J Plant Growth Regul
DOI 10.1007/s00344-015-9547-0

Promotion of Peanut Growth by Co-inoculation with Selected Strains of *Bradyrhizobium* and *Azospirillum*

Julio C. Vicario[1] · Emiliano D. Primo[1] · Marta S. Dardanelli[1] · Walter Giordano[1]

Received: 29 April 2015 / Accepted: 12 August 2015

Abstract The ability of inoculated rhizobial strains to increase root nodulation of host legumes often depends on their competitiveness with existing native soil strains. Results of studies to date on rhizobial inoculation for improvement of peanut (*Arachis hypogaea* L.) production in Argentina have been inconsistent and controversial. In many cases, nodulation and yield of peanut crops have been increased by inoculation of specific rhizobial strains. Native peanut-nodulating strains are generally present in soils of agricultural areas, but their growth-promoting effect is often lower than that of inoculated strains. Many species of the genus *Bradyrhizobium* interact in a host-specific manner with legume species and form nitrogen-fixing root nodules. Other free-living rhizobacteria such as species of the genus *Azospirillum* are facultatively capable of interacting with legume roots and promoting plant growth. We evaluated and compared the effects of various single inoculation and co-inoculation treatments on peanut growth parameters in greenhouse and field experiments. In the greenhouse studies, co-inoculation with various *Bradyrhizobium* strains (native 15A and PC34, and recommended peanut inoculant C145), and *Azospirillum brasilense* strain Az39 generally resulted in increases in the measured parameters. The growth-promoting effect of 15A was similar to or higher than that of C145. In the field studies, 15A-Az39 co-inoculation had a greater promoting effect on measured growth parameters than did C145-Az39 co-inoculation. Our findings indicate that careful selection of native rhizobacterial strains adapted to peanut soils is useful in strategies for growth promotion, and that 15A in particular is a promising candidate for future inoculant formulation.

Keywords *Arachis hypogaea* · Co-inoculation · *Bradyrhizobium* · *Azospirillum* · Nodulation

Introduction

Peanut (*Arachis hypogaea* L.) is the only species of economic importance in the genus *Arachis* (family Leguminosae, subfamily Papilionoideae) (Lavin and others 2001; Doyle and Luckow 2003). Peanut plants are annuals with indeterminate growth. The underground seeds are a popular food item and are also used as a raw material in the confectionery and oil industries (Cholaky and others 1983).

In Argentina, approximately 87 % of peanut production takes place in Córdoba province. The peanuts are high quality, and most of the crop is exported to the European Union, Indonesia, and Canada (SAGPyA 2008). Domestic consumer demand is fairly low. Argentina has surpassed China, India, and the U.S. as the world's predominant peanut exporter (www.camaradelmani.com.ar).

In agriculture, routine and frequent application of various chemicals for increasing food grain production has adverse long-term effects on soil properties and rhizospheric bacterial populations (Ahemad and others 2009). An important alternative strategy is inoculation with plant growth-promoting rhizobacteria (PGPR) and reduced use of agrochemicals.

PGPR comprise a mixed population of naturally occurring soil microorganisms (Singh and others 2011) which are associated with plants and stimulate plant growth either directly or indirectly. Direct growth-promoting mechanisms

✉ Walter Giordano
giordanow@gmail.com

[1] Departamento de Biología Molecular, Universidad Nacional de Río Cuarto, Río Cuarto, Córdoba, Argentina

Published online: 11 November 2015

include phytohormone production and facilitated uptake of environmental nutrients such as nitrogen, phosphorus, and iron. Indirect mechanisms include blocking of the deleterious effects of phytopathogenic organisms by antibiosis, siderophore production, increased competitiveness for space and nutrients, and induction of systemic resistance (Podile and Kishore 2006; Lugtenberg and Kamilova 2009; Figueredo and others 2014).

Arachis hypogaea is frequently nodulated by slow-growing strains of rhizobacteria in the genus *Bradyrhizobium* (Bogino and others 2006); in many cases, species names are not yet assigned (Fabra and others 2010). The mechanism of rhizobial infection in peanut differs from that in other legume species. Nodulation of *A. hypogaea* by *Bradyrhizobium* spp. induces root hair deformation, but only in locations where lateral roots emerge. The infection sites are the junctions between root hair cells and epidermal and cortical cells. The bacteria multiply rapidly inside cortical cells, and the infected cells divide rapidly to form nodules (Boogerd and van Rossum 1997).

