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Original Research

Effects of PGPR Co-inoculation on Growth, Phosphorus Nutrition and Phosphatase/Phytase Activities of Faba Bean under Different Phosphorus Availability Conditions

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Abstract

Plant growth promoting rhizobacteria (PGPR) may improve crop yield and reduce or optimize the use of chemical fertilizers. We investigated the effect of co-inoculation on growth, phosphorus nutrition, and phosphatase-phytase activities of *Vicia faba* under different phosphorus availability conditions. The studied strains possess several PGPR traits such as nitrogen fixation ability, solubilization of phosphate and potassium, and production of exopolysaccharides, auxins, and siderophores. Based on their 16S rDNA sequences, the strains were identified as *Rahnella aquatilis* (PGP30), *Pseudomonas brassicacearum* (PGP291) and *Rhizobium* sp. (RhOF57A). In a greenhouse experiment, plants were inoculated with an individual strain or co-inoculated with two strains in addition to uninoculated controls. Each setup was supplemented either with tricalcium phosphate $Ca_3(PO_4)_2$ (TCP treatment) or irrigated with potassium phosphate KH_2PO_4 (PO_4 treatment). Regardless of the applied phosphorus source, co-inoculation significantly increased biomass and phosphorus concentrations in plants as well as in bean pods. Moreover, especially co-inoculation increased phosphatase-phytase activities in roots supplemented with tricalcium phosphate. This study suggests that co-inoculation of plants is a means to prevent phosphorus limitation and for judicious use of chemical fertilizers.

Keywords: PGPR, rhizobia, phosphatase-phytase, *Vicia faba*, phosphate solubilization

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Introduction

After nitrogen, phosphorus is the most limiting nutrient for plant growth. Depending on the soil pH, phosphorus is sequestered and precipitated by reactions with cations, especially iron, aluminum, calcium, and plants absorbing only a small amount of available phosphorus [1, 2]. For this reason, a significant amount of phosphate fertilizer is required to correct phosphorus deficiency in the soil. Extensive fertilizer use might negatively affect soil microbial function and plant yield [3]. Moreover, the cost of phosphate fertilizers has rapidly increased due to the decrease in high-quality phosphate reserves [4, 5]. Therefore, the use of phosphate fertilizers must be as judicious as possible. Plant growth promoting rhizobacteria (PGPR) actively participate in the transformation of phosphate in the soil and make phosphorus available to the plant. The use of PGPR solubilizing soil phosphate in agronomic practices is advocated for several reasons. They improve soil fertility and increase crop yield through the increase of nutrient availability [6]. They do not pollute the environment, do not affect soil health, protect plants against phytopathogens and low-cost technology is used for their production [7]. Nitrogen-fixing bacteria may be the most promising PGPR group, due to their ability to improve nitrogen nutrition in either symbiotic or non-symbiotic interactions. Co-inoculation with such strains constitutes an alternative approach to improving nitrogen and phosphorus nutrition of crops, especially in deficient soils. For example, co-inoculation with rhizobia and *Bacillus* sp. significantly increased the yield of wheat [8]. Similarly, co-inoculation with *Rhizobium leguminosarum* and a *Pseudomonas* strain stimulated plant growth and yield of chickpea more than the inoculation with one strain only [9].

The majority of Mediterranean soils are characterized by low phosphorus availability, which often limits legume production. Plants have developed several strategies to escape soil phosphorus limitation. They can modify the architecture of their roots to optimize phosphorus acquisition from the soil. They can also hydrolyze or mobilize phosphate compounds by excreting organic acids and acid phosphatase [10, 11]. Symbiotic associations with mycorrhizal fungi enhance the uptake of soil nutrients such as phosphorus and potassium [12]. Some symbiotic rhizobial combinations improve phosphorus availability of plants through the excretion of nodule phosphatases [13].

