International Journal of Agriculture and Crop Sciences.
Available online at www.ijagcs.com
IJACS/2015/8-5/732-737
ISSN 2227-670X ©2015 IJACS Journal

Effects of nitrogen fertilization, inoculation with Rhizobium sp. on the production of biomass, nitrogen content and yield of bean in Oran, Algeria.

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ABSTRACT: Inoculation trials of Vicia faba Aguadulce by Rhizobium sp. strains were conducted in the field during the rainy season in the Misserghin training center in western Algeria. We studied the effect of nitrogen fertilization and inoculation with three symbiotic bacteria (FM.24, FR36, FR26.4.2) previously selected in the greenhouse as the most effective (FM.24 strain), moderately effective (the FR36 strain) and less effective (the FR26.4.2 strain) on the basis of the results of the production of biomass and yield of the variety aguadulcée Vicia faba L under controlled conditions. The obtained results shown that the nitrogen fertilization caused an increase in aboveground biomass and a decrease in biomass nodular unlike inoculation with strains of Rhizobium sp. which caused a significant increase in root and aboveground biomass, increased the height of the aerial parts, and the yield of pods and seeds. In this test, the results obtained were similar to those of green house; the strain FM20.4 native of Mostaganem region remains the most efficient compared to other strains.
Keywords: Vicia faba L, inoculation, nitrogen fertilization, Rhizobium

INTRODUCTION

Legumes were the major direct source of proteins for both man and livestock, especially in poor countries, where animal protein is expensive (Hubbell and Gerald, 2003). They also supplied fat and carbohydrates, besides legumes were rich in minerals for bones construction and essential vitamins to be healthy (Porres et al., 2003). The importance of legumes is that they can fix nitrogen helping symbiotic association with rhizobia, and so they increased the soil nitrogen content (Poth et al., 1986). The bean is one of the main legumes cultivated in Algeria with 58000 hectares wich is equivalent to 44, 3 % of the total surface reserved for this culture (Bousaad and Doumandji, 2004).

The nitrogenous nutrition of legumes was difficult to study because it resulted from the combination of two very different pathways, the fixation of the atmospheric nitrogen and the assimilation of the mineral nitrogen. The contribution of each of these two pathways to nitrogen uptake by plant varied according to the species and culture conditions (Wery, 1987). The improvement of the production of legumes and their use as the cultural precedent thus required a better knowledge of relations between the assimilation and the fixation of nitrogen, on one hand, and the influence of environmental factors and the cultural techniques on these two pathpathways, on the other hand.

The objective of this study is to determine the effects of the biological fertilization on the yield efficiency of Vicia faba L.

MATERIALS AND METHODS

A field experiment on bean was conducted during the 2013-2014 crop year in Oran in western Algeria. The selected site was Misserghine training center. Culture was conducted on an experimental plot of 210 m^2, the ground was prepared by deep plowing, harrowing and leveling and ridging. Soil composite sample was collected from 0 to 15 cm depth and analyzed to study its chemical and physical properties. The plot was then divided into 20 sub-plots of 6 m^2 separated by an alley of 1 m.

The test included five treatments repeated four times in completely randomized blocks. The first one consisted in a supply of nitrogen by using ammonium nitrate at 33.5%, which represented 60 kg of nitrogen per hectare. The second, made up of non-inoculated plants was considered as negative control. The third, fourth and fifth treatments were performed by using inoculation with Rhizobium sp. Strains were selected from the collection of Laboratory of Biotechnology of Rhizobia and Plant Amelioration for their symbiotic performance in greenhouse. The most efficient strain under controlled conditions FM.24 was originated from Mostaganem, the moderately efficient strain FR36 and the least efficient strain FR26.4.2 were from Mascara region. The used variety of Vicia faba aguadulce was provided by Misserghine training center (originated from Spain). Three seeds were placed in a hole on the top of the ridge spacing with 1m (between holes) and 0.5 m (between ridges). The plots were irrigated immediately after sowing and then irrigated every 5 days. Weeding and hoeing were performed manually.

