Phosphate Solubilizing Rhizobacteria Could Have a Stronger Influence on Wheat Root Traits and Aboveground Physiology Than Rhizosphere P Solubilization

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Limited P availability in several agricultural areas is one of the key challenges facing current agriculture. Exploiting P-solubilizing bacteria (PSB) has been an emerging bio-solution for a higher rhizosphere P-availability, meanwhile the above- and below-ground interactions that PSB would trigger remain unclear over plant growing stages. We hypothesized that PSB effects on plant growth may be greater on root traits that positively links with aboveground physiology more than the commonly believed rhizosphere P bio-solubilization. In this study, five contrasting PSB (*Pseudomonas* spp.) isolates (low "PSB_1", moderate "PSB_2 and PSB_4" and high "PSB_3 and PSB_5" P-solubilizing capacity "PSC") were used to investigate above- and below-ground responses in wheat fertilized with rock P (RP) under controlled conditions. Our findings show that all PSB isolates increased wheat root traits, particularly PSB_5 which increased root biomass and PSB_3 that had greater effect on root diameter in 7-, 15- and 42-day old plants. The length, surface and volume of roots significantly increased along with higher rhizosphere available P in 15- and 42-day old plants inoculated with PSB_4 and PSB_2. Shoot biomass significantly increased with both PSB_2 and PSB_5. Root and shoot physiology significantly improved with PSB_1 (lowest PSC) and PSB_4 (moderate PSC), notably shoot total P (78.38%) and root phosphatase activity (390%). Moreover, nutrients acquisition and chlorophyll content increased in inoculated plants and was stimulated (PSB_2, PSB_4) more than rhizosphere P-solubilization, which was also revealed by the significant above- and below-ground inter-correlations, mainly chlorophyll and both total ($R = 0.75$, $p = 0.001$**) and intracellular ($R = 0.7$, $p = 0.000114$*) P contents. These findings demonstrate the necessity to timely monitor the plant-rhizosphere continuum responses, which may be a relevant approach to accurately evaluate PSB through considering below- and above-ground relationships; thus enabling unbiased interpretations prior to field applications.

Keywords: phosphate, *Pseudomonas*, rhizosphere, phosphatase, root traits, solubilization, wheat

INTRODUCTION

After nitrogen (N), phosphorus (P) is the most important nutrient that plants need at an adequate rate from the early stages of their development. This nutrient plays key roles in root development, root traits anatomy modifications and root hair density with a significant contribution in increasing yield of crops and plants resistance against multiple diseases (Kondracka and Rychter, 1997; Ma et al., 2001). At a cellular level, P is vitally important, owing to its involvement in cells division, growth of new tissues and nucleic acid structure which all regulate protein synthesis, energy transfer, and photosynthesis (Vance et al., 2003). Notwithstanding, low P availability in agricultural soils is a pressing issue that affects over two billion hectares worldwide (Oberson et al., 2001). For instance, P deficiency was reported to cause a significant reduction (5–15%) of crop yield (Shenoy and Kalagudi, 2005) with P-deficient plant symptoms characterized by reddish leaves and necrosis in old leaves tips (Luiz et al., 2018). Generally, P availability in most soils depends on multiple factors, notably the ions concentration and soil pH (Hinsinger et al., 2018). In calcareous soils, P is often precipitated with Ca and in acidic soils P binds to Fe and Al (Tariq et al., 2014), resulting in little P bioavailable forms for root absorption, which affects the plant growth and production.

To overcome the low P availability in soils, use of P-efficient crops along with reasonable application of different P sources is paramount to secure crop productivity. For example, using plant species with higher ability to take up and use P in a soil with a low P availability has been an efficacious plant-based strategy. Findings by Djadjaglo and Richter (2008) and Gao et al. (2016) demonstrated that leguminous plants such as *Medicago sativa*, *Vicia faba* and *Phaseolus vulgaris* yielded better under P deficient conditions and had increased P available fraction, P uptake and soil acid phosphatases (APase) activity as a consequence of a more developed root system compared to other crops such as *Sorghum bicolor*. In addition, crop diversification such as the case of legume–cereal intercropping systems have been reported to stimulate P uptake due to a higher belowground biochemical and morphological functional heterogeneity, notably faster root growth and higher nodulation (Hauggaard-Nielsen et al., 2009; Bargaz et al., 2017). Stimulation of plant growth, availability and acquisition of P by roots is likely due to numerous rhizosphere-induced changes including rhizosphere acidification (Betencourt et al., 2012; Latati et al., 2016), exudation of organic acids and P-hydrolyzing enzymes (Hakeem et al., 2014), soil respiration (Ibrahim et al., 2013; Latati et al., 2014), and modulation of the microbial activity in the vicinity of the rhizosphere (Morgan et al., 2005; Song et al., 2007; Sun et al., 2009). Agriculturally beneficial microorganisms commonly known as plant growth promoting microbes (PGPM) have been adopted as a potent microbial strategy (e.g. inoculants, biofertilizers, biopesticides, biostimulant) that may stimulate plant growth *via* direct and/or indirect mechanisms (Mishra and Sundari, 2013; Gupta et al., 2015; Mishra et al., 2017). Direct effects attributed to plant growth promoting rhizobacteria (PGPR) rely on several physiological and biochemical pathways that improve plant nutrition and which encompass most mechanisms related, among other, to solubilization and uptake of nutrients (e.g. P, K, Zn, etc.), biological N_2 fixation and production of phytohormone and siderophore molecules (Fankem et al., 2006; Panhwar et al., 2011). Furthermore, PGPR may indirectly stimulate plant growth by modulating local and systemic plants defense mechanisms or by producing secondary metabolites (allelochemicals) behaving as plant-immunity inducing signals against phytopathogen attacks (Kumar et al., 2018).

Multiple beneficial effects of soil microorganisms have widely been identified as key drivers for a better plant growth and increased soil P availability (Kumar, 2016; Pérez et al., 2016; Bargaz et al., 2018). PGPR exhibiting high PSC have been described to benefit plant growth and yield when associated with roots and even within other plant parts such as leaves (Fahad et al., 2015; Jambhulkar et al., 2016; Tang et al., 2018). For example, application of efficient phosphate solubilizing bacteria (PSB) such as *Bacillus megaterium* increased soil P availability by nearly 30% (Alzoubi and Gaibore, 2012). Likewise, other bacterial species belonging to multiple genera such as *Pseudomonas* (Sharma et al., 2013), *Azotobacter* (Kumar and Singh, 2001), *Xanthomonas* (De Freitas et al., 1997), *Rhodococcus*, *Arthrobacter*, *Serratia*, *Chryseobacterium*, *Gordonia*, *Phyllobacterium*, and *Delftia* sp. (Wani et al., 2005; Chen et al., 2006) are known to exhibit high PSC. In addition to a single use of PSB as bio-inoculants, dual use of the PSB and P-based mineral fertilizers including sparingly insoluble P forms also provided evidences for a profitable integrated plant nutrition system that may lead to a successful "microbial–P mineral" alliance (Adnan et al., 2017; Bargaz et al., 2018; Tahir et al., 2018). Studies by Panhwar et al. (2011) and Bakhshandeh et al. (2015) measured higher yield in rice and sunflower in response to co-application of different mineral P such as triple super phosphate (TSP) and inoculation with various PSB (*Bacillus*, *Rahnella aquatilis*, *Enterobacter* sp., *Pseudomonas fluorescens* and *Pseudomonas putida*) isolates. Such a positive dual use of both resources was confirmed at both physiological and grain yield plant developmental stages consisting of multiple functional traits including photosynthetic pigments -Chl a, Chl b, Chls and Car-, growth parameters, plant height, number of panicles hill, stems hill, grain weight, biological yield, seed oil yield, nutrient concentrations in seeds and oil.

