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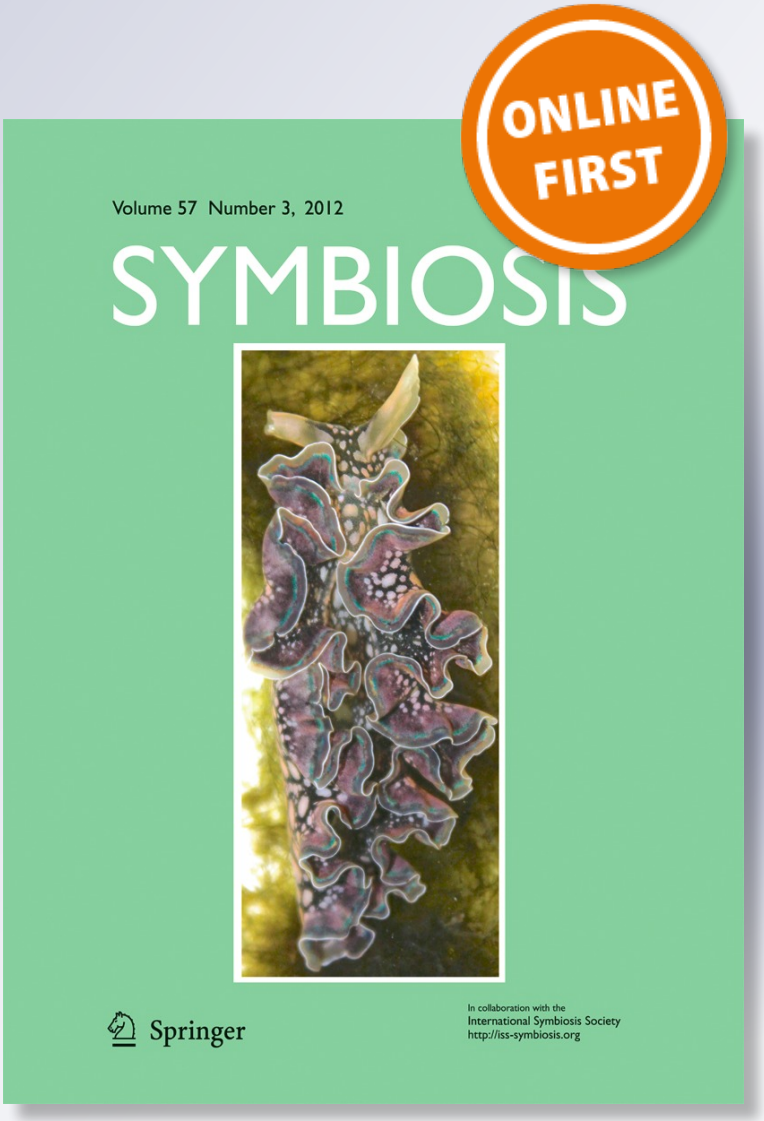

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Effects of single and co-inoculation with native phosphate solubilising strain *Pantoea* sp J49 and the symbiotic nitrogen fixing bacterium *Bradyrhizobium* sp SEMIA 6144 on peanut (*Arachis hypogaea* L.) growth

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Abstract In the present study, attempts were made to analyze the effect of co-inoculation with an efficient phosphate solubilising native isolate *Pantoea* sp J49 and the symbiotic nitrogen fixing *Bradyrhizobium* sp SEMIA 6144 strain on *Arachis hypogaea* L. plants growth. Single and co-inoculation of peanut plants growing in plastic pots containing soil with low P content were developed. Plants were harvested at R1 and R4 growth stages and were analyzed in different growth parameters. Survival of strain *Pantoea* sp J49 was analyzed in soil samples and in root tissues. Plants inoculated only with *Pantoea* sp J49 showed the highest shoot and root weight in both reproductive growth stages evaluated. Plants co-inoculated with this strain and *Bradyrhizobium* sp SEMIA 6144 showed increase in aerial dry weight at R1 stage. Survival assays demonstrated that *Pantoea* sp J49 survives not only in the peanut rhizosphere but also inside plant tissues, including nodules formed when it was co-inoculated with *Bradyrhizobium* sp SEMIA 6144. Results obtained in this study confirm the great potential of the native *Pantoea* sp J49 isolate in the promotion of peanut plant growth, probably related with its capacity to solubilise phosphate.