There have been many studies and differing opinions regarding the most effective agricultural strategies for peanut inoculation. Michelena and others (1996) reported that inoculation of certain rhizobial strains resulted in increased yield of seeds with high protein content. This and similar findings reflect the ability of particular rhizobial strains to occupy nodules on host plants in the presence of other symbionts.

Soils in the peanut-producing areas of Córdoba province contain native populations of rhizobia that are capable of nodulating peanut but are not always highly effective. These native symbionts are limited by environmental factors, production technology, effects of mycotoxins produced by soil fungi, and other factors (Bogino and others 2008). Agricultural practices are varied and complex, and our knowledge of *Bradyrhizobium*-peanut symbioses (particularly the early interaction steps) is limited. New approaches to elucidating these symbioses will provide important knowledge to help us optimize biofertilizer usage and sustainable peanut production.

Studies of colonization ability, competitiveness, and fixation efficiency of various microsymbiont strains in relation to a particular host species allow us to increase nitrogen fixation and productivity. In the legume ecosystem, establishment and persistence of inoculated microbial strains are affected by the presence of native strains that are already adapted to the soil environment, and by various other factors that directly or indirectly impact the competitiveness of the inoculated strain (Bottomley 1992). The biotic factors include microbial antagonism and parasitism (Dowling and Broughton 1986).

Many early studies of legume inoculation involved a single experimental microorganism. Recent studies have increasingly considered mixed inoculation protocols using two or more microorganisms (Dardanelli and others 2008; Sanchez and others 2014).

Among well-known PGPR, *Azospirillum* is a genus of free-living nitrogen-fixing rhizobacteria that have beneficial effects on various legume species (Bashan and de-Bashan 2010). Their growth-promoting effects include increased numbers and diversity of flavonoids present in plant exudates (Burdman and others 1996; Dardanelli and others 2008; Cassán and others 2009). A little is known regarding *Azospirillum* effects on peanut roots in the co-presence of symbiotic *Bradyrhizobium* spp.

In the present study, we examined the effects of co-inoculated *Bradyrhizobium* and *Azospirillum* on growth and nodulation parameters in peanut. Our findings are interpreted in the context of mixed inoculation strategies and enhancement of agricultural food production.

Materials and Methods

Bacterial Strains and Growth Conditions

The five *Bradyrhizobium* strains studied included three recommended for use as inoculants [C145 (Niftal; USA), SEMIA6144 (IPAGRO; Brazil), USDA4438 (USDA, ARS; USA)] and two native strains (15A, PC34) (Bogino and others 2010; Vicario and others 2015). 15A and PC34 are the subjects of studies in progress by our group, and have therefore not yet been deposited into American Type Culture Collection (ATCC). PC34 has been deposited into GenBank under accession number JF317681 (Nievas and others 2012). *Bradyrhizobium* cells were isolated from peanut root nodules and grown in Yeast Extract Mannitol (YEM) medium (Vincent 1970) for 8 days at 30 °C. Strains were selected based on their importance as described by Bogino and others (2010). *Azospirillum brasilense* strain Az39, isolated from roots of wheat plants in the central region of Argentina and often applied as an agricultural inoculant (Rivera and others 2014), was used in co-inoculation experiments.

The five *Bradyrhizobium* strains, inoculated singly or co-inoculated with Az39, were evaluated for effectiveness in a runner Virginia-type peanut (Granoleico) cultivar kindly donated by "Criadero El Carmen," Córdoba, Argentina, in plastic pots filled with sterilized coarse grain vermiculite. This cultivar has a "runner" growth habit and a long (~150 day) cycle (Giayetto and others 1999). Seeds were surface sterilized by immersion in 15 % H_2O_2 for 7 min, washed in sterile distilled water, aseptically pre-germinated, planted, and inoculated with 1 mL bacterial suspension. Plants were grown in a chamber at 28/24 °C under a 16/8-h light/dark regime and supplied with nitrogen-free Jensen solution (Vincent 1970) as needed. Inoculated and uninoculated (control) plants were harvested 32 days after planting. Root systems of the harvested plants were washed, and nodules were picked, counted, and dried (70 °C, 72 h) for determination of dry weight. Plant shoots and root systems were dried and weighed. The period that plants were allowed to grow prior to harvesting/growth parameter measurement was 32 days for controlled greenhouse experiments (because this is the standard period used in a long series of our previous studies) and 150 days for field experiments (based on the field growth cycle of the Granoleico cultivar). All experiments were performed in triplicate.

