Article

Effect of biofertilizers on the assimilation of nitrogen by the wheat crop

Oscar Arath Grageda-Cabrera[1§]
Sarahyt Santamaría González-Figueroa[1]
José Antonio Vera-Nuñez[2]
Juan Francisco Aguirre-Medina[1]
Juan José Peña-Cabriales[2]

[1]Bajío Experimental Field-INIFAP. Celaya-San Miguel de Allende Road km 6.5, Celaya, Guanajuato, México. CP. 38110. Tel. 01(461) 6115323, ext. 233. (sarahytgonzalez@hotmail.com). [2]Center for Research and Advanced Studies of the National Polytechnic Institute. Irapuato Unity. Irapuato, Guanajuato, México. (jpena@ira.cinvestav.mx; jvera@ira.cinvestav.mx).

[§]Corresponding author: grageda.oscar@inifap.gob.mx.

Abstract

The crops absorb an average of 20 to 40% of the applied fertilizer, the remaining percentage is lost from the agricultural system by various mechanisms, causing considerable economic losses and environmental contamination. Among the benefits of the use of microorganisms in agriculture is their ability to improve the assimilation of nutrients. A field experiment was carried out whose objective was to evaluate the effect of inoculation of bacterial and fungal biofertilizers on the efficiency of assimilation of nitrogen fertilizer in wheat cultivation. The experimental design was randomized complete blocks with four repetitions and nine biofertilization treatments using the ^{15}N isotopic dilution technique to determine the N in the fertilizer derived plant and the fertilizer utilization percentage N. The chemical fertilization produced the highest grain yields and ensures its quality. The inoculation of wheat with HVA significantly increased the yield of grain up to 1 291 kg ha^{-1}, the amount of N in the plant from the fertilizer up to 15 kg and the utilization efficiency of nitrogen fertilizer up to 11% compared to the witness without inoculating. There were significant differences in plant-microorganism interaction in biomass production and assimilation of N.

Keywords: *Triticum aestivum*, ^{15}N isotope dilution, fertilizers, inoculants.

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Introduction

In the central region of Mexico known as "The Bajio" (*ca*. 1.1 x 10^6 ha) 40 years ago, 150 kg N ha^{-1} was applied to the wheat crop as a synthetic fertilizer and yields of 5 Mg ha^{-1} were obtained. Currently they apply up to 350 kg N ha^{-1} and the yields have not increased significantly. Studies conducted in the region show that the wheat crop absorbs an average of 20 to 35% of the N applied as fertilizer (Grageda *et al*., 2011), the remaining percentage is lost from the agricultural system by various mechanisms, causing economic losses and contamination environmental, such as eutrophication, acid rain, destruction of the stratospheric ozone layer and increase of the greenhouse effect (Duxbury, 1994; Franzluebbers, 2005; Grageda-Cabrera *et al*., 2005).

The increase in the price of nitrogen fertilizers from $2 049 in the year 2000 to $6 800 in 2015 (t of urea) makes wheat production more expensive and reduces profitability. Faced with this problem, it is necessary to develop technologies that reduce the use of synthetic fertilizers, which can be achieved through the application of beneficial microorganisms. The development and use of biofertilizers is considered as an important alternative for the partial or total substitution of synthetic fertilizers (Caballero *et al*., 1992; Aghilia *et al*., 2014). Currently there is a wide variety of biofertilizers made with microorganisms such as bacteria and fungi, with various functions and according to the type of crop (Pooja *et al*., 2007; All-Taweil *et al*., 2009).

On the other hand, the estimation of the assimilation by plants of soil nutrients and fertilizers is necessary to know the effects of the specific interactions between genotype-microorganism-environment. However, the estimation is difficult because once the nutrient is part of the plant it is usually impossible to determine its origin. There are several methods to quantify the assimilation of N and all have their advantages and disadvantages. However, the ^{15}N isotopic techniques are considered as the most reliable to provide quantitative and integrated values of the N assimilated by the plant. For the aforementioned, the objective of the present study was to quantify the effect of the inoculation of bacterial and fungal biofertilizers on the assimilation of nitrogen fertilizer by wheat cultivation.