Combinatory use of PSB and rock phosphate (RP) that are considered to be natural resources has been successful through a number of applied research investigations that demonstrated an improved agronomic RP efficiency (Gomes et al., 2014; Abbasi et al., 2015; Giro et al., 2016; Bargaz et al., 2018). Exploitation of microbial functional traits related to P solubilization is paramount as to propose microbial-based strategies enabling increased RP use efficiency required in many high P-retention agricultural soils (Kumar, 2016). Many experimental studies provided evidence that synergies may occur when combining both PSB strains and RP that may lead to a cost-effective P-based biofertilizer for a direct application in high P-retention soils. For instance, dual application of RP and PSB (e.g. *Klebsiella*, *Azotobacter*, *Azosporillum* and *Rhizobium*) significantly improved P nutrition in both cereal and

legume crops (Del Pilar López-Ortega et al., 2013; Kaur and Reddy, 2015; Midekssa et al., 2016; Adnan et al., 2017; Manzoor et al., 2017; Ditta et al., 2018). Several above- and below-ground plant parameters are used to quantify such positive effects; however, PSB effects may be complementing nutritional features of RP whose solubilization should occur, owing not only to PSB themselves, but also to which extent they can tightly modulate both functional traits and root activities. As per current knowledge, bacterial *in vitro* assays and plant inoculation experiments have been majorly adopted in order to make decisions on efficient PSB bacterial isolates to be formulated as potent bio-inoculants. Nevertheless, PSB behavioral properties at a temporal scale during plant growth stages need to be mechanistically unraveled and timely monitored either for a single strain or a consortium. This will help understand how tight the relationship between the PSB of interest and the rooting system under sparingly P forms is and whether it always remains tight throughout the different plant growth stages, considering that highly performing PSB *in vitro* are presumably the most efficient *in planta*. Another important aspect of a successful PSB–root interaction would be the best fit in terms of rooting stimulation in addition to rhizosphere P solubilization that most studies have focused on as only few investigations (Bakhshandeh et al., 2015; Sarsan, 2016; Rezakhani et al., 2019) described a positive influence on specific root functional traits. This is in line with the objective of this study to assess the effect of five P solubilizing rhizobacteria exhibiting different PSC "low, medium and high" on *durum wheat* morphological root traits and associated rhizosphere P solubilization in order to shed light on how tight does inoculation link rhizosphere parameters with plant aboveground morphological and physiological traits under RP fertilization.

MATERIALS AND METHODS

Microbial Experiments

Plant Sampling and Rhizobacteria Isolation

In this study, five PSB isolates (*Pseudomonas* spp.) with contrasting PSC were used as inoculant in order to investigate above- and belowground physiological responses in RP-fertilized wheat. They were among 42 PSB isolates that were captured from the rhizosphere soils of several crops (wheat, barley, maize, oat, faba beans, peas, etc.) from two main agricultural areas (Haouz and Erhamna) regions and from the rhizosphere soils of naturally grown plants in the P mining area of Benguerir in Morocco. For PSB isolation, the National Botanical Research Institute's phosphate growth medium (NBRIP)-agar was used with either tricalcium phosphate (TCP, $Ca_3 (PO_4)_2$, 5g/l) or RP (5g/l containing P_2O_5: 30.65%, CaO: 48.51%, MgO: 0.63%, K_2O: 0.09%, Fe_2O_3: 0.25%) as the only source of P added with (per liter) glucose: 10 g; $MgCl_2 \cdot 6H_2O$: 5 g; $MgSO_4 \cdot 7H_2O$: 0.25 g; KCl: 0.2 g and $(NH_4)_2 SO_4$: 0.1 g). Bacterial isolates with clear P solubilisation halos were kept as PSB prior to quantitative analyses of P solubilization rates in NBRIP liquid medium added with either TCP or RP after 7 days of incubation at 28°C. In addition to P solubilization trait, isolates were also screened for other PGP-traits such as medium acidification,

N fixation, indole acetic acid (IAA) production, ammonium production, hydrogen cyanide (HCN) production, and salinity tolerance. Based on their PSC, all PSB isolated (including PSB tested in this study) were sorted out into three groups (low, moderate and high PSC).

DNA Isolation and Molecular Identification

Prior to DNA isolation, PSB isolates were cultivated under gentle agitation in 10 ml of Luria–Bertani broth for 24 h at 28°C. One millilitre of the bacterial culture was placed in micro-tubes and pelleted by centrifugation for 2 min. For genomic bacterial DNA isolation, the GenElute™ Bacterial Genomic DNA kit was used following the manufacturer's instructions. Bacterial DNA from five isolates (PSB_1 to PSB_5) were visualized by agarose gel electrophoresis (0.8%) and spectrophotometrically quantified using the NanoDrop TM ND-1000 V3.7.0 (Thermo Fisher Scientific Inc., Wilmington, USA) prior to PCR amplification of the 16S rDNA. The taxonomic identification of isolates was done by 16S rRNA gene sequencing using the following primers: 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3'). The BLAST analysis of the five PSB isolates (PSB_1 to PSB_5) belongs to *Pseudomonas plecoglossicida*, *Pseudomonas reinekei*, *Pseudomonas koreensis*, *Pseudomonas japonica* and *Pseudomonas frederiksbergensis*, respectively. The 16S rRNA gene sequences were deposited in GenBank under accession numbers MT362706–MT362710.

Determination of P Solubilization Rate

PSB isolates (PSB_1 to PSB_5) were tested for their ability to solubilize TCP by determining the P solubilization index (PSI) in NBRIP agar medium after 7 days of incubation at 28°C. PSI was calculated as the sum of the colony diameter and the clearing zone divided by the colony diameter (Iqbal et al., 2016). Quantitative estimation of either TCP or RP solubilization by each bacterial isolate was done in NBRIP liquid medium in which pH variations were also monitored. The NBRIP medium was inoculated with 0.1 ml of a liquid bacterial culture (10^8 CFU ml^{-1}), incubated at 180 rpm for seven days at 28°C and the supernatants of each PSB suspension was obtained by centrifugation (3,000g for 10 min). The available P fraction were estimated spectrophotometrically using molybdenum blue method against standards that were plotted using spectrophotometer at 880 nm. The absorbance of samples was measured by means of the standard curve using the same wavelength and converted into P concentrations expressed as $\mu g \cdot ml^{-1}$ (Fernández et al., 2007).

Determination of Bacterial Plant Growth Promoting Traits

Free N_2 fixation was confirmed in N-free Ashby medium composed of (per l): agar (15 g), mannitol (15 g), K_2HPO_4 (0.4 g), $CaCl_2 \cdot 2H_2O$ (0.1 g), NaCl (0.2 g), $MgCl_2$ (0.1 g), $FeSO_4 \cdot 7H_2O$ (3.0 mg), $NaMoO \cdot 2H_2O$ (3.0 mg). After 7 days of incubation at 28°C, PSB isolates developed in Ashby medium were considered as free N-fixer isolates and their ability to produce ammonium was verified using Nessler's reagent according to Geetha et al. (2014).

Qualitative analysis of IAA production was first determined (pink colour indicates IAA production) in NBRIP liquid medium added with tryptophan using Salkowski's method (Biswas et al., 2018). Secondly, IAA-producing isolates were then selected to estimate IAA production using bacterial cultures that were grown in 50 ml medium and gently shacked for five days at 28°C. Two millilitres of Salkowski reagent (mixture of 0.5 M ferric chloride ($FeCl_3$) and 35% perchloric acid ($HClO_4$)) were added to 1 ml of culture supernatant and the mixture was incubated in dark at room temperature for 30 min. The development of a pink colour indicating IAA production that quantified (estimation) spectrophotometrically at 535 nm using an IAA concentration curve made with 0, 10, 20, 50, and 100 $\mu g \cdot ml^{-1}$ of synthetic IAA (Barra et al., 2016).

Siderophore production by PSB isolates was revealed on blue CAS (chrome azurol S) agar medium according to Pérez-Miranda et al. (2007). After incubation at $28 \pm 2°C$ for 5 days, the change of CAS agar colour from blue to orange around PSB colonies is an indication of siderophore production. Production of hydrogen cyanide by PSB isolates was carried out in tryptone soya agar medium added with 0.44% of Glycine (Geetha et al., 2014). After two days of incubation at 28°C, HCN production was visually indicated by a color change from yellow to reddish-brown. Salinity tolerance was tested by growing the PSB isolates on Luria–Bertani medium supplied with increasing NaCl concentrations (e.g. 2, 5 and 8%) incubated for three days at 28°C and tolerance to salinity was determined by simple visualization of bacterial growth on Luria–Bertani agar medium (Sarkar et al., 2018).

Plant Inoculation Experiment
Inoculation of Wheat and Plant Growth Conditions
Effect of Inoculation with PSB Isolates on Seedlings Radicles
Five bacterial isolates exhibiting high (PSB_3 and PSB_5), moderate (PSB_2 and PSB_4) and low (PSB_1) PSC and multiple other PGP-traits were used. Their ability to improve wheat seedlings growth, was also determined in 7-day old radical seedlings. Wheat seeds were surface sterilized with sodium hypochlorite (6°, 1 min) and ethanol (96%, 1 min) and then washed thoroughly with sterile distilled water. Inoculum for each PSB isolate was prepared in Luria–Bertani liquid medium at 28°C for 48 h (10^8 CFU ml^{-1}), centrifuged and cell bacterial pellet were used to seed inoculation, which was applied by soaking the seeds in 20 ml of inoculum for 1 h under a gentle shaking. Inoculated seeds were germinated in sterilized germinating paper wherein seeds were evenly spaced, moistened by 2 ml of sterilized water mixed with RP and rolled up in vertical standing of paper. Seeds were incubated for germination in a growth chamber (phytotron) under controlled conditions (28°C, 70% humidity, 16/8 h photoperiod and an illumination intensity of 240 $\mu mol \ m^{-2} s^{-1}$). Radicles of the 7-day old seedlings were measured using the root scanner WinRhizo (Regent Instruments Inc., Quebec City, Canada).

