



Advances in biology through agronomy, aquaculture, coastal and environmental sciences

— Volume II —

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**Advances in biology through
agronomy, aquaculture, coastal and
environmental sciences**
Volume II



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Advances in Biology through Agronomy, Aquaculture, Coastal and Environmental Sciences, Volume II, is an e-book that continues the work of the first version (<https://editorapantanal.com.br/ebooks/2023/advances-in-biology-through-agronomy-aquaculture-coastal-and-environmental-sciences/ebook.pdf>), published in 2023 by Leandris Argente-Martínez and Ofelda Peñuelas-Rubio.

Our e-book compiles 25 manuscripts that present scientific results and reviews based on biological aspects. All of them contribute to explaining the responses of living organisms to agricultural, aquaculture, and coastal ecosystems, as well as those facing fragility or threat of climate change. This e-book was edited by Leandris Argente-Martínez, Ofelda Peñuelas-Rubio, and Joe Luis Arias-Moscote, members of the Consolidated Academic Group ITVAYA-CA-3.

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Impact of biofertilizers on wheat (*Triticum aestivum* L.) yield in different environments of the Bajío region of Guanajuato

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ABSTRACT

The excessive use of synthetic chemical fertilizers in wheat cultivation increases production costs, degrades the physical and chemical properties of the soil, and affects crop profitability. The objective of this study was to identify the effects of combining synthetic chemical fertilizers with biofertilizers on wheat yield, yield components, and physiotechnical variables (NDVI and SPAD values) under conditions in Bajío, Guanajuato, Mexico. The experiments were conducted at two groups of locations during the 2017–2018 fall–winter growing season. The first group consisted of four locations, and the second consisted of two locations. Two microbial compounds were evaluated: 1) the first formulated on the basis of *Azospirillum brasilense* + a consortium of the arbuscular fungus *Rhizophagus irregularis* (BIO1) and 2) the second on the basis of *Streptomyces* spp., *Bacillus thuringiensis*, *Trichoderma longibrachiatum*, *Pseudomonas*, *Mycorrhiza*, and *Azospirillum* (BIO2). In the first group of locations, the following treatments were evaluated: a) application of 100% NPK chemical fertilizer (280–98–20); b) application of 75% NPK chemical fertilizer (210–74–15); c) application of 75% NPK chemical fertilizer + BIO1; d) application of 75% NPK chemical fertilizer + BIO2; and e) a control (no application of chemical fertilizer or biofertilizer). In the second group of locations, the following treatments were evaluated: a) application of 100% of the NPK chemical fertilizer dose (280–98–20); b) application of 50% of the NPK chemical

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fertilizer dose (140–49–10); c) application of 50% of the NPK chemical fertilizer dose + BIO1; d) application of 50% of the NPK chemical fertilizer dose + BIO2; and e) the control plot. In the first group of locations, the results of the SREG analysis revealed statistically significant differences because of the interaction between environments and treatments, where treatments explained 97.5% of the variance in grain yield, whereas locations accounted for 1.64%. Two contrasting response patterns were observed, which were associated mainly with the environmental conditions at each location. In terms of the first response pattern, the yield was greater at the three sites; the yield was particularly enhanced by 100% in response to chemical fertilization and the two types of biofertilizers. A second response pattern was observed at a single location, which was situated in a different sector from the rest of the sites and where the treatment with 75% chemical fertilization yielded the highest results. In general, the treatments with the highest yields were the treatments with 100% chemical fertilization (6.6 t ha⁻¹) and those with 75% chemical fertilization supplemented with BIO2 and BIO1 (6.4 and 6.5 t ha⁻¹, respectively). In the second group of sites, fertilization with 50% chemical fertilizer was evaluated; however, no significant interaction was observed. The BIO2 + 50% chemical fertilizer treatment performed statistically the same as the 100% treatment and better than the 50% chemical fertilizer treatment did.

Keywords: interactions between locations and treatments, microbial compounds, synthetic chemical fertilization.

INTRODUCTION

Fertilizers significantly increase global food production. Research shows that between 30% and 50% of crop yields are attributed to fertilizer application (Yan et al., 2022). This contribution explains why many farmers believe that applying more fertilizer will lead to higher yields (Verhulst et al., 2010). In the Bajío region of Guanajuato, over the past ten years, an average annual production of 318,988.43 t has been achieved, representing 10% of national production (SIAP, 2026). A large proportion of producers in the region make excessive use of inorganic fertilizers, which increases production costs (Gonzales-Figueroa et al., 2022). The use of excessive doses of inorganic fertilizer contributes to environmental impacts that harm the physical, chemical, and biological properties of the soil (Orosco-Corral et al., 2016). The amount of fertilizer applied per unit of cultivated land has increased nearly eightfold since 1961 (Lu; Tian, 2017), and excessive nitrogen input has led to significant losses in the soil through ammonia (NH₃) volatilization, nitrous oxide emissions, surface runoff, and nitrate leaching (Zhou et al., 2016; Huddell et al., 2020). Therefore, it is necessary to utilize technological innovations that help reduce environmental impact as well as lower production costs. One alternative is the use of biofertilizers made from bacteria or fungi, which enable plants to acquire nutrients to improve their growth and yield (Aghili et al., 2014). The term “biofertilizer” has a very broad interpretation, encompassing the effects of microorganisms, green manure, manure, and even plant extracts containing microorganisms. When inoculated, these

agents can live in association or symbiotically with plants, aiding in nutrient assimilation, protection, and increased yield across multiple crops (Nagrle et al., 2023). The objective of this chapter was to identify the effects of the use of inorganic fertilizers combined with biofertilizers on wheat yield, yield components, and physiotechnical variables (NDVI and SPAD values) in the Guanajuato Bajío region.

MATERIALS AND METHODS

Study sites

The evaluation was conducted in five municipalities within the Bajío Guanajuatense region. The evaluation sites were plots of land belonging to cooperating farmers who participated in the MasAgro Guanajuato Project. During the 2017–2018 fall–winter growing season, trials were conducted at various locations within the municipalities of Irapuato (20°30'33"N, 101°17'46"W, 1,730 m), Pénjamo (20°18'53"N, 101°50'09"W, 1,689 m), Salamanca (20°30'31.1868"N, 101°13'27.066"W, 1,721 m), Yuriria (20°20'72.059"N, -101°16'79.928"W, 1,776 m) and Villagrán (20°16'52"N, 101°10'04"W, 1,726 m).

Soil sampling and conditions

Prior to setting up each experiment, soil samples were collected using the “zigzag” method, with 15 subsamples taken to a depth of 30 cm. The subsamples were mixed to obtain a 1-kilogram composite sample, which was sent to the FERTILAB laboratory in the city of Celaya, Guanajuato. In general, the soils are characterized as vertisols with a clay texture; the organic matter concentration ranges from low to moderately high, the chemical reaction is moderately alkaline (with the exception of Irapuato), and the nitrate concentration ranges from low to moderately high; the phosphorus concentration is extremely high except in Villagrán and Yuriria, and the potassium concentration ranges from moderate to very high. The remaining soil fertility parameters are shown in Table 1.

