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DEVELOPMENT AND FIELD EVALUATION OF LIQUID INOCULANTS WITH NATIVE *Bradyrhizobial* STRAINS FOR PEANUT PRODUCTION

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ABSTRACT

A critical process in the leguminous crops cycles is biological nitrogen fixation (BNF). Application of inoculants with N fixing bacteria is economically and environmentally favourable. The aim of this work was to select competitive native peanut microsymbionts, evaluate their survival in inoculant support and assess their impact on peanut (*Arachis hypogaea* L.) production under field conditions at Córdoba province in Argentina. The efficient N fixing *Bradyrhizobium* sp. J-81 and *Bradyrhizobium* sp. J-237, previously obtained from peanut nodules in the region of Cordoba, Argentina, were evaluated. In microcosm assays, plants inoculated with these isolates demonstrated better symbiotic parameters than those inoculated with reference strains. Different bacterial growth media and inoculant stabiliser solutions were evaluated. Balanced medium and arabic gum stabilising solution had optimal bacterial growth and the highest bacterial concentration and viability, respectively. Inoculation with either inoculants resulted in 44% greater peanut pod yield at Pizarro compared to the non-inoculated plants, although no significant differences were found with respect to commercial inoculants treatments.

Key Words: *Arachis hypogaea*, Argentina, biological nitrogen fixation

RÉSUMÉ

La fixation biologique de l'Azote (FBA) est un processus important dans le cycle de vie des légumineuses. L'application d'inoculum de bactéries fixatrices d'azote est favorable au double plan économique et environnemental. Le but de cette étude était de sélectionner des bactéries symbiotiques de l'arachide natives et compétitives, évaluer leur temps de survie dans support d'inoculum et évaluer leur impact sur la production en plein champ de l'arachide (*Arachis hypogaea* L.) dans la province de Córdoba en Argentine. Les bactéries fixatrices d'azote *Bradyrhizobium* sp. J-81 et *Bradyrhizobium* sp. J-237, extraites de nodules collectés sur des plants cultivés dans la région de Cordoba en Argentina, ont été évaluées. Dans des essais de microcosme, des plants inoculés avec ces isolats ont exhibés de meilleurs paramètres symbiotiques que les plants non inoculés. Différents média de culture bactérienne et supports inoculums ont été testés. Medium mixte et solution stabilisée à la gomme arabique ont respectivement exhibés la croissance optimale des bactéries et la meilleure conservation et viabilité des bactéries. L'application de n'importe quel inoculum produisit 44% plus de rendement en gousses d'arachides à Pizarro par rapport aux plantes non-inoculées, et ceci bien qu'aucune différence significative n'a été observée en comparaison avec les traitements à l'inoculum du commerce.

Mots Clés: *Arachis hypogaea*, Argentine, fixation biologique de l'azote

INTRODUCTION

Peanut (*Arachis hypogaea* L.) is an important crop all over the world, that provides food for direct human subsistence and is used in several other food products. Argentina is one of the major peanut producers in the world, and about 85% of its production is concentrated in the Córdoba Province (Cámara Argentina del Maní, 2014). It is generally accepted that peanut is native to the Americas; it was grown first in Central and South America and domestication probably took place in a region north of Argentina and south of Bolivia and Paraguay (Krapovickas, 1969; Kochert *et al*., 1996).

Peanut is a legume with high N requirement, which may be obtained from the soil or from biological N fixation (BNF) (Fabra *et al*., 2010). The organic matter availability in soils of the peanut-growing region in Argentina is becoming low (Castro *et al*., 2006); therefore, there is great need to explore biological and sustainable means to improve soil quality. The ability of peanut to fix atmospheric N, as well as to assimilate soil N, provides the opportunity to balance N exports in grain with input from the atmosphere.

In natural rhizobial populations, there are strains that differ in N fixation ability. The low frequency of highly efficient strains could be a disadvantage to legume production (Stowers and Elkan, 1980). Most soils of the peanut-growing region contain a high ($1.6x10^3$ CFU g^{-1}) number of rhizobia cells, but they vary considerably in their symbiotic capability. Generally, highly effective strains occur at low frequencies (Hiltbold *et al*., 1983). The isolation and selection of elite strains allows for effective rhizobial strains to be inoculated for effective nodulation (Dudeja and Khurana, 1988). However, competition of native strains from homologous rhizobia represents a constraint to obtaining an increase in the seed yield from inoculated plants. Selected rhizobial strains, inoculated on peanut plants, often fail to compete with indigenous soil rhizobia (Castro *et al*., 1999; Bogino *et al*., 2006, 2008). This failure may be overcome by introducing not only effective but also competitive strains.