Field Experiments

Field experiments were performed in a field located in the Dalmacio Vélez Sarsfield area of Córdoba province. The in-furrow technique was used for co-inoculation experiments with Az39 and *Bradyrhizobium* C145 (reference) or 15A and PC34 (native). Treatments involved a randomized complete block design with four replications. Each plot was 6 rows wide and 5 m long, with a 1-m alley between blocks. The Granoleico cultivar was hand-planted during November 2013 in rows 0.7 m apart, with 16 seeds/m^2. Seeds were treated with the commercial fungicide Vitavax Flo (Crompton Química, SACI, Argentina) (carboxin 20 % + thiram 20 %; 250 mL per 100 kg seed). The inoculants (applied directly to the seedbed) were 1.5 L *Bradyrhizobium* per ha and 1 L Az39 per ha, equivalent to 10^9 CFU/mL in each case. At harvest, plants were taken from 1-m^2 sample areas and evaluated for nodule number, nodule dry weight, shoot dry weight, and root dry weight.

Statistical Analysis

A randomized design was used for all experiments. Values shown are mean ± SD from three independent pairs of duplicate experiments. Data were subjected to Analysis of Variance (ANOVA) with multiple comparison variables using Fisher's least significant difference (LSD) test. Differences between means were considered to be significant at $p \leq 0.05$. The software program used was Infostat 1.0 (Dept. of Statistics and Biometry, Faculty of Agricultural Sciences, National University of Córdoba).

Results

Co-inoculation Experiments in Greenhouse

Growth Parameters

Singly inoculated or co-inoculated plants were collected at day 32, and growth and nodulation parameters were measured. The highest stem length values were observed for the co-inoculated SEMIA6144-Az39, singly inoculated SEMIA6144, and co-inoculated USDA4438-Az39 groups. For root length, differences between uninoculated (control) and inoculated groups were less striking, and were significant only for PC34-Az39 and SEMIA6144-Az39 (data not shown).

An increase in shoot dry weight relative to control was observed for all experimental groups except Az39. For root dry weight, C145, USDA4438, PC34, and SEMIA6144-Az39 did not differ significantly from control, and the experimental groups that did show an increase relative to controls did not differ among themselves (data not shown). In general, differences in the measured parameters for the singly inoculated versus co-inoculated groups were not statistically significant, but values for the co-inoculated groups tended to be higher.

Nodulation Parameters

Total nodule number was higher for singly inoculated USDA4438 and 15A than for C145 (Fig. 1a). Co-inoculation with Az39 in each case resulted in increased nodule number relative to single inoculation, but the increases were not statistically significant. Among the co-inoculated groups, a significant difference was observed only for USDA4438-Az39 versus C145-Az39.

For nodule dry weight, values for single inoculation were higher for native strains than reference strains (Fig. 1b). Again, values were higher for the co-inoculated groups.

Of the two isolated native strains, 15A had a growth-promoting effect stronger than that of C145, a strain recommended by INTA for peanut inoculation. Values of the tested growth parameters were generally higher in the co-inoculated groups, but the differences were usually not statistically significant because of the variability of data within each treatment. Such variability may have arisen from the use of different batches of seeds and different supports during the 1.5-year period that greenhouse experiments and assays were conducted. That is, although seeds were always the Granoleico cultivar and the support was always coarse grain vermiculite, there may have been variation in the batches of seeds or vermiculite over time. Differences in color, length, and weight of inoculated versus control plants were evident (Fig. 2). Such differences were more evident from visual observation of plants than from summary results shown in Figs. 1, 4, and 5, because the large variability of data within each treatment precluded statistically significant differences between the treatments.

