

Comunicación breve

Fruit yield of strawberry plants inoculated with *Azospirillum brasilense* RLC1 and REC3 under field conditions

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Abstract

The beneficial effect of inoculating with *Azospirillum brasilense* on crop productivity has been widely described in different crops, but its extensive use in strawberry typical agricultural field environments is scarcely documented. The objective of this study was to quantify the fruit yield of strawberry plants (*Fragaria ananassa* Duch.) inoculated with *A. brasilense* (strains RLC1 and REC3) under typical dryland farming conditions. The study was performed during 2006-2009 growing seasons, evaluating root inoculated and non-inoculated strawberry plants cultivar ‘Camarosa’ in Tucumán, Argentina. The inoculated plants exhibited more vigorous vegetative growth and increased the total fruit yield by 8.5% and marketable yield by 7%, as compared with non-inoculated plants.

Keywords: *Azospirillum brasilense*, inoculation, plant growth-promoting bacteria, *Fragaria ananassa*.

Resumen

El efecto benéfico de la inoculación con *Azospirillum* en la productividad de los cultivos ha sido ampliamente descrito, pero su uso en cultivos agrícolas en condiciones ambientales de campo está poco documentado. El objetivo de este estudio fue evaluar el rendimiento frutal de plantas de frutilla (*Fragaria ananassa* Duch.) inoculadas con *A. brasilense* (cepas RLC1 y REC3) bajo condiciones típicas de cultivo en el campo. El estudio se realizó durante las campañas del 2006 al 2009, evaluando al cultivar ‘Camarosa’ inoculado en raíces o no inoculado, en Tucumán, Argentina. Las plantas inoculadas mostraron un crecimiento vegetativo más vigoroso e incrementaron el rendimiento promedio total de fruta en 8,5% y el rendimiento promedio de fruta comercial en 7%, respecto a las plantas no inoculadas.

Palabras clave: *Azospirillum brasilense*, inoculación, bacteria promotora crecimiento vegetal, *Fragaria ananassa*.

Bacteria of the genus *Azospirillum* are considered plant growth-promoting bacteria (PGPB) due to their capacity for stimulating plant growth through direct or indirect mechanisms (Bashan and de-Bashan, 2005; 2010). Field inoculation with *Azospirillum* spp. has been evaluated worldwide in different crops, soils and climatic regions (Okon and Labandera-González, 1994). However, response to plant inoculation with *Azospirillum* has not always been successful, and the factors affecting the crop response are not completely understood (Okon and Labandera-González, 1994; Bashan and Holguin, 1997).

In previous works, we have determined the natural occurrence of *Azospirillum brasilense* colonizing different tissues of strawberry plants cultivated in Tucumán, Argentina (Pedraza *et al.*,

2007). Different strains of *A. brasilense* had the characteristics of nitrogen fixation, total indoles and siderophores production, positive chemotaxis toward strawberry root exudates, were able to colonize the rhizosphere and inner tissues of strawberry, and promoted the growth of the same plants under controlled environmental conditions (Pedraza *et al.*, 2007; 2010). Also, some strains produced salicylic acid and exerted protection effect of strawberry plants against anthracnose disease, produced by *Colletotrichum* spp. (Tortora *et al.*, 2011; 2012), and were able to colonize new-born plants via stolons from an inoculated mother-plant of strawberry (Guerrero-Molina *et al.*, 2012). However, the performance of these strains inoculated under field conditions in strawberry crop was not assessed until now.

Strawberries are cultivated in different parts of the world, including tropical, subtropical, and temperate areas. Argentina produces strawberry during the 12 months of the year, being the province of Tucumán one of the most important producers (Kirschbaum and Hancock, 2000; Rodríguez *et al.*, 2009).

Considering the benefits that could arise from the interaction between *Azospirillum* and strawberry plants, and its possible application as inoculants as an alternative to reduce chemical fertilization, the aim of this work was to evaluate the inoculation effect of two *A. brasilense* strains on the fruit yield of a commercial cultivar of strawberry under field conditions during 4 years of cropping.

To achieve with that objective, bare-root transplants of strawberry (*Fragaria ananassa* Duch.) cultivar 'Camarosa' (intermediate harvest date) was used. They were obtained from commercial nurseries of El Maitén, Argentina. The roots were inoculated with two local strains of *A. brasilense*, named RLC1 and REC, both isolated from strawberry plants and previously characterized (Pedraza *et al.*, 2007). They were selected based on their capacity to fix nitrogen and to produce siderophores and indoles, considered as beneficial traits within the PGPB (Pedraza *et al.*, 2007).

Pure cultures of each strain were firstly grown in NFb N-free semisolid medium (Baldani and Döbereiner, 1980) for 48 h at 30°C; then, a loopfull from each culture was transferred separately to 100 ml NFb liquid medium, supplemented with 1% NH₄Cl (w/v) (without bromothymol blue), and incubated at 30°C, without shaking, for 72 h. After incubation, the cells were centrifuged at 7000 x g for 10 min and washed twice with buffer phosphate pH 7 to remove any residue from the culture medium that could interfere on the growth promoting effect on strawberry plants. The bacterial concentration for inoculation was about 10⁶ CFU.ml⁻¹, and the roots were inoculated once by immersion into the bacterial suspension for 30 min at the moment of implantation.