Table 1. Soil fertility parameters of the evaluation sites in the autumn–winter cycle from 2017–2018 in the Bajío region, State of Guanajuato, Mexico.

Site	pH	NO ₃ (mg kg ⁻¹)	P (mg kg ⁻¹)	K (mg kg ⁻¹)	POM (%)	HC (cm/h)	PSI (%)	PTC (%)
Irapuato	6.4	14.8	98	622.8	3.2	0.4	3	0
Pénjamo	7.6	4.4	66.5	793.8	3.2	0.1	6.7	2
Salamanca	8	22.5	95.8	1179.3	3.2	0.1	18	4.6
Villagrán	7.7	6.2	41.5	578.2	1.3	0.5	4	2.3
Yuriria	8	3.9	2	699	1.4	0.2	6.7	2.2

pH = Hydrogen potential; NO₃ = Nitrates; P = Phosphorus; K = Potassium; POM % = Percentage of organic matter; HC = Hydraulic conductivity; ESP = Exchangeable sodium percentage; PTC % = Percentage of total carbonates.

Soil management

Conservation agriculture (CA) was implemented in the Irapuato, Villagrán, and Salamanca experiments in 2013, as well as in Pénjamo and Yuriria in 2014. Planting was carried out in 150 cm wide permanent raised beds, each with six planting rows spaced 15 cm apart. Before planting, the beds were reshaped by reworking the furrows. The crop rotation at the evaluation sites consisted of corn during the spring–summer cycle and wheat during the fall–winter cycle. On average, 13.2 t ha⁻¹ of corn residue and 7.15 t ha⁻¹ of wheat residue were produced per cycle. All of this crop residue was left on the soil surface.

Weather conditions

The average maximum, minimum, and mean temperatures at the monitoring sites were 26.6 °C, 8.2 °C, and 17.2 °C, respectively (Table 2). The cumulative precipitation during the cycle was 25.5 mm, and the cumulative solar radiation was 2494.4 MJ m⁻².

Table 2. Climate conditions at the evaluation sites during the 2017–2018 fall–winter cycle in the Bajío region, State of Guanajuato, Mexico.

Site	Temperature (°C)			Accumulated precipitation (mm)	Accumulated solar radiation (MJ m ⁻²)
	Maximum	Minimum	Mean		
Irapuato	27.6	8.3	18.0	19.6	2530.1
Salamanca	26.9	8.0	17.4	2.2	2754.1
Pénjamo	25.9	8.4	17.1	20.8	2299.1
Yuriria	26.6	5.4	16.0	51.8	2305.5
Villagrán	26.2	10.9	18.6	33.2	2583.0

Experimental design

A completely randomized block design with three replicates was used. The experimental unit (EU) had an area of 24 m² and consisted of three beds that were 1.6 m wide and 5 m long. Two microbial mixtures were evaluated: 1) a mixture based on *Azospirillum brasilense* + a consortium of the arbuscular mycorrhizal fungus *Rhizophagus irregularis* (BIO1) and 2) a mixture based on *Streptomyces* spp., *Bacillus thuringiensis*, *Trichoderma longibrachiatum*, *Pseudomonas*, mycorrhizal fungi, and *Azospirillum* (BIO2, CreCR®) (Tierra del Monte®, Querétaro, Mexico). The microbial mixtures were evaluated under the following treatments: a) application of 100% NPK chemical fertilizer (280–98–20); b) application of 75% NPK chemical fertilizer (210–74–15); c) application of 75% NPK chemical fertilizer + BIO1; d) application of 75% NPK chemical fertilizer + BIO2; and e) a control (no application of chemical fertilizer or biofertilizer). In the localities of Yuriria and Villagrán, the following were evaluated: a) application of 100% NPK chemical fertilizer (280–98–20); b) application of 50% NPK chemical fertilizer (140–49–10); c) application of 50% NPK chemical fertilizer + BIO1; d) application of 50% NPK chemical fertilizer + BIO2; and e) the control treatment.

The arbuscular mycorrhizal consortium (BIO1) consists of *Glomus dimorphicum*, *Glomus monosporum*, *Rhizophagus proliferus*, *Rhizophagus fasciculatus*, *Funnelformis mosseae*, and *Scutellospora cerradensis*, formulated as a soil-like granule, and *Azospirillum brasilense*, formulated as a granular inoculant. The microbial compound (BIO2) consists of *Streptomyces* spp., *Bacillus thuringiensis*, *Trichoderma longibrachiatum*, *Pseudomonas*, mycorrhizal fungi, and *Azospirillum*, formulated as a powdered suspension.

Management of treatments using microorganisms

Treatment BIO1: Inoculation was performed prior to sowing. A wetting agent solution (water + wetting agent contained within the product) was prepared and poured over the seed; subsequently, *Azospirillum brasilense* and the arbuscular mycorrhizal consortium were added to the seed and mixed until the biofertilizer dose adhered to the seed. A foliar booster application of the same mixture was performed after the second supplementary irrigation (Table 3).

BIO2: The first application was made 15 days after planting and was applied as a drench, targeting the base of the plant. A second application was made 60 to 70 days after planting, also as a drench.

Table 3. Method and dose of application of treatments at the evaluation sites in the autumn–winter cycle 2017–2018 in the Bajío region, State of Guanajuato, Mexico.

Treatments	Application mode	Dose	Water (L)
mycorrhizal fungi + <i>Azospirillum brasilense</i> (BIO1)	Inoculation	1 kg of mycorrhizal fungi+ 380 gr of <i>Azospirillum brasilense</i> ha ⁻¹	12
mycorrhizal fungi + <i>Azospirillum brasilense</i> (BIO1)	Drench	3 kg of mycorrhizal fungi + 1 L of <i>Azospirillum brasilense</i> ha ⁻¹	200
Microbial formulation (BIO2)	Drench	1 kg ha ⁻¹	200

FERTILIZATION MANAGEMENT

The fertilizer application rate ranged from 280–98–20 (NPK). The fertilizer sources were ammonium sulfate [(NH₄)₂SO₄ (20.5-0-0)], MAP [NH₄H₂PO₄ (11-52-0)], and potassium chloride [KCl (0-0-60)]. The fertilizer was applied manually and incorporated using a hoe, which was then covered with the same soil.

Test run

Sowing was carried out dry; the seed was placed by hand at a seeding rate of 130 kg ha⁻¹. The genetic material used was the wheat variety Cortazar S94. Weed control was achieved by applying 30 g ha⁻¹ of sodium flucarbazone 30 days after sowing. Pest control was carried out by applying 300 ml ha⁻¹

imidacloprid at stage Z4.3 to control aphids [*Diuraphis noxia* (Mordvilko)]. To prevent yellow rust (*Puccinia striiformis* f. sp. tritici), 1 L ha⁻¹ tebuconazole was applied at the Z4.3 stage.