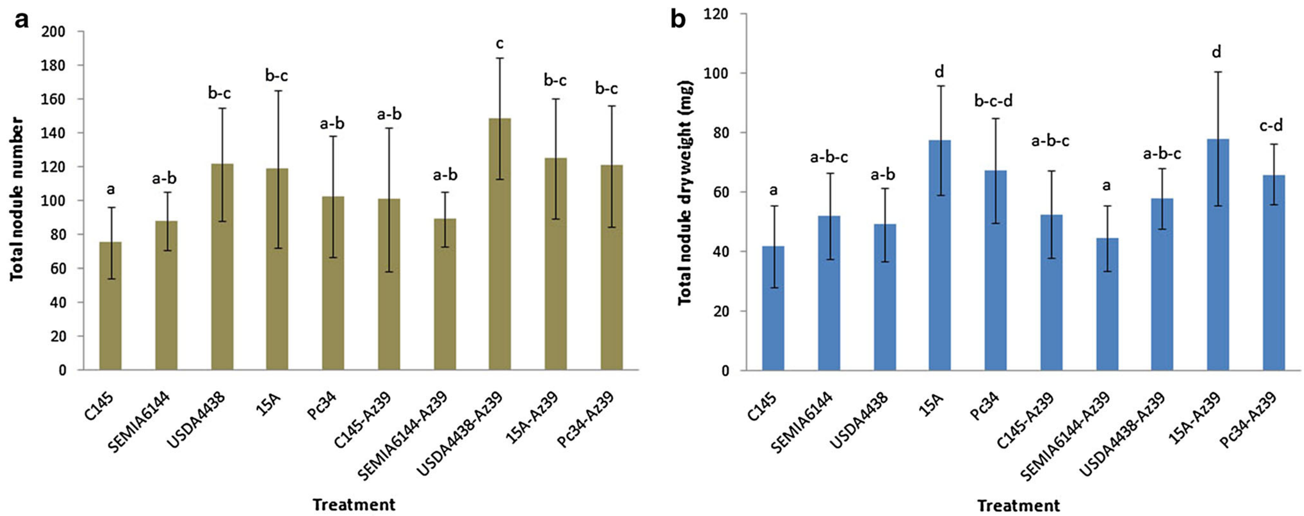

Fig. 1 Effects of single inoculation and co-inoculation of peanut with various *Bradyrhizobium* strains and *A. brasilense* Az39 on total nodule number (**a**) and total nodule dry weight (**b**) at day 32. Data shown are mean ± SD of three independent replicate experiments. *Different letters above bars* indicate statistically significant differences between means ($p < 0.05$, Fisher's LSD test)

Multivariate Statistical Analysis of Principal Components

To place the variables measured in the greenhouse inoculation experiments within a framework allowing more rigorous interpretation, we performed multivariate statistical analysis of principal components. The major purpose of this analysis was to compare the effects of different inoculation strategies on growth and nodulation parameters. The analysis took into account the two types of inoculation strategy (single vs. multiple) and the various measured parameters of growth and nodulation.

The effects of co-inoculation with Az39 in comparison with single inoculation and controls are shown in Fig. 3. The co-inoculation treatments are mainly grouped together in this figure, as indicated by the red circle. In contrast, the single inoculations had a more scattered distribution and a smaller contribution to the measured parameters. No zero or negative correlations among the variables were observed. The strongest correlation was between total nodule dry weight and nodule number. The nodulation parameters showed stronger correlations with dry weights than with lengths of stems and roots. There was a marked distance between inoculation treatments versus controls. Among the single inoculations, Az39 had the least impact on the measured parameters, consistent with the concept that nitrogen provided by PGPR is a key nutrient determining peanut crop development.

Fig. 2 Photographs of typical plants from C145-Az39 co-inoculation, C145 single inoculation, and control groups at day 32

Field Experiments

Application of in-furrow inoculation technique in soil ecosystems is a useful tool that strengthens our conclusions in regard to peanut crop development. On the ground or in the soil is where experimental inoculated strains are exposed to various environmental conditions, compete with established native rhizobial populations, interact with other typical ecosystem species, and are affected by human activities such as application of chemical products. We recently demonstrated (Vicario 2015) a mixed response by peanut rhizobial symbionts to events associated with early-stage interactions with the host plant. It is often problematic and complex to obtain results relevant to formulation of an improved biofertilization strategy. In view of the numerous factors that affect legume/rhizobia interactions, it is important to perform experiments in the field as well as the greenhouse.