Effect of Inoculation With PSB Isolates on 15- and 42-Day Old Wheat Plants
Wheat seeds were surface sterilized and inoculated as described above for seedlings germination parameters. Briefly, the experiment was conducted in plastic pots (20 cm in depth and 15 cm in diameter) that were previously sterilized (6° sodium hypochlorite) and filled with sterilized mixture of sand, soil and peat (2:0.5:0.5). Five bacterial-inoculated (PSB_1 to PSB_5) treatments *versus* two control treatments were tested. Control treatments correspond to 1) non-inoculated wheat plants supplied with rock P (157 kg ha^{-1}) and 2) non-inoculated wheat plants supplied with TSP (85 kg ha^{-1}) a readily available P form (estimated based on wheat P requirement according to Kaur and Reddy, 2015). Inoculated treatments (single inoculation with PSB_1 to PSB_5 isolates) had the same amount of either RP or TSP that were mixed sterilely with the plant growth substrate prior sowing. The experiment was conducted under controlled conditions (28°C, 70% humidity, 16/8 h photoperiod and an illumination intensity of 240 $\mu mol \ m^{-2} s^{-1}$) in a complete randomized design of four replicates per treatment with each replicate consisting of a pot with eight wheat plants. The irrigation was done once a week with P-free Hoagland's solution and watered twice with sterile distilled water to maintain adequate soil field capacity. Six weeks after sowing, two non-destructive analyses (e.g. chlorophyll fluorescence, and stomatal conductance) were measured (Zeng et al., 2013) before plants and rhizosphere soils were harvested for additional above- and below-ground analyses.

DETERMINATION OF PLANT AND RHIZOSPHERE PARAMETERS

Measurement of Morphological Root Traits and Plant Biomass
At both 15 and 42 days after germination, plants were harvested and separated into shoots and roots. The rhizosphere growth substrate was obtained by carefully separating roots from the loosely adhering soil, which was then sieved (2 mm) prior to measurements of Olsen P concentration. Root morphological traits were measured using the automated image analysis software WinRhizo (Regent Instruments Inc., Quebec City, Canada). Each root sample was evenly spread apart in a water layer on a Plexiglas transparent tray and imaged at a resolution of 300 dpi with an Epson Expression 836 L scanning system. Root images were analyzed for total root length (RL), root surface area (RSA), average root diameter (RD) and root volume (RV). Subsequently, dry weights of shoots (SDW) and roots (RDW) were determined before they were ground to a fine powder for analyses of P and N concentrations.

Determination of Rhizosphere Available P and Nutrients (P and N) Acquisition
Available P content in the rhizosphere soil was measured according to Fernández et al. (2007). Total P contents in shoots and roots were determined in finely ground dried samples (0.5 g) that were incinerated at 600°C for 6 h followed by ash solubilization in hydrochloric acid (10N). Obtained filtrates (1 ml) were added to 5 ml of a reaction mixture consisting of ammonium molybdate (2.5%) and hydrazine sulfate (0.15%) and absorbance was read at 820 nm (Majeed et al., 2015). Roots and shoots (100 mg fresh weight (FW)) were

ground with an extraction mixture consisting of 500 µl of 0.1M sodium acetate buffer (pH 5.6) containing 1 mM dithiothreitol. Homogenates were centrifuged at 13,000g at 4°C for 30 min and aliquots of 50 µl of supernatant were used for quantification of inorganic P (Pi) (Bargaz et al., 2012; Bargaz et al., 2017).

Shoot and root Pi contents were measured following the ascorbic acid method as described by Zheng et al. (2009). The finely ground shoot subsamples (0.5 g) were also used for total N analysis using Kjeldahl method (Magomya et al., 2014). The root P acquisition efficiency (RPAE), which reflects the capacity of roots to absorb P from soil, was calculated as the ratio of plant P content to root dry weight (Pan et al., 2008).

Protein and Chlorophyll Contents in Wheat Shoots

Samples of 100 mg fresh weight were ground in 4 ml Tris–HCl buffer (0.1 M pH 7.5) and centrifuged at 15,000g for 20 min. Protein content was determined using Bradford method. Protein concentration was determined based on a bovine serum albumin standard curve. Total chlorophyll concentration was measured according to Pérez-Patricio et al. (2018). An aliquot of 100 mg of fresh leaf tissue was ground in 5 ml of acetone (80%, v/v). Total chlorophyll was determined using the following formula:

$$Chlt = 8.02*(DO663) + 20.20*(DO645).$$

Acid Phosphatase Activity in Wheat Roots

Roots APase activity was measured according to Bargaz et al. (2017). Root fresh weight (100 mg) samples were ground with an extraction mixture consisting of 500 µl of 0.1M sodium acetate buffer (pH 5.6) containing 1 mM dithiothreitol. Homogenates were centrifuged (13,000g at 4°C for 30 min) and supernatant (50 µl) was used for quantification of root APase activity. p-nitrophenyl phosphate (pNPP) was used as a substrate and the enzyme activity was defined as the amount hydrolyzing 1 nmol of pNPP per min per g of root fresh weight.

Statistical Analysis

The statistical data analysis was carried out by IBM® SPSS® Statistics V. 24 software. One-way ANOVA (analysis of variance) was used, followed by Tukey *post hoc* test to determine the significant difference among means of the treatment at 0.05 significance level. PCA analysis was performed using Minitab V.18 statistical software.

RESULTS

PSB Biochemical Properties and Effects on Wheat Seedlings Root Growth
PSB Identification and *In Vitro* Properties
Based on 16S rRNA gene sequencing, PSB isolates used in this study belong to *Pseudomonas* genera. PSB isolates had different PSC from TCP ranging from 113 to 121.2 mg P l^{-1} for PSB_3 and PSB_5 (high PSC), 88.79 to 99.88 mg l^{-1} for PSB_2 and PSB_4 (moderate PSC) and up to 41.37 mg P l^{-1} for PSB_1 (low PSC) (**Table 1**). Clear P solubilizing halos around bacterial colonies were observed in all isolates and varied from 4.9 to 5.8. Medium acidification with either RP or TCP showed a sharp drop from an initial value of 7 to 4.34, except for the PSB_1 isolate whose pH medium was kept around neutrality over five days of incubation. In addition, PSB were assessed to be IAA-producing isolates (10.46–36.41 µg ml^{-1}), N_2-fixers (ammonia production from 0.02 to 0.19 µmole ml^{-1}), siderophore-producers, HCN producers and also salt tolerant growing at up to 0.86 M NaCl.

PSB *In Vivo* Effects on Root Growth of Wheat Seedlings
Measurement of root growth morphological traits in both 7- and 15-day old inoculated seedlings indicated significant increase of most radicle traits with the exception noted for RD showing no difference as compared to non-inoculated seedlings (**Table 2**). Specifically, PSB_1 and PSB_3 improved significantly RL (34.40%), RSA (34.04%) and RV (32.5%) of the 7-day old seedlings; meanwhile, it is the PSB_4 that significantly increased RL (58.54%), RSA (65.55%) and RV (77.77%) of the 15-day old seedlings. This isolate had the highest effect on 15-day old seedlings, notably for RSA and RV that significantly increased by 126.33 and 60% as compared to 7-day old seedlings.

TABLE 1 | Properties of phosphate solubilizing bacterial isolates (PSB_1 to PSB_5) related to P solubilization, solubilization index in agar plate, available P in inoculated soil (ppm), medium acidification, IAA production, ammonia production, siderophore index, HCN production and salinity tolerance.

Pseudomonas strains		SI	P available µg·ml⁻¹		P available in inoculated soil (ppm)	pH		IAA µg·ml⁻¹	NH₃nmol·ml⁻¹	Siderophore index (mm)	HCN	Salinity 5%
			TCP	RP		TCP	RP					
Non-inoculated		–	8.77[e]	0.6[e]	75.1[f]	6.8	6.9	–	–	–	–	–
PSB_1	*P. plecoglossicida*	5	41.37[d]	6.41[d]	219.6[c]	6.56	7.51	29.65[ab]	90[a]	1.64[b]	0.05[a]	+
PSB_2	*P. reinekei*	5.8	99.88[bc]	45.65[c]	179.6[e]	4.91	4.58	28.34[ab]	80[a]	3.66[a]	0.03[ab]	+
PSB_3	*P. koreensis*	4.9	113.2[ab]	59.31[b]	223.9[b]	4.74	4.66	36.41[a]	130[a]	1.25[b]	0.03[ab]	+
PSB_4	*P. japonica*	4.9	88.79[c]	41.42[c]	192.7[e]	4.60	4.34	24.91[ab]	20[a]	4.01[a]	0.02[b]	–
PSB_5	*P. frederiksbergensis*	5.5	121.2[a]	66.02[a]	252.7[a]	4.69	4.92	10.64[b]	190[a]	2.58[ab]	0.02[b]	+

Data are means of four replicates after seven days of incubation. Mean values labeled with the same superscript letter were not significantly different at p < 0.05. SI, Solubilization index; TCP, Tricalcium phosphate; RP, Rock phosphate; IAA, Indole acetic acid; HCN, Hydrogen cyanide; +: tolerance and –: No tolerance.