Variables evaluated

Harvesting was conducted when the grain reached physiological maturity. All spikes were manually harvested from a plot measuring 1.5 m wide by 2 m long. A one-meter-long section of the remaining experimental plot was selected to cut the plants at ground level; 100 stems were randomly selected to estimate yield components (Pietragalla; Pask, 2013). The stems were dried to constant weight in an oven at a temperature of 80 °C and subsequently threshed in a single-plant threshing machine. The grain yield was adjusted to 12% moisture content (yield, kg ha⁻¹); grains per square meter (Grains, m⁻²); ears per square meter (Ears m⁻²); grains per ear (Grains ear⁻¹); thousand-grain weight (TGW, g); harvest index (HI); biomass (Biomass, t ha⁻¹); and grain protein content (PCG, %). In addition, the plant height (cm) and SPAD® value (Soil and Plant Analysis) were evaluated at 57, 64, and 86 days after sowing (das, SPAD57, SPAD64, and SPAD6, respectively). The SPAD value was obtained using a SPAD-502 Plus optical sensor (Konica Minolta Inc., Tokyo, Japan). Ten readings were taken per experimental unit in the middle adaxial zone of the last ligulate leaf of the measured plants. The normalized difference vegetation index (NDVI) values were recorded at 39, 57, and 86 DAS. The NDVI value was obtained using the Green Seeker® optical sensor. Two readings were taken along the central furrows of each experimental unit.

Statistical analyses

The data were subjected to a combined analysis of variance, and tests were conducted to compare means using the least significant difference (LSD, $P \leq 0.05$). Principal component analyses were performed to reduce the dimensionality of the original set of variables (Greenacre et al., 2022); these analyses were conducted using SAS 9.3 software (SAS Institute, 2002). Treatment*environment interaction analysis (IGA) for yield was used to identify the response to treatments across environments (Alvarado et al., 2015), for which the site regression model (SREG) was employed. A stability analysis was conducted for grain yield across locations to identify the yield response to each treatment and across locations (Dyke et al., 1995).

RESULTS AND DISCUSSION

Evaluations with 75% chemical fertilization at four locations

Analysis of variance

Statistical analysis (Tables 4 and 5) revealed that the variability in yield and yield components generated by each study site significantly differed across locations and treatments, except for grain weight per 1,000 grains and the harvest index. With respect to the physio-technical variables, significant

differences were found across locations for the NDVI 39 and the NDVI 86, as well as for the SPAD 64 and the SPAD 86. Furthermore, there was a significant interaction between location and treatment for yield and the NDVI 39, indicating that the behavior of these two variables differed among the evaluation sites. In terms of yield, the treatments accounted for 63% of the total variance, while the evaluation environments accounted for 14%. These findings indicate that the effects of the treatments were more significant than those of the evaluation environments were.

Treatments

When chemical fertilization was reduced to 25%, a statistically significant 5.2% decrease in yield was observed (Figure 1). However, the treatments with 75% chemical fertilization and the addition of biofertilizer (BIO1 and BIO2) performed statistically the same as the previous treatments did. In other words, the incorporation of microorganisms tended to improve yield despite the overall reduction in fertilization. On the other hand, omitting fertilization during the growing cycle resulted in a significant 42.4% reduction in production compared with the maximum dose evaluated during the study.

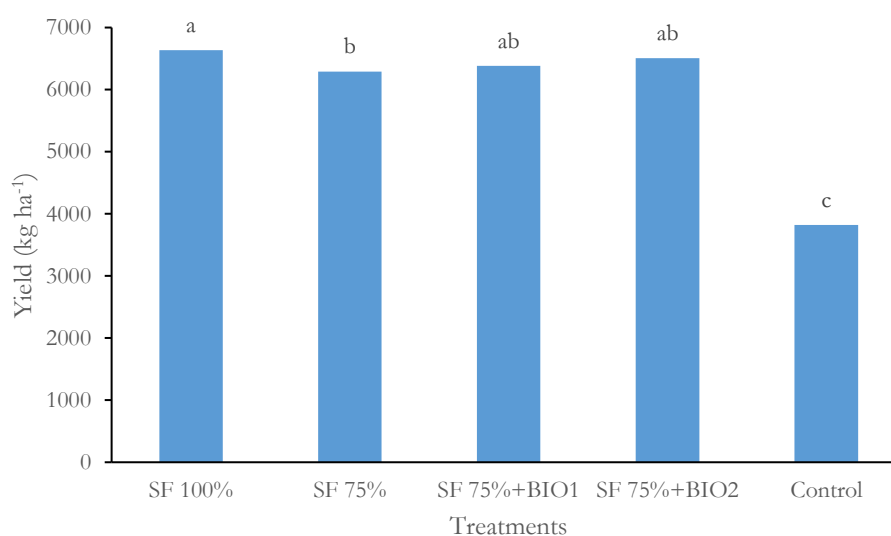


Figure 1. Wheat grain yield of treatments with synthetic fertilization and biofertilizers in four locations in the Bajío region, State of Guanajuato, Mexico. SF: synthetic fertilization; BIO1: microbial formulation 1; BIO2: microbial formulation 2.

Table 4. Mean squares of the yield variables, i.e., Grains m⁻², Ears m⁻², Grains ear⁻¹, TGW, HI and Biomass, in treatments with a 75% reduction in chemical fertilization at four locations in the Bajío region, State of Guanajuato, Mexico.

Source of variation	DF	Yield	Grains m ⁻²	Ears m ⁻²	Grains ear ⁻¹	TGW	HI	Biomass
Sites	3	3839013.15**	8049355.08**	18680.167*	376.1**	55.381	0.0066	18.569**
Blocks (Blk)	2	5513680.53**	15899788.7**	14631.875*	0.686	11.204	0.0054	24.648**
Blk/Site	6	52717.56	432597.8	2537.16	20.859	17.998	0.0025	0.916
Treatments (Treats)	4	16843569.43**	66598368.6**	9772.644*	285.228**	19.618	0.0056	52.336**
Treats*Sites	12	429255.64**	1577984.5	14631.875	13.342	11.204	0.0031	2.099
Residual error	32	162026	1850308.6	3378.697	18.3	25.732	0.0036	1.744
CV Treatments (%)		6.8	12.5	17.2	13.1	9.7	12	11.8
CV Sites (%)		3.9	6.1	14.9	13.9	8.1	9.9	8.5

DF: degrees of freedom; Yield: grain yield; Grains m⁻²: grains per square meter; Ears m⁻²: ears per square meter; Grains ear⁻¹: grains per ear; TGW: thousand-grain weight; HI: harvest index; * and **: significant differences with $P \leq 0.05$ and $P \leq 0.01$, respectively.

Table 5. Mean squares of the variables NDVI at 39, 57 and 86 days after sowing (das) and SPAD values at 57, 64 and 86 DAS in treatments with a 75% reduction in chemical fertilization in the Bajío region, State of Guanajuato, Mexico.

