South American Sciences

Anais do XVIII Encontro Sobre a Cultura do Amendoim

Plant Growth Promoting Rhizobacteria: Effects on root growth and yield of peanut (*Arachis hypogaea* L.) crop

Subject – 07/04/2021 Approved – 07/31/2021 Published – 09/30/2021

http://dx.doi.org/10.52755/sas.v.2i(edesp1)126

Ezequiel Darío Bigatton

Ing. Agr. MSc. Fac. Cs. Agropecuarias - Un. Nac. de Córdoba, Argentina. E-mail: ebigatton@agro.unc.edu.ar.

Ibrahim Ayoub

Ing. Agr. Fac. Cs. Agropecuarias - Un. Nac. de Córdoba, Argentina. E-mail: ibracba@gmail.com.

Francisco Palmero

Ing. Agr. Fac. Cs. Agropecuarias - Un. Nac. de Córdoba, Argentina. E-mail: fpalmero@unc.edu.ar.

Agustín Berdini

Estudiante de Ingeniería Agronómica. Fac. Cs. Agropecuarias - Un. Nac. de Córdoba, Argentina. E-mail: agustin.berdini@mi.unc.edu.ar.

Jorge Javier Baldessari

Ing. Agr. Dr. Instituto Nacional de Tecnología Agropecuaria (INTA)- Estación Experimental Agropecuaria Manfredi, Argentina. E-mail: baldessari.jorge@inta.gob.ar.

María Angeles Castillejo Sánchez

Biol. Dra. Departamento de Bioquímica y Biología Molecular, Un. de Córdoba, España. E-mail: bb2casam@uco.es.

Enrique Iván Lucini

Ing. Agr. Dr. Fac. Cs. Agropecuarias – Un. Nac. de Córdoba, Argentina. E-mail: eilucini@agro.unc.edu.ar.

Ricardo Javier Haro

Ing. Agr. Dr. Instituto Nacional de Tecnología Agropecuaria (INTA)- Estación Experimental Agropecuaria Manfredi, Argentina. E-mail: haro.ricardo@inta.gob.ar.

ABSTRACT

Córdoba province accounts for 88% of peanut (**Arachis hypogea*** ***L.) grown area in Argentina. The use* of plant growth-promoting rhizobacteria (PGPR) is an alternative to reduce synthetic supplies and improve crop yields*. Pseudomonas and Bacillus are the main crop growth-inducing genera when associated with the rhizosphere. The objective of this work was to evaluate the impact of PGPR on some traits associated with growth, both under field and chamber conditions. PGPR stimulated root growth, total biomass, and yield of peanut crops in the field. Root growth was between +10 and +150% relative to the control. PGPR increased yield between 26-44% over the control. The results suggest positive effects of PGPR on the peanut crop.***

Keywords: Bacillus; Pseudomonas; Peanut yield; Growth stimulation.

Rizobactérias promotoras do crescimento vegetal estimulam o crescimento das raízes e o rendimento da cultura do amendoim (*Arachis hypogaea* L.)

RESUMO

A província de Córdoba concentra 88% da área de cultivo de amendoim (Arachis hypogea L.) na Argentina. O uso de rizobactérias promotoras de crescimento de plantas (PGPR) é uma alternativa para reduzir os suprimentos sintéticos e melhorar o rendimento das colheitas. Pseudomonas e Bacillus são os principais gêneros que induzem o crescimento da cultura quando associados à rizosfera. O objetivo deste trabalho foi avaliar o impacto do PGPR em alguns traços associados ao crescimento, tanto em condições de campo como de câmara. O PGPR estimulava o crescimento das raízes, a biomassa total e o rendimento das culturas de amendoim no campo. O crescimento radicular estava entre +10 e +150% em relação ao controle. O PGPR aumentou o rendimento entre 26-44% em relação ao controle. Os resultados sugerem efeitos positivos do PGPR sobre a cultura do amendoim.

Palavras-chave: ***Bacillus; Pseudomonas; Rendimento; Estimulação do crescimento***.

Rizobacterias promotoras del crecimiento vegetal, Aumentan el crecimiento de las raíces y el rendimiento en el cultivo de maní (*Arachis hypogaea* L.)

RESUMEN

Córdoba es la principal región productora de maní (Arachis hypogaea L.) en Argentina, concentrando más del 88% de la superficie sembrada. El uso de rhizobacterias promotoras del crecimiento de plantas (PGPR) es una alternativa para reducir el uso de insumos sintéticos e impulsar mejoras en el rendimiento. Pseudomonas y Bacillus son los principales géneros inductivos del crecimiento del cultivo cuando se asocian a la rizósfera. El objetivo de este trabajo fue evaluar el impacto de la aplicación de PGPR sobre algunos rasgos asociados al crecimiento bajo condiciones de crecimiento a campo y en cámara de cultivos. PGPR estimularon el crecimiento de raíces, la biomasa total y el rendimiento del cultivo de maní a campo. El crecimiento radicular fue entre +10 y +150% respecto al control Las PGPR incrementaron el rendimiento entre 26-44% respecto al control. Los resultados sugieren efectos positivos de PGPR sobre el cultivo de maní.