TABLE 2 | Variations in morphological root traits at 7-, 15- and 42-day old of durum wheat fertilized with rock P in response to inoculation with five PSB isolates *versus* P (RP and TSP) treatments alone.

	7-day old seedlings				15-day old seedlings				42-day old plants			
	RL (cm)	RSA (cm^2)	RV (cm^3)	RD (mm)	RL (cm)	RSA (cm^2)	RV (cm^3)	RD (mm)	RL (cm)	RSA (cm^2)	RV (cm^3)	RD (mm)
RP	44.85[c]	6.58[c]	0.08[c]	0.47[b]	115.97[b]	11.99[b]	0.09[c]	0.32[b]	4468[c]	482[c]	4.01[b]	0.65[c]
TSP	–	–	–	–	137.99[ab]	14.26[ab]	0.11[abc]	0.32[b]	5472[bc]	633[bc]	5.87[ab]	0.71[bc]
PSB$_1$+RP	69.80[a]	10.37[a]	0.12[ab]	0.47[ab]	157.8[ab]	17.71[ab]	0.16[ab]	0.35[ab]	6208[abc]	652[abc]	5.50[ab]	1.01[ab]
PSB$_2$+RP	52.22[bc]	7.48[bc]	0.09[bc]	0.45[ab]	129.3[ab]	14.23[ab]	0.12[abc]	0.34[ab]	8434[a]	887[a]	7.49[a]	0.98[abc]
PSB$_3$+RP	59.02[ab]	9.64[ab]	0.13[a]	0.52[a]	124.3[ab]	15.14[ab]	0.14[abc]	0.38[a]	7602[ab]	804[ab]	6.82[ab]	1.10[a]
PSB$_4$+RP	62.77[ab]	8.77[bc]	0.10[abc]	0.45[ab]	183.87[a]	19.85[a]	0.16[a]	0.34[ab]	73.57[ab]	741[ab]	6.18[ab]	0.86[abc]
PSB$_5$+RP	57.59[ab]	7.84[bc]	0.09[bc]	0.43[ab]	154.79[ab]	15.96[ab]	0.12[abc]	0.32[b]	59.38[bc]	604[bc]	4.93[ab]	0.88[abc]

Data are means of four replicates and each replicate consists of eight wheat plants per pot. Mean values labeled with the same superscript letter were not significantly different at p < 0.05.
RL, root length; RSA, root surface area; RV, root volume; RD, root diameter.

Effects of PSB Isolates on 42-Day Old Durum Wheat Plants Supplied With RP

Effects on Wheat Plant Growth Parameters

Inoculation of RP-fertilized wheat plants with all PSB improved growth parameters, notably SDW, RDW and shoot height (**Table 3**). For all PSB isolates, this positive effect was significant as compared to wheat plants fertilized with RP alone. PSB$_2$ significantly increased both SDW and RDW as compared to either RP- or TSP-fertilized wheat plants. This increase by PSB$_2$ showed the highest SDW as compared to RP (48%) rather than TSP (34%). Differences were also noted among PSB isolates in terms of RDW (though not significant), notably with PSB$_5$ and PSB$_4$ exhibiting the highest and the lowest RDW, respectively.

Effect on Wheat Morphological Root Traits

Morphological root traits (e.g. RL, RSA, RV, RD, Number of tips (Ntips), Number of crossing (Ncross) and Number of froks (Nfroks)) of the 42-day old inoculated plants markedly improved as compared to non-inoculated plants (**Table 2**). Obvious differences were noted between PSB isolates, notably PSB$_2$ whose effect on root traits (except RD) appeared to be the most significant as compared to both RP- and TSP-fertilized and non-inoculated plants. Similar effects were noted for the remaining PSB isolates, but to a lower extent than PSB$_2$. Significant variations were found with PSB$_3$ and PSB$_4$, particularly the significant increase in RL (by 37 and 34%) and RSA (by 66.57 and 53.56%) over RP rather than TSP application.
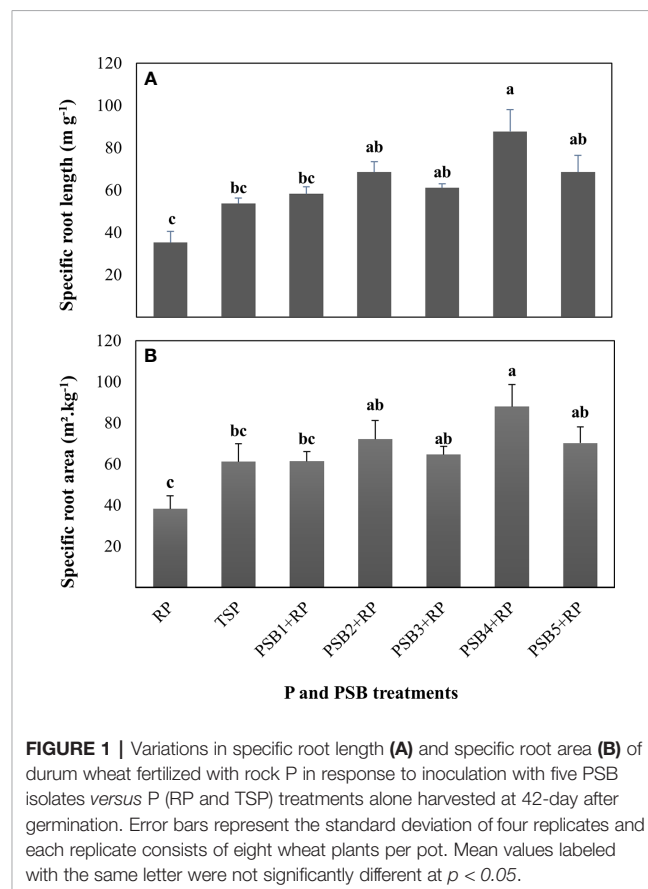
TABLE 3 | Variations in durum wheat growth fertilized with rock P in response to inoculation with five PSB isolates *versus* P (RP and TSP) treatments alone.

	SDW (g)	RDW (g)	SH (cm)	RDep(cm)
RP	2.16[c]	0.66[c]	35.35[bc]	29.63[b]
TSP	2.72[bc]	0.68[c]	31.99[c]	34[ab]
PSB$_1$+RP	3.68[ab]	1.08[abc]	40.5[ab]	37.74[a]
PSB$_2$+RP	4.20[a]	1.23[ab]	45.25[a]	33.95[ab]
PSB$_3$+RP	3.05[bc]	1.21[abc]	38.5[abc]	36.22[a]
PSB$_4$+RP	3.04[bc]	0.87[abc]	44[a]	36.29[a]
PSB$_5$+RP	3.36[ab]	1.30[a]	42.5[a]	36.49[a]

Data are means of four replicates and each replicate consists of eight wheat plants per pot harvested at 42-day after germination. Mean values labeled with the same superscript letter were not significantly different at p < 0.05. SDW, shoot dry weight; RDW, root dry weight; SH, shoot height; RDep, root depth; RP, rock phosphate; TSP, triple super phosphate.

In addition, both "PSB$_1$ and PSB$_5$" isolates also positively affected wheat root traits, albeit differences remain insignificant as compared to either RP- or TSP-fertilized wheat plants.

Furthermore, specific root traits such as specific root area (SRA) and specific root length (SRL) revealed significant differences in response to PSB inoculation (**Figure 1**). Both SRL and SRA were highest in wheat inoculated with PSB$_4$ as compared to application of both RP (increase of 59 and 56%) and TSP (increase of 33 and 30%). The remaining PSB isolates also presented similar trends for SRL and SRA as compared to RP rather than TSP application, though to a lower extent than PSB$_4$.

FIGURE 1 | Variations in specific root length **(A)** and specific root area **(B)** of durum wheat fertilized with rock P in response to inoculation with five PSB isolates *versus* P (RP and TSP) treatments alone harvested at 42-day after germination. Error bars represent the standard deviation of four replicates and each replicate consists of eight wheat plants per pot. Mean values labeled with the same letter were not significantly different at $p < 0.05$.