Source of variation	DF	NDVI 39 das	NDVI 57 das	NDVI 86 das	SPAD 57 das	SPAD 64 das	SPAD 86 das
Sites	3	0.38315819**	0.00728222	0.02859278**	39.6626667	35.5237778*	201.6575556*
Blocks (Blk)	2	0.00121625	0.001185	0.00018667	3.9161667	4.322	27.6551667
Blk/Sites	6	0.00272069	0.00286722	0.00171111	10.6801667	4.2237778	23.0473889
Treatments (Treats)	4	0.03061729**	0.08228917**	0.0593875**	126.7139167**	128.0179167**	176.73725**
Treats * Sites	12	0.0077634*	0.00088917	0.00079972	3.9178056	8.9539167	9.5365833
Residual error	32	0.00336281	0.00139667	0.00233417	3.9583333	9.449792	9.160375
CV Treatments (%)		9.6	4.7	7.2	4.4	6.6	6.3
CV Sites (%)		8.6	6.7	6.2	7.2	4.4	10

DF: degrees of freedom; Yield: grain yield; Grains m⁻²: grains per square meter; Ears m⁻²: ears per square meter; Grains ear⁻¹: grains per ear; TGW: thousand-grain weight; HI: harvest index; * and **: significant differences with $P \leq 0.05$ and $P \leq 0.01$, respectively.

Principal component analysis

Locations

The responses of all the variables evaluated across the four locations are shown in Figure 2. The first two principal components explained 87.8% of the total variance contributed by the thirteen variables. Using the eigenvectors of each principal component (CP) revealed that the most important variables for CP1 were the GPMC, GPE, and NDVI at 57 and 86 days and that for CP2, the most important variables were the Mil, NDVI at 39 days, yield, and EPMC. Therefore, (Figure 3) Pénjamo stood out for having higher GPMC, GPE, and NDVI values at 57 and 86 days. Moreover, Villagrán stood out for having higher Mil and NDVI values at 39 days and EPMC.

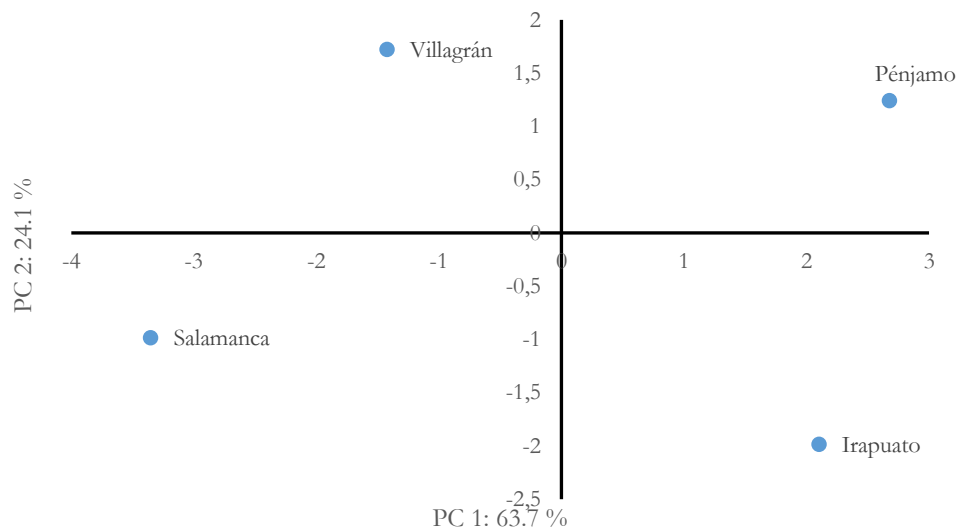


Figure 2. Principal component analysis for the four wheat assessment locations in the Bajío region, State of Guanajuato, Mexico. PC 1 and 2: principal components 1 and 2.

Treatments

The responses of the 13 variables evaluated under the four environmental conditions, as well as the dispersion of the treatments, are shown in Figures 3 and 4. The analysis revealed that the first and second principal components together explained 92.25% of the total variance contributed by the 13 variables studied. The behavior of the variables within the principal components is shown in Figure 3. The variables Mil, IC, and EPMC are more dispersed within the quadrants, indicating that their importance is lower than that of BIO, GPMC, GPE, and the physicochemical variables (NDVI and SPAD). These same variables were distributed within an angle of less than 45°, so their responses were similar, indicating that each exhibited high interdependence because of the magnitude and direction of their vectors with grain yield. Therefore,

when the characteristic vectors of each principal component (CP) were used, most variables, with the exception of IC and EPMC, were the most important for CP1, whereas for CP2, it was IC; however, the same analysis allows us to identify that this component—and thus, the dispersion of IC—was not particularly significant (0.05%).