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1 Introduction

Peanut (*Arachis hypogaea* L.) is a widespread oilseed crop of great agricultural and economic significance. Peanut seeds contain 44–56 % of oil and 22–30 % proteins on dry weight seed basis (Reddy et al. 2003). Argentina is one of the major peanut producers in the world, and about 90 % of its production takes place in the province of Córdoba (S.I.A. 2011). Due to intensity of agricultural practices, peanut soils of Córdoba have decreased their content of soluble phosphorus (P) available to plants (Bonadeo et al. 1997, 1998; Bosch and Da Veiga 2002; Severina 2006).

The rhizosphere, volume of soil surrounding roots influenced chemically, physically and biologically by the plant root, is a highly favourable habitat for the proliferation of microorganisms that exerts a potential impact on plant health and soil fertility (Sorensen 1997). It is well known that a considerable number of bacterial species, mostly those associated with the plant rhizosphere, are able to exert a beneficial effect upon plant growth and thus they have been termed "plant growth promoting bacteria" (PGPB) (Bashan and Holguin 1998). Therefore, their use as "biofertilizers" or control agents has been a focus of numerous researchers for a number of years since this is considered a healthier

alternative to the chemical fertilizers application (Davison 1988; Lemanceau 1992; Glick 1995; Rodriguez and Fraga 1999; Rodriguez et al. 2006).

The solubilisation of phosphates in the rhizosphere is one of the most common modes of action of PGPB (Rodriguez and Fraga 1999; Richardson et al. 2001; Chen et al. 2006). The bacterial release of P from insoluble organic compounds involves enzymatic processes (Rossolini et al. 1998) meanwhile mineral phosphate solubilization is widely associated with the production of low-molecular-weight organic acids, mainly gluconic and 2-cetogluconic acids (Goldstein 1995; Kim et al. 1997; Rodriguez and Fraga 1999; Rodriguez et al. 2006). These acids chelate the cations (Al, Fe, Ca) bound to the insoluble forms of phosphate and convert them into soluble forms with the consequent decrease in the pH of the medium (Kpomblekou and Tabatabai 1994; Stevenson 2005).

Atmospheric N_2-fixation by rhizobia is the most studied direct mechanism involved in plant growth promotion, and rhizobial inoculants for legumes have been worldwide used (Anandham et al. 2007). This process has a high requirement of P since nitrogenase, enzyme responsible for N_2 reduction to ammonia, uses 16 ATP molecules in this reaction. Peanut is efficiently nodulated by bradyrhizobial strains, and *Bradyrhizobium* sp SEMIA 6144 is a bacterium recommended as inoculant for this crop from Instituto de Pesquisas Agronómicas (IPAGRO), Brasil (Taurian et al. 2002).

Although PGPB are common soil inhabitants, usually their numbers are not enough to compete with other bacteria widely established in the rhizosphere. Therefore, to increase the soil number of target microorganisms taking advantage of their beneficial properties for plant yield, their inoculation is generally necessary. A prerequisite to introduce these beneficial bacteria in the environment is that, in addition to plant growth promotion, the effects on soil microflora should be negligible. Thus, it is important to isolate and characterize native bacteria to be used as potential inoculants in the same area where they were obtained.

In spite of many studies on plant growth promotion (PGP) by various P solubilising microorganisms (Nahas 1996; Rodriguez and Fraga 1999), this PGP mechanism has not been extensively studied in peanut. Nevertheless, it has been reported that the rhizosphere of this legume harbour a high diversity of beneficial bacteria with great potential to be used as inoculants (Kishore et al. 2005; Ibañez et al. 2009; Taurian et al. 2010; Tonelli et al. 2010). We have previously obtained a collection of native phosphate solubilising bacteria from field peanut plants and the effect of their inoculation on plant growth was evaluated in microcosm assays (Taurian et al. 2010). Among these isolates, the Gram negative bacterium *Pantoea* sp J49 isolated from inside peanut nodules, demonstrated promising abilities since an increase in shoot dry weight (>92 %) of inoculated plants was determined.

Co-inoculation practices for crop yield improvement have been extensively used since the past decade. Moreover, synergistic interactions on plant growth by co-inoculation of phosphate solubilising bacteria with other beneficial bacteria have been reported (Belimov et al. 1995; Kundu and Gaur 1984; Toro et al. 1998). The aim of this work was to investigate the effect on peanut plant growth of co-inoculation of *Bradyrhizobium* SEMIA 6144 and the phosphate solubilising *Pantoea* sp J49 strain.

