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Effects of inoculation with plant growth promoting rhizobacteria on photosynthesis, antioxidant status and yield of runner bean

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Abstract:

Plant Growth Promoting Rhizobacteria (PGPR) play an important role in agricultural systems, especially as biofertilizer. Therefore, two rhizobacterial strains positive for phosphorous solubilization and siderophore production (S4) and for indoleacetic acid production (S7) were used to determine the effects of seed inoculation on growth of runner bean plants. The tested strains, alone or in combination, increased photosynthesis, transpiration, water use efficiency and leaves chlorophyll content. Also an increase of superoxide dismutase and peroxidase activity was recorded during periods with intense photosynthesis. S7 inoculation significantly increase the grains yield with 41.40 %. The PGPR strains improve the nutritive value of the harvested grains by enhancing the soluble protein content up to 16.24 % and total reducing carbohydrates content up to 49.28%. The inoculation response was comparatively higher at the early growth stages. Our study suggests that the two PGPR strains may be used as biofertilizer for vegetable production in sustainable and ecological agricultural systems.

Key words: runner bean, plant growth promoting rhizobacteria, seed inoculation, photosynthesis, antioxidant status, yield enhancement, biofertilizer

1. Introduction

The scarlet runner or runner bean (*Phaseolus coccineus* L.) is a climbing perennial vegetable often grown as an annual crop for dry seeds or immature pod production, and also as an ornamental vine [1]. Although of minor importance in the United States, the crop is of importance in some parts of Europe. In the United Kingdom runner bean is frequently grown in order to produce a reliable crop of green beans for commercialization [2]. In Romania the lack of scientific knowledge about the biology and ecology of runner bean was an important consequence that contributed to the "slower" progress of this species [3]. *P. coccineus* has a good ecological plasticity and a relative high tolerance to some pathogens that makes it quite suitable for sustainable agricultural systems.

Surrounding plant roots there is an extremely important and active area for root activity and metabolism which is known as rhizosphere [4]. Bacteria inhabiting the rhizosphere and beneficial to plants are termed plant growth promoting rhizobacteria - PGPR [5]. A rhizobacteria is qualified as PGPR when it is able to produce a positive effect on the plant upon inoculation [6]. These bacteria significantly affect plant growth by: providing the host plant with fixed atmospheric nitrogen [7], solubilization of soil phosphorus compounds [8], producing biologically active substances such as auxins and other plant hormones [9], suppressing pathogens by producing antibiotics and siderophores. However, the mechanisms used by these bacteria to produce the effects mentioned are not enough understood [10].

Conventional farming practices that warrant high yield and quality require intensive use of chemical fertilizers, which are costly and have a high pollution effect [11]. Therefore, more recently there has been a resurgence of interest in environmental friendly, sustainable and organic agricultural practices [12]. The use of PGPR offers an attractive way to replace chemical fertilizer, pesticides and supplements. Some PGPR have been produced commercially as inoculants for agriculture to improve plant growth through supply of plant nutrients and may help to sustain environmental health and soil productivity [6; 13].

Many environmental stresses affect crop productivity and impair electron transport system leading to the formation of activated oxygen, such as H_2O_2, O^-_2 and OH^-, which may accumulate and damage the photosynthetic apparatus [14]. In plants, the highly energetic reactions of photosynthesis and an abundant oxygen supply make the chloroplast a particularly rich source of reactive oxygen intermediates [15]. To protect against oxidative stress, plant cells produce both antioxidant enzymes such as superoxide dismutase, peroxidase and catalase as well as non-enzymatic antioxidants such as ascorbate, glutathione and α-tocopherol [16]. Levels of antioxidant enzyme activity and antioxidant concentrations are frequently used as indicators of oxidative stress in plants.

Little is known about the effects of inoculation with PGPR strains on runner bean. Therefore, the main objective of this study was to determine the effect of two rhizobacterial strains on the antioxidant status, physiological activities and yield of runner bean in low-input systems.