Multifactor analysis of variance (ANOVA) was performed to determine the effect of each treatment on the measured parameters (Aerial parts height, Length of root parts, the aerial parts dry weight, Nodular biomass, Seed and pods yield). Comparisons between means of treatments for various measured parameters were made by calculating the standard error (Gomez and Gomez, 1984). The objective of the statistical analysis was to separate the variation due to treatments, the variation due to soil, climate and site in addition to other intrinsic factors.

RESULTS

Misserghine station is located in western Algeria, it is characterized by a semiarid temperate climate, the average temperature is 19 ° C. The rainy season in which planting was performed extends from September to June, with annual rainfall of 550.9 mm.

The results of the physicochemical analysis (Table 1) showed that soil showed clay loam texture. The organic matter and the nitrogen were of 1.12 and 1.9% respectively. Also the concentration of phosphorus was 14.50 ppm. The soil was moderately alkaline with pH 7.9.

Table 1. Results of physico-chemical analyzes of soil sampled from Misserghine region.

Clay (%)	Silt (%)	Sand (%)	pH	Limestone (%)	Organic matter (%)	Nitrogen (g/kg)	Total phosphor (mg/kg)	available phosphorus (mg/kg)
39.66	37.09	23.25	7.90	21.15	1.12	1.90	269	14.50

After 2 months of culture corresponding to the full flowering when nodulation is potentially optimal (Drevon et al., 2003), we harvested randomly twelve plants per treatment using spade deeply depressed and agitated around the foot for the entire root party where was localized the majority of nodules

Aerial parts height

The results of variation of stem height according to the treatments were shown in Figure (1). This figure showed that inoculation application was beneficial for the development and growth of plants comparing to nitrogen fertilization However, the analysis of variance did not reveal a significant difference at the 5% level between the different studied treatments. Noted that the highest growth in plant heights were obtained with inoculated strains FM.24 and FR 26.4.2 (125 cm).

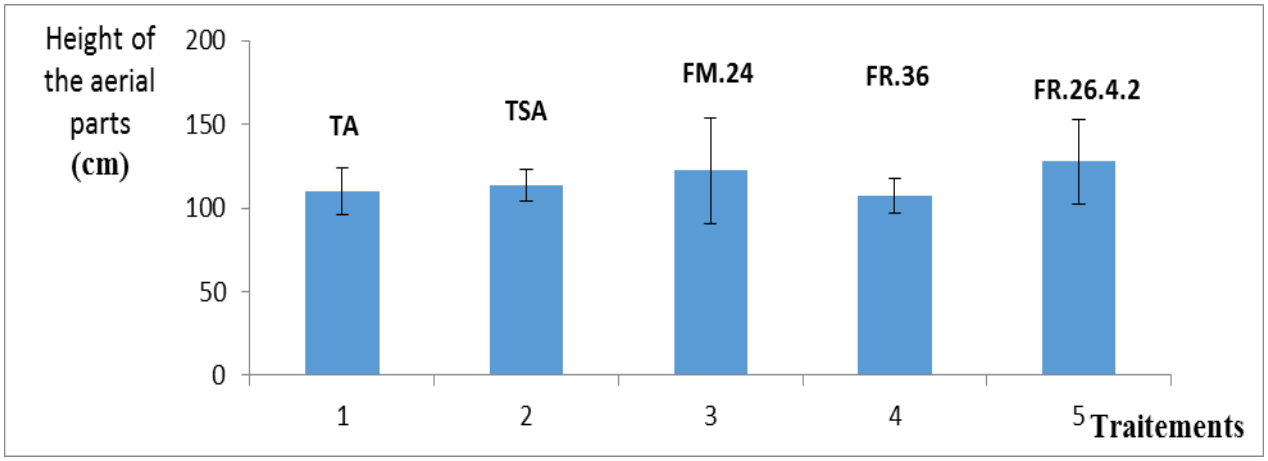

Figure 1. Effect of nitrogen fertilization and inoculation with Rhizobium sp. on aerial parts height of Vicia faba Aguadulce (TA: nitrogen control; TSA: negative control).