2 Material and methods

2.1 Bacteria and culture media

Pantoea sp J49, *Bradyrhizobium* sp SEMIA 6144 and a *Pseudomonas fluorescens* PMT1 strain were used. *Pantoea* sp J49 is a native phosphate solubilising bacteria isolated from peanut tissues (Taurian et al. 2010). *Bradyrhizobium* sp (*Arachis hypogaea* L.) SEMIA 6144 is recommended as peanut inoculant by IPAGRO. *Pseudomonas fluorescens* PMT1, used in this study for comparison purposes, is a phosphate solubilising strain used in the commercial formulation of a biofertilizer for maize and wheat. Bacteria were grown in TSA (trypticase soy agar) (Britania), YEMA (yeast extract mannitol agar) (Vincent 1970) or LB (Luria-Bertani) media (Miller 1972), respectively, and maintained in glycerol stock at 20 % (*v/v*) at −80 °C.

2.2 Phosphate solubilisation in liquid media

The ability of the bacteria to solubilise inorganic phosphate in liquid medium was determined in NBRIP-BPB medium (National Botanical Research Institute´s phosphate growth medium) (Mehta and Nautiyal 2001). Amount of phosphate solubilised was determined by modified Fiske and Subbarow (1925) method. One ml of an overnight culture in LB medium (10^9 cfu/ml) was transferred to 25 ml of NBRIP-BPB. Two ml of bacterial cultures were sampled after 24, 48, 72 hs and 7 days of growth and centrifuged at 10,000 rpm for 12 min. The amount of soluble phosphorus released to the medium was quantified spectrophotometrically by measuring absorbance at 660 nm. The cfu/ml and supernatant pHs of each sample were also measured.

2.3 Bacterial coexistence in plate assays

To evaluate possible bacterial antagonism, the coexistence on plates of both phosphate solubilising bacteria (*Pantoea* sp J49 or *Pseudomonas fluorescens* PMT1) and *Bradyrhizobium* sp SEMIA 6144 was determined. Fresh culture of each species

was streaked on one of the two halfs of YEMA plates. The plates were further incubated at 30 °C and growth was inspected periodically during 7 days (Olmedo 2002).

2.4 Greenhouse assays

Seeds of *Arachis hypogaea* L cv. Tegua were surface sterilized in 70 % ethanol for 5 min, rinsed in sterile water, dipped in H_2O_2 15 % during 10–15 min, and washed 5 times in sterile water. Then, they were germinated at 28 °C in sterilized Petri dishes with one layer of Whatman Nº1 filter paper and moist cotton, until the radicle reached approximately 2 cm length (Taurian et al. 2002). Peanut seedlings were transferred to sterilized plastic cups (11 cm-diameter, 0.9 m^3-volume) each containing 2,5 kg per pot of sterilized soil (Labeda et al. 1975) with low phosphorus content obtained from peanut growing area in Córdoba (organic matter: 1,57 % (Walkley-Black method), pH: 5,50 (Potenciometry 1:2,5), phosphorus: 9 μg/g (Kurtz and Bray I method)) and supplemented with $Ca_3(PO_4)_2$ 0,2 %*w/v* to obtain a concentration of 40 mg/Kg.

The following treatments were conducted:

(1) Uninoculated peanut plants (control)
(2) Uninoculated peanut plants watered regularly with PO_4KH_2 (2 mM) (phosphorus fertilized peanut plants)
(3) Uninoculated peanut plants watered regularly with KNO_3 (0,05 %*w/v*) (nitrogen fertilized peanut plants)
(4) Peanut plants inoculated with *Pantoea* sp J49
(5) Peanut plants inoculated with *Bradyrhizobium* sp SEMIA 6144
(6) Peanut plants inoculated with *Pseudomonas fluorescens* PMT1
(7) Peanut plants co-inoculated with *Pantoea* sp J49 and *Bradyrhizobium* sp SEMIA 6144
(8) Peanut plants co-inoculated with *Pseudomonas fluorescens* PMT1 and *Bradyrhizobium* sp SEMIA 6144

Inoculums were prepared by harvesting overnight cultures of each bacteria growing at 30 °C and 120 rpm. Each culture was washed with 0,9 % NaCl by centrifugation (8000 rpm 10 min) in an Eppendorf Centrifuge 5804 R (Eppendorf-Netheler, Hamburg, Germany), suspended in 0,9 % NaCl and adjusted to the final concentration of 10^8 cfu/ml. Co-inoculation treatments were performed in a ratio of 1:1.

