

Background, Results, and Prospects of Microbial Biofertilizers in Mexico

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ABSTRACT

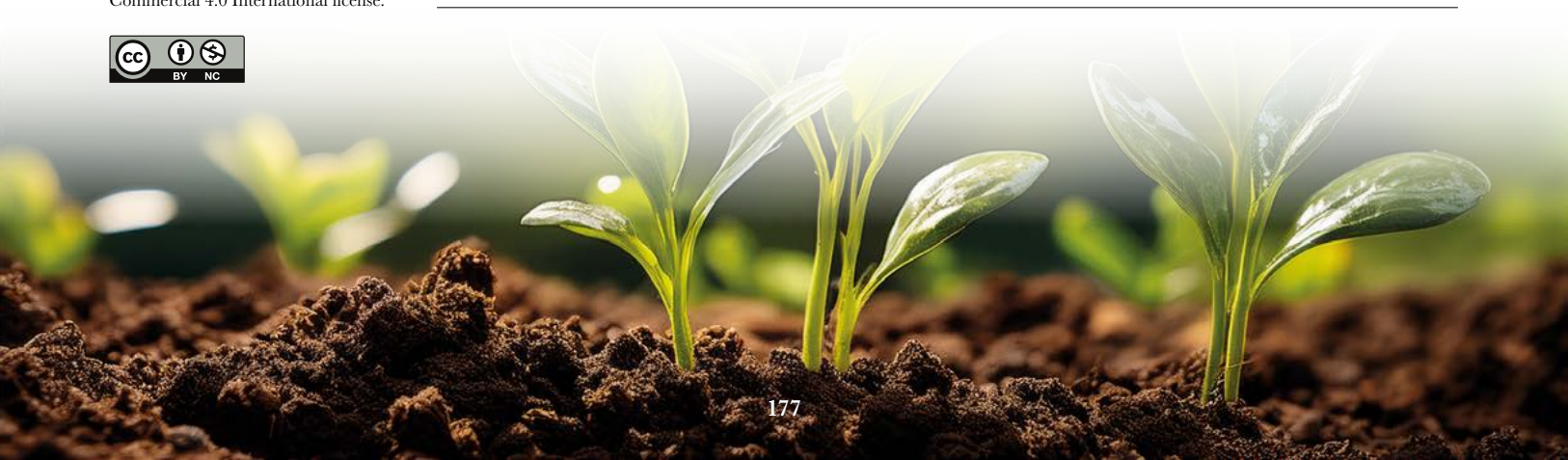
Objective: To convince Mexican producers and authorities about the potential of biofertilizers in different regional agricultural systems. In addition, using validation and demonstration plots with different crops, INIFAP trained producers in the appropriate use of biofertilizers.

Design/methodology/approach: The actions were carried out throughout the country since the 1999 SS cycle. The microorganisms used in this project were *Azospirillum brasilense*, *Rhizobium etli*, *Bradyrhizobium diazoefficiens*, and *Rhizopagus intraradices*. The training courses were attended by 9,229 producers. Biofertilization was carried out in 1,882,236 ha and 3,370,440 doses of biofertilizers were applied during three agricultural cycles. In conclusion, biofertilizers (individually or in co-inoculation treatments) can increase crop yield under various environmental and management conditions.

Results: In most crops, bacteria/fungi co-inoculation promoted a greater growth and yield in host crops. According to the data obtained by the biofertilization program of INIFAP, in Mexico, biofertilization can feasibly achieve more sustainable and profitable agricultural systems than conventional agricultural systems that use agrochemicals. In addition, the effective symbiosis of rhizobia promoted bean growth.

Findings/conclusions: Extensive and readily available research about biofertilizers proves that these microorganisms can increase yield, while providing enough nutrients to crops.

Keywords: rainfed agriculture, microorganisms, yield, technology transfer.