Materials and methods

An experiment was established under field conditions in the Bajio Experimental Field (CEBAJ) of the National Institute of Agricultural and Cattle Forestry Research (INIFAP) in Celaya, Guanajuato, Mexico, located at 20° 35' 06.59'' north latitude, 100° 49' 46.84'' west longitude and altitude of 1 769 m. In the region an average annual rainfall of 650 mm is recorded between june and august and an average annual temperature of 18 °C (maximum 28 °C and minimum 10 °C). The physical and chemical analysis of the soil showed that it is a pelvic vertisol with pH (1:2 water) of 7.9, organic matter content of 1.85% and loamy clay texture (FAO, 1994).

The Nana wheat variety was used and nine treatments were evaluated, which are described in Table 1. The experimental design was randomized complete blocks with four repetitions per treatment; each experimental unit was assigned five rows with a double row of 0.76 m in width and 3 m in length. The agronomic practices were carried out according to the recommendations proposed by the INIFAP for the sowing of wheat in furrows.

Table 1. Treatments to evaluate the effect of biofertilizers on the assimilation of N derived from the fertilizer (N ddf) by the wheat crop.

Inoculant	Fertilization N-P-K (240-60-00) (%)
T1. Without biofertilizer	100
T2. Without biofertilizer	50
T3. Bacterium 2709; species: *Pseudomonas* sp.; origin: State of Guanajuato; concentration: 10^{11} UFC g^{-1} of product.	50
T4. Bacterium T4; species: *Bacillus* sp.; origin: State of Guanajuato; concentration: 10^{11} UFC g^{-1} of product.	50
T5. Mycorrhiza INIFAP^{MR}; species: *Rhizophagus intraradices*; origin: State of Nuevo Leon; concentration: 75-80 spores g^{-1} product.	50
T6. Mycorrhiza CH1; species: consortium *Gigaspora* sp. and *Glomus* sp.; origin: State of Chiapas; concentration: 75-80 spores g^{-1} of product.	50
T7. Mycorrhiza DA18; species: *Scutellospora calospora*; origin: State of Guanajuato; concentration: 75-80 spores g^{-1} of product.	50
T8. Mycorrhiza PI63; species: *Acaulospora scrobiculata*; origin: State of Guanajuato: concentration: 75-80 spores g^{-1} of product.	50
T9. Mycorrhiza PR82; species: consortium *Gigaspora albida* and *Rhizophagus sinuosum*; origin: State of Hidalgo; concentration: 75-80 spores g^{-1} of product.	50

The evaluated biofertilizers were selected by previous studies of biological effectiveness in greenhouse conditions and are protected in the Microbial Cepario of the CEBAJ-INIFAP. The treatments with biofertilizers were inoculated 2 h before sowing. In the case of bacterial inoculants, the seed was treated with the biofertilizer containing 10^{11} CFU g^{-1} of product at a dose of 1.5 kg of inoculant per 50 kg of seed. In the case of mycorrhizae, the seed was treated with the biofertilizer containing 75-80 spores g^{-1} product at a dose of 1 kg of inoculant per 50 kg of seed.

The 100% fertilization was 240-60-00. The source of fertilizer N was ammonium sulfate and it was divided into two applications, 50% at planting and 50% at 40 days after sowing (dds), all P was applied at the time of planting as triple superphosphate. A linear 1 m isotopic plot was installed in each furrow in each treatment and ammonium sulfate enriched with 3% of atoms in excess of ^{15}N was applied.

The variables flowering, physiological maturity, dry weight of grain, straw and total, harvest index, N in grain, straw and total, N derived from fertilizer (N ddf), harvest index of N ddf, and efficiency in the use of N ddf (UN ddf).

The determination of total N was made by the Kjeldahl method (Bremner and Mulvaney, 1982). In the samples containing ^{15}N, the enrichment was determined by optical emission spectrometry (NOI-6e spectrometer), according to the procedure described by Faust *et al*. (1987). Isotope calculations of 15N were determined by the isotope dilution method (Zapata, 1990).