2. Materials and Methods

2.1. Bacterial strain and inoculant preparation

Two bacterial strains (S4 and S7) were selected to asses their potential to enhance the physiological activities and yield of *Phaseolus coccineus* L. in a low-input organic system. The strains were identified using API 50 CHB system and Apiweb software (Biomerieux, France) as follows: strain S4: *Bacillus pummilus* (99.8 % ID), strain S7: *Bacillus mycoides* (88.5 % ID). Based on a preliminary screening test, strain S4 was found positive for phosphate solubilization and siderophore (catechol type) production and S7 was found positive for IAA production (4.86 μg IAA/ml) [17]. The two rhizobacteria were cultivated from slant material in Bunt Rovira nutrient medium (agar-agar free) and incubated in 2000 ml flasks on an orbital shaker at 210 rpm at 27^0 C. After five days the strains were subcultured under the same conditions as described above. The cell densities were adjusted to approximately 1.5 x 10^9 CFU/ml. The strains were further used to inoculate runner bean seeds just prior to sowing.

2.2. Field experiments

Experiments were carried out with runner bean plants using a local population (C2) and sited at the experimental farm of University of Agriculture Sciences and Veterinary Medicine Iasi, during 2011. The farm is located in North-east of Iasi, Romania (lat. $47^0$10' N and long. $27^0$30' E). The local climate is characterized by an average annual temperature of 9.6 °C (49.3 °F) and a total average rainfall of 521 mm·year^{-1}. In terms of the morphological and systematic soil conditions, the soil is classified as chernozem (Cz), with an average supply of nutritive elements, 3.8% organic matter and a pH of 5.8.

Bacterial application of S4 and S7 strains, as well as their combination (S4+S7 in equal parts), was performed using the seed coating method. Runner bean seeds were surface-sterilized in sodium hypochlorite (2 % solution containing 4 ml/l Tween 20) and rinsed five times with distilled water [18]. Seeds were coated for a total time of 1 minute with the inoculum prior to sowing. The control seeds were coated with sterilized tap water.

The experiment was organized using a randomized block design with three replications, each plot covering an area of 27.9 m^2 (3.1 x 9 m). The crop was established by direct sowing on the 10th May 2011 using two seeds per nest. A 40 cm distance between nests from the same row was used. A density of about 32.258 nest/ha (64.516 plants/ha) was obtained using two rows per band, 80 cm distance between rows from the same band and 155 cm total width of a band [19]. In each plot 90 nests (180 plants) were placed. The plants were tied with synthetic strings, one wire for two rows. The plants were growth according to organic cultivation recommendations, including no chemical fertilizers and pesticide applications. During vegetation period, some observations were accomplished regarding the main physiological and yields characteristics.

2.3. Physiological measurements

Physiological measurements were started on 30 of May 2011 (20 days after inoculation – DAI). On this date, nine tagged plants per plot were tested for photosynthesis, transpiration, water-use efficiency and ten tagged plants per plot for chlorophyll content, always using the top leaflet of the last fully developed leaf. Water-use efficiency (WUE) was calculated as the ratio of photosynthesis to transpiration [20]. Gas exchange was measured with an ADC LC*pro+* advanced photosynthesis system (ADC BioScientific Ltd, UK) using a broad leaf chamber with a 6.25 cm^2 window area; the measurements (mean values of six measurements taken during one minute) were performed at 240-310 μmol s^{-1} m^{-2} P.A.R. incident on leaf surface, between 07.00 and 10.00 AM. Leaf chlorophyll content measurements were performed using a CCM-200 plus chlorophyll content meter (ADC BioScientific Ltd, UK). The physiological measurements were repeated on 7th June 2011 (28 DAI), 22nd June (42 days DAI), 9th July (59 days DAI) and 12th August 2011 (96 days DAI), following the main phenophases. In the days of measurements, air temperature varied from a minimum of 19.2^{o}C (7th June 2011) to a maximum of 30^{o}C (12nd August 2011).

2.4. Enzyme assays

Leaf tissues samples were collected during the main phenophases (vegetative, flowering and fruit setting stages) and kept at -20^{0}C until further processing. One g of leaf tissue was grinded for 3 minutes using a mortar and pestle and extracted with 10 ml of 40 mM HEPES, 200 mM NaCl, pH 7.4. After a sequential centrifugation at 14,000 rpm for 30 minutes, the supernatant was used as enzyme source for assessing the activity of two antioxidant enzymes: superoxide dismutase and peroxidase.

2.4.1. Superoxide dismutase

The activity of superoxide dismutase (SOD) was assayed by monitoring its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT). Each 1.5 ml reaction mixture contained 100 mM Tris/HCl (pH 7.8), 75 mM NBT, 2 μM riboflavin, 6 mM EDTA, and 200 μl of enzyme extract. Monitoring the increase in absorbance at 560 nm followed the production of blue formazan. One unit of SOD is defined as the quantity required to inhibit the rate of NBT reduction by 50% [21].