INTRODUCTION

Around the middle of the last century, the use of synthetic fertilizers was fundamental to meet the food demand of the growing Mexican population. Consequently, the Mexican government created Guanos y Fertilizantes de México (GUANOMEX) in 1943 to commercialized synthetic fertilizers at low prices. By the 1970s, the name of this company changed to Fertilizantes Mexicanos S. A. (Fertimex) (Grageda-Cabrera *et al.*, 2012). The Green Revolution arrived in Mexico during the first years of GUANOMEX. Its purpose was to increase agricultural productivity through monocrops (Cecon, 2008), intensive agriculture (Herrera, 2006), improved seeds, agrochemicals, irrigation, technical support, and research (Pichardo, 2006). The results were encouraging (Hernández, 1988). As a consequence of the 1970s Energy Crisis, the price of energy sources used to manufacture fertilizers (such as oil, natural gas, and coal) increased, resulting in a rise in the cost of fertilizers. In addition, the privatization of state-owned companies in the late 1980s put an end to Fertimex S.A., which produced >3 million tonnes of fertilizers per year. Therefore, input purchasing and importation was difficult, particularly for small and medium producers (Sacristán-Roy, 2006). Despite the immediate increase in crop yield obtained by the application of synthetic fertilizers, their excessive use led to environmental pollution, increasing greenhouse gases and depleting the ozone layer (Bohloul *et al.*, 1992). In addition, NO₃ and NO₂ leaching polluted water sources (Martínez-Nieto *et al.*, 2011). Furthermore, the use of these products decreased organic matter decomposition and modified exchangeable cation concentration (Demiraj *et al.*, 2018; Bowden *et al.*, 2019; Mehdizadeh *et al.*, 2019). Likewise, Caballero-Mellado and Martínez-Romero (1999) reported alternations in soil microbiota. However, neither environmental pollution, nor changes in the microbial populations of the rhizosphere, helped to reduce the application of synthetic fertilizers. The reduction of the use of synthetic fertilizers in the 1990s was a consequence of price increase (Ávila Dorantes *et al.*, 2002). By 2000, Mexico was the main net importer of urea, buying 63% of fertilizers used in agriculture (Grageda-Cabrera *et al.*, 2012). This situation and the low profitability of agriculture, led the government—through the Ministry of Agriculture, Animal Production, and Rural Development (SAGAR), currently known as the Ministry of Agriculture and Rural Development (SADER)—to drive technologies developed by domestic research. These technologies were aimed to develop plant nutrition alternatives, using soil beneficial microorganisms studied by several national research and educational institutions. This agricultural practice consisted of adhering the microorganisms to the seeds in order to improve plant nutrition. Sustainable agriculture is based on the use of beneficial microorganisms that maintain soil microbial diversity and drive the association between these microorganisms and plants, promoting growth, productivity, and high yields. Microorganisms, fungi, and bacteria can improve plant growth, increasing nutrient availability (Mazid *et al.*, 2011). Therefore, the use of microbes as bioinoculants—along with chemical fertilizers—is the best strategy to increase plant growth and soil fertility (Prisa *et al.*, 2023). In 1999, SAGAR implemented and developed a biofertilization program through INIFAP. At the beginning, the Programa Nacional de Biofertilizantes was developed for marginal producers who grew maize and bean under rainfed systems, had no access to synthetic fertilizers, and only practiced

subsistence agriculture; however, producers who applied chemical fertilizers to their crops were later included in the program. During the implementation of the program, farmers included biofertilization in their agronomic management —*i.e.*, they adhered microorganisms to seeds and used conventional local crop management. The use of biofertilization in the process helped to reduce the doses of chemical fertilization, without a negative impact on yield. In many cases, it actually increases, with lower production costs. Seed inoculation favors the nutrition of host crops and strengthens the root system through several action mechanisms, consequently promoting a greater plant growth. Meanwhile, the initial objective was the expansion of the research and technology validation program for different crops of interest, in various agroecological regions of the country. In addition, promoting and spreading the use of biofertilizers through training and demonstration plots were fundamental, because, at the time, these technologies were almost unknown by farmers, technicians, and civil servants of the Mexican agricultural sector. These programs had the objective of driving the adoption of biofertilizers in the Mexican countryside and to concomitantly reduce the application of synthetic fertilizers.