The experiments were conducted on a silt loam soil (pH = 5.9; EC = 0.6 mS/cm), during four annual production cycles (2006, 2007, 2008, and 2009) at INTA's Estación Experimental Agropecuaria Famaillá (27° 03 S, 65° 25 W, 363 m elevation) in Tucumán, Argentina. Cropping beds consisted of raised beds 1.25 m apart, 0.40 m high, 0.50 m wide, covered with black polyethylene mulch, with two rows of plants (50,000 plants/ha). A rate of 200 kg.ha⁻¹ of 15-15-15 (N, P, K) fertilizer was applied as pre-planting fertilizer every year. In all four years, fresh-dug bare-root transplants were planted between April 26th and

May 3rd.

The experimental design was a randomized-complete block, with three replications of 30 plants each. First flowers of bare-root transplants were removed until plants developed five fully expanded leaves. Fruit were harvested from July through November, two or three times a week, according to fruit maturity. Fruits were graded into marketable (>10 g per fruit) and no marketable (<10 g, with disease symptoms, or deformed). The threshold value for marketable fruit was 10 g since fruits over this weight are sold for either fresh consumption (larger fruit sizes) or for processing (smaller fruit sizes). Variables measured were total yield total (Kg.ha⁻¹), and marketable yield (Kg.ha⁻¹).

The results were evaluated statistically with an analysis of variance by using the software Infostat version 2012 (Di Rienzo *et al.*, 2012). To assess the differences between mean values, LSD Fisher test at the 5% significance level was used.

As result, strawberry total fruit yields indicated that plant inoculation with strain REC3 of *A. brasilense* increased that yield by 3,276 Kg.ha⁻¹ in 2006, 481 Kg.ha⁻¹ in 2008, and with the strain RLC1 by 2,302 Kg.ha⁻¹ in 2007, and 2,432 Kg.ha⁻¹ in 2009 (Table 1).

Table 1. Total fruit yield of strawberry plants inoculated with *Azospirillum brasilense* in Famaillá, Tucumán, Argentina, during the years 2006, 2007, 2008 and 2009.

Treatment	Total fruit yield (Kg.ha ⁻¹)			
	2006	2007	2008	2009
Control	52,939 b	45,827 b	33,677 a	23,159 b
RLC1	52,766 b	47,392 a	34,137 a	26,304 a
REC3	56,215 a	46,018 b	34,774 a	25,501 a

Mean within columns followed by the same letter are not significantly different ($p \leq 0.05$).

Total production of plants inoculated with RLC1 was 3% higher than non-inoculated plants in 2007 and 14% higher in 2009, with values statistically different ($p \leq 0.05$). However, no statistical difference in total fruit yield among treatments was observed in 2008 (Table 1). Total fruit production was improved when cv. 'Camarosa' was inoculated with *A. brasilense* REC3, but lower values were detected when using the strain RLC1, compared with the control in 2006.

In 2007 and 2009, the application of *A. brasilense* strain RLC1 increased total fruit yield (Table 1) and also marketable fruit yield (Table 2), with significant differences at $p \leq 0.05$. As average, total yield per plant increased by 8.5%, within a range of 3.4% to 13.6%. However, the lack of statistical differences in fruit yield observed in 2008 could be due to the weather conditions, as

during winter time the temperatures were lower than the other years of assay, including days with snow.

As shown in Tables 1 and 2, regardless of the treatment applied, fruit yield decreased from the first year of trail (2006) to the last one (2009). Considering that the plantation of strawberry was carried out always in the same lot, and with most of the soil surface covered with plastic mulching, probably the soil microbial population and activity would play a role to explain that behavior.

Table 2. Marketable fruit yield of strawberry plants inoculated with *Azospirillum brasilense* in Famaillá, Tucumán, Argentina, during the years 2006, 2007, 2008 and 2009.

Treatment	Marketable fruit yield (kg.ha ⁻¹)			
	2006	2007	2008	2009
Control	42,440 b	38,120 b	27,742 a	17,333 b
RLC1	43,490 b	40,422 a	27,812 a	19,795 a
REC3	45,550 a	38,778 b	28,223 a	19,455 a

Mean within columns followed by the same letter are not significantly different ($p \leq 0.05$).

Complex interactions exist between plant genotypes and *Azospirillum* strains. It has been observed in wheat that different *A. brasilense* strains colonize differently a single cultivar, and that the same strain colonizes in different numbers when inoculated to different cultivars (Saubidet and Barneix, 1998).

Information about the beneficial effects of *Azospirillum* sp. inoculation on the growth of cultivated plants is not new and was originally developed under greenhouse and laboratory conditions. The results of individual field studies showing the effects of *Azospirillum* spp. inoculation in agricultural crops are now available, but knowledge of those impacts within the context of more extensive inoculation in large on-farm trials is still quite limited. Limited on-farm work is often justified by the reportedly low consistency of field results conducted in a context of more realistic crop management production conditions (Döbbelaere *et al.*, 2001).

The variability in the results could be due to the interaction of the inoculation practice with environmental conditions (e.g. soil type, water balance) and/or crop management practices (e.g. fertilization, chemical disease control, plant genotype).

The results of this assay suggest that early season inoculation with *Azospirillum* allowed plants to remain actively growing during the period of flower differentiation; probably enhanced N mobilization to crown and roots, increased the number of flowers, and contributed to greater season fruit production. Furthermore, plants treated with

Azospirillum did not exhibit excessive vegetative growth, transplant stress or susceptibility to diseases.

Our results demonstrate the importance of *Azospirillum* inoculation in early stages of strawberry cropping and an easy method of application (root immersion), and application timing. Further research should be conducted to adjust these results to other cultivars and environments, as well as to explain the fruit yield reduction after cropping strawberry in the same lot several years.

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