2.4.2. Peroxidase

Peroxidase activity was assessed according to the method presented by [22]. The reaction mixture contained 200 μl enzyme substrate, 500 μl buffered substrate (50 ml phosphate buffer 0.4 M, pH 5.9 and 2 ml ortho-dianisidine 1 % alcoholic solution), 50 μl H_2O_2 0.05 %. After 20 min at room temperature, 750 μl of 50 % H_2SO_4 were added. The intensity of color was determined spectrophotometrically at 540 nm. The enzyme activity is expressed as peroxidase units (UP) which corresponds to the quantity of enzyme capable to decompose 1 micromole of H_2O_2 per minute.

2.5. Seed nutritive value estimation

The harvested grains were used to asses the influence of tested rhizobacteria on their nutritional value by monitoring some biochemical parameters as following: total reducing carbohydrates, total soluble proteins and total lipid content.

2.5.1. Total reducing carbohydrates were measured using 3,5-dinitrosalicylic acid method [23], with glucose monohydrate solution (30 to 300 μg/ml) as standard; the absorbance was measured at 500 nm using an Beckman Coulter DU 720 UV-VIS spectrophotometer.

2.5.2. Total soluble proteins were extracted using cold phosphate buffer saline (PBS) pH 7.4 [24] in 1 g seed/10 mL buffer ratio. The homogenate was further centrifuged at 14,000 rpm for 30 min. The protein amount in the supernatant was assayed by the dye-binding micro-method of Bradford, using the Roti-Quant reagent from Roth (Karlsruhe, Germany). Total soluble protein content was expressed as mg bovine serum albumin (BSA) per g harvested seeds;

2.5.3. Total lipid content

Approximately 20 grams of beans were dried at 105^0 C until constant weight and grinded to powder using a Waring laboratory blender; exactly 2 grams of powder were extracted with diethyl-ether for 8 hours with a Soxhlet extractor [25], followed by a gravimetric measurement of the remaining material. The differences of the powder weight before and after extraction were considered as sample's total lipid amount and expressed as mg per g harvested seeds.

2.6. Statistical analysis

The experimental data concerning physiological measurements were statistically processed using ANOVA: two-factor with replication [26] followed by a post-hoc analysis using Duncan's multiple range test. All results are expressed as means ± S.E.M. F values for which $p<0.05$ were considered significant. Experimental data regarding the biochemical assays and grain yield were statistically processed using Student (t) test.

3. Results and Discussion

In the present study two rhizobacteria isolates were tested *in vivo* in order to assess their plant growth promoting potential for *P. coccineus* C2 plants. Because at the present time little is known about the PGPR's interactions in the rhizosphere, the experiments were carried out using a single strain (S4 or S7) or a combination of strains (S4+S7) to establish which version is more suitable to enhance runner bean plants growth.

3.1. Photosynthetic rate and related parameters

The physiological measurements were performed following the main phenophases on 30 May 2011 (20 DAI – vegetative stage), 7 June 2011 (28 DAI – vegetative stage), 22 June (42 days DAI – early flowering stage), 9 July (59 days DAI – late flowering + fruit setting stage) and 12 August 2011 (96 days DAI – fruit setting stage).

Net leaf photosynthesis

The values of net photosynthesis in runner bean leaves ranged from 2.12 to 8.19 μmol C m^{-2} s^{-1} (Fig. 1). Initially during vegetative stage photosynthetic activity was higher but declined with time. According to Duncan's test, the photosynthetic rate was significantly influenced by the utilization of S4 and S7 rhizobacterial strains. However, the effect of rizobacterial inoculation on photosynthesis was not consistent across treatments and growth stages. Thus, inoculation with S4 resulted in higher photosynthetic rate compared to control plants at 28 DAI ($p<0.025$) and 42 DAI ($p<0.006$). The S7 treatment showed significantly the highest photosynthetic rate value (8.19 μmol C m^{-2} s^{-1}) during vegetative stage (20 DAI)

compared to the other variants. Co-inoculation significantly increase photosynthetic activity only at 42 DAI ($p<0.04$) versus control, but the influence is milder compared with S4 and S7 inoculated plants. PGPR inoculation could not show any positive influence on photosynthesis at 59 and 96 DAI when no significant differences were recorded between different treatments used (S4, S7 and S4+S7) compared to control.