Competition for nodulation is a quantitative phenotype, which determines the ability of certain rhizobial strains to dominate in the nodules of a given legume host in competition with other strains present in the root rhizosphere (Dowling and Broughton, 1986). In some cases, ineffective indigenous strains are more competitive than the introduced strain (Godar *et al*., 2010). Therefore, a screening procedure would select strains not only highly effective in atmospheric N fixation and able to survive under the ecological conditions of the area in which they are introduced, but also highly competitive. One method for obtaining rhizobial strains, with improved properties, has been the selection of naturally occurring field isolates that best exhibit the trait desired (Martínez-Romero and Rosenblueth, 1990). Then, the practice of rhizobial inoculation is important because it places a large number of pre-selected rhizobia in the immediate vicinity of growing root, so that early nodulation is dominated by the selected bacteria. Taking this into consideration, one of the best sources of rhizobial strains for inoculants production is the soil, where they will be introduced (Jardim-Freire, 1997; Denton *et al*., 2002).

It is known that continuous use of nutrients, especially in an inept nutrient management system may result in reduced soil and water quality. Therefore, use of fixing-N bacteria as biofertilisers should be adopted to leverage from the multiple beneficial impacts on soil (Berg, 2009). The objective of this study was to select the most efficient and competitive native peanut microsymbionts, evaluate its ability to survive and reach high cellular concentration in inoculant support, and evaluate its effectiveness under field conditions.

MATERIALS AND METHODS

Bacterial strains. Native isolates *Bradyrhizobium* sp. J-81 and *Bradyrhizobium* sp. J-237, previously obtained from nodules of peanut plants growing in the field in the region of Cordoba, Argentina, and characterised by 16S RNA sequencing, were selected considering their effectiveness in BNF (Angelini *et al*., 2011). Reference strains, recommended as peanut inoculants *Bradyrhizobium* sp. SEMIA 6144 (FEPAGRO collection, Porto Alegre, RS) and *Bradyrhizobium* sp. C-145 (INTA, Argentina) were also used. Bacterial intrinsic antibiotic

resistance was determined, following a standard method (Josey *et al*., 1979). Growth of single colonies on YEM agar medium (Yeast Extract - Mannitol) (Vincent, 1970), supplemented with the antibiotics kanamycin (100 µg mL^{-1}), nalidixic acid (20 µg mL^{-1}), gentamicin (100 µg mL^{-1}), ampicillin (100 µg mL^{-1}), chloramphenicol (40 µg mL^{-1}), spectinomycin (80 µg mL^{-1}), streptomycin (100 µg mL^{-1}), and neomycin (50 µg mL^{-1}), was evaluated.

Plant growth conditions. Peanut seeds (cvTegua) were surface-sterilised with ethanol (70%) for 30 seconds, followed by 15% (V/V) H_2O_2 for 15 minutes, and then washed six times with sterile distilled water. They were germinated at 28 ºC in sterilised Petri-dishes with one layer of filter paper and moist cotton wool, until the radicles reached approximately 2.0 cm. Seedlings were then sown in sterilised plastic cups, filled with vermiculite (Taurian *et al*., 2006). Plants were grown in a controlled environment (light intensity of 200 µE m^{-2} $second^{-1}$, 16-hr day/8-hr night cycle), at a constant temperature of 28 ºC and a relative humidity of 50%; watered regularly with sterilised tap water and, twice a month, with Hoagland´s N-free medium (Hoagland and Arnon, 1950).

Bacterial competitiveness evaluation. To evaluate the competitiveness of bacterial strains, the method described by Vincent (1970) was followed. Mixtures (1:1) of bacterial cultures in YEM broth medium (1.10^7CFU mL^{-1}) were prepared: (a) SEMIA 6144 + C-145; (b) J-237 + C-145; (c) J-237 + J-81; (d) C-145 + J-81; (e) SEMIA 6144 + J-237; and (f) SEMIA 6144 + J-81. One millilitre of the bacterial mixture was added to 5-days-old seedlings, at the junction between stems and roots. After 45 days in greenhouse conditions, as described above, 20 nodules per plant ($n = 5$ plants) were individually sampled, surface-sterilised and crushed in 250 µL of sterile water. An aliquot of 20 µL was plate cultured in YEM agar medium (Vincent, 1970) supplemented with the appropriate antibiotics.

Evaluation of bacterial growth. Five liquid media were evaluated: YEM broth (Vincent, 1970), balanced (Balatti, 1992), G5 (Singleton *et al*., 2002), YG (0.5 g L^{-1} PO_4HK_2, 0.2 g L^{-1} SO_4Mg, 0.1 g L^{-1} NaCl, 1 g L^{-1} yeast extract, 10 g L^{-1} glycerol), and YLG (0.5 g L^{-1} PO_4HK_2, 0.2 g L^{-1} SO_4Mg, 0.1 g L^{-1} NaCl, 0.4% barm, 10 g L^{-1} glycerol). Bacterial cultures were incubated on a shaker (150 rpm at 28 ºC) until the stationary phase of growth was reached (5-7 days). Optical density (OD) at 620 nm and culture medium pH were measured once a day. The growth rate was calculated as: $\mu = [\ln(OD\ t_1/OD\ t_2)]/(t_1-t_2)$ (Vincent, 1970).The number of CFU mL^{-1} was measured during the stationary phase of growth in plates containing YEM agar medium, following the method described by Somasegaran and Hoben (1994).

