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Plant growth promotion of barley with *Azospirillum brasilense* and *Bacillus subtilis* to achieve sustainable agriculture in arid regions

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Abstract

Plant growth-promoting rhizobacteria (PGPR) are a group of beneficial soil bacteria that colonize the root surface and promote plant growth and health via multiple mechanisms. PGPRs are considered an eco-friendly alternative to hazardous chemical fertilizers. The use of PGPRs as biofertilizers is a biological approach toward the sustainable intensification of agriculture. Therefore, the current study investigated the possibility of two PGPBs, *Azospirillum brasilense* and *Bacillus subtilis*, to increase the bioavailability of Nitrogen (N) and phosphorus (P) in a calcareous soil fertilized with Ammonium Nitrate (AN) and single superphosphate (SSP) and its absorption by the barley (*Hordeum vulgare* L.). The pot experiment, a trial using calcareous soil, was conducted on December 1st, 2022 for 60 days. The bacterial strains were mixed in the ratio (1:1) and tested in combination with four levels of Ammonium Nitrate and single super phosphate 0%, 50%, 75% and 100% of recommended dosage for barley (300 kg Fed^{-1}. Ammonium Nitrate and 150 kg Fed^{-1}. Single super phosphate). Treatments significantly increased the plant's dry weight, N and P absorption and bioavailable N and P in the soil compared to un-inoculated. Also, plants inoculated with bacteria had a significant impact on total amino acids as compared with un-inoculated plants. Accordingly, we can reduce Ammonium Nitrate and single super phosphate application by inoculation with these PGPR to have a major positive impact on barley growth and maintain environment and soil health.

Keywords: Barley, PGPR, available phosphorus, nitrogen fixation, free amino acids

1. Introduction:

Adopting sustainable agricultural practices that involve gradually reducing the use of synthetic agrochemicals, increasing the utilization of biowaste-derived substances, and harnessing the biological and genetic potential of crop plants and microbes is a viable strategy to combat rapid environmental degradation, ensure high agricultural productivity, and improve soil health (Basu et al., 2021). In this context, the use of Plant growth-promoting rhizobacteria (PGPR) inoculants in agriculture represents a friendly alternative environment method compared to mineral fertilizers. PGPR promotes crop growth and health in a variety of ways. They've been linked to nitrogen fixation pathways, mineral solubilization (Zn, Fe, P), and increased tolerance to biotic and abiotic stressors (Santos *et al.,* 2019; and Ramakrishna *et al.,* 2020).

Nitrogen (N) is one of the limiting factors that could reduce barley production in Egyptian soil, while its over-application causes leaching and off-site deposition that leads to the eutrophication of water bodies and greenhouse gases emission. In addition, excessive application of N fertilizers could increase the cost of production, but a low-dose application can affect the performance and productivity of barley, especially under the calcareous soil conditions in Egypt. Therefore, proper N management is required to optimize N use efficiency and improve barley productivity without harming the environment and to meet the increasing demand for cereals consumption.

Plants require phosphorus (P) as the second most important macronutrient after nitrogen. P is one of the lithosphere's less abundant elements (with the exception of nitrogen), and as a result, it is frequently viewed as a limiting nutrient in agricultural soils when compared to other key macronutrients (with the exception of nitrogen). Therefore, using mineral phosphorus fertilizers to increase plant P nutrition has become relatively widespread (Maharajan *et al.,* 2018). Phosphorus is rapidly fixed in the soil after the application of P fertilizers by producing an unavailable complex Ca in calcareous soils (Toro, 2007). Inorganic P is abundant in calcareous soils, but due to P-fixation, only a small amount is accessible for crop use. On the other hand, frequent use of P fertilizers is well-known to be both expensive and unfavorable in the agroecosystem.

The use of plant growth-promoting bacteria (PGPBs) is being recognized as one of the alternative techniques that could promote plant growth, N use efficiency, biological nitrogen fixation (BNF) and phosphate solubilization in a sustainable way to reduce N and P fertilization. Among microbial consortia, A. brasilense and B. subtilis are the most predominant PGPBs in different crops, soils and climatic conditions to improve nutrient acquisition along with better plant growth and yield.