Improving the adaptation of faba bean to phosphorus-deficient soils by inoculation with native PGPR strains might be an appropriate agricultural practice. The main objectives of this study were: (a) to determine whether complex phosphate fertilizer (TCP) coupled with bacterial inoculants would produce levels of growth, yield and nutrient uptake equivalent to those obtained with available phosphorus fertilizers (KH_2PO_4), (b) and to compare the impact of individual inoculation and co-inoculation on the activities of enzymes (phosphatase and phytase) that are involved in plant adaptation to phosphorus stress conditions.

Material and Methods

Isolation of Phosphate-Solubilizing Bacteria

Samples of the rhizospheric soils of faba bean were taken from the cultivated fields of the Marrakech region (Ait Ourir, Morocco). 10 g of soil samples were transferred into 90 ml of sterile physiological water with continuous shaking (180 rpm). 100 µl of the soil solution were plated on agar medium of the National Botanical Research Institute's phosphate medium devoid of yeast extract (NBRIY) [14] containing 5 g of $Ca_3(PO_4)_2$ (tricalcium phosphate: TCP) as a source of complex phosphate. The incubation of the plates was carried out at 28ºC for 48 h to 72 h. Strains producing a clear halo on the NBRIY medium were counted and purified by repeated streaking on the NBRIY medium. As a final step, the strains were purified on TSA (Trypticase Soy Agar) medium and were stored in 25% glycerol at -20ºC. Rhizobia were isolated from nodules of faba bean as described by Benidire et al. [15].

PGPR Activities and Molecular Identification of Rhizobacterial Strains

The rhizobacterial strains were tested for several PGPR activities, such as the solubilization of tricalcium phosphate in an agar medium, which was calculated and presented as diameter halo (cm)/diameter colony (cm) ratio (DH/DC). In the liquid medium, the pH and the P available were calculated for each strain as described by Maghraoui et al. [16]. Other PGPR traits were also conducted, including potassium solubilization (according to Alikhani et al. [17]), siderophore [18], exopolysaccharide [19] and indole acetic acid production [20], as well as nitrogen fixation (N_2) [21]. The molecular identification of the tested rhizobacterial strains was carried out as described by Benidire et al. [15].

Plant Material and Growth Condition

We used for the greenhouse experiment the commercial Aguadulce variety that is widely used in Morocco. One hundred and ten homogenous bean seeds were service-sterilized with 1/3-diluted 12% sodium hypochlorite solution. After a series of successive rinses with sterile distilled water, the seeds were germinated for 72 h at 28ºC. The inocula of the bacterial strains PGP30, PS291 and RhOF57A were prepared by growing each strain in trypticase soy broth or YEM broth (for rhizobia) at 28ºC for 2 to 3 days. Bacterial cells were harvested, washed several times with sterile physiological water (NaCl 9 g/l) and resuspended in the adequate volume of sterile physiological water to obtain a final OD = 1 at $\lambda = 600$ nm (approximately 10^9 CFU/ml). The experiment

included four treatments of faba bean: i) inoculation with PGP291 alone; ii) inoculation with PGP30 alone; iii) co-inoculation with PGP291+RhOF57A; iv) and co-inoculation with PGP30+RhOF57A. For inoculation, the germinated seeds were submerged for 30 min in the inoculum. For co-inoculation, strains were mixed at a 1:1 V/V ratio. Previously disinfected two-liter pots were filled with damp perlite and inoculated seeds were then transferred into the pots (two seeds/pot). Second and third inoculation of the roots with 2 ml of every inoculum were done two and three weeks after planting of the seedlings, respectively.