Development of the Mexican Biofertilization Program

Through the INIFAP, SADER invited several research and educational institutions to be part of the work group in charge of the production of biofertilizers. Both the Universidad Nacional Autónoma de México (UNAM) and the Instituto Politécnico Nacional (IPN) agreed to participate in this project. On the one hand, UNAM participated through its Centro Nacional de Fijación de Nitrógeno (currently known as Centro de Ciencias Genómicas): Mr. Jesús Caballero Mellado (ScD) —who isolated and developed the strain *Azospirillum brasilense* Tarrand, Krieg et Döbereiner— and Mr. Jaime Mora Celis (ScD) —who provided the strain *Rhizobium etli* (Segovia *et al.*, 1993)— took part in the project. On the other hand, IPN —through the participation of Mrs. María Valdés Ramírez (ScD) from the Escuela Nacional de Ciencias Biológicas— provided the strains *Bradyrhizobium japonicum* (currently *B. diazoefficiens*) (Delamuta *et al.*, 2013) for soy (*Glycine max* L. Merr) and the endomycorrhiza fungus *Glomus intraradices* (currently *Rhizophagus intraradices* (Schenck & Sm.) Walker & Schüßler (Schüßler and Walker, 2010)). The initial program was funded by the Fundación Mexicana para la Investigación Agropecuaria y Forestal A.C. (FUMIAF A.C.).

Actions Included in the Biofertilization Program

The implementation of the program included validation and technology transfer activities, as well as biofertilizer training, production, and distribution. The theoretical-practical training started with researchers from the INIFAP, in three Mexican agroecological regions: central region (Puebla), northern-central region (Coahuila), and southeastern region (Tabasco). Subsequently, technicians and producers were trained on the fundamentals of microorganism reproduction. Emphasis was put on caring, transporting, and storing the microorganisms, as well as in the different ways to apply biofertilizers. Training included audiovisual presentations and in person lessons. In addition, practices regarding the adherent (5% carboxymethylcellulose) used to attach the said microorganisms

to seeds, both for hand sowing or drilled seeds were conducted in each region. Overall recommendations were issued for producers that required to inoculate large quantities of seeds. All the participants received written information about the different features of the microorganisms. A total of 231 courses were offered throughout the country: 208 courses during the 1999 spring/summer (SS) cycle and 23 courses during the 2000 autumn/winter (AW) cycle. Mass production of nitrogen fixing organisms (*Azospirillum brasilense*, *Rhizobium etli*, and *Bradyrhizobium diazoefficiens*) required specialized equipment and, consequently, a commercial service provider from Mexico City was hired. The mixture of the substrate used to soak in the bacteria, the preparation of the carboxymethylcellulose-based adhesive, and the packaging were carried out in the Centro Nacional de Investigación Disciplinaria de Microbiología-INIFAP. The final product of each bacteria had a 100×10^6 bacteria g of peat⁻¹ concentration in 400 g bags. This quantity was enough to inoculate medium-sized seeds used to sow 1 ha of maize and/or bean. The endomycorrhizal fungi *R. intraradices* increased through a in vivo production system, using different host plants to contribute to their multiplication. The process was carried out in sterile substrate composed of a mixture of sand and soil with low phosphorous content. The strategy was implemented in experimental fields from four different representative agroecological regions of the country: Valle de México (State of Mexico), Cotaxtla (Veracruz), General Terán (Nuevo León), and Rosario Izapa (Chiapas) (Durán-Prado *et al.*, 2001; Aguirre-Medina *et al.*, 2012). The final product recorded a minimum colonization root efficiency of 95% in the host plant and 40 spores per g of soil⁻¹ plus propagule. The packaged product contained 1 kg INIFAP™ mycorrhiza. This quantity was enough to inoculate medium-sized seeds require to sow 1 ha (Figure 1). During this period, the producer demands of the product were integrated, which facilitated the planning of the distribution of the mycorrhiza throughout the different states of Mexico.

The requests and distribution of biofertilizers had just one (and sometimes two) microorganisms per crop, including *Azospirillum brasilense* and *Rhizobium etli*. The Coordination and Liaison directors of INIFAP were in charge of this stage. These directors were the links between the FUMIAF A.C., the SAGAR-Alianza para el Campo, the district chiefs of SAGAR, and the producer associations. Without their intervention, the



Figure 1. Packaging presentation of microbial biofertilizers distributed to producers from 1999. a) 1999 and b) 2008.