Effects of PSB Isolates on Above- and Below-Ground Physiological Modifications of Durum Wheat Supplied With RP

Effect on P Nutrition of Wheat Supplied With RP

P content of both shoots and roots increased in response to PSB inoculation compared to non-inoculated plants in both 15- and 42-day old wheat plants (**Table 4**). For both "shoot and root" Pi contents, a significant increase was noted in response to PSB_1 and PSB_2 (and PSB_5 for shoot Pi) inoculation as compared to RP-, but also to TSP-fertilized plants in terms of root Pi. This trend tended to vary in 42-day old wheat plants whose Pi content variations were the most significant in shoots (highest) and roots (lowest) in response to PSB_3 inoculation compared to RP-fertilized plants. However, inoculation with PSB_1 induced (42-day old plants) significant root Pi accumulation compared to either RP- or TSP-fertilized plants. In terms of shoot total P content in 42-day old plants, PSB_5 induced higher accumulation compared to all inoculated and non-inoculated treatments. Reversely to root Pi accumulation being significantly low as compared to that TSP-fertilized plants.

Effect on Rhizosphere Available P and Root APase Activity of Wheat

Results in **Table 4** show that the rhizosphere available P increased in all inoculated RP-fertilized 15-day old seedlings, though not significant either between isolates or TSP treatments. However, this parameter significantly decreased in 42-day old plants inoculated with PSB_1, PSB_3 and PSB_5 that exhibited a better root P acquisition as this was confirmed by a higher shoot P content as compared to RP-fertilized plants (**Table 4**). However, TSP-fertilized plants presented the highest rhizosphere available P fraction as compared to all treatments. Additionally, P-hydrolyzing APase in wheat roots varied in response to inoculation and plant growth stage, particularly in the 42-day old wheat plants in which root APase activity increased significantly along with a decrease in rhizosphere P availability (**Table 4**). This trend was mainly noted in roots inoculated with PSB_1, PSB_2 and PSB_5 whose APase activity were almost five times higher as compared to non-inoculated RP- and TSP-fertilized wheat plants.

Effect on Root P Acquisition Efficiency

TSP-fertilized plants have the highest RPAE as compared to the lowest efficiency in RP-fertilized plants (**Figure 2A**). Inoculation of RP-fertilized plants with PSB_1, PSB_3, PSB_4 and PSB_5 did not affect RPAE whose variations remain insignificant to that in RP-fertilized plants. Only PSB_2 significantly enhanced (129.59%) RPAE as compared to non-inoculated RP-fertilized plants. Such a notable increase was also significantly higher as compared to the remaining PSB isolates, but to a lesser extent with PSB_4. On the other hand, inoculated plants expressed a highly significant and positive correlation ($R = 0.6$, $p = p = 0.0014**$) between the inorganic "Pi" content and total P, indicating that PSB isolates likely contribute to a better internal P use efficiency (**Figure 2B**).

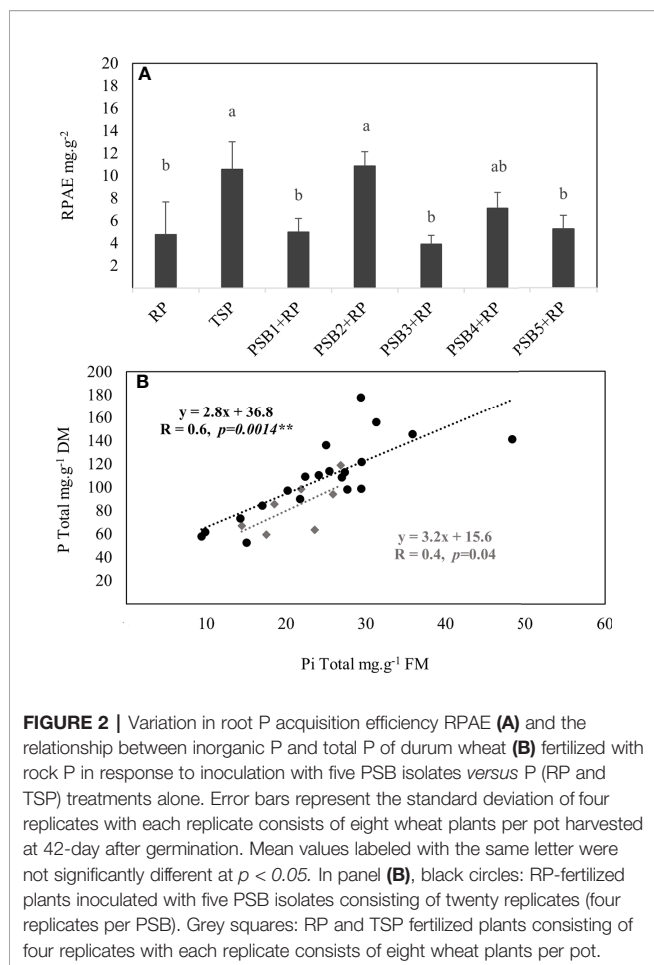### Effect on Chlorophyll (a and b) Content and Stomatal Conductance

An overall increase in chlorophyll content (Chl a and Chl b) was observed in response to inoculation of wheat with all PSB isolates with differential effects found between isolates (**Figures 3A, B**). Only inoculation with PSB_1 and PSB_5 significantly improved Chl b content compared to TSP- and RP- fertilized plants (**Figure 3A**). Similarly, only PSB_1 increased significantly Chl a content and exhibited, among all PSB isolates, the highest Chl a content as compared to controls (**Figure 3B**). Of note, PSB_2 and PSB_3 also increased significantly Chl a content in comparison to non-inoculated plants fertilized with RP only. Likewise, stomatal conductance (gs) was significantly higher in inoculated wheat plants regardless of PSB isolates with an average increase of 71.71 and 58.62% as compared to both RP- and TSP-fertilized wheat plants, respectively (**Figure 3C**).

Significant correlations were found between total Chl content and contents of both Pi ($R = 0.5$, $p = 0.001**$) and total P ($R = 0.6$, $p = 0.001**$) of inoculated RP-fertilized wheat plants (**Figures 4A, B**). Such positive correlations may refer to both use and physiological efficiency of RP for a better photosynthesis activity in inoculated than in non-inoculated plants. This positive interdependency may be estimated up to 36 and 22% based on the slope of the regression model ($y = ax + b$) of Chl content as a function of Pi and total P plant contents, respectively.

TABLE 4 | Variations in P content (total and inorganic) in both root and shoot, available P in rhizosphere growth substrate and APase activity in roots at two growth stage of wheat plants fertilized with rock P and inoculated with five PSB isolates versus P (RP and TSP) treatments alone.

	15-day old seedlings				42-day old plants					
	Shoot Pi ($mg \cdot g^{-1}$)	Root Pi ($mg \cdot g^{-1}$)	Rhizosphere available P (ppm)	Root APase ($nmol \cdot g^{-1} \cdot min^{-1}$)	Shoot P ($mg \cdot g^{-1}$)		Root P ($mg \cdot g^{-1}$)		Rhizosphere available P (ppm)	Root APase ($nmol \cdot g^{-1} \cdot min^{-1}$)
					Pi	Pt	Pi	Pt		
RP	0.05[c]	0.0132[c]	4[c]	3.67[a]	1.29[bc]	2.36[e]	0.37[bc]	1.52[d]	6.01[c]	29.58[b]
TSP	2.67[a]	0.0139[c]	33[b]	11.43[a]	1.34[ab]	3.82[b]	0.36[c]	2.78[a]	29.02[a]	17.62[b]
PSB_1 + RP	1.41[b]	0.0362[b]	22[b]	2.55[a]	1.13[bc]	3.47[c]	0.49[a]	1.86[cd]	6.33[c]	145.189[a]
PSB_2 + RP	1.38[b]	0.2257[a]	24[b]	5.45[a]	1.24[abc]	2.99[d]	0.44[ab]	2.38[ab]	19.41[b]	126.928[a]
PSB_3 + RP	0.84[bc]	0.0115[c]	54[a]	1.9[a]	1.5[a]	3.27[c]	0.25[c]	2.06[bc]	7.37[c]	78.677[ab]
PSB_4 + RP	1.061[bc]	0.0108[c]	47[a]	8.39[a]	1.39[ab]	3.5[c]	0.47[ab]	2.46[ab]	16.86[b]	77.589[ab]
PSB_5 + RP	1.29[b]	0.0151[c]	41[a]	2.39[a]	1.26[ab]	4.21[a]	0.41[abc]	2.26[bc]	5.22[c]	138.53[a]

Data are means of four replicates and each replicate consists of eight wheat plants per pot harvested at 15- and 42-day after germination. Mean values labeled with the same superscript letter were not significantly different at $p < 0.05$.

