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Seed inoculation with plant growth promoting rhizobacteria enhances photosynthesis and yield of runner bean (*Phaseolus coccineus* L.)

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ABSTRACT

Growth promoting potential of two rhizobacterial strains positive for phosphorous solubilization and siderophore production (S4) and for indoleacetic acid production (S7) was assessed on runner bean plants cultivated in organic crop system. Seed inoculation with rhizobacterial strains resulted in an increase of photosynthetic activities, water-use efficiency and chlorophyll content. The positive effect exerted by the two rhizobacterial strains was more pronounced during vegetative and early flower setting stages. Also S4 + S7 treatment significantly increase the grains yield with 27.58%. The results indicate that co-inoculation produced a more pronounced influence, while inoculation alone showed a lower effect, probably due to a synergistic effect between rhizobacterial inoculated strains. Our results suggest that S4 and S7 strains may be utilized as biofertilizer for vegetable production in sustainable and organic agricultural systems.

1. Introduction

Scarlet or runner bean (*Phaseolus coccineus* L.) has many important features that can make it one of the most important legume crops in the world. The plant is especially cultivated for its grains (dried or green), but also as an ornamental plant. The species is used as a germplasm source, with application in genetic breeding (Giurcă, 2009). Although less studied in Romania where is cultivated only on small areas, the runner bean has received special international interest, being grown on large areas in Central and South America, as well as in Europe (Munteanu, 1985). The lack of scientific knowledge about the biology and ecology of runner bean, under the specific circumstances of our country was an important consequence that contributed to the "slower" progress of this species (Munteanu et al., 2007). *P. coccineus* has a good ecological plasticity and a relative high tolerance to some pathogens, a fact that makes it quite suitable for sustainable agricultural systems, including organic systems.

Plant rhizosphere is normally populated with a wide diversity of bacterial populations. Root bacteria are common inhabitants of both the surfaces and the internal tissues of most plants and may have diverse effects on health, growth and development of plants (Kuklinsky-Sobral et al., 2004). Plant growth promoting rhizobacteria (PGPR) are a heterogeneous group of bacteria that can be found in the rhizosphere, at root surfaces and in association with roots, which can improve the extent or quality of plant growth, either directly or indirectly (Ahmad et al., 2008). The direct effect of PGPR refers to the production of plant growth regulators like indoleacetic acid (IAA), gibberellic acid, cytokinins and ethylene (Zahir et al., 2003), providing the host plant with fixed atmospheric nitrogen (Zhang et al., 1996) or to the solubilization of soil phosphorus compounds (De Freitas et al., 1997; Rodríguez and Fraga, 1999). The indirect promotion of plant growth occurs when PGPR reduce or prevent the deleterious effect of one or more phytopathogenic microorganisms through competition for nutrients, siderophore-mediated competition for iron (Neilands, 1995), antibiosis or induction of systemic resistance in the host plant (Fridlender et al., 1993; Wilson, 1997), reduce the concentration of heavy metals available to plants, contributing to the ecological restoration of polluted sites (Marian et al., 2012). However, the mechanisms used by these bacteria to produce the effects mentioned are not enough understood (Lucas García et al., 2004).

Conventional farming practices that warrant high yield and quality require intensive use of chemical fertilizers, but which are costly and have a high pollution effect (Orhan et al., 2006). Therefore, more recently there has been a resurgence of interest in environmental friendly, sustainable and organic agricultural

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practices (Esitken et al., 2006). To the maximum extent feasible, organic agricultural systems rely upon bio-fertilization, crop rotations, crop residues, animal manures, legumes, green manures, off-farm organic wastes and mineral-bearing rocks to maintain soil productivity. Given the negative environmental impacts of chemical fertilizers and their increasing costs, the use of PGPR is advantageous in the sustainable agricultural practices (Chen et al., 2006). Use of bio-fertilizers containing beneficial microorganisms instead of synthetic chemical is known to improve plant growth through supply of plant nutrients and may help to sustain environmental health and soil productivity (O'Connell, 1992).

Biotechnology has opened up new possibilities concerning the application of beneficial bacteria in the soil for the promotion of plant growth and the biological control of soil-borne pathogens (Kloepper et al., 1989). Since the large scale release of genetically engineered bacteria to the environment faces a number of regulatory hurdles, the need to isolate and select superior, naturally occurring rhizosphere bacteria continues to be of interest (Bashan et al., 1993). The utilization of rhizobacteria in agricultural production depends on our knowledge about bacteria–plant interaction and our ability to maintain, manipulate and modify beneficial bacterial populations under field conditions (Hammer et al., 1999). However, to date, there have been no investigations regarding the positive effects of PGPR strains on runner bean. Therefore, the main objective of this study was to determine the potential of two rhizobacterial strains to enhance physiological activities and yield of *P. coccineus* plants in low-input systems, in relation to their mineral phosphate solubilization, IAA and siderophore production capabilities.