Evaluation of bacterial survival. Cultures (10^{10} CFU mL^{-1}) in balanced medium (Balatti, 1992) were diluted (1:1) with four stabiliser solutions: (a) distilled water; (b) 0.6% arabic gum; (c) 0.2% peat and (d) 0.6% arabic gum + 0.2% peat. Then, they were stored in dark at room temperature (25 °C) during 6 months, in a glass bottle. Bacterial viability was checked once a month, by counting CFU mL^{-1} in YEM agar plates (Somasegaran and Hoben, 1994). At these times, the inoculants were cultured on plates containing TSA (tryptic soy agar, Leavitt *et al*., 1955) medium (in which the rhizobia are unable to growth) to evaluate the presence of non-rhizobial microorganisms (Wagner and Skipper, 1993).

Field experiment. To evaluate field performance of inoculants, a field experiment was carried out at four locations across two growing seasons (December to March in 2007/2008 and 2008/2009) in the peanut-growing region of Córdoba Province, Argentina. During the first growing season, a field trial was implemented at Chaján (33°33'17.3"S, 65°00'52.4"W, altitude 510 m) on a silt loam Haplustoll soil, and at Modestino Pizarro (34°05'35.8"S, 65°03'40.0"W, altitude 403 m) on a loamy sand Usthortent soil. During the second growing season, field trials were implemented at Sampacho (33°22'48.4"S, 64°42'31.2"W, altitude 514 m above sea level) on a sandy loam Haplustoll soil, and at Suco (33°26'12.4"S, 64°50'18.4"W, altitude 514 m) on a silt loam Haplustoll soil. The average annual rainfall in this peanut-growing region is 600 mm (Bolsa de Cereales de Córdoba). Physical and chemical soil properties were determined, at the beginning of the experiments,

according to standard methods (Pavan *et al.,* 1992) from samples taken from the 0 to 20 cm depth (Table 1). The research fields had no history of peanut cultivation.

For inocula preparation, a bacterial culture in balanced medium at the stationary phase of growth was diluted in the same volume of the stabiliser solution. The number of CFU mL^{-1} was measured following the method described by Somasegaran and Hoben (1994), and then it was stored at 4 ºC until use. Treatments included (a) inoculated with commercial inoculants, (b) inoculated with *Bradyrhizobium* sp. J-81, (c) inoculated with *Bradyrhizobium* sp. J-237, and (d) non-inoculated. Treatments were laid out in a randomised complete block design, with five replications.

Planting occurred on 24 October 2007 in Chajan, on 5 November 2007 in Pizarro, on 21 October 2008 in Sampacho and on 4 December 2008 in Suco, using cultivar "Granoleico" for all assays. A seed drill Migra® (Vasselli S.A., Santa Fe, Argentina) was used in a no-tillage system, with a seeding rates of 16-18 seeds m^{-1} and a planting depth of 3 cm. The size of each experimental plot was 1 ha (20 rows x 600 meters long) with 0.7 m between rows.

Seeds were treated with the commercial fungicide Maxim® (fludioxonil and metalaxyl-*M*, to control fungi of the classes *Ascomycetes*, *Deuteromycetes and Basidiomycetes*). Weeds were controlled using pre-emergence herbicides application immediately following planting (*S*-metolachlor and diclosulam), followed by sequential foliar application of broad spectrum post-emergence fungicides (azoxystrobin and cyproconazole) at about 40 days after planting.

The inoculant/water mixture, in a ratio of 1.5 L ha^{-1} / 50 L ha^{-1}, was sprayed into the furrows immediately after seed drop, but prior to furrow closure using an equipment water-injection system adapted to the seed drill.

At growth stage R2 (flowering, about 45 days after planting) (Boote, 1982), plants obtained from 1 m^2 were harvested by hand and evaluated for nodule number (NN), percentage of red nodules, shoot dry weight (SDW), total nodule dry weight (NDW), and nodule size (NS)**.** To obtain dry weight, plant tissues were dried in a forced-air drying oven (Debrecen, Buenos Aires, Argentina) at 60 °C, to a constant weight.

At harvest (about 145 days after planting), plants obtained from 1 m^2 were placed in an oven at 80 °C to constant weight and analysed for pod and seed yield. The seeds harvested were classified as 'extra class' size, if they did not pass through a screen with 7.5-mm openings (Bogino *et al*., 2006). Only 1 m^2 from each plot was harvested due to the experiments were conducted at farmer´s fields and using the crop that they planted. Five samples, distributed along the plot, were taken to comprise the 1-m^2 sample. None of the experiments were irrigated and, therefore, growth was conditioned by cumulative rainfall.

Statistical analysis. Data from laboratory experiments were subjected to Analysis of variance (ANOVA), followed by comparison of treatment means, using Least Significant Difference (LSD) at 5% level of significance. Statistical analyses were performed using Infostat software version 2014 (Di Rienzo *et al*., 2014).