FIGURE 2 | Variation in root P acquisition efficiency RPAE **(A)** and the relationship between inorganic P and total P of durum wheat **(B)** fertilized with rock P in response to inoculation with five PSB isolates *versus* P (RP and TSP) treatments alone. Error bars represent the standard deviation of four replicates with each replicate consists of eight wheat plants per pot harvested at 42-day after germination. Mean values labeled with the same letter were not significantly different at $p < 0.05$. In panel **(B)**, black circles: RP-fertilized plants inoculated with five PSB isolates consisting of twenty replicates (four replicates per PSB). Grey squares: RP and TSP fertilized plants consisting of four replicates with each replicate consists of eight wheat plants per pot.

FIGURE 3 | Variations in leaf chlorophyll b content **(A)**, leaf chlorophyll a content **(B)**, and stomatal conductance (gs) **(C)** of durum wheat fertilized with rock P in response to inoculation with five PSB isolates *versus* P (RP and TSP) treatments alone. Error bars represent the standard deviation of four replicates and each replicate consists of eight wheat plants per pot. Mean values labeled with the same letter were not significantly different at $p < 0.05$.

Effect on N and Protein Contents in Durum Wheat Shoots

Wheat plants N content enhanced in response to PSBs inoculation with an average increase of 10.04% compared to non-inoculated plants fertilized with RP alone (**Table 5**), although no significant difference ($P < 0.05$) was noted among inoculated and non-inoculated plants. However, TSP fertilization increased significantly N content by 19 and 30.66% compared to PSB inoculation and RP fertilization, respectively. On the other hand, although N content in inoculated plants did not significantly increase compared to RP-fertilized plants, NUE revealed significant effects due to inoculation with PSB_3 (78%), PSB_5 (50%) and PSB_1 (37.5%) compared to TSP (highest N content) and RP fertilization. Similar to N content, TSP-fertilized plants had the highest protein content as compared to all treatments (**Table 5**). Nevertheless, compared to non-inoculated plants, a statistically greater amount of protein content was increased in response to PSB_2, PSB_3, PSB_5 and PSB_4 by 134.75, 131.52, 57.60 and 40.76%, respectively.

Effect of PSB Inoculation on the Interdependency Between Above- and Below-Ground Traits

Multi-parameter correlation analyses between all above- (e.g. biomass, physiological traits, P and contents, etc.) and below-grou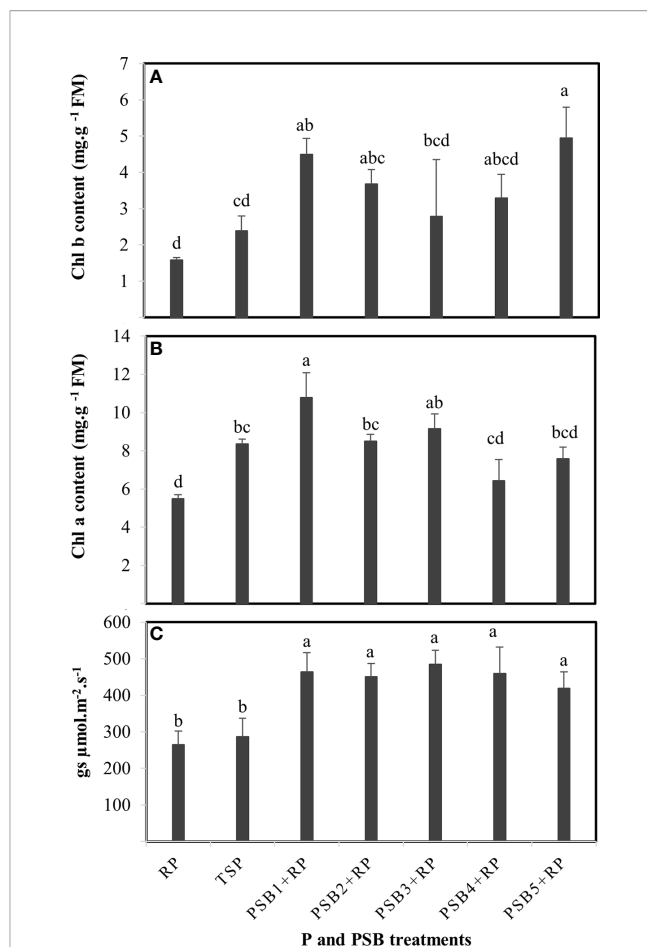nd (e.g. root traits, root APase, available P) parameters revealed significant differences between inoculated and non-inoculated plants (**Figure 5**; **Supplementary Table S2**). The principal component analysis revealed that clustered groups wherein specific root traits (e.g. SRL, SRA, RDW, Ntips) were closely related to N and P content in both shoot and root of inoculated wheat plants (**Figure 5A**), which is also confirmed in the correlation matrix (**Supplementary Table S2**) showing differential responses between PSB isolates. Moreover, inoculated plants presented a second group clustering parameters related to root morphological traits (e.g. RL, RV, RD, Ncross and NForks), physiological traits (e.g. protein and N contents, gs), and rhizosphere available P, which all indicate the importance of root morphological traits in root nutrient acquisition and absorptive capacity (**Figures 2 and 5A**; **Supplementary Table S2**). In addition, inoculation with PSB isolates seems to have positive interdependency between total Chl, root Pi content, SDW and root APase. Unlike inoculated plants, in non-inoculated (fertilized with either RP or TSP) plants morphological root traits (e.g. RV, SRL, RSA and Nfroks) seemed
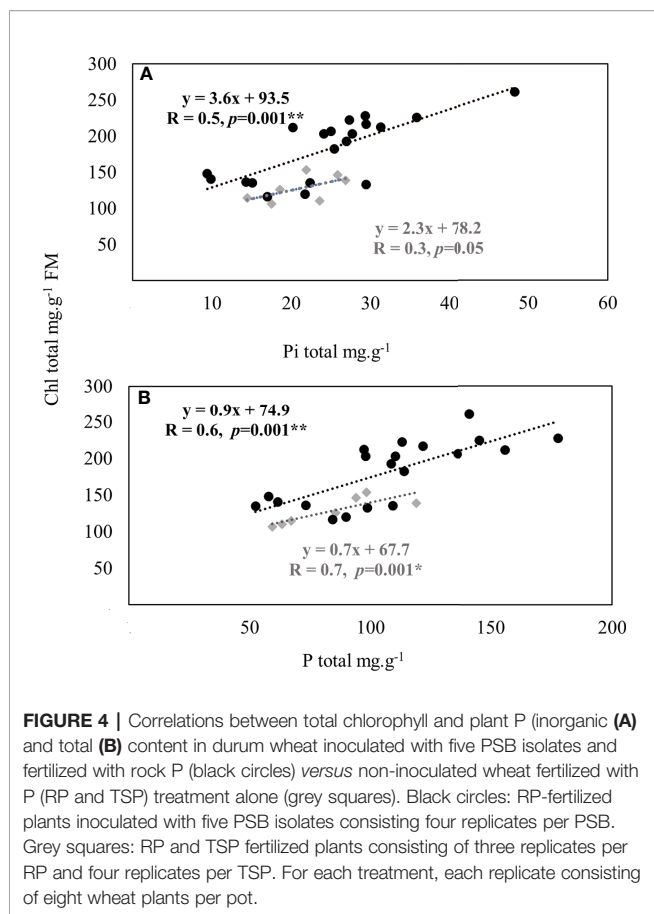
FIGURE 4 | Correlations between total chlorophyll and plant P (inorganic **(A)** and total **(B)** content in durum wheat inoculated with five PSB isolates and fertilized with rock P (black circles) *versus* non-inoculated wheat fertilized with P (RP and TSP) treatment alone (grey squares). Black circles: RP-fertilized plants inoculated with five PSB isolates consisting four replicates per PSB. Grey squares: RP and TSP fertilized plants consisting of three replicates per RP and four replicates per TSP. For each treatment, each replicate consisting of eight wheat plants per pot.

TABLE 5 | Variations in nitrogen and protein contents of durum wheat fertilized with rock P in response to inoculation with five PSB isolates *versus* P (RP and TSP) treatments alone.

	N (mg·g^{-1})	NUE (g^2·mg^{-1})	Prot (mg·g^{-1} FM)
RP	32.25^b	0.08^b	1.84^d
TSP	42.14^a	0.07^b	4.8^a
PSB$_1$ + RP	33.79^b	0.11^a	2.02^d
PSB$_2$ + RP	38.08^{ab}	0.06^b	4.31^b
PSB$_3$ + RP	34.95^b	0.14^a	4.26^b
PSB$_4$ + RP	37.99^{ab}	0.08^b	2.59^c
PSB$_5$ + RP	33.02^b	0.12^a	2.9^c

Data are means of four replicates (each replicate consists of eight wheat plants per pot) harvested at 42 days after germination. Mean values labeled with the same superscript letter were not significantly different at p < 0.05. N, Nitrogen content; NUE, Nitrogen use efficiency; Prot, Protein content.

to correlate with shoot P content, but with no correlation between morphological root traits, APase activity, root Pi content or even RDW that clustered alone (**Figure 5B**). Moreover, the second group clustering physiological and growth parameters (Chl content, SDW, protein content) had a positive correlation with rhizosphere soil P availability. It was also noted that most functional traits involved in P uptake (e.g. RDW, available P, APase and root traits are scattered in the inverse direction of both root morphological and physiological traits (e.g. Chl, protein and N contents, gs) plausibly

indicating an unbalanced deployment of these traits responsible of P acquisition and growth performance.