We used KH_2PO_4 as an available source of phosphorus (PO_4 treatments) and tricalcium phosphate ($Ca_3(PO_4)_2$) as a complex source of phosphorus (TCP treatments). Tricalcium phosphate was supplied at a quantity of 1 g/seed. To irrigate plants of the PO_4 treatment, 0.5 g KH_2PO_4/l of nutrient solution was used. Control treatments without inoculation were also conducted for this experiment: Control 1 (C1): plants were grown under the same conditions without any source of phosphorus; control 2 (C2): plants were supplemented with tricalcium phosphate; control 3 (C3): plants were irrigated with KH_2PO_4 in the nutrient solution. The pots were placed in individual trays in the greenhouse of the Semlalia Faculty of Sciences (Marrakech, Morocco) under natural conditions characterized by a temperature between 20 and 25ºC, 47% relative humidity and the duration of the day was 11 hours. The inoculated pots with PGP30 and PGP291 were separated from the RhOF57A pots and the co-inoculation treatments. The control pots were separated from inoculation treatments. Plants were irrigated two or three times with 250 ml of the nutrient solution of Rigaud and Puppo [22], according to their need. The harvest was carried out after 4 months at the seed maturity stage. Roots were thoroughly rinsed with tap water and separated from the shoots. Shoots and roots of faba bean were dried at 70ºC for 48 h to determine their dry weight. Bean pods were also collected and dried (70ºC, 48 h). For measuring phosphorus concentrations, plants (shoots and roots) and bean pods were ground and ashed at 550ºC. 3 ml of HCl (6 N) were added to every sample and directly placed on a hot plate for evaporation at 330ºC for at least one hour. Finally, 3 ml of hot distilled water were added. The obtained solutions were filtered using Whatman paper of 0.45 µm pore size, and the extracts were added to 20 ml of distilled water and stored at 4°C until measurement of the phosphorus concentration as described by Olsen and Sommers [23]. The results are given in mg of phosphate per g of dry matter.

Phosphatase-Phytase Activities

0.2 g of frozen fresh roots of each plant were ground in an Eppendorf tube containing 500 µl of acetate-buffer (0.1 M pH 5.6), 33% of Polyvinylpyrrolidone (PVP) and 5 µl of β-mercaptoethanol. The tube was centrifuged at 13000 g at 4ºC for 30 min. 100 µl of the supernatant were taken for phosphatase-phytase activity assays. The phosphatase activity in the roots was measured using p-nitrophenyl phosphate as substrate, according to the method described by Asmar and Gissel-Nielsen [24]. 100 µl of the root extract were incubated at 37ºC for 30 min in a mixture of 200 µl acetate-buffer and 200 µl p-nitrophenyl phosphate (p-NPP). The reaction was stopped by adding 1 ml of NaOH (0.5 N). The acid phosphatase activity was determined by measuring the formation of para-nitrophenolate at 410 nm wavelength. The result is given in nmole of para-nitrophenol (PNP) product per min per g of fresh roots. Sodium phytate was used for measuring phytase activity. In brief, 100 µl of root extract were added to a mixture containing 0.1 M sodium acetate buffer (pH 5) and 1 mM sodium phytate. The mixture was incubated for 90 min at 37ºC and the reaction was stopped by the addition of 0.5 ml 10% of HCl. The mixture was centrifuged for 5 min at 13000 rpm. The supernatant was analyzed for Pi liberation [25]. The phytase activity of the extract was determined by spectrophotometry at $\lambda = 630$ nm.

Statistical Analysis

We used a completely random block assay design. Registered growth values of strains (*in vitro* tests) were means of three replicates per treatment. Regarding the greenhouse experiment, results were means of eight biological replicates. All results were subjected to analysis of variance, with a Student-Newman-Keuls (SNK) method for the comparison of means using SPSS software. Analysis of variance (ANOVA) was performed for comparison of means. Standard errors (SE) were also calculated and are presented in the tables. Biomass values, enzyme activities and phosphorus concentrations and their correlation with treatments were used for principal component analyses (PCA) using XLStat software.

Results and Discussion

PGPR Activities and Molecular Identification of Rhizobacterial Strains

The strains differed in their solubilization capacity of tricalcium phosphate on agar plates (Table 1). Halo formation for PGP30 began within three days of incubation, while PGP291 and RhOF57A began to solubilize complex phosphate and produce a clear halo after about 10 days of incubation. After 15 days of incubation, all strains showed a huge clear zone around their colonies. PGP30 exhibited the highest DH/DC value (Table 1), suggesting that PGP30 is the most powerful phosphate solubilizer of the three strains. However, in liquid medium PGP30 released the smallest amount of available phosphorus within 4 days, while RhOF57A recorded the highest quantity

Table 1. Different plant growth-promoting activities of the three bacterial strains.