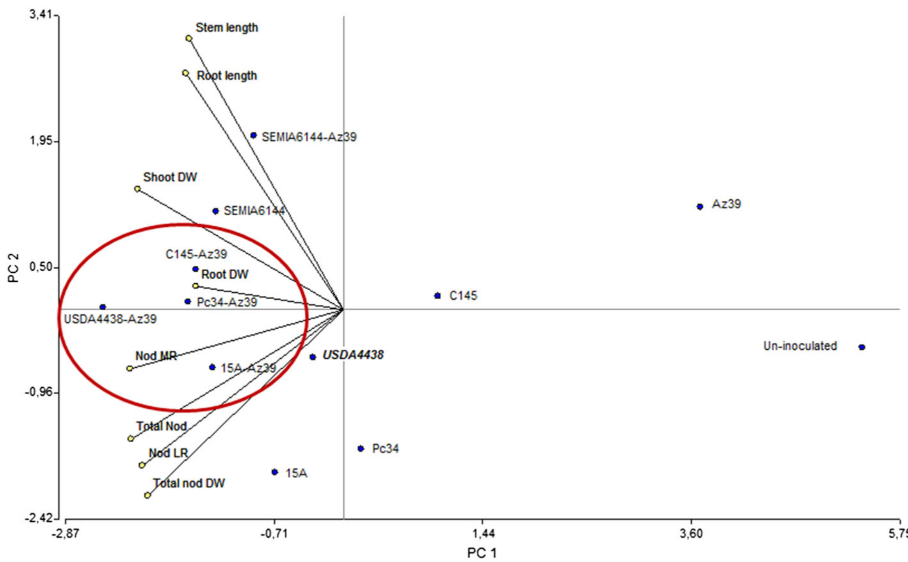

Fig. 3 Principal component analysis plot generated by InfoStat software program V. 1.0 (see "Multivariate Statistical Analysis" section). *Straight lines* represent correlations between stem length, root length, shoot dry weight (DW), root dry weight, main root nodules (Nod MR), lateral root nodules (Nod LR), total nodule dry weight (total nod DW), and total nodule number (total Nod). Isolated points represent different inoculation treatments. *PC1 and PC2* principal component 1 and 2

We conducted our experiments in a field located in the Dalmacio Vélez Sarsfield area of Córdoba province (32°34′34″S, 63°36′47″W) that had a history of agricultural peanut production but not of inoculation.

On the basis of preliminary greenhouse findings on behavior of PGPR mixtures and preliminary field tests (data not shown), we designed a series of field experiments to evaluate the activity of inoculant strains in a dynamic environment affected by multiple factors. Isolated native strains 15A and PC34 were selected for comparison with INTA-recommended reference strain C145, based on efficiency data obtained in the greenhouse experiments.

Growth and Nodulation Parameters at Harvest

Plants were grown 150 days, harvested from 1-m^2 areas, and subjected to determination of shoot dry weight, nodule number, nodule dry weight, and pod yield dry weight (Figs. 4, 5).

For shoot dry weight, the value for the 15A-Az39 co-inoculated group was about 2 times that of control (Fig. 4a). No other experimental group showed a significant difference from control.

For nodule number, three experimental groups had values different from that of control (90) (Fig. 4b). Native strain 15A had the highest value (>300). The value for C145 was not statistically different from that of 15A.

For nodule dry weight, the value for 15A-Az39 co-inoculation was the highest among the experimental groups, and the only one statistically different from control (Fig. 4c). This finding was consistent with the greenhouse experiment showing the highest nodule dry weight for 15A single inoculation (Fig. 1b).

For pod yield dry weight, the values for the three co-inoculation groups were similar to each other, and about 2 times that of control (Fig. 5).

In summary, values for all measured parameters were higher for co-inoculated groups than for control. Co-inoculated native strain 15A generally had the greatest promoting effect, and is a promising candidate for future inoculant bioformulations for peanut crops.

Discussion

Vessey (2003) proposed that the beneficial effects of PGPR strains used as biofertilizers arise mainly from their effects on plant growth regulation, as manifested by modifications of host plant morphology and physiology, for example, changes in root architecture and nutrient assimilation patterns. Species of the genus *Azospirillum* are PGPR that induce root development in both legume and non-legume plants by synthesizing plant hormones that stimulate growth (Dobbelaere and others 2001). The results of our present experiments on co-inoculation of isolated native and reference N_2-fixing *Bradyrhizobium* strains with *A. brasilense* Az39 in peanut are consistent with results of previous studies on other crop species. Native strain 15A shows particular promise as a candidate for future commercial inoculant formulations to improve peanut production in Argentina.