RESULTS AND DISCUSSION

Bacterial competitiveness. In a previous evaluation of 220 native isolates, obtained from nodules of peanut plants, growing in different

TABLE 1. Soil physical and chemical properties at the locations of the field trials

Location	Organic matter (%)	Texture	NO_3-N (ppm)	Water (%)	Bray I P (ppm)	pH (H_2O)
Pizarro	1.30	Loamy sand	11.7	5.0	28.1	6.65
Chaján	1.75	Silt loam	23.1	5.5	36.5	6.70
Sampacho	1.16	Sandy loam	24.7	5.0	35.9	6.00
Suco	1.12	Silt loams	29.9	5.5	13.2	6.34

location in Córdoba, Argentina, we selected strains *Bradyrhizobium* sp. J-81 and *Bradyrhizobium* sp. J-237 considering their symbiotic effectiveness (Angelini *et al*., 2011). In this work, the competitiveness of these strains was evaluated and compared with the reference strains *Bradyrhizobium* sp. SEMIA 6144 and *Bradyrhizobium* sp. C-145. *Bradyrhizobium* sp. J-237 showed the greatest occupancy when compared with all other strains, while J-81 showed a greater occupancy than the reference strains (SEMIA 6144 and C-145) (Table 2). These results support the hypothesis that among indigenous populations, rhizobia that are more competitive than reference strains may be selected, since they are adapted to local ecological conditions (Svenning *et al.*, 2001; Rangin *et al*., 2008; Duodu *et al*., 2009). Comparison between the reference strains reveals that SEMIA 6144 was more competitive than C-145, occupying almost all the nodules formed in co-inoculated plants (Fig. 1).

Selection of bacterial culture medium and inoculant stabiliser solution. The development of improved culture media and carrier material for enhancing survival of rhizobial inoculants is important for ensuring the maintenance of inoculant quality during storage and transport to the field (Bashan *et al*., 2013). For production of *Bradyrhizobium* inoculant, a low-cost medium is preferable. Currently available culture media use yeast extract and manitol as essential components (Balatti, 1992; Bashan *et al*., 2013). In this study, the greatest number of viable cells and growth rate for all strains were reached in the balanced medium (Table 3; Fig. 2). Furthermore, J-81 reached the stationary phase faster than the other tested strains, when growing in this medium. Therefore, the balanced medium was chosen for bacterial growth. An advantage of the use of balanced medium, instead of YEM medium is that it is less expensive.

Peat is the most frequently used carrier in the rhizobial inoculant industry due to its large water holding capacity and surface area that support rhizobial growth and survival (Singleton *et al*., 2002). However, peat is not available in many countries, and peat-based inoculant production requires a significant amount of processing before use in a commercial production system (Tittabutr *et al*., 2007). Liquid inoculant formulation is a solution to the problems associated with processing solid carriers, since it can be sprayed onto the seed. This formulation may use various broth cultures amended with additives that promote cell adhesion to seed and enhance rhizobial survival during storage and after exposure to extreme environmental conditions (Deaker, 2004). Different inoculant stabiliser solutions were evaluated in this work to determine which maintain greatest J-81 and J-237 bacterial viability after storage. The greatest post-storage bacterial viability (2.10^8 CFU mL^{-1}) occurred with arabic gum as stabilising solution. The number of J-81 viable cells was maintained during 6 months, while J-237 could be maintained for 2 months (Fig. 3).

Gum arabic is a biopolymer with adhesive properties that limit heat transfer, and possess high water activity (a_w) (Mugnier and Jung, 1985).

TABLE 2. Antibiotic resistance profiles for strains of *Bradyrhizobium* sp.

Strain	Antibiotic†							
	µg ml^{-1}							
	Km 50	Na 25	Gm 40	Ap 100	Cm 40	Sp 80	Sm 100	Nm 50
SEMIA 6144	-	+	-	+	+	-	-	+
C-145	+	+	+	+	+	-	-	+
J-237	-	+	-	+	+	-	-	-
J-81	-	+	-	+	+	+	-	+

† Ap = ampicillin; Cm = chloramphenicol; Gm = gentamicin; Km = kanamycin; Na = nalidixic acid; Nm = neomycin; Sm = streptomycin; Sp = spectinomycin; (+) resistant; (-) sensitive