Palabras clave: ***Bacillus; Pseudomonas; Rendimiento; Estimulación del crecimiento.***

Introduction

Córdoba is the largest peanut producing province in Argentina, with approximately 350,000 hectares and an average seed yield of 2.57 t.ha-1 (CAMARA ARGENTINA DEL MANÍ, 2021). Market trends force farmers to modify their production systems. The use of plant growth-promoting rhizobacteria (PGPR) is an alternative to reduce synthetic supplies and improve crop yields (REHMAN *et al*., 2020).

Plant Growth-Promoting Rhizobacteria (PGPR) are edaphic microorganisms that interact with the plant rhizosphere and could enhance growth through direct or indirect mechanisms (KUMAR *et al*., 2019; REHMAN *et al*., 2020; BASU *et al*., 2021). The direct mechanisms are related to the crop bio-stimulation and the indirect effects lie in the control of phytopathogens. *Pseudomonas* and *Bacillus* are PGPR genera that increase meristematic activity at the root level and crop growth improving the yield (KUMAR *et al*., 2019). However, their effects on peanut crops need further investigation specially under field conditions. The objective of this work was to determine the PGPR effects on root growth, biomass production, and yield of peanut crops.

Material and Methods

Microorganisms in Field and Pot Trials

Field and Pot trials were performed to evaluate the effects of PGPR strains on peanuts (*Arachis hypogaea*). The cultivar 'Granoleico' was used, and the treatments were: (i) *Bacillus* sp. (RI3), (ii) *Bacillus* sp. (SC6), (iii) *Pseudomonas* sp. (PSE10), (iv) control without PGPR application, and (v) *Bradyrhizobium* spp. as a traditional peanut biofertilizer. All strains were adjusted to $1x10^9$ CFU.mL^{-1} (colony forming unit) and were obtained in the exponential growth phase in tryptic soy broth.

Field experiments (Exp_n) were conducted during two seasons at INTA Manfredi, Argentina. The sowing dates were 10/05/2019 (Exp_1) and 10/15/2020 (Exp_2) using a stand density of 14 plants m^2 at 0.70 m. row spacing. For each treatment, 100 g of seeds were treated with a combination of Thiabendazole (15 g.L^{-1}), Fludioxonil (2.5 g.L^{-1}), and Methalaxil-M (2 g.L^{-1}).

After, seeds were pelleted with a PGPR at a concentration of $1x10^9$ CFU.mL^{-1}. To verify an apparent concentration of 8-9 x 10^9 CFU.seed^{-1}, 10 g of the treated seeds were resuspended in Peptone Water, and serial dilutions were released for bacterial plate count on Tryptic Soy Agar medium for *Bacillus* sp., King F medium for *Pseudomonas* sp., and nitrogen-free medium for *Bradyrhizobium* spp.

At seeding, an application of 190 L.ha^{-1} of PGPR was made at the bottom of each furrow. The apparent concentration of inoculant was $1x10^9$ CFU.mL^{-1}. At the beginning of pod (R3), PGPR was sprayed in each row at the same rate and contraction used at sowing. In both years, the layout of the experiment was a complete randomized block design with three replications.

Each plot had a total surface of 36 m^2. At R3, root biomass was determined according to the procedures of FRASIER *et al*. (2016). Total (TB) and reproductive (RB) biomass were determined at the beginning of seed filling (R5) and maturity harvest (R8).

Furthermore, pod and seed yield were quantified at R8. At each sampling date, plants in 1 m2 were harvested from the two central rows. In controlled conditions trials, pots of 3L were filled with sterilized mixed soil (1:1 V/V) (silty loam Typic Haplustoll soil and sand). Fifteen pots were used per treatment. Seeds were surface-sterilized following the process described by CAMILETTI et al. (2018) and then inoculated as described above (ii-v treatment).

An additional application of 80 $\mu L.seed^{-1}$ of PGPR was made at the bottom of the seed socket (3cm depth) to complete the doses performed for the field trials. Peanut seedlings were grown at 28°C in a chamber with a 16:8 (light/dark) hours of photoperiod, and the soil had maintained at field capacity. Fifteen days after emergence, grow parameters were determined: root length, plant height, and aerial and root biomass.

Results and Discussion

Root growth

An increased range (10%-150%) of root biomass was measured into the first 20 cm of soil under field conditions (**Figure 1a**), with a similar behavoir under pot trial (7%-66%) (**Figure 1b and figure 2**). In addition, root growth under PGPR was higher than *Bradyrhizobium* spp., showing, on average, an increment around 67% and 32% in field and pot trials, respectively. These responses were similar to those previously reported by BASU *et al*. (2021).