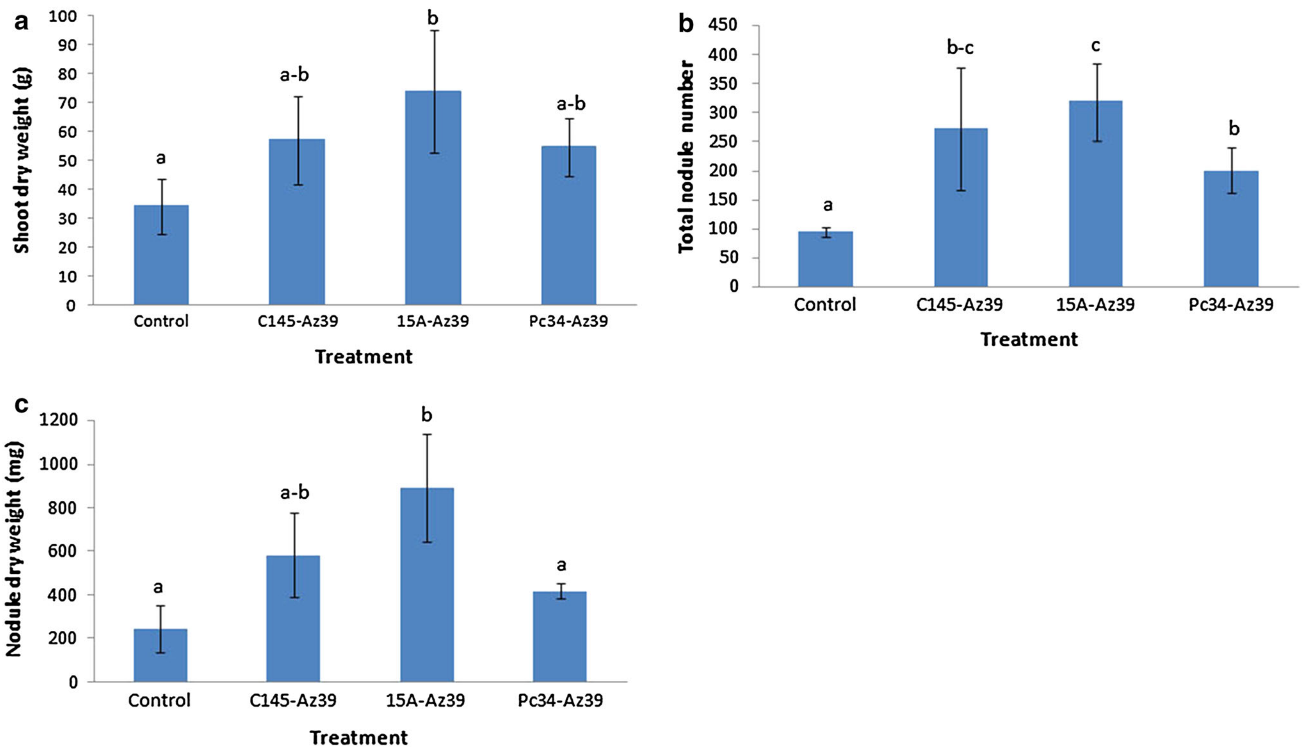

Fig. 4 Effects of co-inoculation treatments (C145-Az39, 15A-Az39, PC34-Az39) on shoot dry weight (**a**), total nodule number (**b**), and nodule dry weight (**c**). Data shown are mean ± SD of three independent replicate experiments. *Different letters above bars* indicate statistically significant differences between means ($p < 0.05$, Fisher's LSD test)

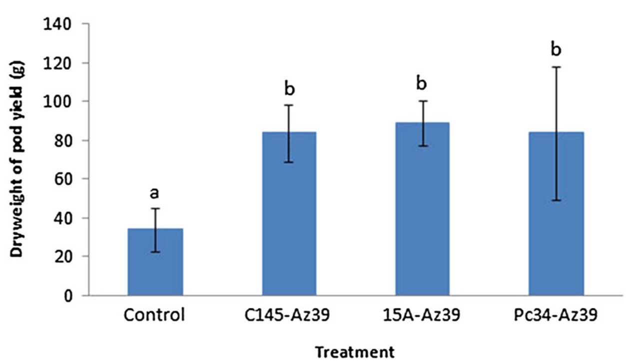

Fig. 5 Effects of co-inoculation treatments on dry weight of pod yield. Data shown are mean ± SD of three independent replicate experiments. *Different letters above bars* indicate statistically significant differences between means ($p < 0.05$, Fisher's LSD test)

In our greenhouse experiments, all plant growth parameters evaluated were enhanced in the groups co-inoculated with *Bradyrhizobium* sp. and Az39. Our field experiments showed improved results for co-inoculation versus control treatments in all measured parameters. Among the three co-inoculation treatments, 15A-Az39 gave the best yield values at harvest, indicating that 15A is the most promising candidate in future inoculant formulation for peanut crops. In conclusion, nodulation and productivity of peanut can be significantly enhanced by co-inoculation of carefully selected native PGPR strains in combination with appropriate biofertilization strategy.

