Article

Agronomic response of *Phaseolus vulgaris* to biofertilization in the field

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Abstract

The use of beneficial microorganisms is a viable alternative to incorporate into legumes to improve fertility and increase nitrogen fixation in the soil. In this work the application of arbuscular mycorrhizal fungi (AMF), *Rhizobium etli* (*Re*) and a reduced dose of inorganic fertilizer in the production and quality of bean (*Phaseolus vulgaris* L.) cv. 'Negro Michigan' under field conditions. The work was carried out in the 'La Bandera' Experimental Field located in the municipality of Actopan, Veracruz, during the spring summer 2016 cycle. An experimental randomized block design with eight treatments was used [T1: (control, T) , T2: (fertilized, F), T3: (inoculated with AMF), T4: (inoculated with *Re*), T5: (inoculated with AMF+*Re*), T6: (inoculated with AMF+50%F), T7: (inoculated with *Re*+50%F) and T8: (inoculated with AMF+*Re*+50%F)], each treatment with three blocks and 500 plants in each. Plant height, stem diameter, number of leaves, flowers, pods and nodules, weight of grains, seed quality variables, total protein content and percentage of root colonization were evaluated. An analysis of variance and Fisher's LSD test with a significance level of 5% were used. The results showed significant differences between treatments ($p \leq 0.05$) for the registered variables, with AMF+*Re*+50%F being the treatment where not only was the quality of the bean grain improved, but the use and fertilization costs would be reduced in favor of the producers' economy.

Keywords: *Rhizobium etli*, arbuscular mycorrhizal fungi, protein.

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Introduction

According to the agro-ecological zoning studies carried out by INIFAP, a large proportion of the area destined for bean cultivation in Mexico is located in rainfed areas where the agro-ecological-productive potential is very vulnerable to droughts, early frosts, discontinuous and unstable distribution. of rainfall during the vegetative cycle of the plant or to the attack of pests and diseases (ASERCA, 1997; SIAP, 2000-2005) and at the state level Veracruz stands out with 1 813 282 ha not suitable for planting and harvesting this legume (SAGARPA-INIFAP, 2012). Therefore, it is not surprising that during the 2017 autumn-winter (residual moisture) and spring-summer (temporary) agricultural cycle, only 0.742 t ha^{-1} was obtained in the Veracruz entity (SIAP, 2017).

However, through the implementation of cutting-edge agrotechnics proposed through genetic improvement (directed hybridization) (Muñoz, 2012) and biotechnology it is feasible that producers not only improve productivity, but also their living conditions and income (FIRA, 2016), since the different varieties of beans (introduced or creoles) could occupy areas destined for sustainable cultivation based on tolerance to pests, diseases and water stress, or by taking advantage of their qualities in biological functions aimed at guaranteeing healthy and nutritious food self-sufficiency (del Valle, 2016; Mora, 2017).

However, several biotic factors, such as pathogens and viruses, cause considerable economic losses in the cultivation areas of Chiapas and Veracruz (López *et al*., 2007) that, together with the indiscriminate application and high cost of the deep nitrogen and phosphate fertilization required in the production of this crop (Martínez-Viera *et al*., 2010; Sánchez-Yañez *et al*., 2014), require the search for alternatives capable of solving problems related to food safety without affecting the levels of productivity, quality and safety desired (Khan *et al*., 2016), or damage the fertility of soils, break the health of consumers, the welfare of farmers and environmental balance (de Souza Vandenberghe *et al*., 2017).

One of the viable options to face this problem lies in the use of microorganisms whose activity improves the absorption of water and nutrients, reduces the spillage of polluting agents and controls the incidence of harmful insects and phytopathogens in the crop (Aguado-Santacruz *et al*., 2012; Yilmaz and Sönmez, 2017). As well as, the symbiotic relationship between the organisms involved occurs in the rhizosphere, the purpose pursued through the research lies in checking which microorganisms are beneficial after associating with their host.