Trait		PGP30	PGP291	RhOF57A
Phosphate solubilization (DH/DC)	3 days	1.76±0.06[a]	-	-
	10 days	2.63±0.13[a]	1.46±0.02[c]	1.6±0.05[b]
	15 days	2.86±0.14[a]	1.29±0.04[b]	1.12±0.05[c]
Auxin production (µg/ml)		13.52±1.26[c]	22.58±2.45[b]	217.56±4.05[a]
Exopolysaccharide production (µg of Congo Red/OD 600)		110.46±4.79[c]	147.78 ± 4.79[b]	894.06±1.67[a]
Siderophore production		(-)	(+)	(+)
Potassium solubilization		(-)	(-)	(+)
Nitrogen fixation		(+)	(+)	(+)

Means±standard errors within the same line followed by different letters are significantly different at $p<0.05$ according to the SNK test. Phosphate solubilization (DH/DC): Average of halo diameter (cm)/Average of colony diameter (cm); Siderophore production (+): Change of medium coloration from blue to orange; Potassium solubilization (+): Halo formation in the medium; Free nitrogen fixation (+): Bacterial development without nitrogen source.

of available phosphorus (2.25 mg/l at 72 h). For strains RhOF57A and PGP291, we noted a decrease in pH values in the first 24 h, which concurred with the release of significant amounts of available phosphorus in the broth (Table 2). Halo formation in agar plates and the pH drop in the liquid medium might be due to the production of organic acids. They may chelate the metal associated with phosphorus and hence make the phosphorus available [26]. RhOF57 might use both organic acids and exopolysaccharides for complex phosphate mobilization as was suggested for *Bacillus marisflavi* by Prabhu et al. [27]. Indeed, RhOF57A showed a massive exopolysaccharide production (Table 1). Moreover, the strains showed other PGPR traits such as nitrogen fixation capacity and the release of indole acetic acid (IAA) with the highest amount recorded for RhOF57A (217.56 µg/ml). According to the blue agar CAS assay, strains RhOF57A and PGP291 were able to produce siderophores, and only RhOF57A had the ability to mobilize complex potassium (Table 1).

Thus, these strains might support plant growth by different mechanisms: i) the production of IAA; a phytohormone that induces plant development and increases the total root surface area for a better nutrient uptake, ii) the production of siderophores responsible for iron chelation and the control of plant pathogens, and iii) the correction of a nutrient unbalance via nitrogen fixation and/or mobilization of complexed minerals such as potassium and phosphate [28]. Accordingly, the studied strains are considered plant growth-promoting rhizobacteria and could be used for plant inoculation.

Table 2. pH values and the amount of available phosphorus (mg/l) released by phosphate-solubilizing strains in NBRIY broth containing tricalcium phosphate.

pH values during incubation time						
Strain	0 h	24 h	48 h	72 h	96 h	120 h
Control	7.19±0.01[a]	7.19±0.03[a]	6.70±0.04[b]	6.96±0.04[a]	6.43±0.02[c]	5.91±0.01[d]
PGP30	7.20±0.14[a]	7.20±0.10[a]	5.53±0.08[b]	7.06±0.24[a]	7.24±0.03[a]	7.25±0.03[a]
PGP291	7.19±0.09[a]	4.8±0.02[d]	4.38±0.10[e]	5.89±0.01[c]	5.82±0.03[c]	6.13±0.03[b]
RhOF57A	7.11±0.01[a]	4.20±0.04[e]	4.58±0.04[d]	6.35±0.10[b]	4.48±0.03[d]	5.25±0.17[c]
P available values in the broth (mg/l) after the incubation time indicated above						
Control	-	-	-	-	-	-
PGP30	0.013±0.00[d]	0.94±0.05[a]	0.62±0.15[b]	0.81±0.12[a]	0.46±0.13[c]	0.015±0.02[d]
PGP291	0.011±0.00[d]	1.84±0.14[a]	1.15±0.15[c]	1.54±0.10[b]	0.89±0.25[c]	0.012±0.01[d]
RhOF57A	0.011±0.00[c]	2.02±1[a]	1.17±0.3[b]	2.25±0.01[a]	1.15± 0.3[b]	0.011±0.04[c]