Inoculation with *Azospirillum brasilense* has been reported as a promising inoculant for promoting plant growth and increasing yield, N uptake and N use efficiency. This inoculant has the capability to colonize the plant rhizosphere and alter root architecture by increasing root branching and volume, which could increase nutrient and water acquisition and N use efficiency. Inoculation with *Bacillus subtilis* allows the plants to grow in abiotic extremes by stabilizing and stimulating plant growth through the solubilization of inorganic mineral phosphate and nutrient uptake. Inoculation with *B. subtilis* also has a positive influence on the grain yield, agronomic traits and root dry mass of cereals, being considered a strategic tool in the agro-ecological production system. Inoculation with *B. subtilis* can reduce NH_3 volatilization up to 44% by decreasing the conversion of fertilizer N into NH_4^+ and increasing the nitrification process, thus increasing the N use efficiency of soil and plants.

The use of inoculants containing PGPRs is increasing day by day due to the high cost of fertilizers and increasing awareness of sustainable and less polluting agriculture. However, research on the effects of co-inoculation with *A. brasilense* and *B. subtilis* on barley crops is still unknown and lacking. Therefore, the objective of the current study was to evaluate the combined effects of different N and P doses and seed co-inoculation with *A. brasilense* + *B. subtilis* on barley (*Hordeum vulgare* L.) growth, N and P uptake and bio-availability of N and P in soil.

2. Materials and methods:

2.1. Soil:

Calcareous soil sample (0–30 cm) was collected from Experimental Farm-City of Scientific Research and Technological Applications (SRTACity) located in Borg Al-Arab, Alexandria, Egypt (30° 53' 33.17" N and 29° 22' 46.43" E). Soil samples were air-dried, grinded, passed through a 2-mm sieve and analyzed. The pH was determined in a 1:2.5 w/v soil: water suspension (Anderson et al., 1982). The EC was measured using an EC meter in saturated paste extracts (Corwin and Yemoto, 2017). The Calcimeter method was used to measure the total calcium carbonate content (Pansu and Gautheyrou, 2006b). Total N was measured by the Kjeldahl digestion method. Available P was extracted with NaHCO3 and measured using a spectrophotometer at a wavelength of 880 nm. Available K was extracted by ammonium acetate solution (1 N) and measured by the flame photometer. The particle size distribution of sand, silt, and clay, as well as the soil characteristics, were examined using the hydrometer method with sodium hexameta-phosphate as a dispersion agent (Gee and Bauder, 1986). Results are presented in table 1.

2.2. Preparation of inoculums and seed sowing

The two efficient PGPR strains, *Azospirillum brasilense* (Accession No. OR607908) and *Bacillus subtilis* strain AEM 1 (Accession No. OR430402.1) were obtained from Land and Water Technologies Department, Arid Lands Cultivation Research Institute, City of Scientific Research and Technological Applications (SRTA-city). They were isolated and identified as PGPR in previous studies. Each strain was grown in 500 ml flasks containing nutrient broth and grown aerobically on a rotating shaker (150 pm) for 48 h at 30 °C, after that cells were harvested by centrifugation at 3000 rpm for 20 min and re-suspended in sterile 0.85% NaCl solution. The bacterial suspension was then diluted in sterile distilled water to give a final concentration 10^8 CFU ml^{-1} (Colony Forming Unit CFU) and used to inoculate barley grains. For mixed inoculation, an equal volume containing (10^8 CFU ml^{-1} of each strain) were mixed (1:1) and used for inoculating barley seeds. Barley grains were soaked (except un-inoculated) with the culture broth of both PGPR inoculants for 10 min before sowing.

2.3. Pot experiment:

A pot experiment was carried out at Experimental Farm-City of Scientific Research and Technological Applications (SRTA-City) located in Borg Al-Arab, Alexandria, Egypt. Pots of 25 cm in diameter and 20 cm in depth were sterilized with 1.5% sodium hypochlorite, then sterile water to remove the excess of hypochlorite and filled with 2 kg of prepared soil. Giza 123 barley seeds were obtained from Agricultural Research Centre, Egypt. The seeds were surface sterilized by soaking in 70% ethanol for 3 min and then in 1% sodium hypochlorite (bleach) for 10 min. To remove the residual bleach, the seeds were washed ten times with sterile tap water and air dried and soaked (except un-inoculated) with the culture broth of *A. brasilense* + *B. subtilis* inoculants for 10 min before sowing. Barley seeds were sown at 1 cm depth (10 seeds pot^{-1}) on 1st, December, 2022. In addition, each pot (except un-inoculated pots) was inoculated with 100 ml of PGPR inoculants containing 10^8 CFU ml^{-1} after sowing to ensure soil inoculation. Seed germination percent was calculated after 7 days of sowing (90-100 %) and then were thinned to five seedlings in each pot. Experiment was set up in an open environment covered with wire. The pots were watered weekly to maintain soil moisture at field capacity 25%.