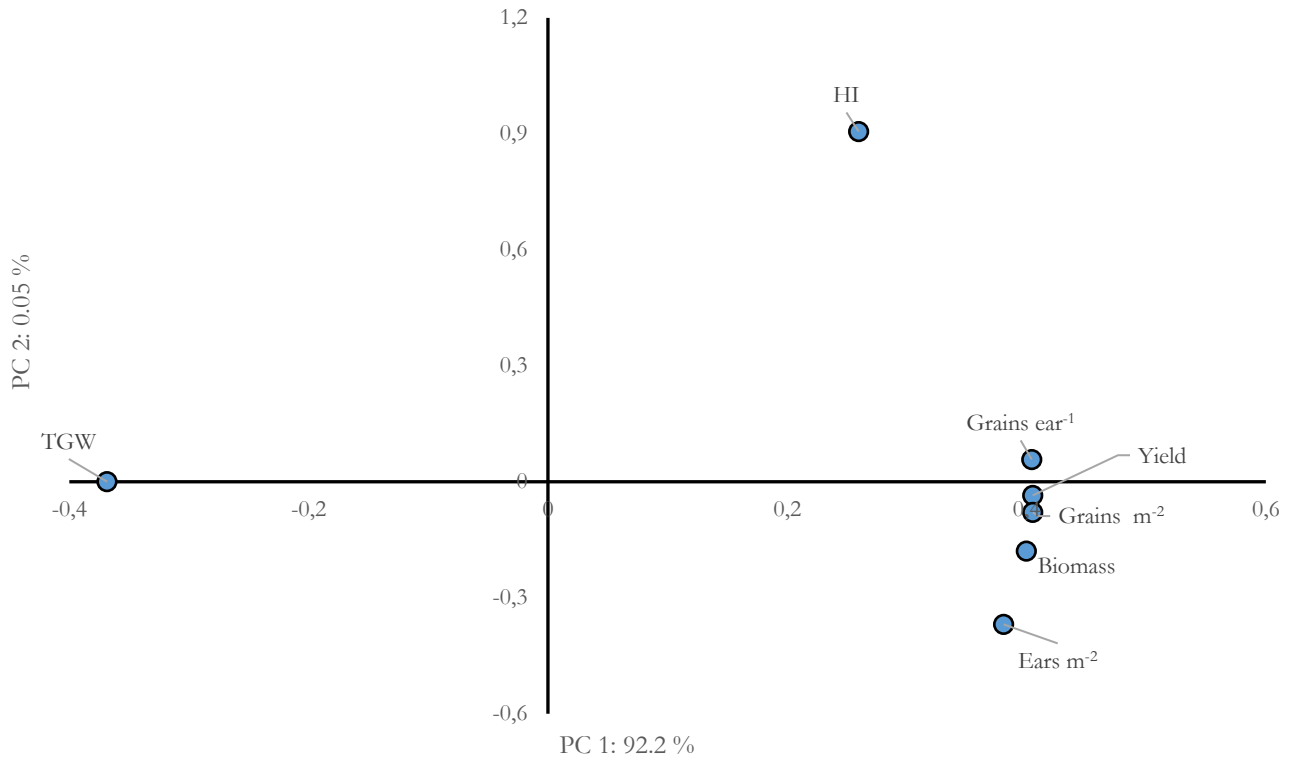


Figure 1. Eigenvectors of the variables evaluated at four locations in the Bajío region, state of Guanajuato, Mexico. PC 1 and 2: Principal component 1 and 2; Yield: grain yield; Grains m⁻²: grains per square meter; Ears m⁻²: ears per square meter; Grains ear⁻¹: grains per ear; TGW: thousand-grain weight; HI: Harvest index.

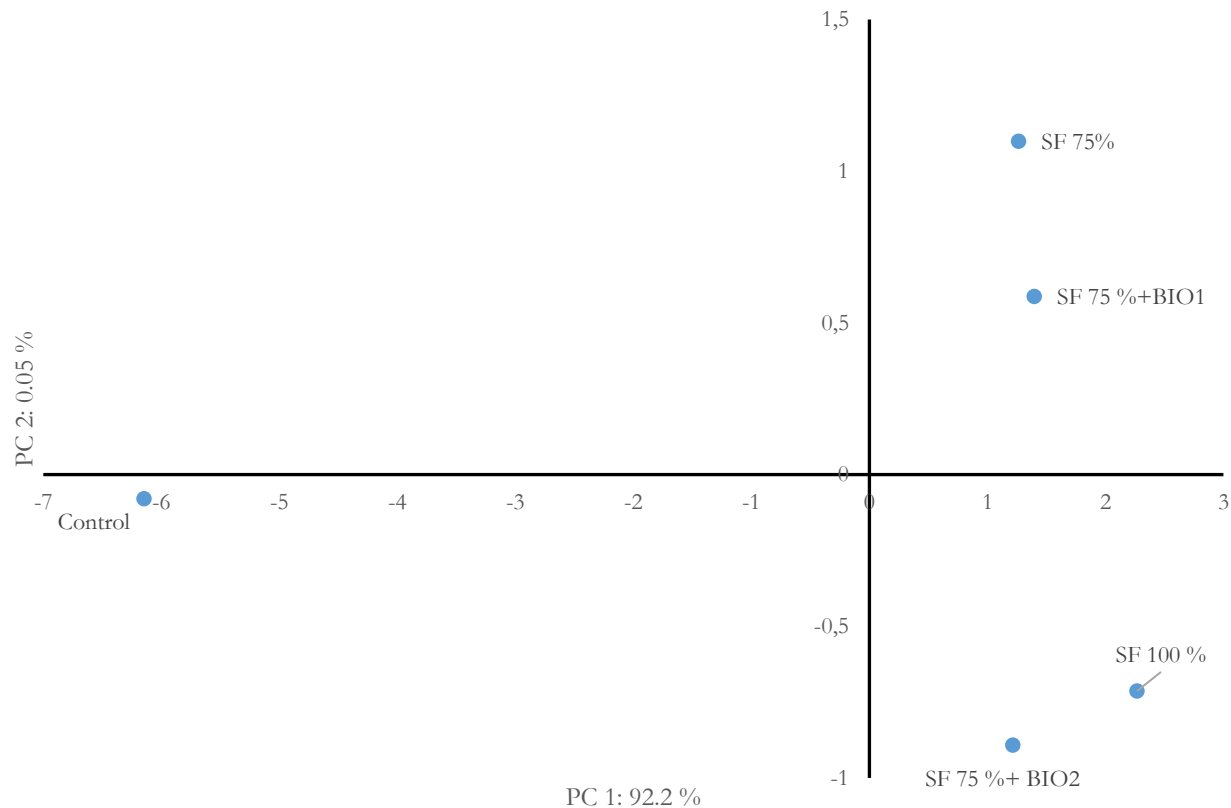


Figure 4. Dispersion analysis of synthetic and biofertilizer fertilization treatments in the Bajío region, State of Guanajuato, Mexico. PC 1 and 2: Principal component 1 and 2; SF 100%: Synthetic fertilization 100%; SF 75%: Synthetic fertilization 75%; SF 75%+BIO1: Synthetic fertilization + Microbial formulation 1; SF75%+BIO2: Synthetic fertilization + Microbial formulation 2.

Interaction between treatments and the environment

As shown in Figure 5, of the total variance observed in the yield data, the first factor—or principal component—related to the treatments explained 97.5%, and the second factor, related to the evaluation environments, explained 1.64%; thus, the SREG model explained almost all of the variation in the treatments and environment (99.1%). Three main sectors were observed, one of which included all the locations except Pénjamo, which was located in another sector of the Cartesian plane. The soil conditions of the first group of locations were characterized by a high percentage of organic matter in two locations (Irapuato and Salamanca), whereas in Villagrán, it was low. The soil pH in these locations was moderately alkaline, unlike that in Irapuato, which was moderately acidic. The phosphorus supply was high except in Villagrán, whose level was moderately low. In all three cases, the potassium supply was moderately high. The salinity percentage was low at two locations (Salamanca and Villagrán), while Irapuato was free of salinity. In terms of the percentage of sodium saturation, two locations had a level less than 5% (Villagrán and Irapuato), whereas Salamanca had a level of 18%.