Length of root parts

Root growth variation in bean cultivation driving under different treatments was shown in Figure (2). The general appearance of the histograms showed that root length varied depending on nitrogen treatment and the strains used for inoculation. The analysis of variance did not reveal a significant difference at the 5% level between the different treatments studied.

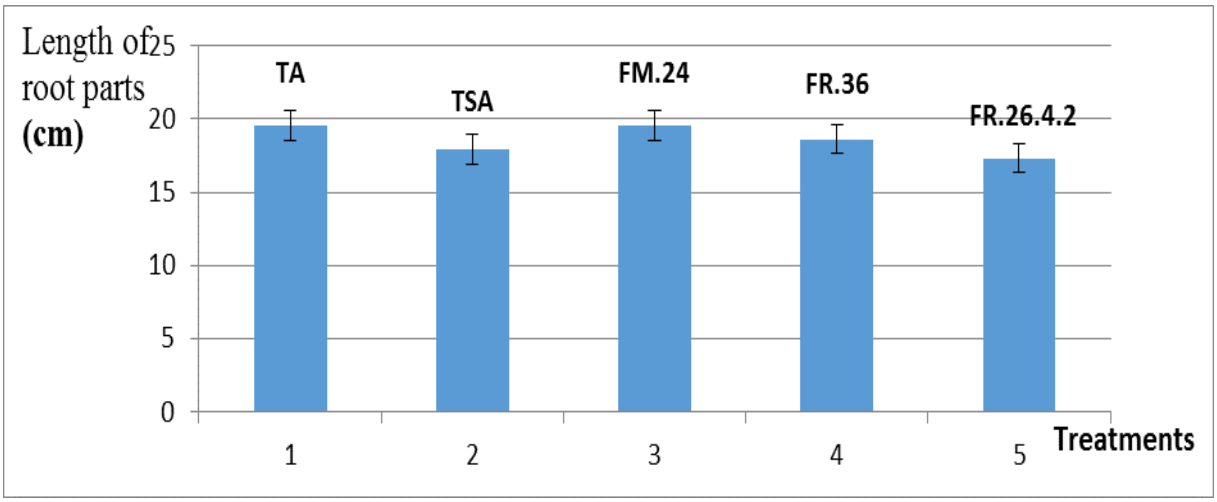

Figure 2. Effect of nitrogen fertilization and inoculation with Rhizobium sp. on root growth of Vicia faba Aguadulce

Indeed, a improvement in root length was observed in response to the supply of nitrogen and the FM.24 inoculation strains (19.58 cm) and FR.36 (18.61 cm), compared with the negative control (17.91 cm).

Measure of the aerial parts dry weight

Aerial dry matter obtained from negative control and sown crops was illustrated in Figure (3) showed that this parameter varied with treatment. According to the histogram, the nitrogen treatment and inoculation with the strain FM 24 had given a better biomass, But this difference was not very important. The statistical analysis of the data did not reveal a significant difference between treatments at 5% level.

