Short communication

Response of strawberry plants inoculated with *Azospirillum* and *Burkholderia* at field conditions

Respuesta de plantas de frutilla inoculadas con *Azospirillum* y *Burkholderia* en condiciones de campo

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Abstract

The use of synthetic fertilizers is a most common practice used to incorporate nutrients in agricultural soils; these are expensive leading to increases in the total crop-production costs. This traditional crop management may turn inefficient and polluting as part of the nutrients can be lost by lixiviation or become insoluble by cation exchange. Therefore, the aim of this study was to evaluate the agronomic response of strawberry plants (*Fragaria ananassa* Duch.) inoculated with two native PGPB as a biotechnological alternative to the plant nutrition. The experiment was carried out under field conditions during 2014, using strawberry plants cv. 'Fortuna Florida' inoculated with the local strains *Azospirillum brasilense* REC3 and *Burkholderia vietnamensis* AFTV3, applied individually and combined. Results showed greater growth index (30%) and relative chlorophyll content in leaves (10%) in inoculated plants compared to non-inoculated controls, and also increased commercial fruit yield in plants inoculated with *A. brasilense* REC3 (12.6%), *B. vietnamensis* AFTV3 (13.4%) and both (7.2%) in comparison with uninoculated controls.

Keywords: *Fragaria ananassa*, *Azospirillum*, *Burkholderia*, PGPB, inoculation.

Resumen

La utilización de fertilizantes sintéticos es la práctica más empleada para la provisión de nutrientes en los suelos agrícolas, a pesar de ser costosos, y de encarecer el costo de producción. Parte de los mismos pueden perderse por lixiviación o tornarse insolubles por intercambio de cationes, haciendo que el proceso tradicional de cultivo sea poco eficiente, además de contaminar el medio ambiente. Es por ello que el objetivo de este trabajo fue estudiar la respuesta agronómica en plantas de frutilla (*Fragaria ananassa* Duch.) empleando 2 cepas PGPB nativas como una alternativa biotecnológica en la nutrición vegetal. El ensayo se realizó en condiciones de campo en 2014. Se utilizaron plantas de frutilla cultivar 'Fortuna Florida' que fueron inoculadas con las cepas locales *Azospirillum brasilense* REC3 y *Burkholderia vietnamensis* AFTV3, aplicadas en forma individual y combinadas. De manera general, se observó que las plantas inoculadas presentaron un mayor índice de crecimiento (30%) y contenido relativo de clorofila en sus hojas (10%), respecto al control no inoculado. También incrementaron el rendimiento de frutos comerciales las plantas inoculadas con *A. brasilense* REC3 (12,6%), *B. vietnamensis* AFTV3 (13,4%) y la mezcla de ambas (7,2%) respecto a los controles sin inocular.

Palabras clave: *Fragaria ananassa*, *Azospirillum*, *Burkholderia*, PGPB, inoculación.

The incorporation of nitrogen into agricultural soils can be done through the decomposition of organic matter, electric discharge during rainfalls, application of synthetic chemical fertilizers, and by the process of biological nitrogen fixation (BNF). Among these possibilities, the application of synthetic fertilizers is the most widely used practice in spite of being expensive, which increases the total crop-production cost. Even more, this traditional crop management may be

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inefficient and polluting as part of the chemicals applied are lost by lixiviation or become insoluble by cation exchange (Pardo *et al*., 2009).

Strawberry is a commercial intensive crop that requires the application of fertilizers during different phenological stages. Nitrogen-fertilizer doses applied are not fixed; they may vary according to the strawberry cultivar demands and nutritional soil conditions from 150 to 200 kg·ha^{-1} (Agüero and Kirschbaum, 2013).

It is well known that plant growth-promoting bacteria (PGPB) are capable of effectively colonize plant roots and promote plant growth throughout many direct and indirect mechanisms, including nutrients solubilization and uptake, phytohormone synthesis and biocontrol of pathogens (Bashan and de-Bashan, 2005; Lucy *et al*., 2004). Thus, the use of PGPB, such as *Azospirillum* and *Burkholderi*a in strawberry cropping would be a biotechnological alternative to partially or totally substitute the use of nitrogen fertilizers and avoid environmental pollution.