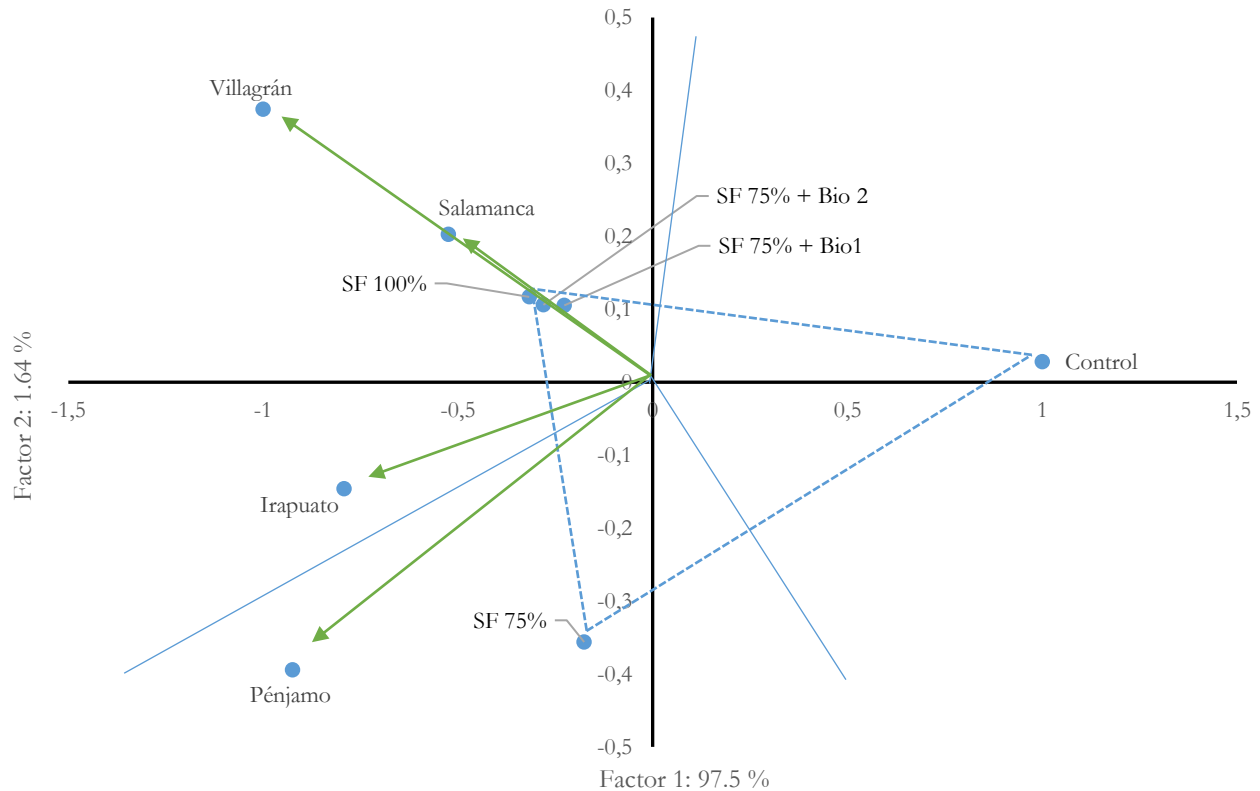


Figure 5. Treatment * environment interaction analysis for wheat yield at four locations in the Bajío region, State of Guanajuato, Mexico. SF 100% and 75%: synthetic fertilization at 100% and 75%, respectively; SF 75%+BIO1: synthetic fertilization at 75%+ microbial formulation 1; and SF 75%+BIO2: synthetic fertilization at 75%+ microbial formulation 2.

On the Cartesian plane, the treatments with 100% chemical fertilization and the two with biofertilizers favored yield performance in this set of locations. On the other hand, the location of Pénjamo was situated in a different sector from the other locations, where the yield of the treatment with 75% chemical fertilization performed best. Climate conditions may be key to understanding these results, as compared with the other locations, Pénjamo had the lowest maximum temperature, lower average temperature and radiation, and the lowest accumulated radiation (Table 2). Among the locations, those with the highest yield on the basis of the length of their vectors were Villagrán and Pénjamo. The treatments with the highest yields were the treatments with 100% chemical fertilization, as well as those with 75% chemical fertilization supplemented with BIO2 and BIO1.

Stability analysis

Although the control treatment was the most stable, it also had the lowest yield compared with all the other treatments and evaluation sites (Figure 6). On the other hand, compared with the regression line, the treatments with 100% chemical fertilization and both treatments incorporating biofertilizer exhibited similar behavior; consequently, their stability across the evaluation sites was similar in terms of regression deviations and higher yields.

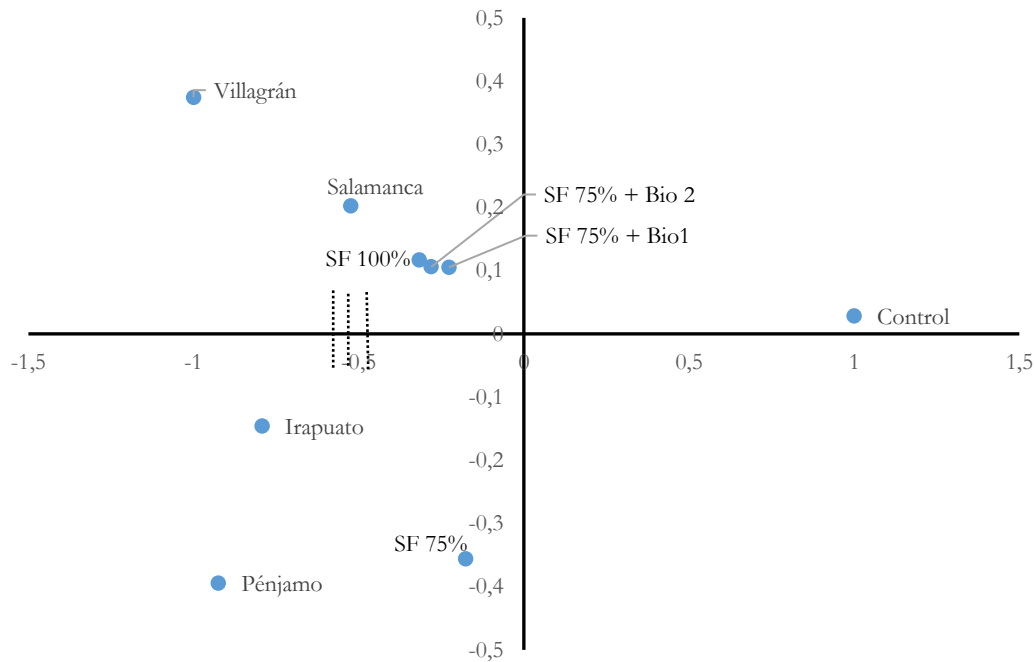


Figure 6. Performance stability analysis in biofertilizer evaluation environments for wheat yield at four locations in the Bajío region, State of Guanajuato, Mexico. SF 100% and 75%: synthetic fertilization at 100% and 75%, respectively; SF 75%+BIO1: synthetic fertilization at 75%+ microbial formulation 1; and SF 75%+BIO2: synthetic fertilization at 75%+ microbial formulation 2.

The treatment with the greatest instability was the 75% chemical fertilization treatment; this means that its performance across the sites is unpredictable, which is likely due to the different soil fertility conditions at the sites. In terms of the sites, Irapuato was the site with the lowest regression deviation, while Pénjamo and Villagrán exhibited contrasting and somewhat unpredictable performance.

Evaluations using 50% chemical fertilizer at two locations.***Analysis of variance***

Among the locations (Table 6), significant differences were observed in yield and its components, with the exception of the numbers of GMC, GPE, and IC, whereas for the physicochemical variables (Table 7), significant differences were observed among the locations for all the variables, with the exception of SPAD at 42 and 57 days after sowing. For the treatments, statistical significance was observed for all the evaluated variables except for the IC (Table 6). With respect to the interaction between treatments and study sites, no significant differences were observed except for the NDVI at 57 days after sowing; therefore, the response of the treatments across locations was similar.