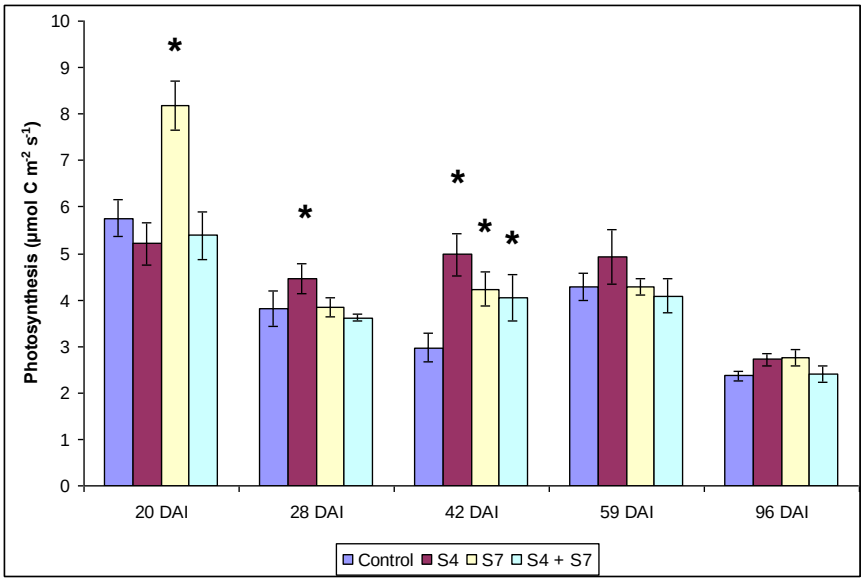

Fig. 1 – Effects of inoculation with PGPR strains S4, S7 and S4+S7 on photosynthesis in runner bean leaves. Values are mean ± S.E.M, (*n* = 9), * p< 0.05 vs. Control. DAI = days after inoculation.

Transpiration

S7 strain was more effective in promoting plant transpiration than S4 and S4+S7 strains. Thus S7 inoculation increased transpiration up to 24.24%, significant differences being recorded at 20, 28 and 42 DAI by comparison to the non-PGPR inoculated plants (Fig. 2). Our results showed no significant differences concerning leaves transpiration between PGPR treatments and control at 59 and 96 DAI. Also no influences of S4 and S4+S7 on transpiration could be recorded during the whole experimental period.

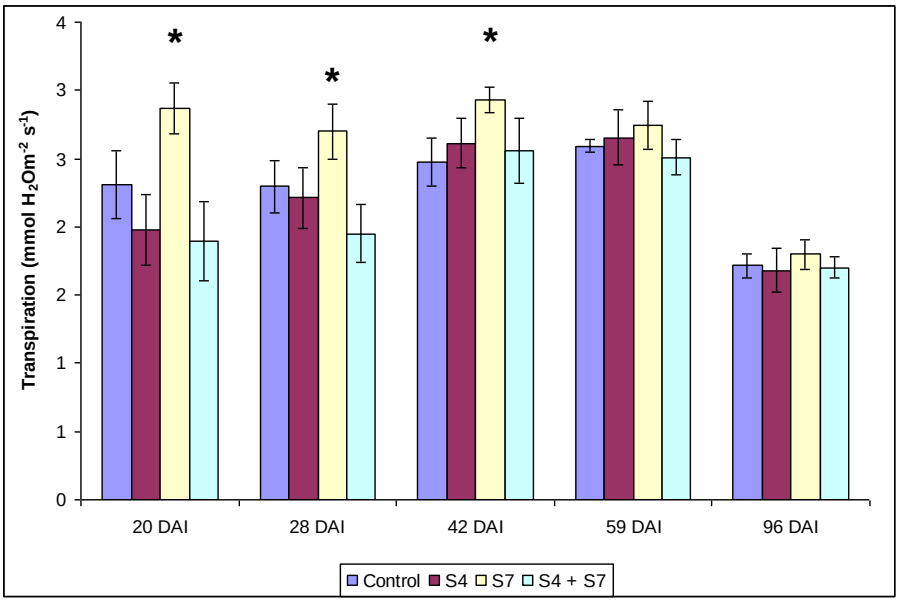

Fig. 2 - Effects of inoculation with PGPR strains S4, S7 and S4+S7 on transpiration in runner bean leaves. Values are mean ± S.E.M, (*n* = 9), * p<0.05 vs. Control. DAI = days after inoculation.