The data were analyzed statistically following the standard variance analysis procedure and Tukey's multiple means comparison test (SAS Institute, 2014).

Results and discussion

Yield

The yield data of dry matter of grain, straw, total and harvest index are presented in Table 2. Statistical differences existed ($p \leq 0.05$) in most of the treatments evaluated, several studies in wheat have shown differences due to the plant-microorganism-environment interaction (Khalid *et al*., 2004; Ferraris and Couretot, 2006; Arias, 2007). Regarding grain production, the highest yield was obtained in the T1 treatment (regional control without biofertilizer and with 100% fertilization) and the lowest in the T2 treatment (control without biofertilizer and with 50% fertilization), in the crop of wheat reduce fertilization causes a reduction in the yield of 1 475 kg ha^{-1} so this practice is not recommended. The application of bacteria in the wheat yield had a small effect if the T3 vs T2 were compared and negative if the T4 vs T2 were compared, the low yields in T4 could be due to the negative effect of certain plant-microorganism relationships in inhibiting the radical development and reduce the efficiency in the intake of nutrients such as N and P, especially in alkaline P soils (Afzal *et al*., 2005; Barea *et al*., 2005; El-Sirafy *et al*., 2006).

Table 2. Effect of the application of biofertilizers on the accumulation of biomass in the wheat crop.

Treatment	Dry weight (kg ha^{-1})			Index of harvest
	Grain	Straw	Total	
T1. Without biofertilizer (F 100%)	6 790 a	9 772 bc	16 563 a	0.41 ab
T2. Without biofertilizer (F 50%)	5 315 e	6 497 g	11 812 e	0.45 a
T3. Bacterium 2709 (F 50%)	5 545 d	10 298 a	15 843 b	0.35 c
T4. Bacterium T4 (F 50%)	5 166 e	8 429 e	13 595 d	0.38 bc
T5. Mycorrhiza INIFAP^{MR} (F 50%)	6 368 b	8 794 d	15 162 c	0.42 ab
T6. Mycorrhiza CH1 (F 50%)	6 606 a	9 506 c	16 112 ab	0.41 a
T7. Mycorrhiza DA18 (F 50%)	5 795 c	8 002 f	13 797 d	0.42 ab
T8. Mycorrhiza PI63 (F 50%)	5 336 e	9 910 b	15 247 c	0.35 c
T9. Mycorrhiza PR82 (F 50%)	5 335 e	8 705 de	14 040 d	0.38 bc
CV (%)	6.48	11.55	8.52	8
DSH	207.9	331.82	539.26	0.04

Averages with different letter in the same column are statistically different Tukey ($p \leq 0.05$).

A greater effect on wheat yield was observed with the mycorrhizae applied in treatments T6 and T5, which coincides with that reported by Kumar *et al*. (2011), in field trials with two wheat genotypes had increases of up to 25.8% when the seeds were inoculated with mycorrhizae. In this case, T6 could be an option to reduce the risk of environmental contamination by applying only 120 kg ha^{-1} of N. In this case, AMF had a greater effect on wheat yield than bacteria, in this respect Peltzer *et al*. (2003), observed a null effect of the inoculation on the yield and weight of wheat grain with *Pseudomonas fluorescens*. In addition, another cause may be the immobilization of nutrients when microorganisms decompose organic waste (Grageda-Cabrera *et al*., 2003).

Bolletta *et al*. (2002), in plots of wheat inoculated with mycorrhizae obtained between 15 to 18% more yield than the unfertilized controls, in this trial the differences disappeared when compared with the treatments fertilized at 100%.

Regarding the production of total dry matter, all treatments exceeded T2, which was related to greater production of straw. The highest production was obtained in treatments T1 and T6. The reduction in fertilization caused a reduction in the total biomass of 4 751 kg ha^{-1}. This is confirmed by the harvest index (IC), which is an indicator that provides information on grain production compared to all the biomass produced. The data show that the inoculation of biofertilizers affected the harvest index, treatments T3 (bacterium 2709) and T8 (mycorrhizae PI63) negatively affected this parameter, the crop produced more straw than grain compared to the other treatments, which leads to an efficient use of nutrients.