distribution of biofertilizers would have failed in Mexico. Researchers from the INIFAP, private companies, and some rural development districts (particularly in Oaxaca) were in charge of the technology validation. Researchers from the INIFAP also established the validation plots for the biofertilization technology: 244 plots for the 1999 SS cycle, 23 for the 1999-2000 AW cycle, and 240 for the 2000 SS cycle, resulting in a total of 507 plots sown with different crops. The plots were established in fields from cooperating producers (minimum area: 1 ha). The producers and researchers selected together the most appropriate biofertilizers for each site, taking into account local conditions and the characteristic of the crop. The producers were in charge of agricultural management. The aim was to drive their interaction through agricultural management and to identify the efficiency and potential of the application, both in the plots and under the specific environmental conditions of the crops of interest. The yield values recorded in each plot was used to develop a graph with the Sigma Plot v. 11.0 software. Each plot group included standard error (\pm) for their agroecological region. Except for Sonora and Baja California, 9,229 agricultural producers and technicians from all the regions of the country took part in this training program (Figure 2).

Training activities increased during the 1999 SS and 1999-2000 AW agricultural cycles. The technicians of the Programa de Capacitación and Extensión of the Alianza para el Campo were also involved in this process. During the 1999-2000 AW cycle, biofertilizers were distributed in 411,907 ha in the states of Colima, Nayarit, Sinaloa, Sonora, and Baja California Norte and Sur. Seventy-six percent of the product was applied to sorghum crops (Table 1). Two treatments methods were applied during the technology validation process carried out in the plots of the producers: one using microorganisms (individual or co-inoculation treatment) and another using microorganisms plus the dose of chemical fertilizers used by the cooperating producer.

Table 2 includes the total number of plots established per agricultural cycle and crop. It also includes the results for maize and beans, the crops which were evaluated with greater frequency by the INIFAP researchers.



Figure 2. Biofertilization theoretical-practical training for agricultural producers and technicians.

Table 1. Microbial biofertilizers distribution and number of hectares.

Agricultural cycle	Hectares	Biofertilizer dose
SS 1999	577 657	753 191
AW1999-2000	411 907	893 764
SS 2000	893 611	723 485
Total	1 882 263	3 370 440

Source: Technical report of the Programa Nacional de Biofertilizantes. SS (Spring-Summer), AW (Autumn-Winter).

The results for maize were divided in three groups: native maize, commercial material, and commercial varieties + synthetic fertilizers. The yield of *Phaseolus vulgaris* L. increased in the 52 plots where microorganisms were used individually or in co-inoculation. The results obtained were higher than control. *R. etli* biofertilization resulted in a lower average yield than the fertilizer treatments used in the center, northeast, and south Pacific regions of Mexico; however, in the Gulf of Mexico, *R. etli* recorded higher results than fertilizers. For its part, the response to *R. intraradices* biofertilization was different between agroecological regions. *R. intraradices* increased yield in the northeastern and the south Pacific regions; however, in the latter region, fertilization surpassed microbial individual biofertilization. Meanwhile, in the Central Gulf of Mexico, *R. etli* biofertilization was 9% higher than *R. intraradices* (Figure 3).

RESULTS AND DISCUSSION

The differential yield obtained by *R. etli* suggested that regional environmental limitations restricted the yield of bean (Zahran, 1999), although bean can establish symbiotic associations with a wide diversity of rhizobia (Bernal and Graham, 2001). In addition, bean varieties strongly depend on nitrogen fertilizers to achieve a high yield in the field. This phenomenon is the result of the considerable variability of its nodulation and nitrogen fixation capacity; consequently, bean is capable of a stronger nitrogen fixation (Rahmani *et al.*, 2011) than other pulses. In the south Pacific, both environmental factors

Table 2. Validation plots per agricultural cycle and crop in Mexico.

Crop	Biofertilizer				Biofertilizer plus chemical fertilizer				Total in three cycles
	SS 1999	AW 1999-2000	SS 2000	Total	SS 1999	AW 1999-2000	SS 2000	Total	
Corn	62	7	32	101	69		24	93	194
Bean	28	3	6	37	26	6	6	38	75
Sorghum	16	6	15	37	14		145	159	196
Wheat	1		1	2	12		1	13	15
Barley	4			4	3			3	7
Oatmeal	3			3	2			2	5
Others	1	1	10	12	3			3	15
Total	115	17	64	196	129	6	176	311	507

Source: Technical report of the Programa Nacional de Biofertilizantes.