Treatments

The results of the multiple comparison test for means (least significant difference) revealed (Tables 8 and 9) that the grain yield was significantly greater when 100% chemical fertilizer was applied than when 50% was applied (Figure 7); however, when BIO2 was added to this fertilization rate (Tables 8 and 9), the yield was statistically the same as that in the 100% treatment and was greater than that in the 50% chemical fertilization treatment.

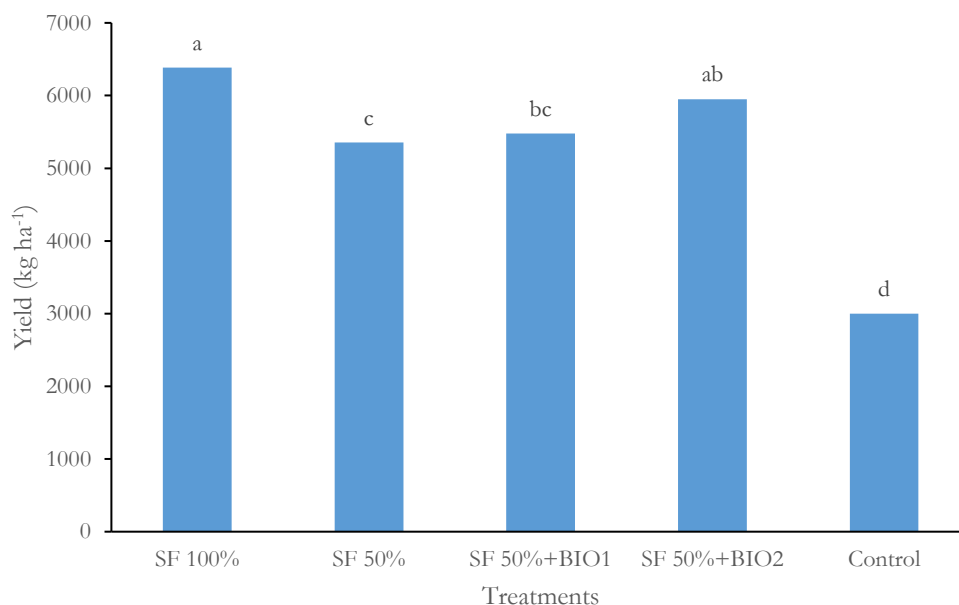


Figure 7. Wheat grain yield in response to chemical fertilization and biofertilizer treatments at two locations in the Bajío region, State of Guanajuato, Mexico. SF: synthetic fertilization; BIO1: microbial formulation 1; BIO2: microbial formulation 2.

Table 6. Mean squares of the yield variables, i.e., Grains m⁻², Ears m⁻², Grains ear⁻¹, TGW, HI and Biomass, in treatments with a 50% reduction in chemical fertilization at two locations in the Bajío region, State of Guanajuato, Mexico.

Source of variation	DF	Yield	Grains m ⁻²	Ears m ⁻²	Grains ear ⁻¹	TGW	HI	Biomass
Sites	1	7090740.833**	3714374.907	42007.692*	131.8803333	246.5333333**	0.00147	16.1773633*
Blocks (Blk)	2	454343.09	1823490.5	2530.489	31.759	13.075	0.00013	0.7235833
Blk/Sites	2	314629.49	1064684.3	881.239	27.0223333	0.0583333	0.00063	0.4425633
Treatments (Treats)	4	10345878.06**	1064684.3**	10083.20417*	136.4553333**	6.575*	0.00038	37.79480**
Treats*Sites	4	154752.08	656876.8	1498.3245	15.0086667	3.9083333	0.00155333	2.484955
Residual error	16	218105.51	596164	3266.9636	9.664	2.0979167	0.00108417	1.2557025
CV Treatments (%)		8.9	8.1	17.7	10.5	2.8	6.7	11.1
CV Sites (%)		10.7	10.9	9.2	17.6	0.5	5.1	6.6

DF: degrees of freedom; Yield: grain yield; Grains m⁻²: grains per square meter; Ears m⁻²: ears per square meter; Grains ear⁻¹: grains per ear; TGW: thousand-grain weight; HI: harvest index; * and **: significant differences with $P \leq 0.05$ and $P \leq 0.01$, respectively.

Table 7. Mean squares of the NDVI and SPAD values measured at 42, 59 and 94 days after sowing (das) in treatments with a 50% reduction in chemical fertilization at two locations in the Bajío region, State of Guanajuato, Mexico.

Source of variation	DF	NDVI 42 das	NDVI 59 das	NDVI 94 das	SPAD 42 das	SPAD 59 das	SPAD 94 das
Sites	1	0.83000333**	0.08008333*	0.22360333**	11.163	6.075	769.1203333*
Blocks (Blk)	2	0.00036333	0.00624333	0.00175	1.8253333	1.393	6.4543333
Blk/Sites	2	0.00996333	0.00384333	0.00196333	4.404	6.871	14.2263333
Treatments (Treats)	4	0.04727167**	0.09378833**	0.03496167**	102.5211667**	41.0555*	163.9353333**
Treats*Sites	4	0.001045	0.03704167**	0.00192833	11.9871667	7.7058333	27.3853333
Residual error	16	0.00308833	0.0068475	0.002715	7.0784167	12.5261667	16.655333
CV Treatments (%)		9.5	11.4	9.7	6.0	7.8	9.7
CV Sites (%)		17.1	8.5	8.3	4.8	5.8	9.0

DF: degrees of freedom; Yield: grain yield; Grains m⁻²: grains per square meter; Ears m⁻²: ears per square meter; Grains ear⁻¹: grains per ear; TGW: thousand-grain weight; HI: harvest index; * and **: significant differences with $P \leq 0.05$ and $P \leq 0.01$, respectively.

Table 8. Comparison of the means of the minimum significant difference for yield, Grains m⁻², Ears m⁻², Grains ear⁻¹, TGW, HI and biomass in treatments with a 50% reduction in chemical fertilization at two locations in the Bajío region, State of Guanajuato, Mexico.

Treatments	Yield (kg ha ⁻¹)	Grains m ⁻² (No.)	Ears m ⁻² (No.)	Grains ear ⁻¹ GPE (No.)	TGW (g)	HI	Biomass (t ha ⁻¹)
SF 100%	6384.7 a	11891.4 a	365.02 a	33.467 a	50.75 b	0.48833	12.3683 a
SF 50%	5352.8 c	9556.9 c	323.63 ab	30.817 a	52.9167 a	0.48833	10.545 b
SF 50%+BIO1	5476.3 bc	9702.7 c	319.32 ab	30.767 a	53.5 a	0.505	10.3133 b
SF 50%+BIO2	5950.8 ab	10843.8 b	346 a	31.533 a	51.9167 ab	0.48833	11.515 ab
Control	3000.9 d	5403.2 d	256.53 b	21.267 b	52.4167 ab	0.485	5.88 c
DMS	430	143	4.1	0.03	0.4		0.23

DMS: least significant difference; means with the same letters per column are not significantly different (LSD, $P \leq 0.05$); Yield: grain yield; Grains m⁻²: grains per square meter; Ears m⁻²: ears per square meter; Grains ear⁻¹: grains per ear; TGW: thousand-grain weight; HI: harvest index.