Total nitrogen

In the Table 3 shows the assimilation of total N. The statistical analysis indicates that there are significant differences similar to those observed in the total dry weight (Table 2). Absorption of N is directly related to performance (Sprent, 1987).

Table 3. Effect of the application of various biofertilizers on the accumulation of N in the wheat crop.

Treatment	N (kg ha^{-1})			Harvest index of N
	Grain	Straw	Total	
T1. Without biofertilizer (F 100%)	159 a	61 b	219 a	0.72 bc
T2. Without biofertilizer (F 50%)	123 e	40 f	163 g	0.75 a
T3. Bacterium 2709 (F 50%)	129 d	70 a	198 c	0.65 e
T4. Bacterium T4 (F 50%)	119 e	55 d	174 f	0.69 d
T5. Mycorrhiza INIFAP^{MR} (F 50%)	150 b	56 cd	205 bc	0.72 bc
T6. Mycorrhiza CH1 (F 50%)	153 b	59 bc	212 ab	0.72bc
T7. Mycorrhiza DA18 (F 50%)	137 c	50 e	186 d	0.73 ab
T8. Mycorrhiza PI63 (F 50%)	124 de	61 b	185 de	0.67 de
T9. Mycorrhiza PR82 (F 50%)	124 de	54 d	178 ef	0.7 cd
CV (%)	4.57	6.43	5.65	2.01
DSH	5.11	3.28	7.59	0.02

Averages with different letter in the same column are statistically different Tukey ($p \leq 0.05$).

Regarding the content of N in grain, the treatments T5 and T6 were statistically equal and surpassed only by the treatment fertilized to 100% of the dose, reason why these biofertilizers contributed to the accumulation of N (protein) in the grain. This same effect was observed for total N in the plant, with values between 205 to 212 kg N ha^{-1}. The treatments that accumulated less amount of N in grain were T4, T8 and T9, which corroborates that the biofertilizers do not work of generic form, these must be generated for specific regions of production.

Regarding the harvest index of N (ICN), it was significantly affected by biofertilizer treatments. The Tukey test establishes five groups of treatments with a significant response to ICN, the largest is associated with ICN> 0.73 and the lowest ICN< 0.67. The highest ICN was 0.75, which indicates that the plant used 75% of its total nitrogen for grain. But it is not associated with the highest grain yields as observed in the fertilized treatment with an ICN of 0.72. The application of biofertilizers influenced the assimilation of N and the content of N in grain; these characteristics are related to the amount of protein, carotene content and quality (Degidio *et al*., 1993). On the contrary, the dilution of the N content in grain causes an increase in the percentage of white belly and reduces the quality (Mahdi *et al*., 1996).

Nitrogen derived from fertilizer (N ddf) and percent utilization of nitrogen fertilizer (% UFN)

The results of the amount of N in the plant derived from the fertilizer are presented in Table 4. The treatment that accumulated the greatest amount of N of the fertilizer was T1 with a total of 83 kg N ddf ha^{-1}, which indicates that the Wheat took 136 kg ha^{-1} N from the soil. This treatment received twice as much nitrogen fertilizer as the other treatments, which was reflected in a greater assimilation of N ddf. T5 and T5 treatments followed with 48 and 53 kg N ddf ha^{-1} each, these treatments take advantage of more N of the soil with 157 and 159 kg ha^{-1}, respectively. The grain presented greater N ddf than straw.

Table 4. Effect of the application of biofertilizers on the accumulation of nitrogen derived from the fertilizer (N ddf) in the wheat crop.