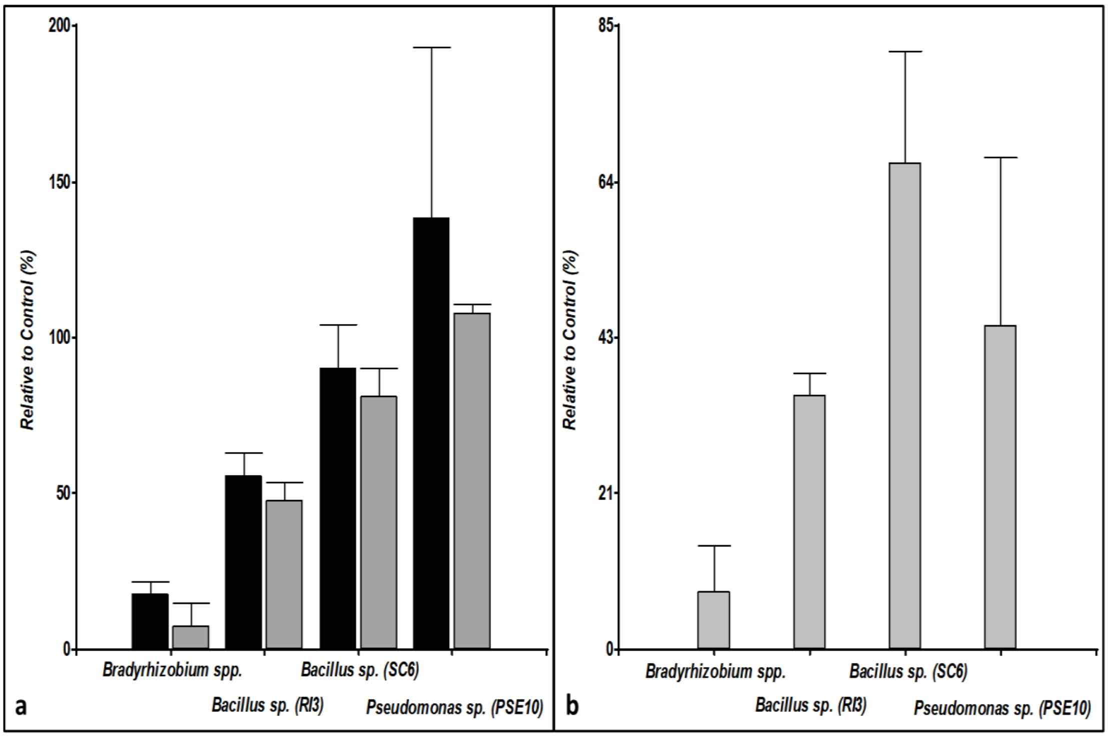

Figure 1. Root biomass (%) related to the Control treatment in (a) field and (b) pot trials. Black and gray bars (a) correspond to Exp_1 and Exp_2, respectively.

Figure 2. Root determination in the pot trial.

Biomass production and Yield

Contrasting biomass production between seasons (2019>2020) reflected variability of environmental conditions (data not shown). However, interestingly, the highest values of TB were found in Exp_2. At R5, there were no differences between treatments in both seasons. At R8, positive effects of PGPR on reproductive biomass were evident. Pod yield of PGPR treatments were higher than control in both field experiments, with *Pseudomonas* sp. (PSE10) showing the highest values (34-31%), followed by *Bacillus* sp. (RI3)

(23-41%), *Bacillus* sp. (SC6) (20-26%), and *Bradyrhizobium* spp. (13-15%) in Exp_1 and Exp_2, respectively (Table 1). Seed yield of PGPR treatments were higher than control in both experiments. *Pseudomonas* sp. (PSE10) (32-19%) and *Bacillus* sp. (SC6) (22-44%) obtained higher values than *Bradyrhizobium* spp. (16-29%) and *Bacillus* sp. (RI3) (10-11%). Recently, similar PGPR effects were reported in peanuts and other crops (IPEK et al., 2014; SOOD et al., 2017; BIGATTON *et al*., 2020).

Table 1. Total biomass (TB) and reproductive biomass (RB) at R5 and R8 stages of peanut crop growing under PGPR treatments. At R8, Reproductive Biomass had determinate as Pod and Seed Yield. Values represent average and standard deviation.