Table 9. Comparison of means using the minimum significant difference for the NDVI and SPAD values at 42, 59 and 94 days after sowing (das) in treatments with a 50% reduction in chemical fertilization at two locations in the Bajío region, State of Guanajuato, Mexico.

Treatments	NDVI 42 das	NDVI 59 das	NDVI 94 das	SPAD value 42 das	SPAD value 59 das	SPAD value 94 das
FS 100%	0.65167 a	0.84333 a	0.60333 a	47.2 a	48.483 a	49.117 a
FS 50%	0.615 a	0.77833 a	0.56167 a	45.317 a	45.333 ab	39.483 bc
FS 50%+BIO1	0.61 a	0.80833 a	0.56167 a	46.517 a	46.1 a	42.383 b
FS 50%+BIO2	0.42833 b	0.535 c	0.405 b	36.933 b	41.25 b	34.95 c
Control	0.61667 a	0.67333 b	0.55333 a	44.35 a	45.783 a	43.583 b
DMS	0.0017	0.03	0.0007	0.55	0.25	1.1

DMS: least significant difference; means with the same letters per column are not significantly different (LSD, $P \leq 0.05$); NDVI: normalized differential vegetation index.

This finding indicates that the incorporation of BIO2 increased the yield. There were no significant differences between BIO1 and BIO2; however, BIO2 performed similarly to the 50% chemical fertilization treatment. With respect to yield components, the number of GMCs increased when 100% chemical fertilization was applied. For EPMC and GPE, the treatments performed similarly, with the exception of the control. With respect to the thousand-kernel weight (TKW), all the treatments performed similarly, with the exception of the 100% chemical application treatment, which had the lowest values and was similar to the 50% treatment with BIO2. For IC, no significant differences were observed among the treatments.

DISCUSSION

Statistical analyses revealed that the treatments accounted for the greatest percentage of the observed variability in yield (80% and 58%, explaining 75% and 50% of the variation in fertilizer application, respectively), whereas the evaluation sites accounted for 18% and 40.3% (evaluating 75% and 50% of the fertilizer dose, respectively), and the interaction between the two accounted for 2% and 0.9% (evaluating 75% and 50% of the fertilizer dose, respectively). These results indicate that fertilizer rate management was more important than environmental conditions for yield expression in the evaluated treatments, which is consistent with the findings of Alotaibi et al. (2024). When 100% and 75% chemical fertilization was evaluated, the effects of biofertilizers on grain yield tended to increase yield; this finding was similar to the results reported by Rangel et al. (2014) and Sánchez de la Cruz et al. (2008) in México and by Marcandalli -Boleta et al. (2020), Alhammad et al. (2023), and Iqbal et al. (2024) in Brazil and the latter in Pakistan and in contrast with the results of Báez-Pérez et al. (2020) in Mexico's Bajío region, as they reported significantly higher yields in treatments with inoculants than in treatments without inoculation, although these latter results were based on evaluations conducted at a single location. Shahwar et al. (2023) reported significant responses in yield components to inoculation with various growth promoters in various crops and at various latitudes. On the other hand, Lemos et al. (2013) reported that inoculation with *Azospirillum brasilense* in conjunction with nitrogen fertilization was successful, as bacterial inoculation contributes to plant development; however, it did not replace nitrogen fertilization but rather eventually allowed a reduction in the nitrogen dose to be applied.

The effect of biofertilizers on the productivity of the treatments was not significant under the conditions in which they were evaluated. These findings suggest the need for further investigation into the behavior of soil microorganisms and their interactions with the environment. Studying soil microorganisms could aid in identifying their abundance in the soil associated with each treatment, which could aid in understanding the effects of biofertilizers on yield (Asoegwu et al., 2020). Shakir et al. (2012) and Jalal et al. (2023) reported that the yield response to inoculation is more pronounced in plants under adverse conditions, such as water deficit and low soil nutrient concentrations; however, Ferreira et al.

(2019) and Tabassum et al. (2017) reported that inoculation with *Azospirillum brasilense* does not always result in a favorable yield response under water stress or low soil nutrient concentrations.

By reducing chemical fertilization by 50%, it was found that the application of BIO 2 biofertilizer significantly increased yield by 10% compared with that in the treatment without inoculation (50% chemical fertilization), a result similar to that reported by El-Akhdar et al. (2024), Shintate-Galindo et al. (2019), and Nunes et al. (2015), who reported significant yield increases in contrast to those in treatments without biofertilizer inoculation. The BIO2 treatment performed statistically the same as the treatment with full fertilization (100%); this result is similar to that reported by Grageda-Cabrera et al. (2018), who reported that mycorrhizal inoculation with 50% chemical fertilization yielded the same yield as full fertilization (100%). The yield tended to improve in the BIO1 treatment; however, it was statistically inferior to full fertilization. This suggests the possibility of conducting evaluations in environments with adverse conditions to identify alternatives that optimize production, as well as the use of chemical fertilizers (Singh et al., 2023).

Furthermore, the results of the periodic measurements of physiotechnical variables such as the chlorophyll concentration and the SPAD and NDVI values revealed that all the treatments with biofertilizers resulted in similar physiological behaviors. The information provided by these variables served as the basis for identifying the effects of biofertilizers on the treatments. These results are consistent with those reported by Quintarelli et al. (2025), who reported no differences among different biofertilizers but reported that these treatments outperformed the absolute control treatment.

Statistical differences in the interactions between treatments and study sites indicated that the effects of biofertilizers varied across different environments; the study by Santillano-Cázares et al. (2022) helps explain these findings, since when 14 biofertilizers were evaluated across five states in Mexico, they reported that the effects of treatments inoculated with biofertilizers were not the same across the evaluated locations and reported a significant yield response to inoculation with *Azospirillum brasilense* and *Glomus* spp. in only two of six locations in Mexico. On the other hand, these results contrast with those reported by Moradi and Siosemardeh (2025), who reported no statistically significant differences in the interaction between environments. The interaction analysis revealed that climatic conditions were also important, with Pénjamo location presenting better conditions for yield expression; this finding is similar to the results of Chavez-Díaz et al. (2020), who highlighted that the treatments evaluated with microorganisms in wheat differed because of interactions among plants, microorganisms, and the environment.

CONCLUSIONS

The interaction effect between location and treatment on yield was significant, indicating that yield performance differed across the evaluation sites. The treatments accounted for 63% of the total

variance, while the evaluation sites accounted for 14%, indicating that the effect of the treatments was more significant than that of the evaluation sites.

The analyses revealed that the treatment with 100% chemical fertilizer significantly outperformed the 75% and 50% treatments in terms of yield. However, when BIO2 was added to the 50% fertilizer treatment, the yield was statistically equivalent to that of the 100% control and greater than that of the 50% baseline. Similarly, when BIO1 or BIO2 was added to the 75% fertilizer treatment, a grain yield comparable to that of the full treatment was achieved (100%).

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