Rhizospheric microorganisms that live in association with roots have been shown to stimulate plant growth and/or reduce incidence of plant diseases. Many recent studies have clearly demonstrated the important role that PGPR play in agriculture (Pérez-Montaño and others 2014). Certain PGPR strains, including *Azospirillum,* are being utilized worldwide for enhancement of crop productivity (Cassán and others 2015). Increased application of PGPR is expected to reduce the global dependence on hazardous agricultural chemicals that have long-term harmful and destabilizing effects on agro-ecosystems. Continued improvement of inoculant delivery systems will enhance the environmental persistence of PGPR, and further reduce the need for chemical fertilizers and pesticides in agriculture.

Acknowledgments This study was supported by grants from the Secretaría de Ciencia y Técnica de la Universidad Nacional de Río Cuarto, the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), and the Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT), Argentina. JCV and EDP hold a CONICET fellowship. MSD and WG are Career Members of CONICET. The authors are grateful to Guillermo Cerioni for assistance in the field experiments, to Mr. H. Primo for making his farm in Dalmacio Vélez Sarsfield available for the experiments, and to Dr. S. Anderson for English editing of the manuscript.

References

Ahemad M, Khan MS, Zaidi A, Wani PA (2009) Remediation of herbicides contaminated soil using microbes. In: Khan MS, Zaidi A, Musarrat J (eds) Microbes in sustainable agriculture. Nova Science Publishers Inc, New York, pp 261–284

Bashan Y, de-Bashan L (2010) How the plant growth-promoting bacterium *Azospirillum* promotes plant growth a critical assessment. Adv Agron 108:77–136

Bogino P, Banchio E, Rinaudi L, Cerioni G, Bonfiglio C, Giordano W (2006) Peanut (*Arachis hypogaea* L.) response to inoculation with *Bradyrhizobium* sp. in soils of Argentina. Ann Appl Biol 148:207–212

Bogino P, Banchio E, Bonfiglio C, Giordano W (2008) Competitiveness of a *Bradyrhizobium* sp. strain in soils containing indigenous rhizobia. Curr Microbiol 56:66–72

Bogino P, Banchio E, Giordano W (2010) Molecular diversity of peanut-nodulating rhizobia in soils of Argentina. J Basic Microbiol 50:27–279

Boogerd FC, van Rossum D (1997) Nodulation of groundnut by *Bradyrhizobium*: a simple infection process by crack entry. FEMS Microbiol Rev 21:5–27

Bottomley PJ (1992) Ecology of *Bradyrhizobium* and *Rhizobium*. In: Stacey G, Burris RH, Evans HJ (eds) Biological nitrogen fixation. Chapman, New York, pp 293–348

Burdman S, Volpin H, Kigel J, Kapulnik Y, Okon Y (1996) Promotion of nod gene inducers and nodulation in common bean (*Phaseolus vulgaris*) roots inoculated with *Azospirillum brasilense* Cd. Appl Environ Microbiol 62:3030–3033

Cassán F, Maiale S, Masciarelli O, Vidal A, Luna V, Ruiz O (2009) Cadaverine production by *Azospirillum brasilense* and its possible role in plant growth promotion and osmotic stress mitigation. Eur J Soil Biol 45:12–19

Cassán FD, Okon Y, Creus CM (2015) Handbook for *Azospirillum*. Springer, Switzerland. doi:10.1007/978-3-319-06542-7

Cholaki L, Giayetto O, Neuman E, Cavaignac S (1983) Respuesta del maní (*Arachis hypogaea* L.) a la inoculación al suelo con *Rhizobium* spp. Revista UNRC 3:173–179

Dardanelli MS, Rodríguez Navarro DN, Megías M, Okon Y (2008) Influencia de la coinoculación *Azospirillum*-rizobios sobre el crecimiento y la fijación de N de leguminosas de interés agronómico. In: Cassán F, García Salamone I (eds) *Azospirillum* sp.: cell physiology, plant interactions and agronomic research in Argentina. Asociación Argentina de Microbiología, Buenos Aires, pp 141–151

Dobbelaere S, Croonenborghs A, Thys A, Ptacek D, Vanderleyden J, Dutto P, Labandera-Gonzalez C, Caballero-Mellado J, Aguirre JF, Kapulnik Y, Brener S, Burdman S, Kadouri D, Sarig S, Okon Y (2001) Responses of agronomically important crops to inoculation with *Azospirillum*. Aust J Plant Physiol 28:871–879