For the specific case of beans, it is reported that *Rhizobium etli* is a rhizobacterium that promotes the growth of legumes and [not legumes] (García-Fraile *et al*., 2012; Soriano and González, 2012; Shameer and Prasad, 2018) through the mutualism, because in addition to nodular roots and produce acetic acid, gibberellins and cytokinins (Mayak *et al*., 2004), converts atmospheric dinitrogen (N_2) into ammonium ion (NH^{4+}) (Meilhoc *et al*., 2011; Santi *et al*., 2013; Miwa and Okazaki, 2017) that fix with great efficiency in the soil (Pérez-Montaño *et al*., 2014) and from there the plant absorbs it in the root cells through different groups of transporters located in the membrane Plasma (Kiba and Krapp, 2016).

As regards the fungi forming arbuscular mycorrhiza (AMF), Labrador (2015) and León-Aroca *et al*. (2017) highlight their ability to maintain an efficient absorption of water and nutrients, favor the uptake of primary elements [especially phosphorus] (Abd-Alla *et al*., 2014; Yadav *et al*., 2018) and other essential non-nutritional benefits for the survival of plants (Halder *et al*., 2015; Delavaux *et al*., 2017) without neglecting their contribution in the suppression of pests and diseases (Vázquez, 2015; Jacott *et al*., 2017).

However, the synergism resulting from the incorporation of two rhizospheric microorganisms (*Rhizobium*-AMF) in the radicular system of legumes in general (Larimer *et al*., 2014; Pierre *et al*., 2014) and bean in particular (*P*. *vulgaris* cv. CC-25-9) (Liriano *et al*., 2012) has confirmed its ability to improve the health and productive potential of plants by establishing a tripartite symbiosis with undeniable ecological significance (Bauer *et al*., 2012; Ossler *et al*., 2015) and agriculture (Tajini *et al*., 2012; Brandan de Weht *et al*., 2013; Martin *et al*., 2015).

For this reason, the objective of this work was to evaluate the effect of dual inoculation (*R*. *etli*+AMF) and reduced inorganic fertilization on the production and quality of *P*. *vulgaris* cv. ‘Negro Michigan’ under field conditions in order to know the feasibility of incorporating microorganisms that contribute to improve the performance and protein efficiency in these production systems.

Materials and methods

Location of area of study

The research was conducted during the spring-summer cycle of 2016 in the Experimental Field ‘La Bandera’, which belongs to the Faculty of Agricultural Sciences of the Veracruzana University, Campus Xalapa, located in the municipality of Actopan, Veracruz, at 19° 27’ 30’’ of north latitude, 96° 34’ 20’’of west longitude and average altitude of 360 masl (Vázquez *et al*., 1992).

Bio-inputs used

The bean seeds (*P*. *vulgaris* cv. ‘Negro Michigan’) evaluated were harvested in the Actopan region, Veracruz, Mexico and were purchased from the facilities of the Cotaxtla Experimental Field (CAECOT) of the INIFAP, which is a decentralized organ of the Ministry of Agriculture, Livestock, Rural Development, Fisheries and Food (SAGARPA). These were washed with water and introduced with adequate moisture in plastic bags to add the rhizobacterial inoculant (*R*. *etli*) or mycorrhizal (AMF).

Description of treatments

The treatments evaluated in this work were: T1: (control, T), T2: (fertilized, F), T3: (inoculated with AMF), T4: (inoculated with *Re*), T5: (inoculated with AMF+*Re*), T6: (inoculated with AMF+50%F), T7: (inoculated with *Re*+50%F) and T8: (inoculated with AMF+*Re*+50%F).

Origin of rhizobacterial inoculum

The bacterial inoculum based on *R. etli* was provided by the Beneficial Organisms Laboratory (LOB) of the Faculty of Agricultural Science of the Veracruzana University, Campus Xalapa.

Origin of the mycorrhizal inoculum

The arbuscular mycorrhizal consortium RIN1-UV was provided by the LOB and consisted of *Rhizoglomus intraradices*, *Claroideoglomus etunicatum*, *Gigaspora albida* and *Glomus* sp., with root colonization capacity of ±85%.