Water use efficiency

Water-use efficiency was increased in the presence of S4 and S7 strains during the vegetative growth stage (Fig. 3). At 20 DAI the most effective strain was S7 (4.83 μmol C m^{-2} s^{-1}/mmol H_2Om^{-2} s^{-1}), while at 28 and 42 DAI the highest values for WUE were recorded in the case of S4 inoculated plants (1.60 respectively 2.51 μmol C m^{-2} s^{-1}/mmol H_2Om^{-2} s^{-1}). Also, co-inoculation increased significantly WUE, compared to control at 42 DAI. Nevertheless, no PGPR beneficial influence could be observed at 59 and 96 DAI.

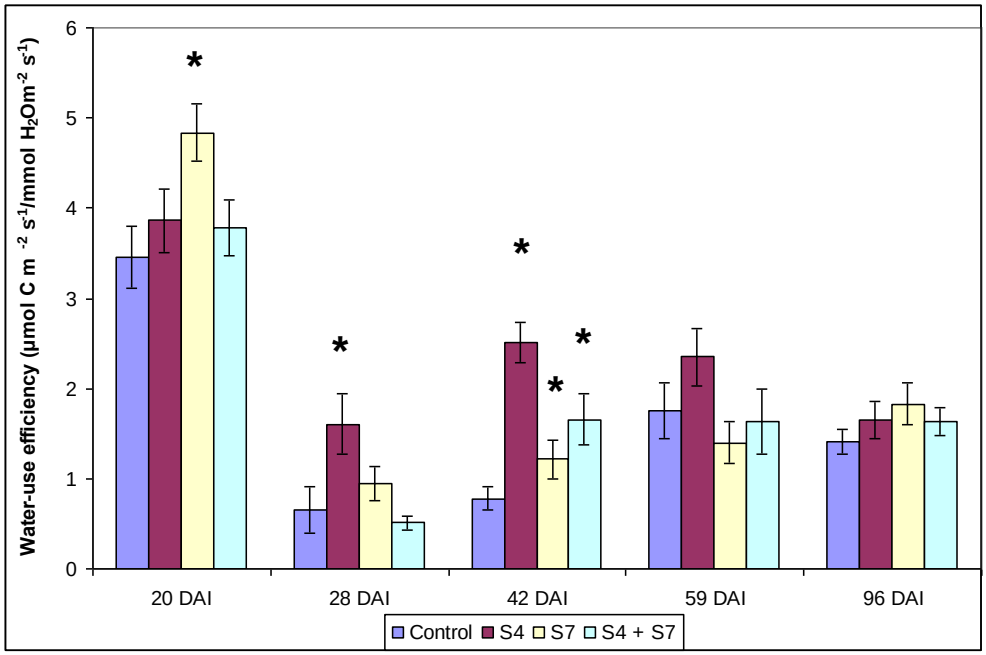

Fig. 3 – Effects of inoculation with PGPR strains S4, S7 and S4+S7 on water-use efficiency in runner bean leaves. Values are mean ± S.E.M, (*n* = 9), * p<0.005 vs. Control. DAI = days after inoculation.

Chlorophyll content

PGPR inoculation with S4 and S7 strains increased significantly the chlorophyll content only at 42 and 59 DAI. No significant differences where observed during the vegetative stage as previously recorded for photosynthesis rate, transpiration and WUE. Also, co-inoculation showed no beneficial influences during the entire experimental period (Fig. 4).

3.2. Enzyme activities

The activity of superoxide dismutase and peroxidase in runner bean plants treated with PGPR was observed at three different times: 20, 42 and 59 DAI. During vegetative stage, significantly higher SOD activity was obtained in the leaves of S7 inoculated plants compared with control. Also, S4 and S4+S7 treatments induced higher SOD activities comparing with the control but the recorded differences where not significant (Fig. 5).

Co-inoculation induced the highest peroxidase activity at 20 DAI (Fig. 6). Also at the same time, plants treated with S7 strain showed a milder, but significant increase of peroxidase activity, compared with the control. At 42 DAI the highest enzymatic activity was recorded for S4 treated plants. No significant differences were recorded for the other treatments versus control at 42 DAI. The PGPRP inoculation of runner bean plants did not influence peroxidase activity at 59 DAI.