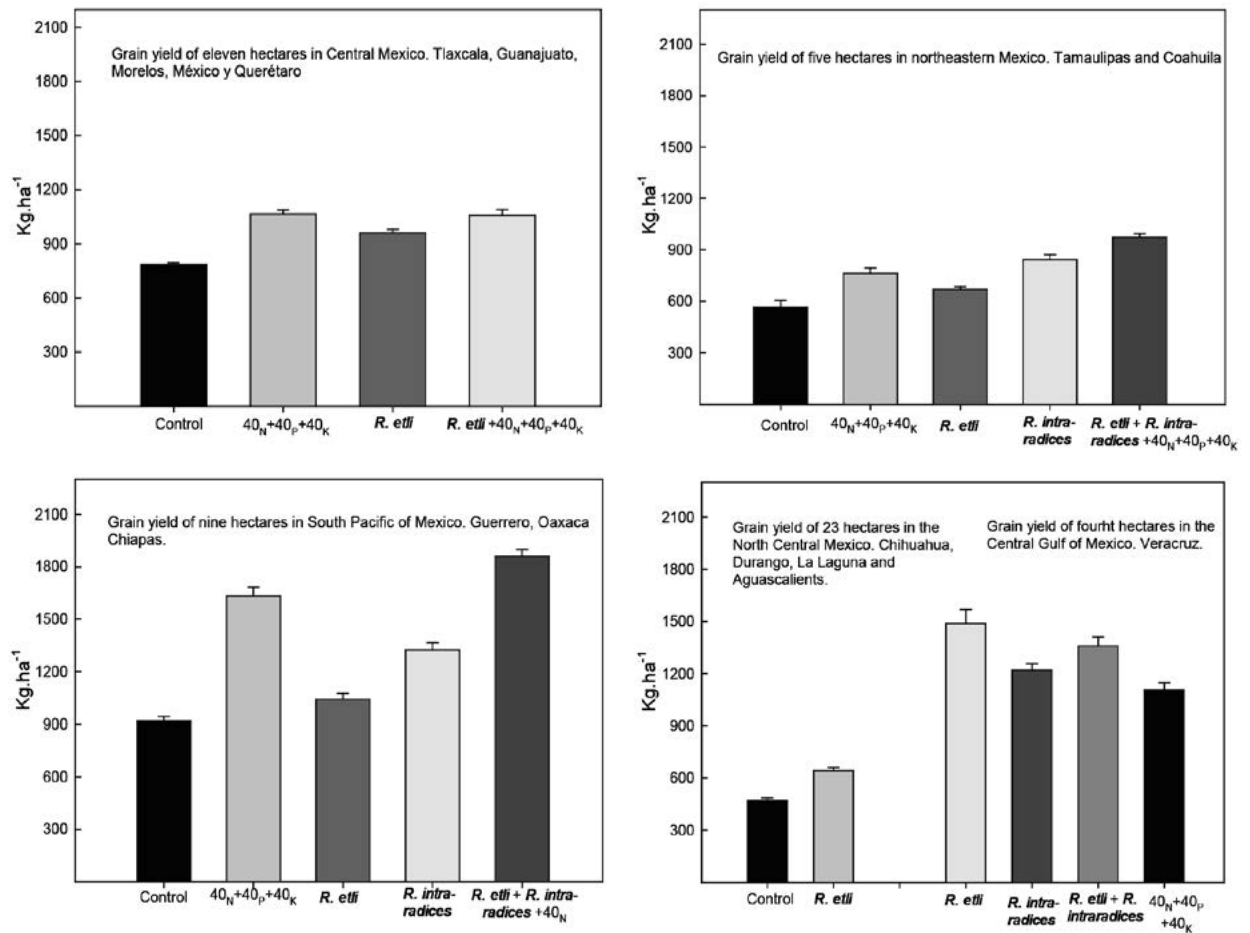


Figure 3. *Phaseolus vulgaris* L. yield obtained using microbial biofertilizers and/or synthetic fertilization in different regions of Mexico. The vertical line indicates the standard error (\pm).

and local crop management can increase bean yield. Bean co-inoculated with *R. etli* and *R. intraradices* recorded an important yield increase in the south Pacific and northeastern regions of Mexico.