Four levels of N fertilizer were applied as Ammonium Nitrate (AN) NH_4NO_3 33.5%, N 100% of the recommended dose (300 kg fed^{-1} of NH_4NO_3 33.5% N at the rate of 100 kg N fed^{-1}), N 75%, N 50%, and N 0% (not amended with Nitrogen fertilizer) with or without PGPR. Doses of N fertilizer were added in three equal doses before irrigation. The first dose was added at the time of sowing, the second was applied after 20 days from cultivation and the third one was after 40 days from planting. The recommended dose of K fertilizer (50 kg fed^{-1} of potassium sulphate 48% K_2O) was applied before sowing of grains.

Four levels of P fertilizer were applied as single super phosphate (SSP) 15% P_2O_5, P 100% of the recommended dose (150 kg fed^{-1}), P 75%, P 50%, and P 0% (not amended with phosphorus fertilizer) with or without PGPR. Phosphorus doses were added and mixed well with soil before sowing.

The experiment was set in a randomized complete block design (RCBD) with three replicates. There were 8 treatments in the experiment (4 levels of AN + SSP 0, 50, 75 and 100% of recommended doses, and 2 inoculation treatments *A. brasilense* + *B. subtilis* and control without inoculation) as the following T1 (control), T2 (Control + 50 % AN, SSP), T3 (Control + 75 % AN, SSP), T4 (Control+ 100% AN, SSP), T5 (*A. brasilense* + *B. subtilis* + 0 % AN, SSP), T6 (*A. brasilense* + *B. subtilis* + 50 % AN, SSP), T7 (*A. brasilense* + *B. subtilis* + 75 % AN, SSP), T8 (*A. brasilense* + *B. subtilis* + 100% AN, SSP)

2.4. Plant analyses:

After 60 days of cultivation, plants were harvested, root and shoot portions of plants were separated and data regarding growth (shoot dry weight and root dry weight) were measured. They were oven dried at 70°C to a constant weight and grinded after drying to determine the leaf N and P concentrations following the methodology (Edje and Burris, 2015).

2.5. Soil analyses

Rhizosphere soil samples were collected from each treatment by uprooting the plants carefully without damaging the root system. Roots were shaken gently to remove loosely adhering soil particles from each treatment and then the soils were analyzed for measuring available P content which extracted by the bicarbonate method and determined using the molybdate blue color method (Olsen et al., 1954). The content of total nitrogen (TN) in soil was determined by the Kjeldahl method (Estefan et al. 2013).

2.6. Free Amino Acids in plants:

The content of free amino acids arginine, proline, and phenylalanine was measured in plants according to Umbreit *et al*., (1972). The same extraction was carried out by grinding dry matter in Macllavaine buffer (sodium citrate buffer, pH 6.8). Homogenized for 3 minutes and centrifuged at 4000 rpm for 15 min. The supernatant was then used to determine the content of some free amino acids such as arginine, proline, and phenylalanine (Umbreit *et al.,* 1972). 0.5 mL of extract, 1 mL citrate buffer (pH 5), 0.5 mL of ninhydrin, and 3.5 mL of isopropanol solution were used for this purpose. The optical density of proline was determined spectrophotometrically at 450 nm, 492 nm for phenylalanine, and 515 nm for arginine. In addition, instead of extract, 0.5 ml of distilled water was utilized in the reference cuvette. The concentration of each amino acid was calculated using a standard curve created for the relevant amino acids.

2.7. Statistical Analysis:

The data were examined using a two-way analysis of variance (ANOVA) at $p \leq 0.05$ with the statistical tools of the Co-Stat programmer for statistics (2004). The Least significant difference (LSD0.05) test was also employed to distinguish between significant and non-significant data.

3. Results and discussion:

3.1. Plant analyses:

The combination treatment of PGP bacteria (*A. brasilense* and *B. subtilis*) have resulted in the increment of growth parameters (shoot and root dry weight), total nitrogen and phosphorus concentration in barley. The analysis of variance revealed the highly significance ($p \leq 0.05$) differences among growth parameters (Table 2). The bacterial consortium in a combination with 75, 100 % AN+SSP have the highest barley shoot and root dry weight, which were statistically not different.

Co-inoculation of PGPBs can stimulate different root activities that may regulate several physiological functions such as root hair elongation and meristems cell multiplication in host plants, thus leading to greater exploitation of soil for nutrient and water uptake and establishing tolerance against abiotic and biotic stress.