Means±standard errors within the same line followed by different letters are significantly different at $p<0.05$ according to the SNK test.

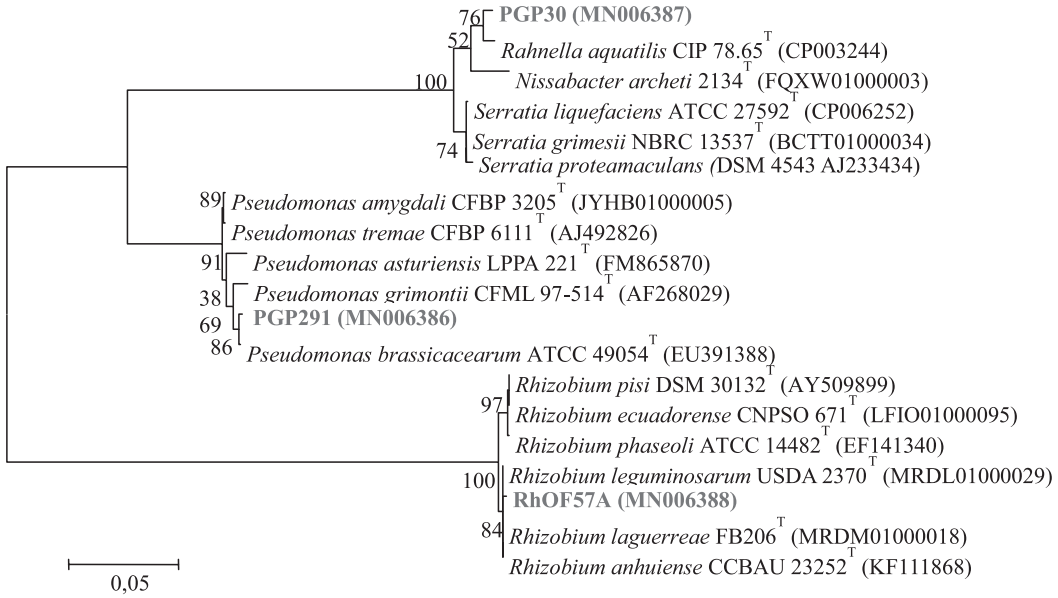

Fig. 1. Maximum likelihood phylogenetic tree of strains PGP30, PGP291 and RhOF57A based on 16S rDNA gene sequences, showing their position with regard to related species. Bootstrap values based on 1500 replications are given at branch points. Accession numbers are given in parenthesis. Scale bar: substitutions per nucleotide position.

Molecular identification revealed that PGP30 is similar to *Rahnella aquatilis*, PGP291 is closest to *Pseudomonas brassicacearum*, while RhOF57A is a *Rhizobium* sp. (Fig. 1). The 16S rDNA nucleotide sequences determined in this work were submitted to the GenBank database and have been assigned the accession numbers MN006387, MN006386, and MN006388 for PGP30, PGP291, and RhOF57A, respectively.

Table 3. Effect of bacterial inoculation on plant parameters with different phosphorus supplies.