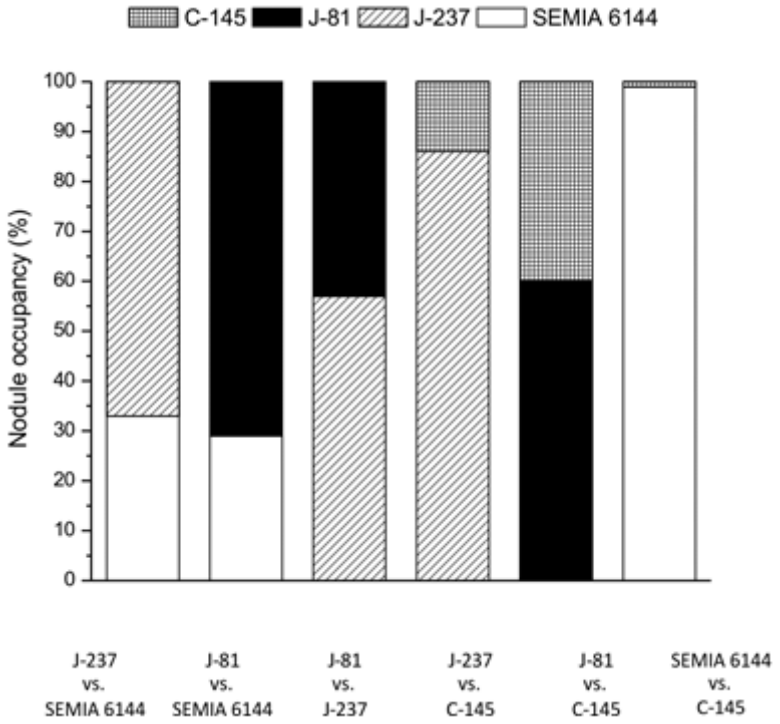

Figure 1. Competitiveness of native and reference bradyrhizobial strains.

TABLE 3. Growth and growth rate of bradyrhizobial strains in different culture media

	Growth[†]			
	CFU mL^{-1} (10^9)			
Medium	SEMIA	C-145	J-81	J-237
YEM	0.69±0.02 ab	2.76±2.17 b	1.42±0.32 b	0.81±0.10 b
Balanced	8.76±2.76a	11.50±1.6 a	23.70±2.90 a	16.70±0.80 a
YG	0.31±0.15bc	2.05±0.82 b	1.84±0.33 b	1.45±0.25 b
YLG	0.05±0.01 c	1.25±0.39 b	2.70±0.35 b	1.56±1.25 b
G5	0.21 ±0.11 c	1.05±0.74 b	1.95±0.05 b	1.45±0.05 b
LSD (5%)	0,4	2,31	4	2
	Growth rate (μ)[†‡]			
YEM	0.17±0.04 bc	0.21±0.06 a	0.19±0.05 d	0.22±0.08 b
Balanced	0.27±0.02 a	0.39±0.02 a	0.34±0.03 a	0.37±0.01 a
YG	0.19±0.07 b	0.28±0.05 a	0.24±0.02 bc	0.23±0.05 b
YLG	0.13±0.06 c	0.27±0.04 a	0.20±0.01 cd	0.19±0.05 b
G5	0.20±0.02 b	0.30±0.01 a	0.25±0.01 b	0.19±0.02 b
LSD (5%)	0.005	0.007	0.003	0.005

[†] Data represent the mean ± SE of three replicates. Means in a same column followed by the same letters are not significantly different (P<0.05, Fisher´s LSD). [‡] Values are data multiplied by 10