Each treatment was replicated 10 times and the assay was repeated twice. Plants were grown under controlled environmental conditions (light intensity of 200 μR m-2 sec −1, 16-h day/8-h night cycle, at a constant temperature of 28 °C and a relative humidity of 50 %), watered regularly with sterilized tap water and, twice a month, with the nutrient solution described by Hoagland and Arnon (1950) but devoid of combined nitrogen and soluble phosphate. Five peanut plants from each treatment were harvested at R1 (60 days) and 5 plants at R4 (120 days) reproductive stages (Nwokolo and Smartt 1996) and analyzed for aerial and root dry weight and length, and, in those inoculated with *Bradyrhizobium* strain (individually or co-inoculated), number and dry weigth of nodules were also determined. In plants harvested on stage R4, pod dry weight and number, aerial N (following the method described by LECO 2008) and P content (by the colorimetric method described by Murphy and Riley 1962) were also analyzed.

2.5 Survival of Pantoea sp J49 in soil, roots and nodules

Survival of inoculated bacteria in soil was determined by streaking serial dilutions of dry soil sample (10 g) from each pot in phosphate buffered saline (PBS) on TSA and YEMA plates. After incubation of plates at 28 °C during 2–8 days, cfu/g dry soil was determined. In order to establish presence of strain J49 within peanut roots and nodules, a sample of root tissue and individual nodules were obtained from plants at R1 and R4 growth stages. Isolation of epiphytic and endophytic bacteria from peanut plants was performed as described by Kuklinsky-Sobral et al. (2004). Peanut plants were washed in running tap water to remove soil, and the roots, stems and leaves were separated. Epiphytic bacteria were isolated from non-disinfected tissue. Three grams of roots, stems or leaves were placed in a 500 ml Erlenmeyer flask containing 25 g of 0.1 cm diameter glass beads and 50 ml of phosphate buffered saline (PBS: NaCl 0.14 M; KCl 0.0027 M; Na_2HPO_4 0.01 M; KH_2PO_4 0.0018 M, pH 7.4) and agitated at 150 rpm for 1 h. To isolate endophytic bacteria, epiphytes were previously removed by surface disinfection using serial washing in 70 % ethanol for 1 min, sodium hypochlorite for 3 min, 70 % ethanol for 30 s and two rinses in sterilized distilled water. The disinfection process was checked by plating aliquots of the sterile distilled water used in the final rinse onto 10 % (*w/v*) TSA and incubating the plates at 28 °C. Then, three grams of appropriate tissue were placed in a 500 ml Erlenmeyer flask containing 25 g of 0.1 cm diameter glass beads and 50 ml of PBS and agitated at 150 rpm for 1 h. Aliquots (100 μl) of serial dilutions (1:10^3 to 1:10^6) were plated onto 10 % (*w/v*) TSA supplemented with cycloheximide (50 $\mu g ml^{-1}$) to control fungal growth and the plates incubated at 28 °C for 7 days. Nodules were surface sterilized by immersion in H_2O_2 20 % (*v/v*) during 2 min, and washed 5 times with sterile distilled water. To check the efficiency of the sterilizing method, a 100 ml aliquot of the last washing solution was incubated on TSA and YEMA plates (Vincent 1970). Surface sterilized nodules were then individually crushed in a drop of sterile water and this suspension was streaked on YEMA or TSA plates and incubated at 28 °C for 10 days.