		R5		R8		
Treatments	Year	Total Biomass ($kg.ha^{-1}$)	Reproductive Biomass ($kg.ha^{-1}$)	Total Biomass ($kg.ha^{-1}$)	Pod Yield ($kg.ha^{-1}$)	Seed Yield ($kg.ha^{-1}$)
Control	2019	10116.8 ± 1203.1	2686.8 ± 145.5	10996 ± 501.4	6093.8 ± 984.8	4824.5 ± 860.3
Control	2020	7830 ± 1077.4	927.9 ± 162.2	11238.2 ± 933.9	5751.6 ± 1001.7	4376.4 ± 738.5
Pseudomonas sp. (PSE10)	2019	8892.8 ± 24.3	2385.8 ± 203.3	14662.5 ± 136.1	8184.5 ± 610.5	6363.3 ± 289.1
Pseudomonas sp. (PSE10)	2020	8395.9 ± 1124.6	1000.5 ± 258.8	13295.4 ± 1117.6	7107.5 ± 1230.3	5556.6 ± 960.3
Bradyrhizobium ssp.	2019	10838.4 ± 538.1	2799.2 ± 100.6	11784.8 ± 770.6	6934.5 ± 315.5	5302.5 ± 272.9
Bradyrhizobium ssp.	2020	8519.3 ± 757.1	1115.9 ± 502.1	13063 ± 705.1	6635.4 ± 1141.6	4871 ± 872.2
Bacillus sp. (RI3)	2019	10980.2 ± 561.2	2754.5 ± 311.5	12828.5 ± 750.7	7490.5 ± 502.5	5907.9 ± 440.8
Bacillus sp. (RI3)	2020	6835.8 ± 173.1	782.5 ± 136.5	14707.6 ± 903.4	8108.9 ± 436.6	6308.9 ± 377.4
Bacillus sp. (SC6)	2019	9348.4 ± 577.1	2465.5 ±148.21	12156.5 ± 927.9	7254.2 ± 201.3	5593.8 ± 76.4
Bacillus sp. (SC6)	2020	7883.9 ± 524.8	945.9 ± 236.5	13472.1 ± 870	7242.8 ± 760.8	5640 ± 466.9

Conclusions

The PGPR stimulated the root growth, increased the total biomass and yield (seed and pod). This behavior was consistent in all experiments and levels of analysis. These results provided evidence that PGPR promotes peanut growth.

Acknowledgments

To the National Council for Scientific and Technical Research (CONICET) of the Argentine Republic that supports the doctorate of Ing. Agr. Bigatton. The authors thank the FCA-UNC and INTA for providing laboratories and the experimental site.

References

CAMARA ARGENTINA DEL MANÍ. **Nueva estimación de siembra 2021**. Disponible en: http://www.camaradelmani.org.ar/espanol/nueva-estimacion-de-siembra-cam/. Acceso: 15 jun. 2021.

CAMILETTI, Boris X. *et al*. Characterization of Argentinian endemic *Aspergillus flavus* isolates and their potential use as biocontrol agents for mycotoxins in maize. **Phytopathology**, v. 108, n. 7, p. 818-828, 2018. https://doi.org/10.1094/PHYTO-07-17-0255-R

BASU, Anirban. *et al*. Plant growth promoting rhizobacteria (PGPR) as green bioinoculants: recent developments, constraints, and prospects. Sustainability, v. 13, n. 3, p. 1140, 2021. https://doi.org/10.3390/su13031140

BIGATTON, Ezequiel Darío. *et al*. Efectos de las Rizobacterias Promotoras del Crecimiento Vegetal sobre la producción de biomasa, rendimento y la calidad física del grano de maní (*Arachis hypogaea* L.). **South American Sciences**, v. 1, n. 2, p. e2058-e2058, 2020. https://doi.org/10.17648/sas.v1i2.58

FRASIER, Ileana. *et al*. Direct field method for root biomass quantification in agroecosystems. **MethodsX**, v. 3, p. 513-519, 2016. https://doi.org/10.1016/j.mex.2016.08.002

IPEK, Muzaffer. *et al*. Plant Growth-Promoting Rhizobacteria (Pgpr) Increase Yield, Growth And Nutrition Of Strawberry Under High-Calcareous Soil Conditions. **Journal Of Plant Nutrition**, v. 37, n. 7, p. 990-1001, 2014. http://dx.doi.org/10.1080/01904167.2014.881857.

KUMAR, Ashok. *et al*. Recent advances of PGPR based approaches for stress tolerance in plants for sustainable agriculture. **Biocatalysis And Agricultural Biotechnology**, v. 20, p. 101271, 2019. http://dx.doi.org/10.1016/j.bcab.2019.101271.

SOOD, Gaurav, *et al*. Effect of conjoint application of indigenous PGPR and chemical fertilizers on productivity of maize (*Zea mays* L.) under mid hills of Himachal Pradesh. **Journal Of Plant Nutrition**, v. 41, n. 3, p. 297-303, 19 set. 2017. Informa UK Limited. http://dx.doi.org/10.1080/01904167.2017.1381116.