Simple inoculation (*Re*) (AMF) and dual (*Re*+AMF)

The simple and dual inoculation of 500 bean seeds cv. 'Negro Michigan' was made in plastic bags where 10 mL of gum arabic was added as adherent, 10 g of mycorrhizal inoculum (AMF), 50 g of the rhizobacterial strain (*Re*, with peat as a carrier and a concentration of 10^5 CFU g^{-1}).) and 50 g *Re* + 10 g of *Re* + AMF, respectively. After 5 min of agitation, the seeds were emptied in unicel trays and dried in the shade for later sowing.

Land preparation and sowing

The experimental unit was conditioned by fallowing and harrowing an area of 93.5 m2 (5.5 mx 17 m), in which two seeds were placed per punch or sowing posture in the groove ridge with spear, at a distance of 25 cm (10 cm bushes $rows^{-1}$) and 25 cm between rows. Each net parcel measured 3.43 m^2 (1.25 m x 2.75 m).

Irrigation

Irrigation rolled to field capacity was applied during the stages of the vegetative and reproductive phase that began after the germination and emergence of the seedlings until before flowering (pre-flowering) and maturation of the first pods.

Fertilization

The first application of the formula 46-00-00 (urea 46% N) was broadcast, when the seedlings had 10 days of germination and the second 30 days later. In the fertilized treatments the application was made at 100% and in the reduced doses only half (50%) of the indicated nitrogen fertilizer was applied.

Experimental design

The experimental design used was a randomized block with eight treatments [T1: (control, T), T2: (fertilized, F), T3: (inoculated with AMF), T4: (inoculated with *Re*), T5: (inoculated with AMF+*Re*), T6: (inoculated with AMF+50%F), T7: (inoculated with *Re*+50% F) and T8: (inoculated with AMF+*Re*+50% F)] and three replications with 500 plants in each where the following variables were measured: height of the plant (cm), diameter of the stem (mm), number of leaves, flowers, pods and nodules (visual count), number of nodes per plant (visual count), weight (g) and grain quality variables (length, width and thickness [mm] and thickness [g]), total protein content (%) (AOAC, 1975) and percentage of root colonization (%) (Giovannetti and Mosse, 1980).

Clearing and staining of roots

The roots harvested at 90 days after sowing (DDS) were fixed in FAA (formaldehyde-acetic acid and alcohol) and their clearing and staining was performed with the technique suggested by Phillips and Hayman (1970).

Percentage of root colonization

This procedure was performed by the grid method of Giovannetti and Mosse (1980), quantifying the presence of fungal structures (hyphae, arbuscules and vesicles) in the vertical and horizontal lines of colonized roots observed with a dissection microscope in each treatment and their respective repetitions.

Statistic analysis

The data obtained were analyzed with the software Statistica (version 8.0, StatSoft Inc., Tulsa, USA) for Windows, and the means were compared by the test of the minimum significant difference LSD of Fisher with a level of significance of 5% (α= 0.05).

Results and discussion

The Anova revealed significant differences in all variables evaluated based on the Tukey test ($p \leq 0.05$). In height, the *Re* and AMF treatments showed increases of 140.35% and 96.85% with respect to control plants, while dual inoculation supplemented with a reduced dose of inorganic fertilizer (AMF+*Re*+50%F) showed greater diameter of the stem (176.39%), number of leaves (201.33%), flowers (329.32%), pods (312.5%) and weight of grains (620.36%) in comparison with control plants (Table 1). These results can be attributed to the fact that the soils where this study was carried out, calcium saturation is high and the assimilable phosphorus content is very low (8-12 ppm) due to the moderately alkaline pH (7.81-8) that immobilizes it and not It is readily available to plants because they are found in insoluble forms (Castañeda, 2000; Zhang *et al*., 2017).

Table 1. Behavior of means for the variables evaluated.