2.6 Genomic fingerprint analysis

Approximately 10–12 colonies from TSA or YEMA plates, obtained from the survival assay, were selected to obtain bacterial DNA template. Total bacterial DNA was obtained by using the procedure described by Walsh et al. (1991). A loopful of a colony was suspended in 300 μl of 1 M NaCl, mixed thoroughly and centrifuged at 14,000 rpm for 4 min. The supernatant was discarded and the pellet was suspended in 300 μl double-distilled sterile water. After the sample was mixed and centrifuged, the supernatant was removed and the pellet was suspended in 150 μl of 6 % (aqueous suspension) resin Chelex 100 (Bio Rad, USA). This suspension was incubated at 56 °C for 20 min, followed by mixing and further incubation at 99 °C for 8 min. DNA concentration of the samples was approximately 5 ng μl^{-1}. The sequences of ERIC (Enterobacterial Repetitive Intergenic Consensus) primers E1 (5′-ATGTAAGCTCCTGGGGATTCAC-3′)/E2 (5′-AAGTAAGTGACTG GGGTGAGCG-3′) used in this study were described by de Bruijn (1992). The ERIC-PCR was performed in 12 μl reaction mixture containing 1x PCR buffer, 1.5 mM $MgCl_2$, 200 μM of each nucleotide (Promega, USA), 0.3 μM of each primer, 1 U of *Taq* DNA polymerase (Promega, USA) and 3.6 μl of template DNA solution. The temperature profile was as follows: initial denaturation at 95 °C for 1 min, 35 cycles of denaturation at 94 °C for 1 min, annealing at 52 °C for 1 min, extension at 65 °C for 8 min and a final extension step at 68 °C for 16 min. PCRs were performed in a Mastercycler gradient block (Eppendorf, Germany). The ERIC amplification products in 12 μl sub-samples were separated according to molecular size by horizontal electrophoresis on 2.5 % (*w/v*) agarose gels stained with ethidium bromide.

2.7 Data analysis

The data were subjected to analysis of variance (ANOVA) and differences among treatments detected by LSD test ($p<0.05$).

3 Results

3.1 Phosphate solubilisation ability in liquid media of *Pantoea* sp J49 and *Pseudomonas fluorescens* PMT1

Amount of phosphate solubilised by native isolate *Pantoea* sp J49 and *Pseudomonas fluorescens* PMT1 was determined at 24, 48, 72 h and 7 days of growth in NBRIP-BPB broth. *P. fluorescens* PMT1 produced the highest amount of soluble phosphate after 24 h reaching 764,7±37,4 μg/ml of culture medium while native isolate *Pantoea* sp J49 produced 385,4±41,4 μg/ml after 7 days of growth (Fig. 1).

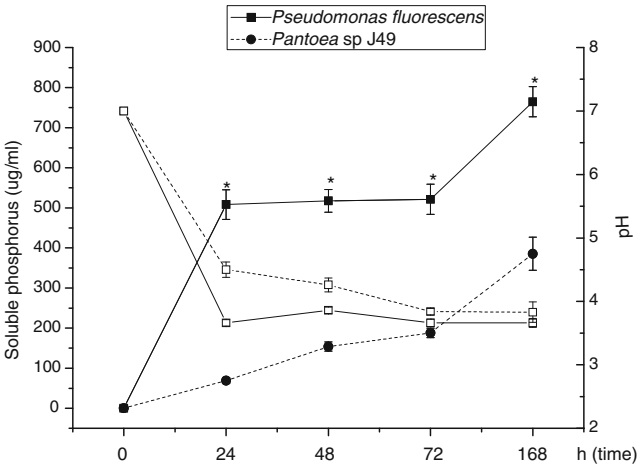

Fig. 1 Levels of soluble phosphorus released by *Pantoea* sp J49 and *Pseudomonas fluorescens* PMT1 and pH values in NBRIP-BPB medium. Fill boxes indicate soluble phosphorus content and open boxes indicate the pH of the medium. * indicates statistically significant difference. Data are means ± S.E. of five replicates $p<0.05$ according to LSD test

Even when the quantity of soluble phosphate produced by *Pantoea* sp J49 was significantly lower than that produced by *P. fluorescens* PMT1, both bacteria acidified NBRIP-BPB medium reaching similar values (pH: 3,8 and 3,6 respectively). Viability of isolates was not affected along the assay until the end of the experiment (data not shown).

3.2 Bacterial coexistence in plate assays

This assay demonstrated that both phosphate solubilising strains did not show antagonism or deleterious effect against nodulating *Bradyrhizobium* strain, either in simultaneous or delayed streaked plates (data not shown).

