Short communication

Total and marketable fruit yield of strawberry plants grown under different levels of nitrogen fertility and inoculated with *Azospirillum brasilense* REC3

Rendimiento frutal total y comercial de plantas de frutillas cultivadas bajo diferentes niveles de fertilidad nitrogenada e inoculadas con *Azospirillum brasilense* REC3

N.C. Lovaisa[1,2,3]; M.F. Guerrero Molina[1,2,3]; P.G.A. Delaporte Quintana[1,2]; S.M. Salazar[1,4]

[1] Facultad de Agronomía y Zootecnia, Universidad Nacional de Tucumán. Avda. Kirchner 1900, (4000), San Miguel de Tucumán, Tucumán, Argentina. *E-mail: lovaisanadia@gmail.com

[2] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina.

[3] Instituto Superior de Investigaciones Biológicas (INSIBIO-UNT-CONICET), Instituto de Química Biológica "Dr. Bernabé Bloj", Facultad de Bioquímica, Química y Farmacia, UNT. Chacabuco 461, (4000), San Miguel de Tucumán. Tucumán, Argentina.

[4] INTA EEA Famaillá. Ruta Prov. 301 Km 32, (4132), Famaillá. Tucumán, Argentina.

Abstract

The aim of this work was to evaluate the agronomic response of strawberry plants (*Fragaria ananassa* Duch.) inoculated with *Azospirillum brasilense* as a biotechnological alternative to reduce or complement the plant nitrogen-fertilization under field conditions. The field trial was carried out in the province of Tucumán, Argentina, inoculating or not strawberry plants (cv. 'Camino Real') with *A. brasilense* REC3 and applying or not nitrogen-fertilization. Treatments consisted in plants cultivated under different nitrogen fertilization (0%, 50%, 100%) with or without bacterial inoculation. The variables assessed were: SPAD relative values, total and marketable fruit yield, and growth index. As a result, it was observed that *A. brasilense* REC3 contributed positively to the nitrogen-nutrition of strawberry plants growing at field conditions, expressed in the values of the variables assessed.

Keywords: Strawberry; *Azospirillum*; PGPB; Fruit-yield; Nitrogen-fertilization.

Resumen

El objetivo de este trabajo fue evaluar la respuesta agronómica de plantas de frutilla (*Fragaria ananassa* Duch.) inoculadas con *Azospirillum brasilense* como alternativa biotecnológica para reducir o complementar la fertilización nitrogenada de las plantas en condiciones de campo. Para ello, se realizó un ensayo en la provincia de Tucumán (Argentina) con plantas de frutilla (cv. 'Camino Real'), inoculadas o no con *A. brasilense* REC3 y aplicando o no fertilización nitrogenada. Los tratamientos consistieron en plantas cultivadas bajo diferentes dosis de fertilización nitrogenada (0 %, 50 %, 100 %), con o sin inoculación bacteriana. Las variables evaluadas fueron: valores relativos SPAD, rendimiento frutal total y comercial, e índice de crecimiento. Como resultado se observó que *A. brasilense* REC3 contribuyó positivamente a la nutrición nitrogenada de las plantas de frutilla cultivadas en condiciones de campo, expresado en los valores de las variables evaluadas.

Palabras clave: Frutilla; *Azospirillum*; PGPB; Rendimiento frutal; Fertilización nitrogenada.

Among the factors considered essential for plant growth, after water, nitrogen is the main limiting factor for plant productivity, being the most influential element on fruit yield and quality in strawberry production (Nestby *et al*., 2005; Ojeda-Real *et al*., 2009). Its content in leaves, which is directly related to chlorophyll content, has been used as a tool for monitoring the status of nitrogen in the plant, considering that it can change with the growth stage and cultivar (Güler *et al*., 2006). It has been observed that with low supplies of nitrogen, leaf area and root size is reduced; while an excess of nitrogen causes fruits of low firmness, slows ripening and promotes excessive vegetative growth, adversely affecting performance and generating favorable conditions for the development of disease conditions (May and Pritts, 1990; Nestby *et al*., 2005).