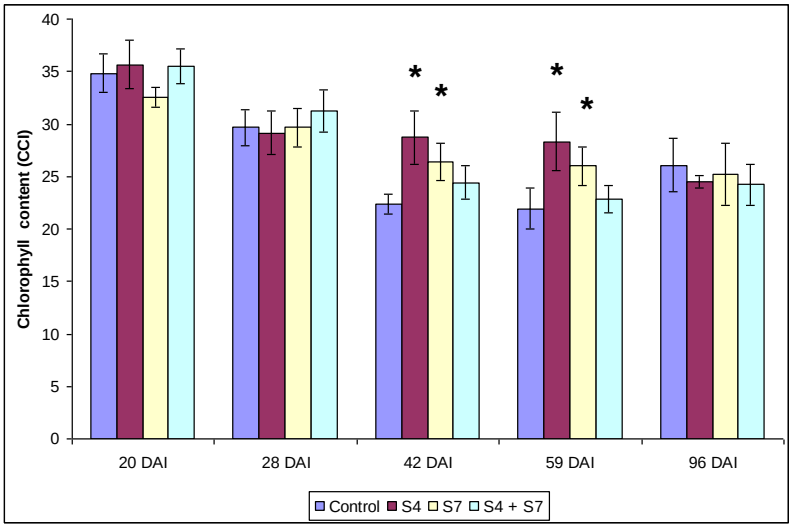

Fig. 4 - Effect of PGPR strains S4, S7 and S4+S7 on chlorophyll content in runner bean leaves. Values are mean ± S.E.M, (*n* = 10). * p<0.005 vs. Control. DAI = days after inoculation.

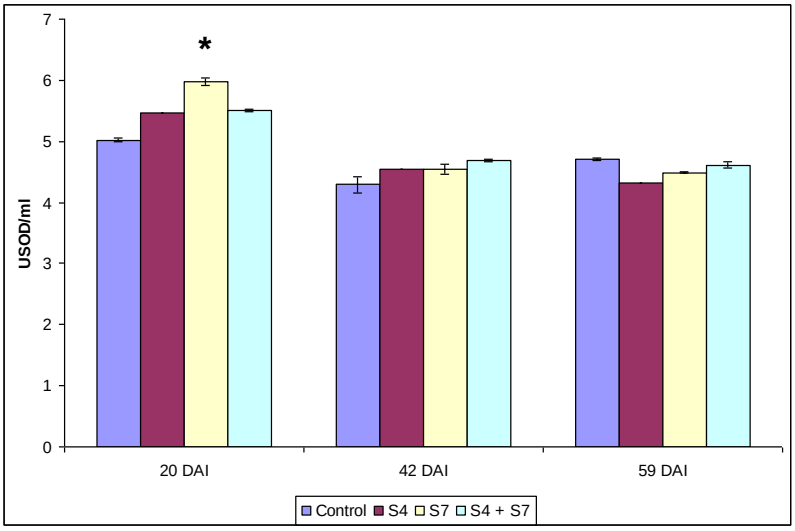

Fig. 5 - Effect of PGPR strains S4, S7 and S4+S7 on superoxide dismutase activity in runner bean leaves. Values are mean ± S.E.M, (*n* = 3). * p<0.005 vs. Control. DAI = days after inoculation.

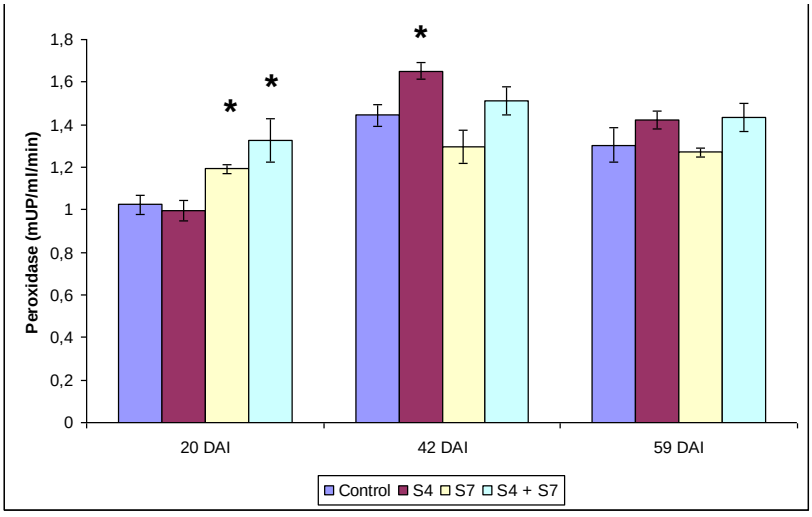

Fig. 6 - Effect of PGPR strains S4, S7 and S4+S7 on peroxidase activity in runner bean leaves. Values are mean ± S.E.M, (*n* = 3). *p<0.005 vs. Control. DAI = days after inoculation.