Treatments	Height (cm)	No. of leaves	Stem diameter (mm)	No. of flowers	No. pods	No. of grain	No. of nodules	(%) root colonization
T	37.86d	15e	3.22b	4.16f	4.16d	4.96e	5.5f	3.61e
F	57.76c	21.66de	4.7b	8e	5.36d	10.2d	0g	2.04e
AMF	74.53b	30.16bc	5.71ab	10.8d	11.23a	14.26c	9.23e	86.33a
Re	91a	26.6b	5.4ab	9.16e	7.13bc	11.7cd	24.13b	13.06c
AMF+*Re*	69.3b	31.16bc	5.46ab	12.86c	8.4bc	18.53b	27.6a	85.54a
AMF+50%F	65.8bc	33.13bc	6.1ab	11.30d	7.66bc	20.93b	12.03d	82.78a

Treatments	Height (cm)	No. of leaves	Stem diameter (mm)	No. of flowers	No. pods	No. of grain	No. of nodules	(%) root colonization
Re+50%F	65.13bc	38.93b	7.88ab	14.4b	9.06b	21.23b	17.86c	8.41d
AMF+*Re*+50F	59.43c	45.2a	8.9a	17.86a	17.16a	35.73a	11.86d	82.78a
SD	18	14	5	4	2	9.02	8	39
CV	28	47	92	37	36	52	66	87

SD= standard deviation; CV= coefficients of variation. The averages with different letters in the same column are statistically different (Tukey, $p \leq 0.05$).

Although it has been shown that AMF and some bacterial genera have the property of solubilizing usable phosphates for plants (Martínez *et al*., 2013; Beltran-Pineda, 2014; Sawers *et al*., 2017) and that the predominant function of the first it is the phosphorus absorption (Wilson *et al*., 2012; Smith and Smith, 2012; Sarabia *et al*., 2017) and in the latter the consequent biological nodulation and fixation of nitrogen available for both legume and non-legume hosts (Meng *et al*., 2015), different authors mention natural adaptations attributable to the symbiotic relationships with these microorganisms where the content or availability of these elements is poor or marginal (Rosas *et al*., 1998; Sarabia *et al*., 2010; Rodríguez-López *et al*., 2015; Zhang *et al*., 2016).

In this case, most of the response variables recorded indicate that the best treatment was the interaction between AMF+*Re*+50%F, which not only agrees with the benefits reported by Romero-García *et al*. (2016) regarding the reduction and optimization in the use of fungicides and chemical fertilizers due to the positive effect registered in the phenology and biomass of *P*. *vulgaris*, since Sánchez-Yañez *et al*. (2014) tested a commercial inoculant composed of several microorganisms, among which the AMF and the atmospheric nitrogen fixing bacteria stand out, and showed that when the application of P is reduced (50% nitrogen fertilization and phosphate, FNP) under a system of protected agriculture, bean cultivation is feasible in health and yield with the support of a mixed inoculant (eg Endospore 33®).

Therefore, it is fair to recommend this biological input to farmers who intend to optimize the dose of FNP without risk of affecting the bean's vegetative cycle, the quality of the grain, or its yield.

In relation to the quality of beans, the Anova (Fisher's LSD, $p \leq 0.05$) showed significant differences between the treatments evaluated, being in treatment 8 (AMF+*Re*+50%F) where the total protein content was higher (Table 2). Regarding the seed weight variables (100 units), thickness, width and length, the best treatments were where the simple inoculation was carried out (only AMF or *Re*) (Table 2).

Table 2. Analysis of quality of bean seed.