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Strawberry is a commercial intensive crop that requires the application of fertilizers during different phenological stages. The usual practices of nitrogen fertilization vary according to the cultivar and environmental conditions. Although this crop occurs frequently in soils with good natural fertility, producers in Tucumán, Argentina, apply nitrogen doses ranging from 120, 280 to 320 kg nitrogen UF ha^{-1} using higher doses in order to ensure high performance, even when the recommended dose for the province do not surpass 155 kg ha^{-1} (Kirschbaum *et al*., 2006). This fact, plus the high impact on the cost for the use of fertilizers, can exert negative effects on the environment, since it is known that the crop does not consume the entire fertilizer applied and part of it is lost through leaching, contaminating groundwater (Ju *et al*., 2006).

An alternative for total or partial replacement of nitrogen fertilizers in the cultivation of strawberries could be the use of plant growth promoting bacteria (PGPB), such as bacteria of the genus *Azospirillum* which is capable of positively affecting the growth and yield of many species of plants of agronomic interest.

The aim of this work was to evaluate the agronomic response of strawberry plants (*Fragaria ananassa* Duch.) inoculated with *Azospirillum brasilense* as a biotechnological alternative to reduce or complement the plant nitrogen fertilization under field conditions.

To achieve this objective plants of strawberry cv. 'Camino Real' were inoculated with *Azospirillum brasilense* REC3, a strain isolated from roots of strawberry cultivated in Tucumán (Pedraza *et al*., 2007). In order to accomplish that, plants were inoculated by submerging their roots in a pure bacterial suspension (10^6 CFU ml^{-1}) for 30 min before implantation as described in Pedraza *et al*. (2010).

The experiment was conducted on a silt loam soil (electric conductivity = 0.6 mS cm^{-1}; pH = 5.7), 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}). Different doses of nitrogen (N) were applied: 0% - 50% and 100%. The fertilization was performed by applying 120 nitrogen, 70 phosphorus oxide (III), 220 potassium oxide, 40 calcium oxide and 20 magnesium oxide (kg ha^{-1}) through drip irrigation, corresponding the nitrogen dose of 100% to 120 N kg ha^{-1}. The experimental design was in complete randomized blocks, with six replications of 40 plants each. Six treatments were evaluated: 1) control plants fertilized with 100% nitrogen (Control-100% N), 2) inoculated plants fertilized with 100% nitrogen (REC3-100%); 3) control plants fertilized with 50% nitrogen (Control-50% N); 4) inoculated plants fertilized with 50% nitrogen (REC3-50% N); 5) control plants without nitrogen (Control-0% N) and 6) inoculated plants without nitrogen (REC3-0% N). Fruits were harvested from May through October, two or three times a week, according to fruit maturity.

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}).

At the end of the cropping season, roots and shoots were oven-dried at 65 °C for 72 h (constant weight) and dry weight of each tissue recorded. Total biomass was calculated as the sum of root and shoot dry weights, and the growth index (GI) as (final biomass – initial biomass) divided by the initial biomass.

To assess the differences between mean values, t test at the 5% significance level was used. The analyses were run using the software Infostat (Di Rienzo *et al*., 2008).

As a result, it was observed that the relative chlorophyll content in the leaves decreased from the vegetative to the reproductive stage of the plants. The highest values were recorded at 60 dpi in the inoculated treatments under different doses of nitrogen fertilization in comparison to uninoculated controls (Table 1).

The mean fruit yield values of cv. 'Camino Real' were increased in all the treatments with plants inoculated with REC3, independently of nitrogen-fertilization. This information is presented in Table 2, considering total and marketable fruit yields.

Table 1. SPAD relative values of plants cv. 'Camino Real' inoculated with REC3 under different doses of nitrogen (N) measured from 30 dpi (days post inoculation). Mean values within columns followed by a different letter are significantly different ($p < 0.05$).