DISCUSSION

The present study contributes to the available knowledge on wheat plant–PSB interaction under low P availability, specifically variations in root morphological traits along with associated rhizosphere modifications and aboveground physiological parameters related to P use efficiency. We show a significant effect of five PSB isolates contrasting in their PSC on the rhizosphere P availability, root morphological traits and improved aboveground parameters whose variations can help advance understanding the highly intricate root–PSB interactions under low available P forms such as rock P. We also address the hypothesis that PSB may have a stronger effect on root biophysical traits (more than localized rhizosphere P solubilization) reverberating positively on root nutrient absorption capacity (including P among other) and the overall crop physiology. In addition, our findings suggest that a highly *in-plate* P solubilizing bacterium does not necessarily indicate important *in-planta* responses given that temporal modifications at the belowground level were found to be PSB-specific regardless of the P solubilization capacity they were first selected for.

P Solubilizing Rhizobacteria Had Different Effects on Durum Wheat Seedlings Development

The ability of PSB isolates to solubilize two different P forms (e.g. RP and TCP) was demonstrated to influence positively wheat seedling development, an improvement that may be related not only to increased rhizosphere P bio-solubilization and modifications in root morphology (**Table 2**; **Supplementary Table S1**), but also to the multiple PGP traits (i.e., IAA, siderophore, NH$_4^+$, etc.; **Table 1**) plausibly contributing to an additional growth improvement. Improvements in seedlings root morphological traits in response to inoculation (specifically PSB$_1$ and PSB$_4$) were probably a consequence of a higher IAA production, which is a plant growth regulator hormone that in addition to boosting growth and root elongation, it also improves photosynthetic capacity, carbohydrate metabolism and the overall plant yield (Li et al., 2019). In this context, a recent study by Marathe et al. (2017) demonstrated the ability of an IAA-producing PSB strain (*Pseudomonas aeruginosa*) to stimulate 2 days earlier seed germination and increase both nutrients uptake (N, P, and K) and chlorophyll content (chl a and chl b). This is fully aligned with the current study's findings as well as a number of previous research investigations (Khiangte and Lalfakzuala, 2011; Linu et al., 2019; Liu et al., 2019), although accurate quantitative analysis will be required to determine either the amount and the type of IAA likely responsible for root growth (Kowalczyk and Sandberg, 2001; Liu et al., 2012). However, it remains unclear why inoculation with PSB$_3$ that exhibit the highest IAA production rate did not affect root traits (especially
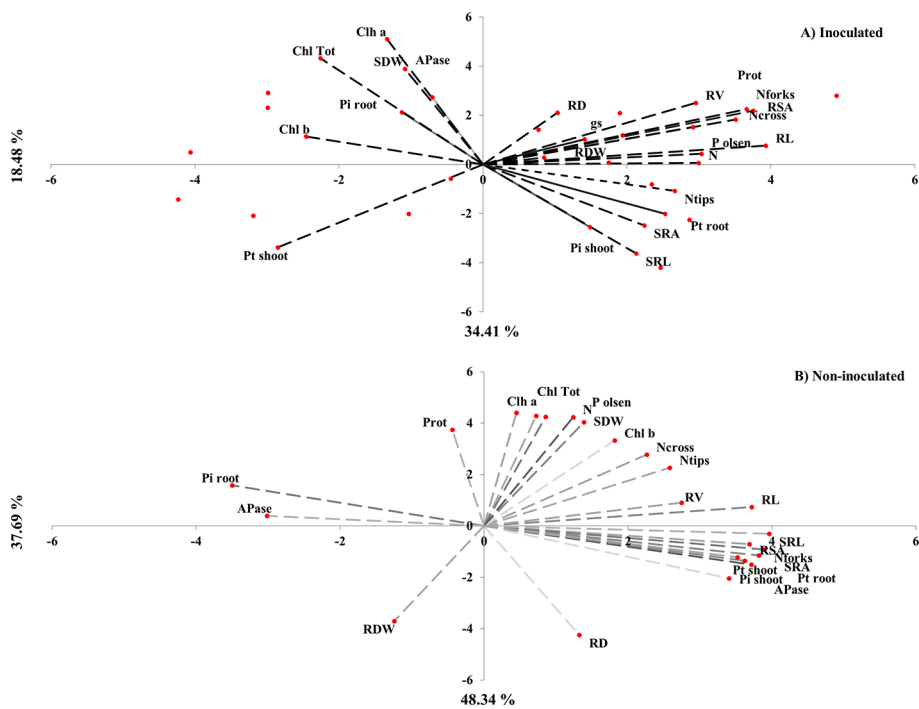
FIGURE 5 | Principal component analysis elaborated based on growth, root traits and physiological parameters measured in durum wheat fertilized with rock P in response to inoculation with five PSB isolates *versus* non-inoculated plants fertilized with P (RP and TSP) treatments alone. Data are means of four replicates and each replicate consists of eight wheat plants per pot harvested at 42-day after germination. Pt shoot, total P of shoot; Pi shoot, intracellular P of shoot; Pt root, total P of root; Pi root, intracellular P of root; N, Nitrogen uptake; P Olsen, P in the rhizosphere; APase, acid phosphatase activity in root; Chl b, leaf chlorophyll b content; Chl a, leaf chlorophyll a content; Chl tot, total Chlorophyll; gs, stomatal conductance; Prot, protein content; RDW, root dry weight; SDW, shoot dry weight; SRL, specific root length; SRA, specific root area; RL, root length; RSA, Root surface Area; RD, Root diameter; RV, Root volume; Ncross, Number of Crossing; NTips, Number of Tips; Nforks, Number of Fork.

in 15-day old seedlings) as compared to either the remaining PSB isolates or to non-inoculated seedlings. Other bacterial traits such as ammonia production, medium acidification and osmotic stress tolerance (**Table 1**) may positively contribute in seedling robustness including important early-stage root nutrient absorptive capacity that could improve RP solubilization and subsequent utilization (Abbasi et al., 2015; Kumar, 2016; Pérez et al., 2016).

The PGP bacterial traits are well documented (Cerozi and Fitzsimmons, 2016; Vandamme et al., 2016; Paul and Sinha, 2017; Suleman et al., 2018; Liu et al., 2019; Parastesh et al., 2019) in terms of increasing plant growth and yield, meanwhile, spatial and temporal modifications of root functional traits in response to PSB inoculation are still not well-known. In this study, rhizosphere modifications at early stage of plant development (7- and 15-day old seedlings) are likely attributable to inoculation with PSB isolates whose *in soil* P solubilization rates were almost three times higher than non-inoculated plant-less soils, which is consistent with the *in-plate* findings (**Table 1**). In addition, variations in terms of P solubilization both *in vitro* and in plant-less soil indicated clear differences between PSB isolates, especially with PSB$_1$ that increased significantly RL and RSA in 7-day old seedlings, while exhibiting the lowest PSC. Such stimulation was also observed

in 15-day seedlings whose P content (especially in roots) significantly increased in response to inoculation with PSB$_1$ (**Table 2**). Inversely, the isolate PSB$_5$ exhibiting the highest PSC had no significant effect on the 7-day old wheat seedlings as compared to non-inoculated seedlings supplied with RP. Furthermore, the observed variations in 15-day old seedlings seemed to be PSB-specific given that only PSB$_4$ (moderate PSC) induced significant improvements in root traits, mainly RL, RSA and RV (**Table 2**). Such early-stage rhizosphere variations that the current study unfolded for the first time could indicate clear differential effects that PSB may have on wheat seedling at an early growth stage. This will lead to new research questions enabling a better understanding of potential modes of actions that the PSB–root interface may evolve rather than the routinely evaluated bacterial P solubilization and currently believed to be crucial in screening efficient PSB.