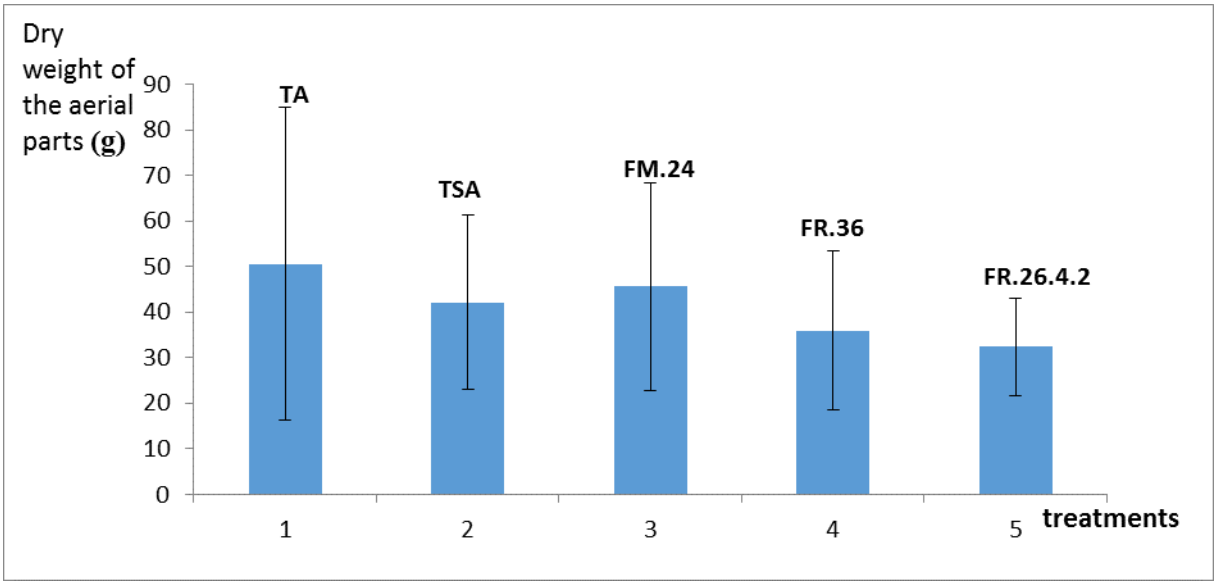

Figure 3. Effect of nitrogen fertilization and inoculation with Rhizobium sp. on the dry weight of the aerial parts of the variety of Vicia faba Aguadulce.

Nodular biomass

Variations, depending on the treatment of nodular biomass recorded during the experimental trial wear shown in figure (4). The inoculated treatment by strain FM24 showed the better nodular biomass but the analysis of variance did not reveal a significant difference at the 5% level between the different studied treatments.

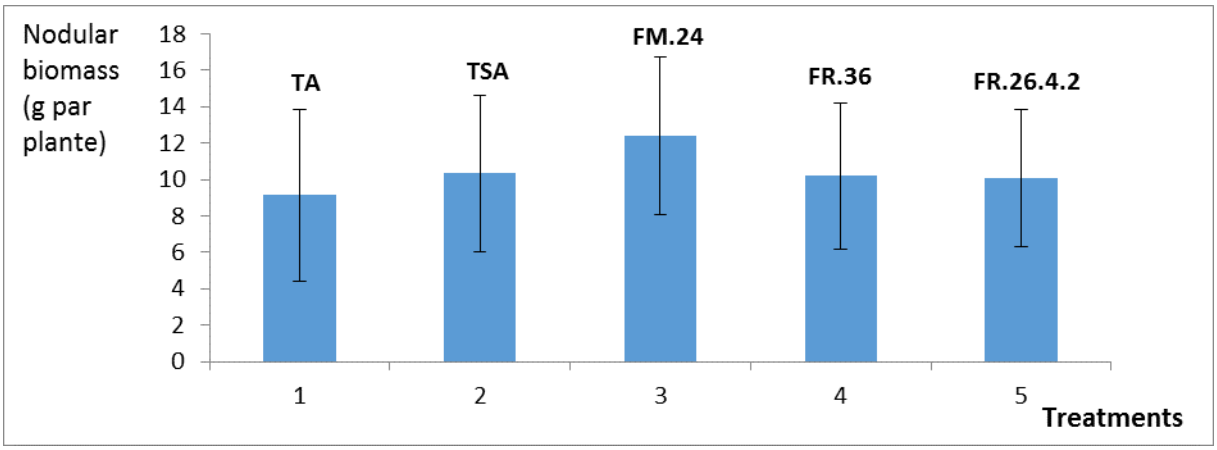

Figure 4. Effect of nitrogen fertilization and Rhizobium inoculation on the nodular biomass of Vicia faba variety of Aguadulce

It appears, first, that culture on soil with non-inoculated control initiated the formation of nodules on the plants roots. Compared with the negative control treatment, the nitrogen treatment resulted in a net decrease in nodular formation.