Treatment	Nitrogen derived from fertilizer (N ddf) (kg N ddf ha^{-1})		
	Grain	Straw	Total
T1. Without biofertilizer (F 100%)	60 a	23 a	83 a
T2. Without biofertilizer (F 50%)	29 d	9 f	38 g
T3. Bacterium 2709 (F 50%)	26 f	14 c	41 e
T4. Bacterium T4 (F 50%)	28 e	13 d	41 e
T5. Mycorrhiza INIFAP^{MR} (F 50%)	35 c	13.5 cd	48 c
T6. Mycorrhiza CH1 (F 50%)	38 b	15 b	53 b
T7. Mycorrhiza DA18 (F 50%)	35 c	13 d	48 c
T8. Mycorrhiza PI63 (F 50%)	28 e	14 c	42 d
T9. Mycorrhiza PR82 (F 50%)	28 e	12 e	40 f
CV (%)	3.7	2.46	0.75
DSH	0.57	6.83	3.87

Averages with different letter in the same column are statistically different Tukey ($p \leq 0.05$).

The results of the percentage of N ddf and UFN are presented in Table 5. Of the total N assimilated by the crop, less than 40% came from the nitrogen fertilizer and the rest of the soil. There was a significant difference ($p \leq 0.05$) in the values of the percentage of N ddf in the crop between biofertilization treatments. The highest values were obtained in T1, with 38% N ddf, which raises the need to review the current application form of the fertilizer to improve its use. Regarding the effect of biofertilizers, those that contributed to the plant assimilating a greater quantity of Nddf were the treatments with mycorrhizae T5 and T6, with 23 and 25% N ddf.

Table 5. Effect of the application of biofertilizers on the accumulation of nitrogen derived from the fertilizer (N ddf) and the percentage of nitrogen fertilizer utilization (% UFN) in the wheat crop.

Treatment	N ddf (%)	UFN (%)
T1. Without biofertilizer (F 100%)	37.75 a	32 c
T2. Without biofertilizer (F 50%)	23.75 cd	29.75 e
T3. Bacterium 2709 (F 50%)	20.75 f	31.25 cd
T4. Bacterium T4 (F 50%)	23.5 de	31 ced
T5. Mycorrhiza INIFAP^{MR} (F 50%)	23.25 de	37.25 b
T6. Mycorrhiza CH1 (F 50%)	25 bc	41 a
T7. Mycorrhiza DA18 (F 50%)	26 b	37 b
T8. Mycorrhiza PI63 (F 50%)	22.75 de	32 c
T9. Mycorrhiza PR82 (F 50%)	22.25 e	30.25 ed
CV (%)	5.09	6.81
DSH	1.26	1.46

Averages with different letter in the same column are statistically different Tukey ($p \leq 0.05$).

The percentage of UFN indicates the amount of N that assimilated the crop of the total of N applied as fertilizer. It was observed that inoculation with T5 and T6 mycorrhiza contributed to the plant assimilating a greater amount of N than applied as fertilizer, improving with this the percentage of UFN compared to T1, which had greater availability of N.

The best treatments in terms of percentage of UFN were T6 (Mycorrhiza CH1), T5 (Micorriza INIFAPMR) and T7 (Mycorrhiza DA18), in these cases the plant assimilated 41, 37 and 37% of the total applied fertilizer, representing 52.8, 48.2 and 48 kg N ha^{-1}, respectively.

Even when inoculation with biofertilizers improved the percentage of UFN, it was low (less than 45%). More 55% of the N applied as fertilizer could not be counted, surely a high proportion was lost as nitrates by leaching or in gaseous form by denitrification, nitrification and volatilization because it is a slightly alkaline soil and retains moisture (Vermoesen *et al.*, 1993; Grageda-Cabrera *et al*., 2011; Wanga *et al*., 2015).

Conclusions

The chemical fertilization produced the highest grain yields and ensures the quality of the mime. The inoculation with HVA increased the grain yield of the wheat crop up to 20% in comparison with the control without biofertilizer with the same dose of fertilization. Inoculation with HVA influenced the crop index of both dry matter and N. The biofertilizers with HVA increased the absorption of nitrogen fertilizer in a range of 2 to 15 kg N ha^{-1} more than the control without inoculation. The efficiency in the use of nitrogen fertilizer (% UFN) was increased with the inoculation of HVA.

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