P Solubilizing Bacteria May Have Greater Effect on Rooting System More Than Rhizosphere P Solubilization

Besides the direct effects that PSB may have on RP solubilization, it seems that changes in root morphological traits over time (e.g. 7-, 15- and 42-old day wheat plants) were associated with higher plant growth (**Tables 2–4**). After 15 days of growth, inoculation

with all PSB isolates significantly improved RDW, whereas rhizosphere available P did not change significantly even though a slight increase may be seen as compared to RP-fertilized plants (**Table 4**). Roots of the 42-day old wheat plants also showed a similar trend when inoculated with PSB_1, PSB_2 and PSB_5. For plants inoculated with PSB_5 (exhibiting the highest PSC), the lower rhizosphere available P fraction may partly be attributed to important RDW and root depth that indicate an important root growth presumably responsible for increased root P uptake and P content in wheat shoots (**Table 4**). In line with these findings, experimental evidences about soil bacterial P bio-solubilization are available (Mamta et al., 2010; Batool and Iqbal, 2019; Emami et al., 2019), meanwhile relationship between PSB and root functional traits and their extension within the rhizosphere are not yet fully deciphered and so more when it comes more particularly to temporal variations throughout crop growth stages. Overall, our findings show for the first time that PSB may exhibit different PSC over growing time (three plant growth stages) and that P solubilization rate at the rhizosphere level likely difficult to estimate while roots continue to grow spatially and temporally. It may be hypothesized that an accurate PSB screening would definitely consider both biochemical features of PSB as well as the root-bacteria interaction responses giving that rhizosphere P assimilation is a root-driven biological process that heavily rely on overall belowground growth performances.

To the best of our knowledge and even though root parameters were measured destructively, this study is the first to describe interesting temporal variations of both morphological and physiological wheat root traits in response to inoculation with PSB that are contrasting in their PSC. Indeed, our findings corroborate with most PSB- and/or PGPR-based research investigations wherein crop growth responses, notably root modifications were measured either at early (germination and seedling) or at late harvesting stages without considering the contrast that PSB may have on P solubilization rate they may express during different plant developmental stages. Overall, at the three plant growth stages (7-, 15- and 42-day), root morphological traits (e.g. volume, surface, diameter, and number of tips and forks) obviously increased in response to inoculation that enabled efficient use of RP, which is consistent with the recent findings by Wang and Chu (2015); Suleman et al. (2018); Liu et al. (2019) and Wang et al. (2019). These authors demonstrated that, in addition to P solubilization, PSB inoculation can modify root functioning through modulation of the expression of auxin-responsive genes, hence playing major role in regulation of endogenous IAA level with positive consequences on P acquisition and plant physiological status. Furthermore, spatial rhizosphere/root heterogeneity may occur due to increased soil exploration leading to a higher solubilization and root absorption of P, which may be a consequence of AIA-producing PSB isolates (notably PSB_1, PSB_2 and PSB_3) likely involved in regulating the root system morphology such as lengthening lateral roots (Raya-Gonzalez et al., 2014). Moreover, vigorous and efficient rooting systems in inoculated wheat plants was associated with higher shoot N and leaf protein contents, notably in response to PSB_2 characterized to be an ammonium-producing (equivalent to 80 nmol ml^{-1}) isolate and plausibly involved in non-symbiotic N_2 fixation during plant growth. Correlation analyses (**Supplementary Table S2**) also indicated tight relationships between shoot N, leaf protein contents and root traits (e.g. RSA, RD, and N tips), which provides evidence of a stronger belowground effect. Particularly, root morphological traits (e.g. SRL, SRA, RDW, Ntips) could heavily contribute to a better acquisition of both N and P (**Figure 5**; **Supplementary Table S2**). However, advanced multidisciplinary approaches are needed, notably combining N_2 fixation methods (i.e. natural ^{15}N abundance), belowground photosynthate allocation and root occupancy of introduced PSB candidates. In this context, it was estimated that up to 60% of the photosynthesis-fixed C in wheat, pea, maize, and tomato, is belowground-translocated where root-associated microorganisms can metabolize it or use it for the benefit of plant growth and the rhizosphere microbiome (Morgan et al., 2005; Hernández et al., 2015; Wang et al., 2016).

Rhizosphere Bacterial Bio-Solubilization of P Presumably Stimulates Positive Above- and Below-Ground Interactions

The majority of the previous studies have largely described the direct effects of PSBs on plant growth based essentially on P solubilization and plant growth (Manzoor et al., 2017; Singh et al., 2018; Liu et al., 2019). Other studies have also reported important beneficial effects on few root parameters (Sarsan, 2016; Suleman et al., 2018; Rezakhani et al., 2019) with no strong linkage with aboveground P-related parameters. However, this study provided new evidence that PSB effects are not basically restricted to P solubilization alone, but extend to multiple known and unknown indirect effects on morphological root traits, thus improving both acquisition and internal use of P. Multiple differential responses at the level of "PSB-plant" interactions are reported herein, notably the significant increase of the rhizosphere P availability due to PSB inoculation (**Table 1**) at an early stage of plant development (7-day old seedlings), which could provide adequate amount of P readily available for the continuously growing roots, thus a better plant growth and P nutrition presumably secured for the subsequent growth stages. Another response is that PSB could play a key role in plant growth through promoting root development in the 15-day old seedlings more than P solubilization that seemed to be pronounced earlier at the 7th day after germination, and tended to decrease in 15-day and 42-day old wheat plants. This is consistent with the observation that in inoculated plant-less soil (results not shown) P solubilization was higher after 7 days of incubation and tended to decrease at the day 15 followed by an important (though not significant) P solubilization recovery at the 42nd day.

In wheat plants inoculated with PSB isolates, most above- and belowground parameters were clustered in one group, particularly root morphological traits such as Ntips indicating root proliferation (Harmer, 1990) that significantly correlated with root and shoot P contents. This relationship could be

explained by the root's ability for a high soil foraging, owing among other, to root proliferation leading to a greater P uptake. In addition, the positive correlation between rhizosphere available P and both root Ntips and RD in response to inoculation may indicate that increased P availability in the rhizosphere soil and its better aboveground translocation occurred either directly or indirectly in response to PSB inoculation, presumably owing to a better deployment of root morphological traits (**Table 2**) that enabled a larger soil surface exploitation (higher RL, RSA, Nforks and specific root length and area). Moreover, in 42-day old plants, PSB tended to stimulate both root APase activity and RL more than P solubilization activity which decreased over time owing to increased root absorption capacity as well as to a possible internal remobilization of cellular P pools due to increased root APase activity. This is consistent with previous studies reporting the ability of PSB strains to produce APase for improving P nutrition (Behera et al., 2017; Chawngthu et al., 2020). However, this trait has never been timely monitored in inoculated roots or properly considered as an essential root trait worth investigation during the *in planta* PSB screening steps.

Moreover, inoculated plants showed clear improvement of aboveground physiological traits exemplified by higher P, N and chlorophyll contents and stomatal conductance compared to non-inoculated plants. Photosynthetic activity (total Chl) coherently improved with increased shoot P (both total and inorganic) content under RP supply and PSB (notably PSB_3) inoculation (**Figure 4**; **Supplementary Table S2**), which is clearly explained by the inoculation positive effects on root proliferation enabling more P acquisition from soil that correlates well with photosynthetic chlorophyll content. Such a finding indicates clear relationships that are likely PSB-triggered under low P availability as compared to adequate (TSP) mineral P nutrition that did not produce such a response. This is consistent with the recent findings by Wang et al. (2015) and Rozier et al. (2019) that PGPR (e.g. *Azospirillum lipoferum*, *Azospirillum brasilense* and *Burkholderia phytofirmans*, notably N_2 fixing) contributed to a better photosynthetic activity in maize, wheat and switchgrass, however neither above- or below-ground mechanistic interactions related to P and PSB were highlighted so far.

CONCLUSIONS

Although PSB-focused research investigations have advanced understanding the complexity of involved mechanisms, it remains unclear how do PSB contribute to below- and above-ground interactions. It is strongly realistic that PSB contribute directly to rhizosphere P solubilization, however the extent in which PSB may contribute to root biophysical properties and aboveground physiological variations remain puzzling, especially owing to intricate root-PSB interactions that may occur and plausibly change over time. Our findings demonstrate that contrast in terms of bacterial P solubilization rate might not be the sole criterion to discriminate at early stage (*in plate* screening) low-rate PSB isolates whose effect *in planta* could be significant due to specific interactions with roots that somehow

enable positive aboveground responses (case of PSB_1 and PSB_4). It may also be suggested that capturing efficient PSB and evaluating their *in planta* promoting growth traits through multiple inoculation experiments is still unavoidable, however plant responses should be monitored timely and spatially in order to point out "in-time & in-space" modifications enabling accurate interpretations of the bacterial effects. Such a strategy, although time-consuming, could provide insightful data on relevant ploy-bacterial "PSB" mixture with complementary and synergistic effects during all plant growth stages. This could crucially complement the routinely "*In plate*" bacterial consortium construction approach, which do not consider timely plant responses during the screening process. It might also be concluded that adopting interdisciplinary approaches, notably phenotyping functional traits both below- and above-ground, is likely necessary for unbiased results interpretations and accurate screening, ultimately leading to successful *in field* applications.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/ **Supplementary Material**.

AUTHOR CONTRIBUTIONS

All authors contributed to the article and approved the submitted version.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2020.00979/ full#supplementary-material

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