Regarding the inoculation treatment, they had led to a marked improvement in nodular growth, compared with that recorded on the control soil. These differences were translated into better efficiency of FM.24 strain, compared with FR.36 FR.26.4.2 strains that hadn't shown any effect on the nodular growth.

Seed and pods yield

At maturing step, 13 weeks after sowing, crops were harvested and seed yield and pods for each treatment was recorded.

Pods and seeds number of Vicia faba Aguadulce variety with the five treatments were shown in Figure (5). The general histogram appearance showed that inoculation by FM.24 strain was accompanied by a significant increase in the number of pods and seeds. For both parameters studied, the analysis of variance revealed a significant difference at the 1% level between the different treatments tested with P< 0,0001for pods number and P<0.02 for Seed number. Thus, the Newman-Keuls test at 5% level revealed three homogeneous groups for pods number and two groups for seeds number. The first group included FM.24 strain with the higher number of pods. Negative and positive controls were grouped, on another hand, treatments with the moderate and lesser efficient strains FR. 36 and FR 26.4.2 respectively were completely different. For seeds number, treatment with FM.24 was apart comparatively with other treatments.

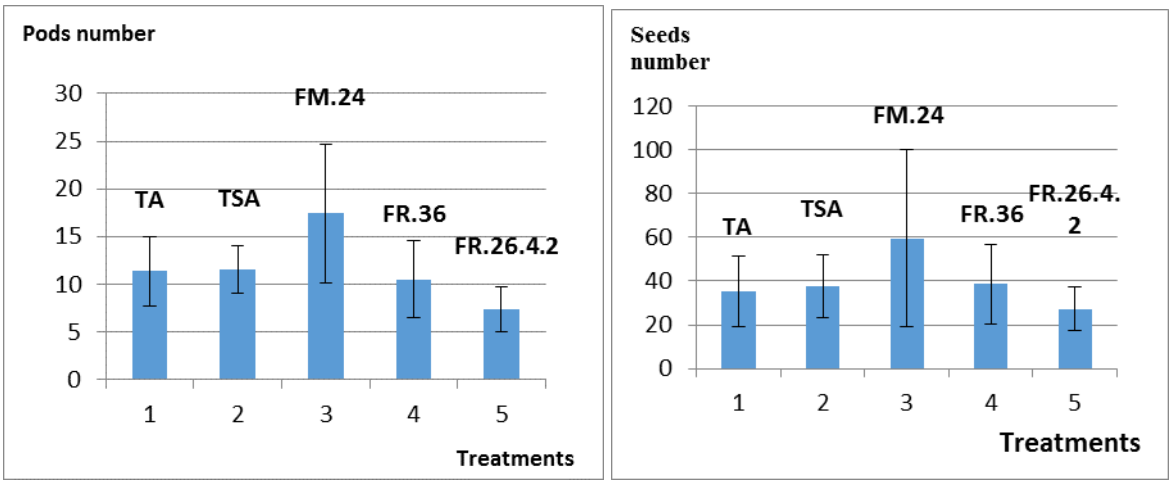

Figure 5. Effect of nitrogen fertilization and inoculation with Rhizobium sp. Performance of Vicia faba Aguadulce variety. Results crop year, 2013-2014, conducted in Misserghine training center. TA: nitrogen control; TSA: negative control, Rhizobium sp. strains. : FM24, FR 36, FR.26.4.2.