Treatment	Plant length (cm)	Shoot dry weight (g)	Root dry weight (g)	Root/shoot ratio	Pod dry weight (g)	Increase of bean pod dry weight over control (%)
C1	70.33±1.15^{d}	2.96 ±0.24^{e}	2.65±0.15^{a}	0.89±0.02^{a}	(*)	-
TCP						
C2	90±1.99^{c}	3.15±0.2^{e}	2.47±0.1^{a}	0.78±0.12^{a}	0.13±0.02^{d}	-
Tr1	93.67±1.7^{b}	4.50±0.24^{c}	2.93±0.19^{a}	0.65±0.08^{a}	0.22±0.04^{c}	69.23
Tr2	96.67±2.4^{b}	4.60±0.23^{c}	2.53±0.32^{a}	0.55±0.04^{a}	0.25±0.03^{c}	92.31
Tr3	98.33±1.1^{b}	6.71±0.23^{b}	0.97±0.16^{b}	0.14±0.09^{c}	0.56±0.02^{b}	330.76
Tr4	105.17±1.7^{a}	7.72±0.25^{a}	0.98±0.16^{b}	0.12±0.05^{c}	0.68±0.01^{a}	423.07
PO_4						
C3	89±2.41^{c}	3.82±0.12^{d}	1.98±0.18^{a}	0.51±0.06^{ab}	0.15±0.02^{c}	-
Tr1	95±2.22^{b}	4.38±0.19^{c}	2.32±0.16^{a}	0.52±0.03^{a}	0.18±0.03^{c}	20
Tr2	97±1.68^{b}	4.97±0.19^{c}	2.33±0.18^{a}	0.46±0.01^{b}	0.20±0.03^{c}	33.33
Tr3	100±1.81^{b}	6.82±0.13^{b}	0.55±0.15^{c}	0.08±0.07^{c}	0.60±0.04^{b}	300
Tr4	110±2.80^{a}	7.42±0.23^{a}	0.88±0.06^{b}	0.11±0.08^{c}	0.75±0.02^{a}	400

C1: uninoculated control plants without phosphorus source; C2: uninoculated control plants supplemented with tricalcium phosphate; C3: uninoculated control plants supplemented with KH_2PO_4; Tr1: plants inoculated with PGP291; Tr2: plants inoculated with PGP30; Tr3: plants inoculated with PGP291+RhOF57A; Tr4: plants inoculated with PGP30+RhOF57A. (*): No pod development for C1 plants. Means (±standard errors) within the same column followed by different letters are significantly different according to the Student, Newmann, Keuls test at $p<0.05$.

Table 4. Effect of bacterial inoculation on phosphorus concentration, phytase and phosphatase activities in faba bean plants with different phosphorus supply.

Treatment	Plant phosphorus concentration (mg/g of dry matter)	Bean pod phosphorus concentration (mg/g of dry matter)	Phosphatase (nmole of Pi/min/g of fresh matter)	Phytase (nmole of Pi/min/g of fresh matter)
C1	5.78±0.05^{f}	(*)	40.95±2.05^{f}	30±2^{f}
TCP				
C2	6.1±0.25^{f}	3.61±0.42^{d}	35.77±4.63^{f}	21±6.2^{f}
Tr1	11.8±0.21^{d}	5.69±0.30^{c}	83.77±5.61^{d}	30±2.2^{f}
Tr2	15.73±0.25^{c}	6.90±0.30^{b}	96.71±7.36^{d}	109±3.2^{e}
Tr3	17.14±0.24^{b}	8.49±0.31^{a}	118.57±3.68^{c}	140±4.4^{b}
Tr4	19.12±0.10^{a}	8.75±0.34^{a}	180.64±5.61^{a}	208±2^{a}
PO_4				
C3	8±0.23e	2.89±0.19^{e}	27.96±2.56^{g}	12±3^{g}
Tr1	12.31±0.28^{d}	4.21±0.2^{d}	56.3±2.23^{e}	24±4^{f}
Tr2	16.77±0.10^{b}	4.90±0.33^{d}	91.51±9.30^{d}	39±2^{e}
Tr3	18.60±0.58^{a}	8.85±0.31^{a}	110.75±4.27^{c}	96±1.4^{d}
Tr4	19.42±0.24^{a}	9.51±0.37^{a}	140.60±9.09^{b}	133±6.2^{b}