3.3 Effect of single and co- inoculation on peanut plant growth

Peanut plants were harvested at 60 (R1 growth stage) and 120 (R4 growth stage) days post inoculation (dpi) and growth parameters were determined (Table 1). All plants treated with *Pantoea* sp J49 (either single or co-inoculated) showed an enhanced effect on peanut plants growth. Growth promotion of peanut plants by this bacterium was observed in aerial and root biomass parameters. In those plants inoculated with *Pantoea* sp J49, the aerial and root dry weights increased significantly in both growth stages (R1 and R4). It was observed an increase of 76,2 % and 49,0 % in aerial biomass and an increase of 112,5 % and 78,6 % in root biomass in R1 and R4 growth stages, respectively. In plants co-inoculated with this bacterium and *Bradyrhizobium* sp SEMIA 6144, the aerial dry weight increased at R1 growth stage (44,5 %). Increase of this

Table 1 Effect of inoculation with phosphate solubilising strains *Pantoea* sp J49 and *Pseudomonas fluorescens* PMT1 separately and in combination with *Bradyrhizobium* sp SEMIA 6144 on stems and roots length and dry weigth, pod number and dry weigth and N and P aerial content

Treatments	Aerial length (cm/plant)		Root length (cm/plant)		Dry weight (g/plant)				Pod		Aerial P content (mg P/plant) **	Aerial N content (mgN/plant)
					Shoot		Roots					
	60 dpi	120 dpi	60 dpi	120 dpi	60 dpi	120 dpi	60 dpi	120dpi	Number	Dry weight		
Uninoculated	38.30±2.28	48.05±2.02	17.27±2.26	14.93±1.55	1.01±0.12	1.68±0.4	0.08±0.02	0.14±0.02	1.13±0.43	0.38±0.14	2.45±0.3	63.3±4.03
Inoculation treatments												
Bradyrhizobium. sp SEMIA 6144	39.95±2.28	42.81±2.16	13.32±2.26	13.70±1.55	1.10±0.07	2.06±0.35	0.11±0.02	0.17±0.03	1±0.8	0.07±0.07	ND	ND
Pantoea sp J49	43.55±1.98	48.58±1.90	17.28±1.92	17.09±1.45	1.78±0.14*[a]	2.87±0.26 *	0.17±0.02*	0.25±0.03*	1.78±0.41	0.61±0.16	3.88±0.25*	72.17±3.68
J49+SEMIA 6144	41.77±2.12	43.79±1.90	13.43±2.09	13.41±1.45	1.46±0.15*	2.16±0.26	0.11±0.02	0.15±0.03	1.56±0.48	0.54±0.12	ND	ND
Ps. fluorescens	36.41±1.98	43.80±2.02	14.66±1.96	15.74±1.45	0.93±0.08	2.25±0.28	0.11±0.02	0.19±0.05	1.88±0.44	0.29±0.1	2.43±0.38	57.1±8.37
Ps. fluorescens + SEMIA 6144	36.14±1.98	45.06±2.02	10.16±1.96*	10.64±1.45*	0.68±0.07*	1.72±0.2	0.1±0.02	0.13±0.02	0.38±0.18	0.3±0.29	ND	ND
Fertilized treatments												
N fertilized	37.10±2.80	44.90±2.55	17.18±2.77	15.12±1.83	1.25±0.16	2.14±0.35	0.18±0.03*	0.17±0.04	1±0.63	0.37±0.05	ND	ND
P fertilized	38.94±2.50	40.63±2.85	17.66±2.48	16.08±2.05	1.4±0.17*	2.07±0.4	0.13±0.02	0.15±0.03	1±0.7	0.55±0.01	3.91±0.23*	57.5±6.92

Data are means ± S.E., $n=5$, $p<0.05$ according to LSD test, * and [a] indicate statistically significant difference compared with non inoculated peanut plants or inoculated with *Bradyrhizobium sp.* SEMIA 6144, respectively, *ND* not determined