DISCUSSION

Bacteria nodulating legume roots are a diverse and ubiquitous group in soils (O'Hara, 2001). In the case of bean, many times, the associated strains presented low infectivity level or were very sensitive to

environmental stresses, which didn't allow effective symbiosis for providing substantial amounts of nitrogen to the host plant and soil. But the great diversity of rhizobia nodulating bean provided an important germplasm natural resource to be exploited for desired characteristics (Sadowsky and Graham, 1998; Dilworth et al., 2001), which could ensure the success of inoculation. Strains with a high ability to survive and great efficiency under various edaphic stresses were attracting more and more interest. Soil type may caused such differences, as has been suggested by Tellawi et al. (1986) who found that this factor significantly affect the number nodule, biomass and absorption of nitrogen. Moreover, it could present little competitiveness with native strains. Indeed, producers need inocula able to survive in disturbed soils, which formed population capable of good nodulation despite edaphic stress. Although the introduction of effective rhizobia of foreign origin presented some success, finding indigenous strains, adapted to local conditions, remains the most potentially effective approach. Indeed, Rodríguez-Navarro et al. (1999) showed that native rhizobia had a higher nitrogen fixation compared to collection strains, which many of them had foreign origin. According to these authors, there was still a good margin of nitrogen fixation enhancement by selection of appropriate native strains.

In this study, the result of physical-chemical analysis of soil showed that nitrogen and organic matter content is moderate for optimal production of field crops (Birhanu Debele, 1980). In addition, phosphorus concentration was adequate for legumes culture (Cottenie, 1980), and sufficient to improve nodulation and nitrogen fixation. The pH value is suitable for the survival of rhizobia (Tate III, 2000).

The variations were observed with the cultivar, the experimental site and strain of rhizobia. Strain Rhizobium sp.FM.24 had proved the higher efficiency on aguadulcée variety of Vicia faba, which resulted in a improvement of aerial parts growth, and root nodule and yield (pods and seeds). In the literature, several studies had reported a positive effect of inoculation leading to a significant improvement in grain yield (Abdel-Ghaffar et al., 1981; Moawad et al., 1994; Mpepereki and Makonese, 1994). The efficiency of strains thus was variable from one region to another. The infectivity of strains FM 24 and FR.36 and FR.26.4.2 probably decreased because of soil and climatic conditions (soil type, temperature and rainfall) or competitiveness with native strains.

Regarding the contribution of nitrogen fertilization, it is well known that the high nitrogen concentration in the soil inhibited nodules formation and N^2 fixation process (Jensen, 1996; Gentili and Huss-Danell, 2003; Xiao et al., 2004; Gentili et al., 2006). Inputs of nitrogen fertilizers in early culture are often necessary to allow an adequate legumes yield but excess inputs usually had an inhibitory effect on N_2 fixation (Zahran, 1999). Salvagiotti et al. (2008) showed a reduction in symbiotic nitrogen fixation (NSF) depending on nitrogen rates made, especially by mineral fertilizer. Nitrates have a greater inhibitory effect on NSF compared to ammonium. It was verified in several works that the presence of nitrate ions inhibited root infection (Abdel-Wahab et al., 1996), nodule development (Imsande, 1986) and nitrogenase activity (Purcell et al., 1990; Sanginga et al., 1996; Arrese-Igor et al., 1997).Our results were according to the previous autors.

We conclude that the inoculation in natura with selected strains from the greenhouse for their infectivity is important to obtained good results in natura. However, the strains selected most be competitive with indigenous strains of the trial region and also support constrains of biotic and abiotic of this region. The strain FM 24 isolated from root nodule of Vicia faba from …. Showed the better result in particular ton the yield of seeds and pods and can be proposed as biological inoculum with to Vicia faba.

ACKNOWLEDGEMENT

Special thanks to Pr. Ighil HARIZ and Pr. Benbayer for their assistance.

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