Total plant N was also significantly influenced by PGPR inoculation and the different levels of fertilization treatments. The highest values (2.71, 2.16 % in shoot and root, respectively) were recorded when plants subjected to *A. brasilense* and *B. subtilis* +100 % AN, SSP, while the lowest values (0.75, 0.53 % in shoot and root, respectively) were obtained in the un-inoculated and unfertilized (control) plants (Table 2).

Total plant P was substantially affected by PGPB inoculation and different fertilization levels. There were significant differences among inoculated and un-inoculated plants, the highest values (0.29, 0.17 % in shoot and root, respectively) obtained from *A.brasilense* + *B. subtilis* +100 % AN, SSP (Table 2).

Several factors are applied in agriculture to increase N use efficiency, such as fertilizer management and improvement in genetic traits (to increase the ability of plants to acquire more nutrients) at the crucial stage of plant development to alleviate limitations in crop growth and nutrients demand, and adequate soil management to improve soil fertility and nutrients availability. The factors initially include more effective fertilizers application methods, site-specific management and highly effective fertilizers (new and modified fertilizers and inhibitors that are leading to slow/controlled release). The present results are based on inoculation and co-inoculation of *B. subtilis* and *A. brasilense*, which could fit into the aforementioned factor to improve crop growth and N use efficiency. It is essential to understand that several technological choices have different influences on crop yields in response to N fertilization, which might be the consequence of such practices that lead to several major benefits.

The benefits of *A. brasilense* and *B. subtilis* inoculation on root development were highlighted by greater root dry mass and root N and P content, which are likely to be the key mechanism to increasing nutrients uptake and lead to greater growth of barley. Studies reported that growth-promoting and diazotrophic bacteria have improved N acquisition by plants through biological N fixation (BNF) and by increasing root hair growth through physiological changes in plants that have increased the production of plant growth hormones such as indole-3-acetic acid, cytokinins, gibberellins and ethylene, which could influence the ability of plant roots to penetrate into the soil for greater water and nutrient absorption.

Recovery of applied fertilizers was linearly increased with co-inoculation of *A. brasilense* + *B. subtilis* under increasing N and P doses (Table 2, 3). The possible explanation for the increase in plant N and P accumulation by co-inoculation with *A. brasilense* and *B. subtilis* may be related to the ability of *Azospirillum* and *Bacillus* to perform biological nitrogen fixation (BNF) and phosphate solubilization. Although BNF is a determining factor for increasing N use efficiency and N uptake by plants, these bacteria are still functionally contributing to some other mechanisms (production of gibberellins, auxins and cytokinins) to increase plant growth. Thus, increasing N and P use efficiency and the recovery of applied N and P in barley with Co-inoculation of A. brasilense + *B. subtilis* contributes to sustainable barley production under reduced N and P fertilization. Previous studies reported that the interaction of microorganisms and plants activates multiple mechanisms to promote growth and improve the yield and nutritional quality of wheat, particularly with inoculation of *A. brasilense* and *B. subtilis*.

3.2. Total nitrogen and available phosphorus in soil:

Bacterial inoculations and mineral fertilizers application significantly affected the total nitrogen and available phosphorus in soil (Table 3). Co-inoculation with *A. brasilense* and *B. subtilis*, with different doses of AN+SSP, increased total nitrogen in soil by 51, 112, 118 % than soil amended with 100 % of AN, SSP. Co-inoculation with *A. brasilense* and *B. subtilis* without chemical AN+SSP increased soil total nitrogen (0.95 g kg^{-1}) than control (0.33 g kg^{-1}), which was statistically not different from the treatments with 100 % AN, SSP (0.97 g kg^{-1}).

Our findings showed an important impact of bacterial inoculation on soil available phosphorus in treatments with or without chemical fertilizers compared with control (Table 3). Regarding soil available phosphorus, inoculation with *A. brasilense* and *B. subtilis* induced a significant ($p \leq 0.05$) increase in this soil parameter compared to un-inoculated soil. Co-inoculation with *A. brasilense* and *B. subtilis*+100 % AN, SSP showed the highest value of soil available phosphorus (16.59 mg kg-1), followed by *A. brasilense* and *B. subtilis*+75 % AN, SSP (16.51 mg kg-1) and *A. brasilense* and *B. subtilis*+50 % AN, SSP (14.07 mg kg-1).

Moreover, Co-inoculation with *A. brasilense* and *B. subtilis* without AN, SSP increased soil available phosphorus by 29 % than soil amended with 100 % AN, SSP without inoculation.