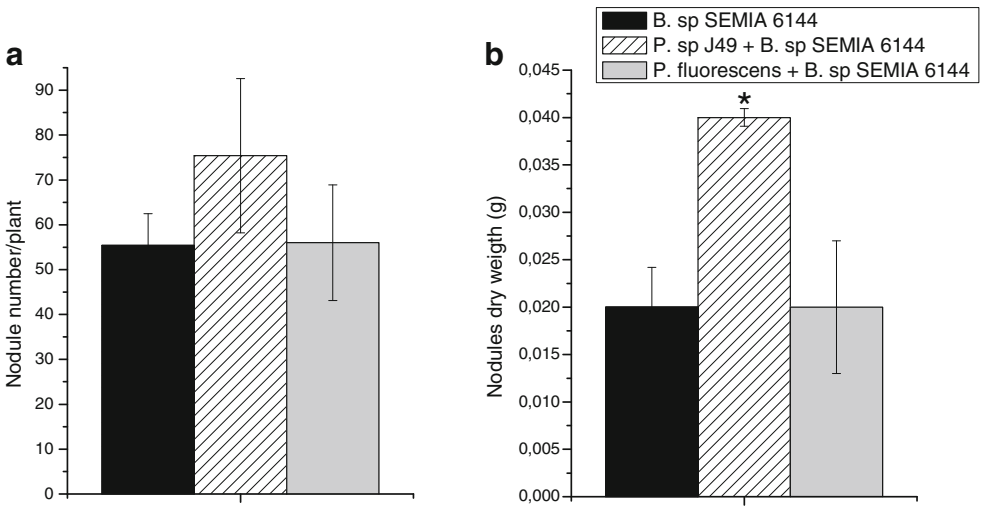

Fig. 2 Effect of co-inoculation of peanut plants on nodule number (**a**) and dry weight (**b**) at R4 reproductive growth stage. Data are means±S.E. of 5 replicates, $p<0.05$ according to LSD test, * indicates statistically significant difference compared with peanut plants inoculated only with *Bradyrhizobium* sp SEMIA 6144

parameter in this growth stage, was also observed in P fertilized plants (38,5 %). On the other hand, N fertilized peanut plants showed in R1 an increase in root growth (125 %). In plants inoculated (single or co-inoculated) with *P. fluorescens* no changes in any of the parameters measured were observed. Further, co-inoculation of this strain with *Bradyrhizobium* sp SEMIA 6144 decreased the plants root length at 60 and 120 dpi and the aerial dry weight at 60 dpi. On the other hand, no changes were observed in aerial length as well as in pod´s number and biomass of peanut plants from different treatments.

Phosphorus and nitrogen content were measured in J49 inoculated peanut plants at 120 dpi, since they showed biomass improvement compared with uninoculated plants. For comparison these parameters were also measured in plants inoculated with *P. fluorescens* PMT1 or fertilized with nitrogen or phosphorus. Phosphorus aerial content of peanut plants inoculated with *Pantoea* sp J49 was significantly higher than that of uninoculated plants reaching similar values to those of P fertilized plants. Increase of P content observed in plants inoculated with this strain and in those fertilized with P was of 58,4 % and 59,6 %, respectively.

When symbiotic parameters were analyzed it was observed that nodule dry weight but not their number, significantly increased in peanut plants co-inoculated with *Bradyrhizobium* sp SEMIA 6144 and *Pantoea* sp J49 (Fig. 2). No differences were observed between treatments in nodule dry weight/nodule number ratios (data not shown).

3.4 Survival of Pantoea sp J49 in soil and in peanut plants

The ERIC–PCR profiles from DNA of bacteria isolated from nodules, inside root tissues, from their surfaces or from soil samples were compared with those from the inoculated

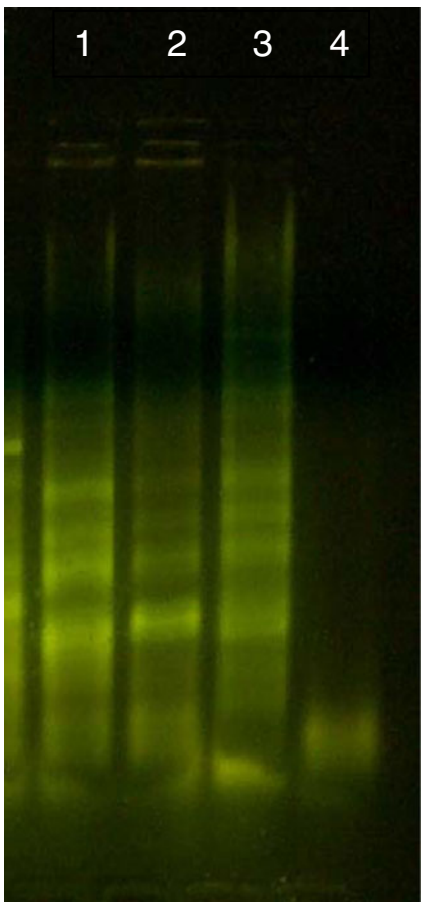

Fig. 3 ERIC-PCR profiles of DNA obtained from colonies recovered from survival assay of *Pantoea* sp J49 single and co-inoculated with *Bradyrhizobium* sp SEMIA 6144. Lane 1: *P.* sp J49 isolated from external root tissue of plants inoculated only with this strain, 2: DNA from inoculated *P.* sp J49 culture, 3: *P.* sp J49 isolated from nodules of plants co-inoculated with *Bradyrhizobium* sp SEMIA 6144, 4: negative control of PCR reaction

strain. Results obtained indicated that *Pantoea* sp J49 survives in the peanut rhizosphere and also inside plant tissues, including nodules induced by *Bradyrhizobium* sp SEMIA 6144 (Fig. 3). Strain J49 was detected in the root surface as epiphyte (log 10^4 cfu/g of tissue) up to 120 dpi. Inside root tissues, including nodules, this bacterium was also recovered (log 10^5 cfu/g of tissue) at 120 dpi.