Treatments	Dpi							
	30	45	60	75	90	105	120	135
Control-0N	45.03 b	48.21 b	53.23 b	55.56 a	54.78 a	55.76 a	50.24 a	49.39 a
REC3-0N	49.28 a	52.33 a	58.35a	56.93 a	56.26 a	56.41 a	52.16 a	49.46 a
Control-50N	46.88 b	51.59 b	55.84 b	56.51 a	55.21 a	56.33 a	54.08 a	48.26 a
REC3-50N	50.02 a	54.85 a	60.32 a	59.71 a	58.32 a	57.75 a	54.86 a	48.76 a
Control-100N	47.94 b	51.31 b	57.25 b	56.59 b	57.81 a	58.32 a	54.39 a	48.75 a
REC3-100N	51.64 a	56.15 a	61.25 a	60.35 a	59.96 a	59.21 a	56.06 a	49.01 a
Phenological stage	Vegetative stage			Flowering stage			Production stage	

Table 2. Marketable fruit yield (MF) and total fruit yield of strawberry plants (TF) growth under different doses of nitrogen (N) and associated or not with *A. brasilense* REC3 in Famaillá, Tucumán (Argentina), during 2014. Mean values within columns followed by a different letter are significantly different ($p < 0.05$).

Treatment	Fruit yield (kg ha^{-1})	
	MF	TF
Control-0N	22360.3 a	23040.7 a
REC3-0N	22964.2 a	23962.3 a
Control-50N	24726.1 a	25203.5 a
REC3-50N	25508.3 a	26463.6 b
Control-100N	30201.7 a	31065.6 a
REC3-100N	32421.5 b	33550.8 b

At the end of the cropping season, the growth index of strawberry plants was determined. As shown in Table 3 it was significantly increased by the inoculation with REC3 only when nitrogen was applied at 50% ($p < 0.05$).

Table 3. Growth index of plants inoculated with REC3 under different doses of nitrogen (N). Mean values within columns followed by a different letter are significantly different ($p < 0.05$).

Treatment	Growth index
Control-0N	4.89 a
REC3-0N	5.47 a
Control-50N	5.01 a
REC3-50N	6.24 b
Control-100N	6.38 a
REC3-100N	6.95 a

According to our results, it was observed that strawberry plants inoculated with *Azospirillum brasilense* REC3 under different doses of nitrogen fertilization increased greenness index. Several studies report that different doses of nitrogen fertilization affect the chlorophyll content of leaves in *Berberidopsis*, tomato and corn, determining the most appropriate level of N-fertilization (Mendoza *et al*., 1998; Novoa and Villagrán, 2002; Latsague *et al*., 2014). Considering that *A. brasilense* REC3 has proven to promote plant growth in different strawberry cultivars (Pedraza *et al*., 2010), we speculate that it could also help to the plant to increase its N content as observed herein with the SPAD relative values.

The incidence of the different treatments applied in this work on strawberry fruit yields is remarkable, considering that the two doses of nitrogen (50%, 100%) were below the level frequently recommended to growers in this cropping region, and besides, that some cases plants did not receive nitrogen-fertilization but just bacterial inoculation. The treatments inoculated with *A. brasilense* REC3 and nitrogen = 50% or nitrogen = 100% increased 5.2% and 7.3% of fruit yield, respectively, as compared to their control plants. Therefore, it could be inferred that the incidence of inoculation with REC3 on fruit production is better expressed when the N availability in the soil is reduced. This is also in line with the growth index values observed in this work. Thus, *Azospirillum* would be providing nitrogen to the plant requirements through its different mechanisms of action. Similar results were obtained in trials with maize inoculated with *A. brasilense* and with different doses of fertilizers, being greater the relative increase in biomass and grain yield at reduced than at complete doses of fertilizers applied (Swędrzyńska and Sawicka, 2000). Also, we have further evidences of the beneficial effects exerted by different microorganisms

in strawberry crop, increasing the plant growth and fruit yield under environmental controlled and field conditions (Pedraza *et al*. 2010; Esitken *et al*., 2010; Salazar *et al*., 2012; Guerrero-Molina *et al*. 2014; Lovaisa *et al*., 2015).

With the results observed in this work, including SPAD relative values, total and marketable fruit yield, and growth index, we can expect that the use of *A. brasilense* REC3 may contribute positively to the nitrogen-nutrition of strawberry plants growing at field conditions as a biotechnological alternative for plant nutrition.

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