It has been reported that *Burkholderia* genus is capable of increasing crop yields in different plant species, e.g. rice, sugar cane and maize (Van *et al.*, 2000; Hernández-Rodríguez *et al*., 2010), but there is not available information on its use in strawberry crop. In a previous work we have isolated and characterized several strains of this genus from strawberry roots, being the strain *B. vietnamensis* AFTV3 the one showing remarkable PGPB features such as N_2-fixation, indoles and siderophores production, and phosphate solubilization (Lovaisa *et al*., 2011).

The genus *Azospirillum* has also been proven to have positive effects on growth and yields of several crops, including strawberry (Pedraza *et al*., 2010; Saikia *et al*., 2012). The strain *A. brasilense* REC3 outstands among other isolates from the province of Tucumán (Argentina) because of its PGPB traits and also because it was able to promote growth, enhance mineral nutrition, and increase fruit yield of different cultivars of strawberry (Pedraza *et al.*, 2007; 2010; Salazar *et al*., 2012; Guerrero-Molina *et al*., 2014).

Therefore, the aim of this study was to evaluate the agronomic response of strawberry plants (*Fragaria ananassa* Duch.) inoculated with two native PGPB as a biotechnological alternative to the plant nutrition.

To accomplish this objective, an experiment using strawberry plants was set at field conditions. For this, plants of strawberry (*Fragaria ananassa*, Duch) cv. 'Fortuna Florida' (short day cultivar), were used. They were purchased from commercial nurseries of El Maitén, Chubut, Argentina. The experiments were conducted on a silt loam soil (pH = 5.7; EC = 0.6 mS·cm^{-1}), during one annual production cycle (2014) 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^{-1}). A rate of 120 kg·ha^{-1} of 15-15-15 (N, P, K) fertilizer was applied as pre-planting fertilizer. The experimental design was a completely randomized block, with six replications of 40 plants each. Fruit were harvested from May through October, two or three times a week, according to fruit maturity.

The bacterial strains used in this study were *Azospirillum brasilense* REC3 and *Burkholderia vietnamensis* AFTV3, both isolated from roots of strawberry cultivated in Tucumán.

Plant roots were inoculated once by immersion into pure bacterial suspension (10^{6} CFU·ml^{-1}) for 30 min before implantation. NFb and LGI liquid media (Baldani *et al*., 2014) were used to grow *A. brasilense* REC3 and *B. vientnamensis* AFTV3, respectively.

Along the whole production period, the relative chlorophyll content was periodically measured every 2 weeks from 30 days of implantation by using a Minolta SPAD-502 chlorophyll-meter. These results were expressed as SPAD values.

Fruits were graded into marketable (>10 g per fruit) and non-marketable (<10 g, either with disease symptoms or deformed). The threshold value for marketable fruit was 10 g since fruits over this weight are sold either for fresh consumption (larger fruit sizes) or processing (smaller fruit sizes). Variables measured were total fruit-yield (kg·ha^{-1}) and marketable fruit-yield (kg·ha^{-1}). One-way ANOVA analysis and Tukey´s multiple comparison tests ($p \leq 0.05$) were done with Statistix Analytical Software 1996 for Windows®.

By the end of the cropping season, roots and shoot were oven dried at 65° C for 72 h (constant weight) and dry weight of each tissue was recorded. The growth index (GI) was calculated with the dry weight data according to the formula:

GI=[(final biomass-initial biomass)/initial biomass]

As result, the relative chlorophyll content values decreased from vegetative to productive stages. Also a positive impact was observed on strawberry chlorophyll content on leaves of inoculated plants in comparison to uninoculated controls (Figure 1).

The growth index of strawberry was significantly increased by the inoculation with REC3, AFTV3 and the combination of both ($p \leq 0.05$). The highest values were observed in the treatments inoculated with AFTV3 (37%) and REC3 (32%), compared with control non-inoculated (Figure 2).

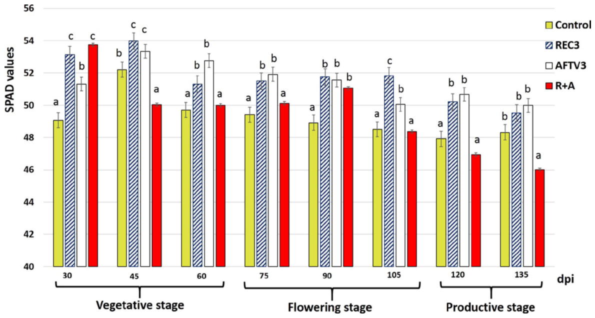

Figure 1. SPAD relative values (mean ± standard error) of plants cv. 'Fortuna Florida' inoculated with REC3, AFTV3 and REC3 + AFTV3 (R+A) measured from 30 dpi (days post inoculation). Bars with the same letter are not significantly different ($p \leq 0.05$).

