Jing Gao and Nan Wang designed the study. Yali Zhang, Rui Li and Wenbo Wang executed the experiments and analyzed data. Yali Zhang drafted the manuscript with the contributions of Gang Zhang, Yonggang Yan and Jiakun Yan. All authors have approved the manuscript.

Conflict of interest

The authors declare no conflict of interest.

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