2. Materials and methods

2.1. Isolation of rhizobacteria

Bacteria were isolated from the rhizosphere of field-grown soybean and runner bean crop from the Experimental Farm Ezareni, University of Agriculture Sciences and Veterinary Medicine, Iasi County, Romania. After the roots were separated from bulk soil, rhizospheric soil and root samples were blended in a sterile Waring blender at high speed for 1 min and serial dilutions (1/10) were made in PBS (phosphate buffer saline, ph 7.4). Aliquots (0.1 ml) were plated on Bunt Rovira nutrient medium (Bunt and Rovira, 1955) and incubated at 28° C for seven days. Isolates were re-streaked on the same nutrient medium, checked for purity and stored on slants at 4° C.

2.2. Plant growth promoting capabilities tests

2.2.1. Phosphorus solubilizing assay

The ability of isolates to solubilize phosphorus (P) was qualitatively assessed using potato-dextrose yeast extract agar (PDYA, pH 7.0), containing freshly precipitated calcium phosphate: 50 ml sterile 10% (wt vol^{-1}) K_2HPO_4 and 100 ml sterile 10% (wt vol^{-1}) $CaCl_2$ were added per liter of sterile PDYA to produce a precipitate of $CaHPO_4$ (Katznelson and Bose, 1959). Each bacterial culture was streaked in the center of a PDYA-CaP plate and incubated at 28° C. After 14 days, phosphate solubilization was assessed visually. The ratio between the diameter of the halo and the diameter of the colony (mm) was calculated.

2.2.2. Colorimetric IAA assay

A 24-h tryptic soy agar (TSA, Applichem) culture of each isolate was suspended in sterile water to an optical density (O.D.) of 0.5 at 500 nm in order to perform a colorimetric IAA assay. The suspension (2 ml) was added to 28 ml of growth medium in a 50 ml test tube. The growth medium contained (in g/l): glucose-5; yeast extract-0.025; L-tryptophan-0.204. Controls were prepared by substituting sterile water for bacterial suspension. Tubes were capped, vortexed and statically incubated in the dark at 28° C for 72 h. Prior to analyses for auxins, individual cultures were adjusted to approximately 10^8 cells/ml with sterile water and filtered through 0.2 μm membranes. Triplicate tubes of each isolate were used for the assays. Samples were assayed for production of auxins (IAA equivalents) using standard method of Gordon and Weber (Gordon and Weber, 1951) in which auxins present in the culture filtrate (3 ml) was reacted with Salkowski reagent (2 ml) to yield a pink-colored product after 30 min incubation, which was quantitatively measured on a Beckman-Coulter DU 730 Life Sciences UV–vis spectrophotometer at 530 nm.

2.2.3. O-CAS assay

CAS medium was prepared according to Schwyn and Neilands (Schwyn and Neilands, 1987). The medium for a liter of overlay was as follows: chrome azurol S (CAS)-60.5 mg, hexadecyltrimetyl ammonium bromide (HDTMA)-72.9 mg, piperazine-1,4-bis(2-ethanesulfonic acid) (PIPES)-30.24 g, and 1 mM $FeCl_3 \cdot 6H_2O$ in 10 mM HCl-10 ml. Agarose (0.9%, w/v) was used as gelling agent. Siderophore detection was achieved after 10 ml overlay of this medium were applied over those Luria Bertani (LB – 1.0% tryptone, 0.5% yeast extract, 1.0% sodium chloride, pH 7.0) plates (standard, 100 mm diameter Petri dishes) containing cultivated rhizobacteria to be tested for siderophore production. After a maximum period of 15 min, a change in color was observed in the overlaid medium, exclusively surrounding producer microorganisms, from blue to purple (as described for siderophores of the catechol type) or from blue to orange (as reported for microorganisms that produce hydroxamates). All these experiments were made at least three times with three replicates for each strain.

2.3. Bacterial strain and inoculant preparation

Two bacterial strains (S4 and S7) which presented the best plant growth promoting (PGP) capabilities at preliminary tests were used to asses their potential to enhance the physiological activities and yield of *P. 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). 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° C. After five days the strains were subcultured under the same conditions as described above. The cell densities were determined at 600 nm (S4 – OD_{600} = 1.715, S7 – OD_{600} = 1.735) and were further confirmed by plate count technique: approximately 1.43×10^9 CFU/ml (S4) and 1.57×10^9 CFU/ml (S7). The strains were further used to inoculate runner bean seeds just prior of sowing.

2.4. Field experiments

Experiments were carried out with runner bean plants using a local population (C3) and sited at the experimental farm of University of Agriculture Sciences and Veterinary Medicine Iasi, during 2011. The farm is located in northeast of Iasi, Romania (lat. 47°10′ N and long. 27°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 applications of S4 and S7 strains, as well as their combination (S4 + S7 in equal parts), were performed using the coating