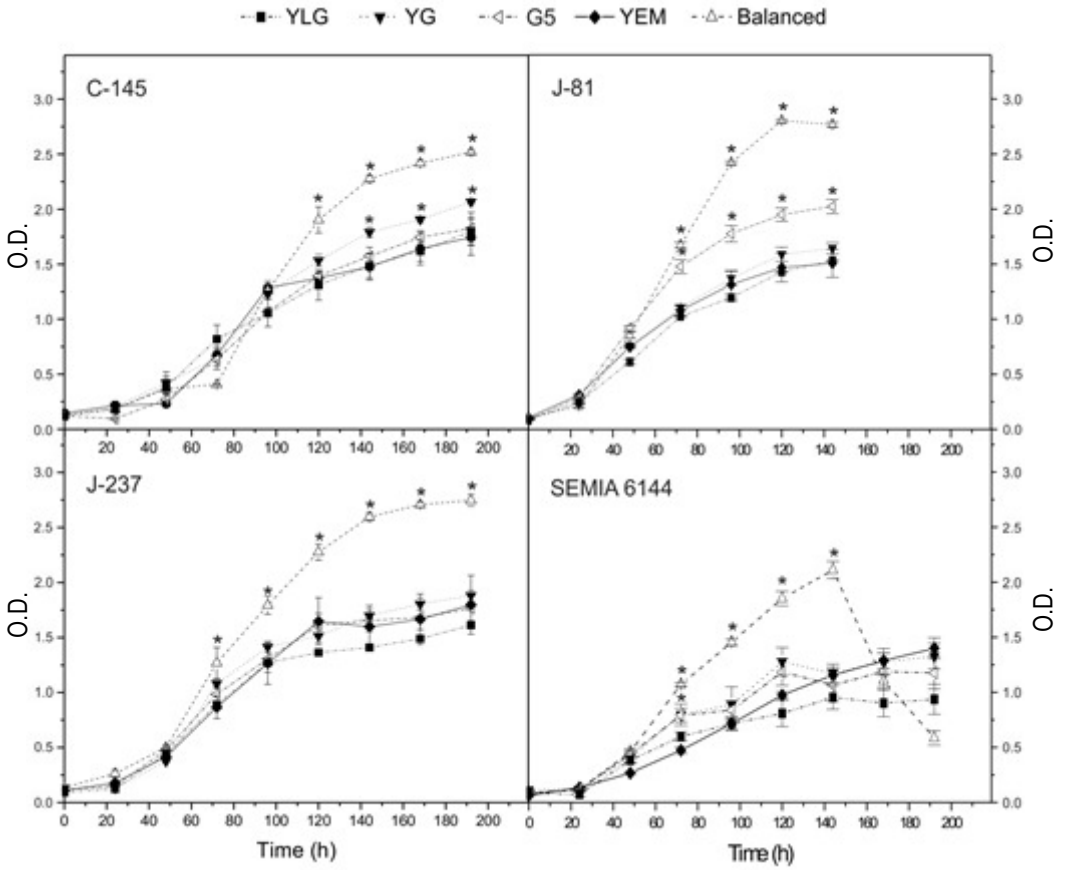

Figure 2. Optical density (O.D) of bacterial strains in different culture media. Data represent the mean ± SE of three replicates. * significantly different (P<0.05, Fisher´s LSD).

Tittabutr *et al.* (2007) evaluated different additives and found that Gum arabic supported most rhizobial strains at 10^8 CFU mL^{-1}, but *Rhizobium phaseoli* TAL1383 and *Sinorhizobium fredii* HH103 did not survive with this additive. They inferred that there is a degree of interaction between strains of rhizobia and additives that may benefit liquid inoculants performance, and that, to maximise performance of liquid inoculants, additives may need to be selected for individual species and strains to optimise the performance of individual inoculants.

Field performance of liquid inoculants. The inoculants formulated with native isolates *Bradyrhizobium* sp. J-81 or J-237 cultures in balanced medium and diluted in gum arabic induced the formation of more nodules than those from non-inoculated plants at all sites. Most of them were red coloured, indicating their high efficiency in N fixation (Vincet, 1970). Moreover, in plants from Pizarro, Suco, and Chaján, strains J-81 and J-237 induced the formation of a greater number of nodules than those induced by commercial inoculants (Fig. 4), indicating that these native strains are more infective than those from the commercial inoculants.

Nodule size induced by these isolates in plants growing at Chaján and Pizarro was smaller than those induced by reference strains or non-inoculated strains (Table 4). In Sampacho and Suco, nodules induced by J-237 or non-inoculated strains, respectively, had the greatest size. However, at almost all the sites, NDW formed by inoculated rhizobia were greater than those induced by non-inoculated strains (except those obtained at Chaján from plants inoculated with J-81). This indicates that inoculation with native

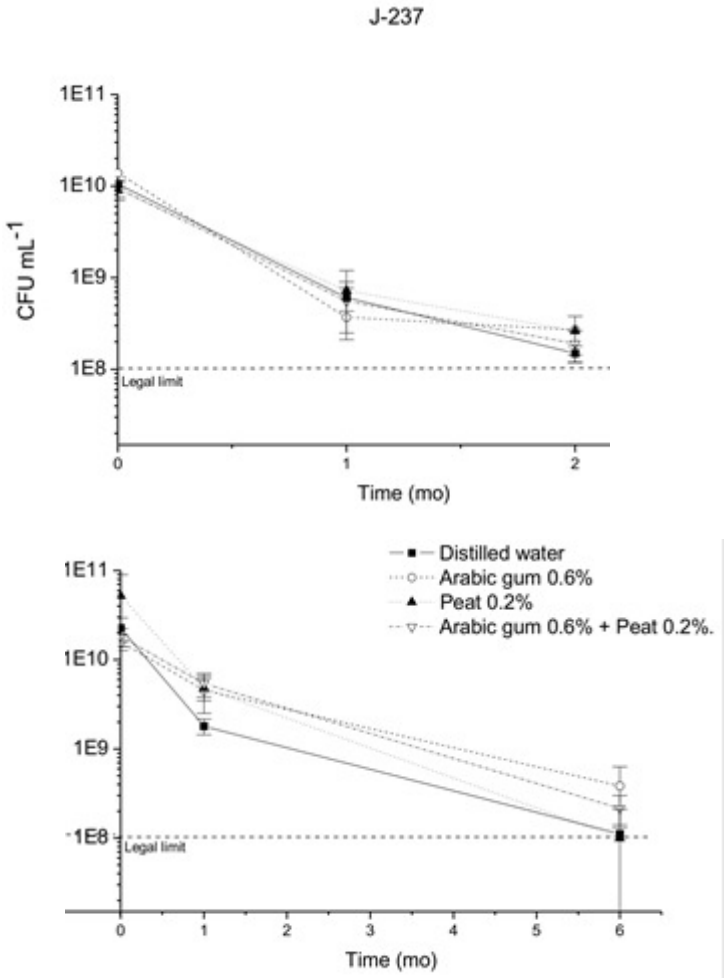

Figure 3. Survival of *Bradyrhizobium* sp. J-237 and J-81 in liquid inoculant from balanced medium containing different stabiliser solutions. Data represent the mean ± SE of three replicates.

strains increases the biomass of the N-fixing tissue. In Pizarro, NDW induced by J-81 and J-237 was also greater than that formed with commercial inoculant (Table 4). However, only plants from Sampacho inoculated with J-81 had greater SDW than plants treated with commercial inoculants, indicating no relationship between NDW and SDW.