Considering the above results, we suggest that inoculated PGPR can transform elements, such as nitrogen and phosphorus, into nutrients that can be absorbed and utilized by plants through nitrogen fixation and phosphorus solubilization (Tagore et al., 2014). Furthermore, we suggest that PGPR can secrete auxins, such as IAA, to directly promote plant growth (Selvakumar et al., 2012). Specifically, the increase in plant root activity promotes the secretion of organic acids and reduces the pH of the rhizosphere. This decrease of soil pH has a certain degradation effect on phosphorus in the soil, which increases the concentration of available nutrients in the rhizosphere leading to an increase the absorption of nutrients by plant roots (Khan et al., 2009). On the other hand, the forms and contents of nutrients, such as nitrogen and phosphorus, in soils are related to the changes in soil enzyme activities, and the enhancement of soil enzyme activity is closely related to the increase of soil nutrient content (Cusack et al., 2011; You et al., 2014). PGPR inoculation can accelerate the decomposition of soil organic compounds, provide substrates for enzymatic reactions, and promote microbial growth, thereby improving soil enzyme activity, increasing soil nutrient content, and providing a thriving soil ecological environment for the growth of plants (Piromyou et al., 2011; Saia et al., 2015). Therefore, PGPR inoculation can improve plant habitats by producing antimicrobial substances, enhancing plant resistance, and improving soil fertility, which indirectly promotes plant growth and increases plant yields (Khadeejath et al., 2017).

Wan et al. (2019) conducted research to evaluate the potential of eight bacterial genera, including *Acinetobacter*, *Pseudomonas*, *Massilia*, *Bacillus*, *Arthrobacter*, *Stenotrophomonas*, *Ochrobactrum*, and *Cupriavidus*, to solubilize phosphorus. The results indicated that *Acinetobacter* exhibited a remarkable ability to solubilize phosphorus, making it a promising candidate for enhancing soil fertility and quality. Liu et al. (2018) have shown that phosphorus solubilizing bacteria have the ability to secrete small molecular organic acids that can dissolve inorganic phosphorus, which in turn can alter soil properties and indirectly influence the microbial community in the rhizosphere. Pantigoso et al. (2020) investigated the effectiveness of bacteria such as Enterobacter cloacae, Pseudomonas pseudoalcaligenes, and Bacillus thuringiensis in solubilizing plant-unavailable P in either inorganic (calcium phosphate) or organic (phytin) forms. The study found that threonine played a vital role in promoting bacterial solubilization and plant uptake of various nutrients. The authors also suggested that specialized compounds exuded by these bacteria could be a promising approach to unlock existing phosphorus reservoirs in croplands. Kour et al. (2021) evaluated the ability of various genera of plant growth-promoting bacteria, including *Bacillus*, *Enterobacter*, *Pseudomonas*, *Staphylococcus*, *Acinetobacter*, *Klebsiella*, and *Proteus*, to solubilize a significant amount of phosphorus from soil samples collected from the Lesser Himalayas ecosystem.

The results indicated that these bacteria demonstrated a remarkable capacity to solubilize phosphorus, suggesting their potential for enhancing soil fertility and plant growth. Thus, these bacteria could be used for reducing the amount of phosphorus fertilizers.

3.3. Free Amino Acids in plants:

Concerning leaves' Arginine, proline and Phenylalanine content, bacterial inoculation had significant effect on inoculated barley plants. Results of Table (4) indicated that there was a significant increase in Arginine, proline and Phenylalanine content with barley plants that inoculated with bacterial strains as compared with control plants. Furthermore, the data illustrated that the inoculated plants +100% AN, SSP recorded a significantly highest increase with the Arginine, proline and phenylalanine content (1.006, 0.676 and 0.950 mg g^{-1} DW, respectively). While the lowest mean value of phenylalanine content was noticed in control plants.

Proline is an essential osmoregulator for membrane steadiness, buffering cellular redox potential, and scavenging free radicals (Sallam et al., 2019). Proline can also aid in activating the detoxification pathway (Khan et al., 2021; Li et al., 2018). A significant increase effect of PGPR on proline content was noted exclusively in stressed plants inoculated with PGPR.

Biofertilizers containing PGPR can improve plant growth and yield by adding nutrients to the soil and enhancing its fertility. This could contribute to establishing more sustainable crop production practices that can withstand changing environmental conditions and contribute to food security. PGPR offer a sustainable and environmentally benign alternative to conventional agricultural practices and have the potential to contribute to developing more sustainable and resilient agriculture.