3.3. Protein, carbohydrate and lipid bean content

Plants inoculated with PGPR showed higher seed protein-content compared to control plants (Table 1). Thus inoculation with S7 strain induced a 16.24 % increase of runner bean soluble protein yield, followed by S4 treatment (139.83 mg/g harvested seeds). Co-inoculation determined also an increase of seed protein yield versus control, but the recorded differences where not significant. Similar pattern was also showed for total reducing carbohydrates seed content (Table 1). In this case both inoculation and co-inoculation with PGPR strains induced an increase of carbohydrates bean content. An increase of 49.28% was achieved by inoculation with S4 strain, followed by S4 + S7 (24.52%) and S7 (23.57%) treatments. PGPR inoculation of runner bean plants did not induced any significant differences regarding the total lipid content of harvested beans compared with non-inoculated plants (Table 1).

Table 1 – Total soluble protein, reducing carbohydrates and lipid content of harvested beans

Treatment	Total soluble protein (mg/g harvested seeds)	Total reducing carbohydrates (mg/g harvested seeds)	Total lipid (mg/g harvested seeds)
Control	123,06 ± 5,00	4,20 ± 0,86	38,17 ± 4,53
S4	139,83 ± 6,29*	**6,27 ± 0,86***	36,51 ± 4,84
S7	**143,05 ± 4,72***	5,19 ± 0,79*	36,93 ± 2,86
S4 + S7	128,10 ± 3,49	5,23 ± 0,63*	38,48 ± 5,24

- values are means ± standard deviation of three separate determinations.
* - significant differences vs. control ($p<0.05$)

3.4. Effect of PGPR on grain yield

Yield increases occurred in all rhizobacterial treatments and where significant compared to control: S4 – 33.86 % ($p<0.008$), S7 - 41.40 % ($p<0.01$) and S4 + S7 – 18.42 % ($p<0.04$) (Table 2). The highest grain yield was recorded in the case of plants inoculated with S7 strain - 906.74 kg/ha, followed by S4 (858.42 kg/ha) treatment.

Table 2 - Effect of PGPR strains S4, S7 and S4+S7 on runner bean yield.

Treatments	Yield (kg/ha)
S4	858.42 ±19.03*
S7	**906.74 ± 13.44***
S4+S7	759.4 ± 28.76*
Control	641.25 ± 24.12

- values are means ± standard deviation of five separate determinations.
* - significant differences vs. control ($p<0.05$)

The effects of PGPR on plant growth are a well documented fact. However, insufficient data are available about the effects of these bacteria on physiology of runner bean plants. Therefore our study was focused on presenting the effects of inoculation with two PGPR strains on photosynthesis, transpiration, water use efficiency, chlorophyll leaves content, antioxidant status and grain yield of runner bean plants grown under organic conditions.

PGPR strains used for seed inoculation induced significant increases of photosynthetic rate at 20 DAI (S7 strain), 28 DAI (S4) and 42 DAI (S4, S7 and S4 + S7 treatments). Similar effects where also showed by the same rhizobacteria on WUE. Regarding transpiration, a significant enhancement of this physiological parameter was recorded only in the case of S7 inoculation during vegetative and early flowering stages. Increased photosynthetic rate as a

consequence of PGPR inoculation was also reported by other authors [16; 27; 28]. Previous studies suggest that increased photosynthetic activity is a consequence of a higher N incorporation which contributed to the formation of chlorophyll [29]. Our results showed significant higher chlorophyll content in leaves of runner bean plants inoculated with S4 and S7 strains versus control only during early flowering and late flowering + fruit setting stages and not during vegetative stage. Moreover, the two rhizobacteria strains were found positive for PGP traits such as phosphate solubilization/siderophore production (S4) and IAA production (S7) and were found negative for nitrogen fixation (data not shown). Therefore we may presume that the main mechanism of photosynthesis enhancement is related to direct effect of the tested rhizobacteria on runner bean plants physiological status rather than to nitrogen fixation. Similar results were previously reported by Zhang and co-workers (1997) who showed direct effects of PGPR on photosynthetic rates of soybean at different growth stages before the onset of N_2 fixation.