Field experiments conducted in Pizarro revealed that inoculation increased peanut pod and seed yields compared to the non-inoculated treatment, reaching values that did not differ from those obtained with a commercial inoculant treatment (Table 5). Similar results were obtained in a study carried out in Chile, in which peanut inoculation with the strains *Bradyrhizobium* sp C-145 or SEMIA 6144, induced a significant increase of plant growth compared with uninoculated plants without nitrogen (Zapata *et al*., 2014). In Egypt, it was also demonstrated that peanut inoculation with bradyrhizobial strains exerted considerable improvement in number and mass of nodules, nitrogen fixation and plant growth (Badawi *et al*., 2011). Differences between inoculated and non-inoculated treatments at Pizarro could be related to low availability of N in the soil (Table 1) and low quantity of native rhizobia as evidenced in the number of nodules formed on non-inoculated plants (Fig. 4).

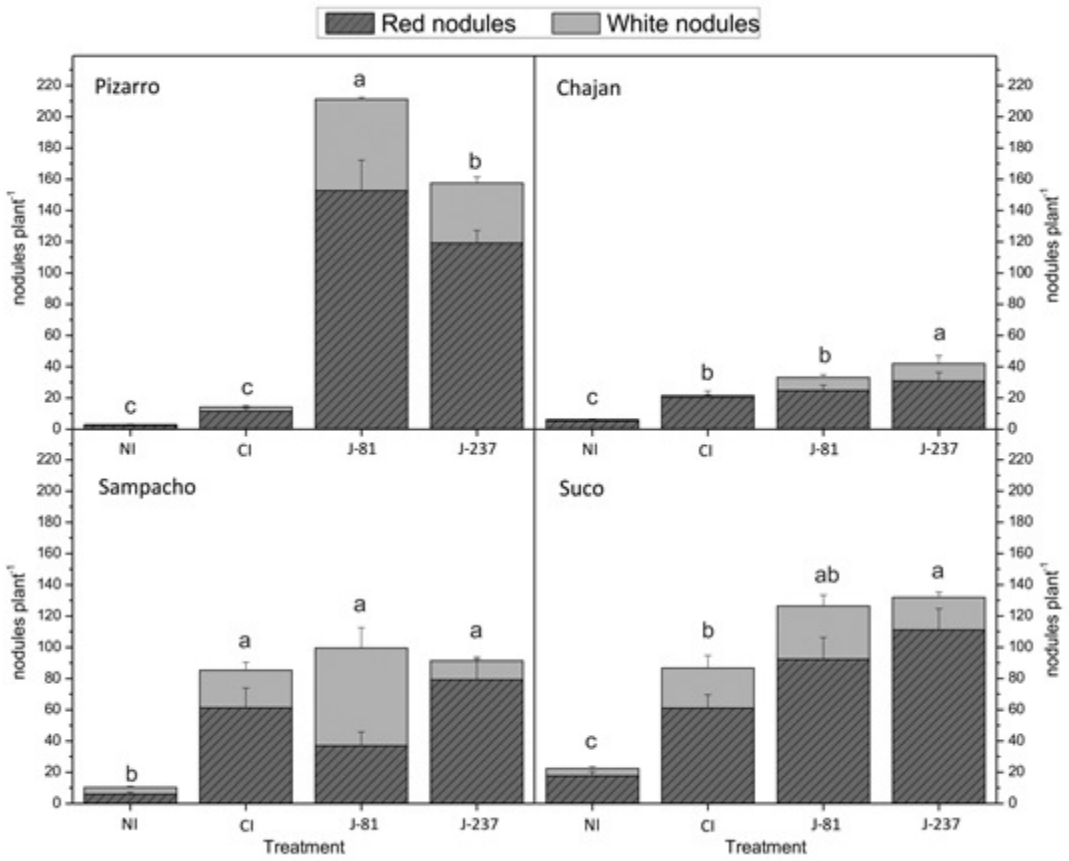

Figure 4. Effect of peanut inoculation with *Bradyrhizobium* sp. on plant nodulation at the R2 growth stage in field assay. NI not inoculated; CI commercial inoculant. Data represent the mean ± SE of three replicates. Means with the same letter within a site are not significantly different at P<0.05 with respect to total nodule number.

At Chaján, Suco and Sampacho, no significant difference in seed yield between inoculated and non-inoculated plants were observed (Table 5). The lack of increase in seed yield could be due to nitrogenase activity inhibition as a consequence of the high soil N concentration (Table 1), as well as water stress during pod filling.