C1: uninoculated control plants without phosphorus source; C2: uninoculated control plants supplemented with tricalcium phosphate; C3: uninoculated control plants supplemented with KH_2PO_4; Tr1: plants inoculated with PGP291; Tr2: plants inoculated with PGP30; Tr3: plants inoculated with PGP291+RhOF57A; Tr4: plants inoculated with PGP30+RhOF57A. (*): No pod development for C1 plants. Means (±standard errors) within the same column followed by different letters are significantly different according to the Student, Newmann, Keuls test at $p<0.05$.

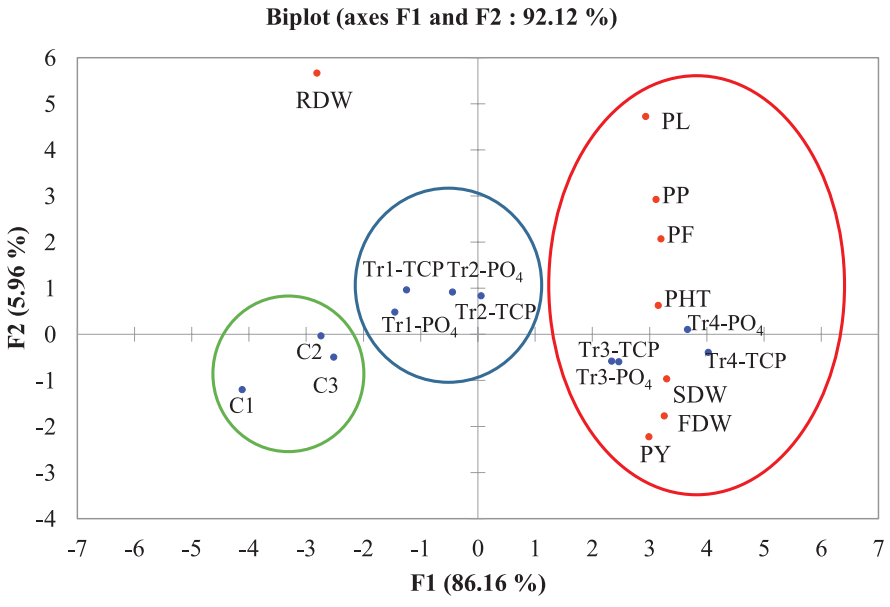

Fig. 2. Principal component analyses (PCA) of faba bean submitted to different treatments given in blue: C1: plants without inoculation and without phosphorus source; C2: plants grown without inoculation and supplemented with tricalcium phosphate; C3: plants without inoculation and irrigated with KH_2PO_4; Tr1-TCP: plants inoculated with PGP291 and supplemented with tricalcium phosphate; Tr2-TCP: plants inoculated with PGP30 and supplemented with tricalcium phosphate; Tr3-TCP: plants inoculated with PGP291+RhOF57A and supplemented with tricalcium phosphate; Tr4-TCP: plants inoculated with PGP30+RhOF57A and supplemented with tricalcium phosphate; Tr1-PO4: plants inoculated with PGP291 and irrigated with KH_2PO_4; Tr2-PO_4: plants inoculated with PGP30 and irrigated with KH_2PO_4; Tr3-PO_4: plants inoculated with PGP291+RhOF57A and irrigated with KH_2PO_4; Tr4-PO_4: plants inoculated with PGP30+RhOF57A and irrigated with KH_2PO_4. Biomass, yield and nutrient concentrations are represented in red. PL: plant length; SDW: shoot dry weight; RDW: root dry weight; FDW: fruit pod dry weight; PP: phosphorus concentration in plants; PF: phosphorus concentration in fruit; PY: phytase activity in plants; PHT: phosphatase activity in plants.