Treatments	Weight of 100 seeds (g)	Thickness (mm)	Width (mm)	Length (mm)	Total protein (%)
Control	10.82e	3.3e	2.44d	6.29d	15.17d
AMF	15.06a	6.51a	6.58a	10.21a	17.88cd
Re	13.34b	5.7a	6.59a	10.37a	19.22bc

Treatments	Weight of 100 seeds (g)	Thickness (mm)	Width (mm)	Length (mm)	Total protein (%)
F	11.55de	4.15d	5.3c	8.24c	16d
AMF+*Re*	13.03bc	4.77c	6.41ab	9.94ab	20.88bc
AMF+50%F	12.88bc	4.72c	6.43ab	10.01ab	23.99b
Re+50%F	12.05cd	4.64cd	6.57ab	10.14ab	23.89b
AMF+*Re*+50%F	12.41bcd	4.67c	6.24b	9.6b	25.5 a
SD	1.31	0.94	1.37	1.37	3.94
CV	10	19	23	14	19.36

SD= standard deviation; CV= coefficient of variation. Averages with different letters in the same column are statistically different (Tukey, $p \leq 0.05$).

The most satisfactory yields were obtained with the AMF+*Re*+50%F treatment and in terms of seed quality (weight, thickness, length and width) the best treatments were when the plants were inoculated with AMF or with *Rhizobium*.

Finally, it should be noted that with the synergistic interaction between these microorganisms and the reduced fertilizer contribution of the AMF+*Re*+50%F treatment, the highest percentages of protein were obtained (25%), a response that according to Ojeda *et al*. (2014) seems justifiable, if it is taken into account that AMF improve phosphorus absorption through its hyphal network, whereas *Rhizobium* (symbiotic and legume specific bacteria) requires a high demand for this element for the biological fixation of nitrogen, which is taken by plants and transformed into proteins.

In the same way, it agrees with that reported by Vargas-Torres *et al*. (2004) for different cultivars of black beans (Tacana, Huasteco, TLP19 and Veracruz) in which they have found values between 18.9 and 24.2% or 23.41 0.16% in Mayocoba (Carmona-García *et al*., 2007), which they coincide with the quantity and range of proteins indicated in Table 2 (between 15.17 and 25.5%).

Without doubt, this nutritional quality, coupled with the contribution of dietary fiber, minerals (calcium, iron, phosphorus, magnesium and zinc) and vitamins (thiamine, niacin and folic acid) in the diet (Ulloa *et al*., 2011), is transcendent since this legume occupies a very important place as a source of world food and human health (Pujola *et al*., 2007).

Microbial colonization

The largest number of nodules (X-bar 27.6 nodules plant^{-1}) was in the treatment AMF+*Re* (Table 1), qualifying its absence in treatment F and that its amount in the treatment T was low (X-bar 5-6 plant^{-1}). On the other hand, the roots of the control plants presented native mycorrhizal colonization (3.61%), those of treatment 3 (AMF) 86.33% and, in the rest, these were 85.54% (AMF+*Re*) and 82.78% (in AMF+50%F and AMF+*Re*+50%F) (Table 1). Regarding these two variables, these percentages were high in the treatments where both rhizospheric microorganisms (AMF and *Re*) interacted with the bean plants, which can be attributed to the synergy coming from the tripartite symbiosis AMF-bacteria-legume where, as reported in the literature, mycorrhizal colonization optimizes phosphorus absorption and this promotes bacterial nodulation and nitrogen fixation (Rabie *et al*., 2005; Mortimer *et al*., 2008; Javaid, 2010).

Although in treatment T bean plants showed low levels of root colonization with native AMF, their efficiency did not produce the expected agronomic ranges. Then, the functioning of the tripartite symbiosis and adaptation to the conditions where this agrosystem was established can be improved by inoculation with previously selected strains, such as Bouizgarne *et al*. (2015); Miransari (2017) suggest it. The same tendency was observed in the formation of nodules by soil rhizobia, which also showed no beneficial effects on their hosts.

Conclusions

Under the conditions under which the experiment was carried out, the best treatment was AMF+*Rhizobium*+50%F where their symbiotic interaction improved the grain quality of *P. vulgaris* cv. 'Negro Michigan', so that this management can be a viable alternative to replace or at least reduce the use of inorganic fertilizers and in this way, minimize costs to agricultural producers in the Actopan region.

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