CONCLUSION

Balanced medium culture allowed the greatest bradyrhizobial strain numbers to be reached, while 0.6% gum arabic was the stabilising solution that maintained the greatest bacterial viability after 6 months of storage. Response to inoculation was only found in Pizarro, and was probably related to the low soil N availability at this site. In this soil, inoculation with the selected strains increase pod and seed yields and the seed yield classified as "extra class" size, indicating that competition by non-inoculated native soil strains was overcome with the selected inoculum strains. Although inoculation is still not a common practice for peanut production in most agricultural systems, this work confirms that inoculation of peanut with selected rhizobial strains may improve yield.

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TABLE 4. Effect of peanut inoculation on shoot dry weight (SDW), total nodule dry weight (NDW), and nodule size (NS)

Site	Treatment	SDW†	NDW†‡	NS†
		— — — — g — — — —		(mg nodule^{-1})
Chaján	Non-inoculated	13.31±1.33 a	0.11±0.02 b	1.38±0.16 a
	Commercial inoculant	11.56±0.97 a	0.38±0.07 a	1.23±0.37 a
	J-237	11.99±1.77 a	0.32±0.04 a	0.69±0.06 b
	J-81	11.61±0.85 a	0.18±0.03 b	0.59±0.06 b
	LSD (5%)	3.68	0.01	0.57
Pizarro	Non-inoculated	8.57±0.78 b	0.07±0.01 c	1.97±0.24a
	Commercial inoculant	10.61±0.63 a	0.58±0.13 b	1.90±0.17a
	J-237	9.10±0.68 ab	2.12±0.16 a	1.23±0.06b
	J-81	8.21±0.60 b	2.17±0.16 a	1.05±0.07b
	LSD (5%)	1.91	0.03	0.43
Sampacho	Non-inoculated	16.78±1.45 b	0.23±0.04 c	1.94±0.31 b
	Commercial inoculant	9.76±1.24c	1.38±0.21 b	1.84±0.20 b
	J-237	10.01±0.82c	2.10±0.17 a	2.96±0.48 a
	J-81	21.09±1.35 a	1.59±0.27 ab	1.59±0.12 b
	LSD (5%)	3.51	0.05	0.87
Suco	Non-inoculated	9.66±1.56b	0.43±0.05 c	2.05±0.18 a
	Commercial inoculant	16.21±2.05a	0.82±0.09 b	1.06±0.15 b
	J-237	12.83±1.49 ab	1.48±0.19 a	1.11±0.04 b
	J-81	14.97±1.55 a	1.09±0.14 ab	1.23±0.15 b
	LSD (5%)	4.73	0.04	0.43

† Data represent the mean ± SE of five replicates. Within a column for a given site, means followed by the same letters are not significantly different (P<0.05, Fisher´s LSD). ‡ Values are data multiplied by 10

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TABLE 5. Effect of peanut inoculation on seed yield (SY), pod yield (PY) and confectionary peanut (CP) percentage.

Site	Treatment	SY†	PY†	CP†‡
		— — — — — kg ha^{-1} — — — — —		%
Chaján	Non-inoculated	5,134.10±783.87 a	6,502.44±947.87 a	84.35±4.52 a
	Commercial inoculant	4,736.42±0564.89 ab	5,991.20±764.14ab	86.15±4.11 a
	J-237	4,149.82±1485.74 b	5,406.28±676.51 b	82.38±1.63 a
	J-81	4,696.43±521.10 ab	5,958.55±753.27 ab	83.43±2.64 a
	LSD (5%)	858.8	1,100.96	6.27
Pizarro	Non-inoculated	1,861.36±82.61 b	2,422±160.74b	59.79±4.03 a
	Commercial inoculant	3,378.60±383.55 a	4,211.40±432.07 a	68.77±7.13 a
	J-237	3,802.70±383.31 a	4,650.12±449.01 a	69.68±3.86a
	J-81	3,374.88±720.12 a	4,195.68±828.61 a	71.43±3.96 b
	LSD (5%)	633.26	734.91	6.62
Sampacho	Non-inoculated	3,514.50±127.24 a	4,671.44±200.73 a	79.25±4.67 a
	Commercial inoculant	3,345.86±781.12a	4,317.90±979.92 a	84.92±2.62a
	J-237	3,665.92±408.12a	4,781.10±530.49 a	81.55±5.85 a
	J-81	3,3561.30±519.38 a	4,631.28±657.67 a	81.15±4.23 a
	LSD (5%)	447.08	612.34	6.02
Suco	Non-inoculated	2,707.10±550.89a	3,987.50±570.68 a	50.92±5.18a
	Commercial inoculant	2,780.90±736.34 a	4,214.76±999.56 a	46.42±3.95ab
	J-237	2,608.90±316.91 a	3,563.96±179.33 a	52.94±4.39a
	J-81	3,126.74±205.31 a	4,297.12±247.05 a	56.41±5.09a
	LSD (5%)	666.42	877.77	6.27

† Data represent the mean ± SE of five replicates. Within a column for a given site, means followed by the same letters are not significantly different (P<0.05, Fisher´s LSD). ‡ Percentage of confectionery peanut seeds (seed size > 7.5 mm)

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