Our study revealed that the activity of investigated antioxidant enzymes was increased in the presence of PGPR strains. SOD activity was significantly higher in the leaves of S7 inoculated plants at 20 DAI, compared with the control. Also, we recorded an enhancement of peroxidase activity at 20 DAI in the case of S4 and S4 + S7 treatments and at 42 DAI for S4 strain inoculated plants. Normally, these antioxidant enzymes tend to detoxify the toxic H_2O_2 accumulated during intense photosynthetic activities as it was recorded in the case of runner bean plants treated with S7 strain at 20 DAI and for S4 inoculated plants at 42 DAI. Efficient remova1 of reactive oxygen intermediates from chloroplasts is critical, since H_2O_2 concentrations as low as 10 μM can inhibit photosynthesis by 50 % [30]. Also it is clearly stated that the over expression of antioxidant enzymes such as SOD in chloroplasts provides increased protection from oxidative stress [15]. Therefore we may assume that S4 and S7 strains can prevent oxidative stress by increasing SOD and peroxidase activities during periods with intense photosynthesis; this elevated activity could be correlated with increased stress tolerance. Similar results concerning antioxidant protective effects of PGPR where previously reported by other authors [14; 16; 31] for different forms of induced oxidative stress.

Inoculation and co-inoculation of runner bean plants with S4 and S7 strains influenced the nutritional value of the harvested grains. Thus, PGPR increased seed total soluble protein content up to 16.24 %. Also both inoculation and co-inoculation enhanced the total reducing carbohydrates content of the beans up to 49.28%, in the case of S4 treatment. Improvement of grain protein yield where also reported by [32] in the case of soybean plants under short season conditions. The protein content enhancement is related to a higher relative increases in nitrogen fixation due to PGPR inoculation. In our case, the two PGPR strains used for seed inoculation were found negative for nitrogen fixation capabilities. Therefore the increase of grains total soluble protein content could be related to enhancement of physiological activities of runner bean plants and subsequent to soybean growth. Similar results were previously reported for soybean plants grown in organic conditions [33]. In this case, inoculation with *Bacillus pumilus* Rs3 strain increased with 66 % the total amount of seed soluble protein, probably due to stimulation of protein biosynthesis processes in soybean plants. Considering that beans provide dietary proteins that play an essential role in human nutrition by complementing other foods [34] we believe that S4 and S7 strains could be used in future agricultural biotechnology in order to improve the nutritive value of runner bean plants.

Seed inoculation with PGPR strains significantly increased the grain yield with 41.40 % in organic cultivation conditions. The yield increase can be attributed to higher photosynthetic activities and also with efficient nutrient uptake and water use [35]. Our study showed that S7 inoculation increased photosynthesis, transpiration and WUE, maintaining in this way the physiological activities at a higher level which is essential to increase the yield. All these

effects might be explained by the capability of the S7 strain to synthesize IAA which is related to enhance root proliferation [36]. This increase root development and further plant mineral uptake, stimulating indirectly plant growth [37].

The inoculation response was comparatively higher at the early growth stages (vegetative and early flowering stages), compared with the other investigated phenophases. This could be related with the root nutrient exchanges that become more intense during vegetative growth [38]. Because rhizobacteria mediates soil minerals and nutrients uptake [39] PGPR's influence is more visible during this stage.

This is the first study to demonstrate that PGPR can increase photosynthesis, WUE, chlorophyll content, activity of antioxidant enzymes and yield of runner bean plants. It is well documented that PGPR can increase plant growth by a combination of mechanisms, which include phytohormone production, increasing the availability of soil nutrients, nitrogen fixation [40; 41; 42]. Since both S4 and S7 strains were found negative to nitrogen fixation trait (data not shown) we may presume that the plant growth promoting effect could be explained by IAA producing and P solubilizing capacities of the tested strains.

4. Conclusions

The results of the present study suggests that S4 and S7 strains alone or in combination have a great potential to increase photosynthesis, transpiration, water use efficiency, leaves chlorophyll content and grain yield. PGPR strains can indirect enhance stress tolerance as a consequence of increasing activity of some antioxidant enzymes during periods with intense photosynthesis. The PGPR strains improve the nutritive value of the beans by enhancing the soluble protein and reducing carbohydrates content. The inoculation response was comparatively higher at the early growth stages. Our study suggests that the two PGPR strains may be used as biofertilizer for vegetable production in sustainable and ecological agricultural systems. However further studies are necessary in order to evaluate the impact of beneficial bacteria introduction into soil ecosystems.

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