Plant Nutrition and Yield Improvement

Plant length and shoot dry weight of inoculated faba bean plants were significantly higher than for uninoculated controls (Table 3). Highest values were obtained for the co-inoculation with PGP30+RhOF57A. In contrast, root dry weights and root/shoot ratios of co-inoculated plants showed the lowest values (Table 3). Balemi and Negisho [12] reported that the higher root/shoot ratio is often a sign of growth under phosphorus limitations. The reduction in plant leaf growth under phosphorus limitation leads to the translocation of photosynthates to the roots in order to improve their development for better soil exploration. The biomass of bean pods was also highest for the co-inoculated treatments with both phosphorus sources (Table 3).

Co-inoculated plants retained more phosphorus compared to plants with individual inoculation and controls (Table 4). Likewise, co-inoculation improved the phosphorus concentrations of bean pots with both phosphorus sources (Table 4). The principal component analyses (PCA) revealed that all the measured parameters correlate with the first factor for faba bean plants (Fig. 2). Higher biomass and phosphorus concentrations corresponded to the co-inoculated treatments under both phosphorus sources (on the right). They are separated from the individual inoculation treatments of faba bean. The lower levels of these parameters (on the left) correspond to the uninoculated controls (Fig. 2). Several other studies show the positive effect of co-inoculation with rhizobia and different PGPR on plant growth and yield of lentils, bean, *Vigna mungo* and chickpea [29-32], indicating that plant growth promotion can be achieved by the selection of suitable strains. Success depends on several factors such as plant genotype and bacterial species used [33]. Plants have developed many mechanisms to adapt to phosphorus-deficient soils, which includes the secretion of phosphatases from roots [34]. This facilitates the hydrolysis of soil organic phosphate at lower pH and thereby increases the availability of orthophosphate [35]. To test, if this is also true for faba bean in our inoculation experiments, we measured phosphatase and phytase activities in the roots of plants under a different phosphorus supply. Indeed, inoculation with PGPR significantly increased the corresponding enzymatic activities – especially in roots supplemented with tricalcium phosphate (Table 4). Co-inoculation treatments yielded the highest values. Such a stimulation of phosphatase/phytase was reported for other plant/bacteria combinations. For example, Ramesh et al. [36] reported that several *Bacillus* isolates stimulated phosphatase and phytase activity in the soybean rhizosphere to different degrees. A dependency of phosphatase activity on the combination of *Phaseolus vulgaris* cultivars and rhizobial strains was found by Mandri et al. [13]. For *Phaseolus vulgaris*, it was shown that a low phosphorus level in the soil leads to an increase both the density and the activity of phytate-mineralizing bacteria [37]. Such a predisposition of bacteria to produce phosphatases would be an advantage for plants growing in phosphorous-deficient soils, as most of the beans are cultivated in Africa in phosphorus-deficient soils [38].

Conclusions

To improve soil fertility and plant growth, effective phosphate-solubilizing bacteria are a promising tool to maintain agricultural resources. We studied the impact of phosphate source and inoculation on faba bean growth, phosphate nutrition, and phosphatase-phytase activities. The tested bacterial strains were able to mobilize insoluble phosphate and potassium and to produce important compounds for plant growth and nutrition such as auxins, siderophores, and exopolysaccharides. Inoculation of faba bean with these strains significantly improved plant length, shoot, root, and bean pod dry weight. Similarly, phosphorus concentrations in the plant and phosphatase/phytase activities were increased. Highest values were obtained with co-inoculation treatments. The PCA analyses showed that the highest values of biomass, phosphorus concentrations, and enzymatic activities are attributed to the co-inoculated plants supplemented with tricalcium phosphate or irrigated with potassium phosphate KH_2PO_4. The ability of the studied strains to increase growth and productivity of bean plants – even in the presence of the insoluble form of phosphorus – encourages their future use in agricultural practices. For validation of the results, studies under natural field conditions are necessary. This might lead to a reduced and optimized use of chemical fertilizer and thus ensure sustainable agricultural resources.

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Conflict of Interest

The authors declare there is no conflict of interest.

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