4 Discussion

In this study it was investigated the effects of co-inoculation of native phosphate solubilising bacterium *Pantoea* sp J49 and nitrogen fixing bacterium *Bradyrhizobium* SEMIA 6144 on peanut growth. Considering that some species of rhizobia are able to solubilise inorganic phosphate (Halder et al. 1991; Chabot et al. 1996; Sashidra and Podile 2010), we previously determined that *Bradyrhizobium* SEMIA 6144 is unable to solubilise tricalcium phosphate. Assays done in liquid NBRIP-BPB medium containing tricalcium phosphate as insoluble phosphate source indicated that *Pseudomonas fluorescens* PMT1 strain produced a higher level of soluble phosphorus than *Pantoea* sp J49. Amounts of soluble phosphorus produced by *Pantoea* sp J49 were similar to those reported for other *Pantoea* and *Enterobacter* strains, such as *Enterobacter asbiriae* (Gyaneswar et al. 1999), *Pantoea agglomerans*, (Chung et al. 2005; Pérez et al. 2007; Son et al. 2006), *Pantoea ananatis* (Pérez et al. 2007) and *Pantoea dispersa* (Selvakumar et al. 2007). Secretion of organic acids to the extracellular medium is considered the main mechanism of inorganic phosphate solubilisation. Direct oxidation of glucose to gluconic acid in periplasmic space, resulting in acidification of the region adjacent to the cell, is the metabolic basis of this process in some gram negative bacteria (Goldstein 1995). In this study, the pH values of culture medium decreased when soluble phosphate quantity increased indicating that acidification of the medium could probably be related with P solubilisation.

Although ability determination of *Pantoea* sp J49 to solubilise P in liquid medium showed that this native strain is less effective than *Pseudomonas fluorescens* PMT1 strain, microcosm assays demonstrated that J49 exerts more beneficial effects on peanut growth.

Considering results obtained, it is possible to state that *Pantoea* sp is a promising plant growth promoting bacteria since it significantly increased the plant biomass, nodule dry weight and aerial P content. These beneficial effects are of great interest, particularly the last one, because inadequate P supply limits N_2-fixation and eventually the N supply to legumes, reducing the plant growth. Considering the increase in the P content of plants inoculated with this bacterium, it is possible to speculate that, under the experimental conditions used in this work, probably the main PGP mechanism involved in the peanut growth promotion previously reported (Taurian et al. 2010) has been the phosphate solubilising ability. The fact that no increase in P content was previously determined in plant at R1 growth stage (Taurian et al. 2010), could be related with a lower requirement of this nutrient compared with plants at R4 growth stage.

Pantoea sp J49, when introduced into the rhizosphere, was able to colonize the ectorhizosphere and endorhizphere. Endophytic state of a bacterium is a desirable trait when looking for PGPB because it ensures survival (Reinhold-Hurek and Hurek 1998) and probable improves the beneficial effects on plant growth (Hardoim et al. 2008). *Pantoea* sp J49 is an endophytic bacterium isolated from peanut nodules. Strains from this genus have been isolated from apoplastic space of sugar cane stems and it has been informed that they are capable of growing under extreme conditions (Loiret et al. 2004). The fact that this microorganism is able to live in a variety of habitats as soil and inside plant tissues, represents an advantage because it confers a better response to abiotic and biotic environmental changes.

5 Conclusions

Results obtained in this study confirm the great potential of the native *Pantoea* sp J49 isolate in the promotion of peanut plant growth, probably related with its capacity to solubilise phosphate. This endophytic bacterium solubilises great amounts of tricalcium phosphate, an insoluble P source common in peanut cultivation area of Córdoba, Argentina. This beneficial property is very important to consider this bacterium to be used as an alternative to phosphorus fertilizers application.

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