4. Conclusion:

Inoculation with plant growth-promoting bacteria is considered one the most feasible, economical and sustainable strategies that could increase crop production to overcome food security challenges and reduce N and P fertilizer dependency. Seed inoculation with Azospirillum brasilense and Bacillus subtilis increased barley biomass, nitrogen and phosphorus uptake, available nitrogen and phosphorus in soil compared to the AN, SSP fertilizer application. Consequently, it was concluded that substitution of inorganic fertilizers by *Azospirillum brasilence* and *Bacillus subtilis* inoculation, can produce excellent results, thus making barley crop cultivation in low inputs systems sustainable in arid regions.

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Tables

Properties	value
Soil texture class	Sandy Loam
Sand %	65.3
Clay %	16.0
Silt %	18.7
Organic matter, %	0.93
E C_e dS/m (saturation extract)	2.31
pH (1:2.5 w/v)	8.31
Field Capacity %	25
$CaCO_3$, %	32.3
Available P, mg Kg^{-1}	5.12
Avalable K, mg Kg^{-1}	475
Total N, mgKg^{-1}	320
Total P, mgKg^{-1}	485

Table 1. Physical and chemical analysis of soil

Treatments		Plant growth parameters					
		Shoot			Root		
		Dry Weight g/plant	N %	P %	Dry Weight g/plant	N %	P %
(T1)	Control (0 % AN, SSP)	0.41	0.75	0.15	0.10	0.53	0.06
(T2)	50 % AN, SSP	0.69	0.91	0.19	0.14	0.66	0.08
(T3)	75 % AN, SSP	0.85	1.07	0.25	0.16	0.78	0.12
(T4)	100% AN, SSP	1.08	1.30	0.26	0.15	0.94	0.14
(T5)	*A.brasilense* + *B. subtilis* + 0 % AN, SSP	1.02	1.03	0.21	0.17	0.88	0.12
(T6)	*A.brasilense* + *B. subtilis* +50 % AN, SSP	1.19	1.99	0.24	0.18	1.49	0.14
(T7)	*A.brasilense* + *B. subtilis* +75 % AN, SSP	1.48	2.57	0.28	0.26	2.06	0.17
(T8)	*A.brasilense* + *B. subtilis* +100 % AN, SSP	1.35	2.71	0.29	0.24	2.16	0.17
	L.S.D (5%)	0.19	0.33	0.035	0.04	0.12	0.027

Table 2. Effects of PGPR, AN, SSP doses on barley dry weight, N and P concentration in shoot and root.

	Treatments	Soil TN g kg^{-1}	Soil Avai P mg kg^{-1}
(T1)	Control (0 % AN, SSP)	0.33	6.57
(T2)	50 % AN, SSP	0.48	7.26
(T3)	75 % AN, SSP	0.82	7.85
(T4)	100% AN, SSP	0.97	8.33
(T5)	*A.brasilense* + *B. subtilis* + 0 % AN, SSP	0.95	10.75
(T6)	*A.brasilense* + *B. subtilis* +50 % AN, SSP	1.47	14.07
(T7)	*A.brasilense* + *B. subtilis* +75 % AN, SSP	2.06	16.51
(T8)	*A.brasilense* + *B. subtilis* +100 % AN, SSP	2.11	16.59
	L.S.D	0.22	2.67

Table 3. Total N and Available P in soil as affected by PGPR co-inoculation and fertilization

	Treatments	Arginine (mg g^{-1} DW)	Proline (mg g^{-1} DW)	Phenylalanine (mg g^{-1} DW)
(T1)	Control (0 % AN, SSP)	0.747	0.546	0.640
(T2)	50 % AN, SSP	0.784	0.572	0.693
(T3)	75 % AN, SSP	0.796	0.569	0.734
(T4)	100% AN, SSP	0.859	0.576	0.767
(T5)	*A.brasilense* + *B. subtilis* + 0 % AN, SSP	0.911	0.590	0.803
(T6)	*A.brasilense* + *B. subtilis* +50 % AN, SSP	0.966	0.650	0.844
(T7)	*A.brasilense* + *B. subtilis* +75 % AN, SSP	0.991	0.667	0.925
(T8)	*A.brasilense* + *B. subtilis* +100 % AN, SSP	1.006	0.676	0.950
	L.S.D	0.077	0.058	0.065

Table 4. Amino acid composition (mg g^{-1} DW) of barley shoot as affected by co-inoculation with PGPR.

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