Dowling DN, Broughton WJ (1986) Competition for nodulation of legumes. Annu Rev Microbiol 40:131–157

Doyle J, Luckow M (2003) The rest of the iceberg Legume diversity and evolution in a phylogenetic context. Plant Physiol 131:900–910

Fabra A, Castro S, Taurian T, Angelini J, Ibañez F, Dardanelli M, Tonelli M, Bianucci E, Valetti L (2010) Interaction among *Arachis hypogaea* L. (peanut) and beneficial soil microorganisms: how much is it known? Crit Rev Microbiol 36:179–194

Figueredo MS, Tonelli ML, Taurian T, Angelini J, Ibañez F, Valetti L, Muñoz V, Anzuay MS, Ludueña L, Fabra A (2014) Interrelationships between *Bacillus* sp. CHEP5 and *Bradyrhizobium* sp. SEMIA6144 in the induced systemic resistance against *Sclerotium rolfsii* and symbiosis on peanut plants. J Biosci 39:877–885

Giayetto O, Cerioni GA, Amin MS (1999) Use of asymptotic model to obtain optimum plant density in peanut (*Arachis hypogaea* L.). J Peanut Sci 32:1–6

Lavin M, Pennington R, Klitgaard B, Sprent J, Lima H, Gasson P (2001) The dalbergioid legumes. (*Fabaceae*), delimitation of a pantropical monophyletic clade. Am J Bot 88:503–533

Lugtenberg B, Kamilova F (2009) Plant-growth-promoting rhizobacteria. Ann Rev Microbiol 63:541–556

Michelena V, Mata A, Pino F (1996) Memorias de la XVIII Reunión Latinoamericana de Rhizobiología. Santa Cruz de la Sierra, Bolivia

Nievas F, Bogino P, Nocelli N, Giordano W (2012) Genotypic analysis of isolated peanut-nodulating rhizobial strains reveals differences among populations obtained from soils with different cropping histories. Appl Soil Ecol 53:74–82

Pérez-Montaño F, Alías-Villegas C, Bellogín RA, del Cerro P, Espuny MR, Jiménez-Guerrero I, López-Baena FJ, Ollero FJ, Cubo T (2014) Plant growth promotion in cereal and leguminous agricultural important plants: from microorganism capacities to crop production. Microbiol Res 169:325–336

Podile AR, Kishore GK (2006) Plant growth-promoting rhizabacteria. In: Gnanamanickam SS (ed) Plant-associated bacteria. Springer, The Netherlands, pp 195–230

Rivera D, Revale S, Molina R, Gualpa J, Puente M, Maroniche G, Paris G, Baker D, Clavijo B, McLay K, Spaepen S, Perticari A, Vazquez M, Wisniewski-Dyé F, Watkins C, Martínez-Abarca F, Vanderleyden J, Cassán F (2014) Complete genome sequence of the model rhizosphere strain *Azospirillum brasilense* Az39, successfully applied in agriculture. Genome Announc 2(4):e00683-14. doi:10.1128/genomeA.00683-14

SAGPyA (2008) Secretaría de agricultura, ganadería, pesca y alimentos http://www.sagpyamecon.gov.ar

Sanchez AC, Gutierrez RT, Santana RC, Urrutia AR, Fauvart M, Michiels J, Vanderleyden J (2014) Effects of co-inoculation of native *Rhizobium* and *Pseudomonas* strains on growth parameters and yield of two contrasting L. genotypes under Cuban soil conditions. Eur J Soil Biol 62:105–112

Singh CS, Pandey VC, Singh DP (2011) Efficient soil microorganisms: a new dimension for sustainable agriculture and environmental development. Agric Ecosyst Environ 140:339–353

Vessey JK (2003) Plant growth promoting rhizobacteria as biofertilizers. Plant Soil 255:571–586

Vicario JC (2015) Estudios fisiológicos, bioquímicos y moleculares en rizobios: aplicacíon como biofertilizantes para la agricultura sustentable de maní. Universidad Nacional de Río Cuarto, Tesis Doctoral

Vicario JC, Dardanelli MS, Giordano W (2015) Swimming and swarming motility properties of peanut-nodulating rhizobia. FEMS Microbiol Lett 362:1–6

Vincent J (1970) A manual for the practical study of root nodule bacteria. In International biological programme, vol 15. Blackwel Scientific Publication, Oxford