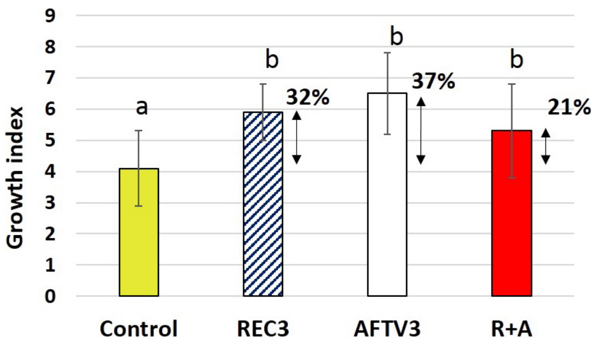

Figure 2. Growth index (mean ± standard error) of plants inoculated with REC3, AFTV3 and REC3 + AFTV3 (R+A). Bars with the same letter are not significantly different ($p \leq 0.05$). Arrows indicate the % increment compared to the control.

The marketable and total fruit yield of strawberry inoculated with the PGPB is shown in Table 1.

Table 1. Marketable fruit yield of strawberry plants (MF) and total fruit yield of strawberry plants (TF) inoculated with REC3, AFTV3, and R+A, in Famaillá, Tucumán (Argentina), during 2014. Mean values within columns followed by the same letter are not significantly different ($p \leq 0.05$).

Treatment	Fruit yield ($Kg \cdot ha^{-1}$)	
	MF	TF
Control	29903,5c	33663,2c
REC3	33693,1a	35296,7b
AFTV3	33919,5a	36558,7a
R+A	32079,3b	36879,4a

After these results, we have observed that single strain inoculation with *A. brasilense* REC3 and *B. vietnamensis* AFTV3, as well as combined inoculation with bacteria strains, had a positive impact on relative chlorophyll values in plants var. Fortuna Florida. This can be interpreted as a greater N content on inoculated strawberry leaves, and this is important since plants were N-fertilized just once before crop-implantation. Hence, PGPB could have played an important role in strawberry plant nutrition through some of their mechanisms (e.g., indole production that may allow a better root development to absorb nutrients from soil; or the biological nitrogen-fixing process).

Also, PGPB- inoculation increased strawberry fruit yield, mainly of marketable type. This was observed in values between 7% and 13% higher than non-inoculated plants. Besides, this represents about 2,000 kg and 4,000 kg of fruit per hectare. Considering that a base fertilization was applied in every treatment when the crop was implanted, we can infer that the PGPB used conferred to the plants some differential advantages in their nutrition. Probably, this enables them to support a better fruit yield.

It is known that the application of *A. brasilense* on wheat caused increases in grain yields (Díaz-Zorita and Grove, 2006), and in corn plants increased their biomass and yield by 27% (Hungria *et al*, 2010). Regarding strawberry crop, it was reported the use of different beneficial microorganisms, such as *Pseudomonas*, *Bacillus*, and *Azospirillum*, indicating that they were able of increasing plant biomass and fruit yield (Esitken *et al*., 2010; Erturk *et al*., 2012; Salazar *et al*., 2012). Also, it was reported the capacity of *A. brasilense* as a biocontrol agent against anthracnose, a fungal disease of strawberry (Tortora *et al*., 2011).

Several species of *Burkholderia* have been reported by their beneficial effects such as increasing biomass in sugar cane (de Oliveira *et al*., 2006), and grain yield in maize and rice (Hernández-Rodríguez *et al*., 2010; Estrada *et al*., 2013). However, there is not information on its use in strawberry crop; therefore, this is the first report of *B. vietnamensis* applied to this crop at field conditions.

Considering that *A. brasilense* REC3 and *B. vietnamensis* AFTV3 promoted strawberry growth and increased strawberry fruit yield at field conditions, these PGPB are promising as a biotechnological alternative for strawberry plant nutrition.

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