When endomycorrhizal fungi associate with *Rhizobium* and *Azospirillum* fixing bacteria establish a synergy with various host plants, which grown even more, increasing their capacity to explore larger soil areas and consequently gaining access to more nutrients and water.

Hidalgo-Rodríguez *et al.* (2019) reported similar results. These authors used a co-inoculation with two symbionts in Peru, achieving a higher protein production (Küçük, 2011) and plant growth increase. Compared with control, the yield of native maize biofertilized with microorganisms (individual or co-inoculation treatment) increased by 19%. *R. intraradices* and *A. brasilense* co-inoculation recorded a similar yield than the individual *R. intraradices* biofertilization (Figure 4).

Microbial biofertilizers use several mechanisms to promote the growth of host plants, including the production of growth regulators (Naiman *et al.*, 2009) which favor root and leaf growth (Dar *et al.*, 2021) and the increase of mineralization and transport of nutrients.

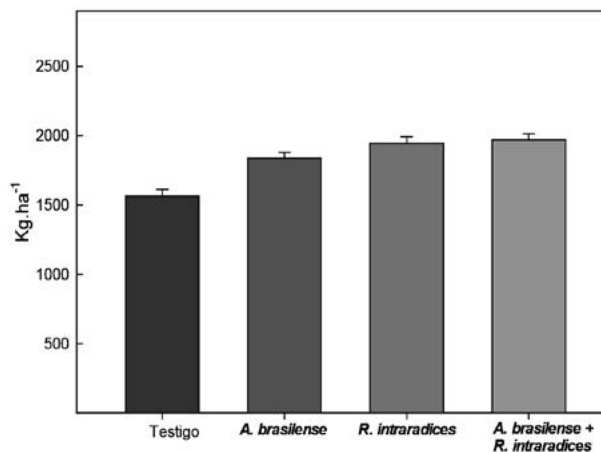


Figure 4. Yield of native maize from different Mexican regions, using microbial biofertilizers. The vertical line indicates a standard error (\pm) in 24 plots.

Likewise, endomycorrhizal fungi and other phosphate solubilizing microorganisms improve the availability of this element when it is found as insoluble phosphate (Djuuna *et al.*, 2022). The response of biofertilizers in the field is influenced by several abiotic factors; however, soil microbiota is also fundamental (Kumar *et al.*, 2017), because some microbes can positively or negatively impact the relationship between plants and beneficial microorganisms (Nannipieri *et al.*, 2003). Contrasting results were recorded with different maize varieties in different agroecological environments. Nevertheless, inoculated maize from the southeastern, central Gulf, and northeastern areas showed that the effect of biofertilizers surpassed control. In addition, the results reported different responses, depending on the microorganism used in the treatment. The highest yields were obtained by *R. intraradices* (individual treatment) and by *R. intraradices* and *A. brasilense* (co-inoculation treatment). In the case of the south Pacific area, no consistent differences were recorded between treatments, although the *A. brasilense* biofertilization obtained a slightly trend towards a higher yield (Figure 5).

In central Mexico, *A. brasilense* and *R. intraradices* (individual treatment) recorded the highest yield increase. Xipeng Liu *et al.* (2023) developed a result meta-analysis of biofertilizers and different crops. They pointed out that individual biofertilization of microorganisms increased the growth of plants by 29%, while the biofertilization with consortia increased the said growth by 48%. The increase of yield in biofertilized maize is the result of the higher mineral absorption produced by the symbiosis. In the maize fields of Mexico, *A. brasilense* biofertilization resulted in an important yield increase (Dobbelaere *et al.*, 2001). In India, wheat yield results obtained by the co-inoculation of *Glomus fasciculatum* and several bacteria from genera *Pseudomonas*, *Bacillus*, *Azotobacter*, and *Azospirillum* were significantly higher than the results recorded by control (Parewa and Yadav, 2014). The different maize responses to *R. intraradices* can be associated with the colonization preferences of the said fungi for this plant (Bonfante and Genre, 2008). In addition, these responses can be very specific to the host plant and the fungi used in the treatments (Gonzalez-Chavez *et al.*, 2004). Endomycorrhizal fungi can increase the

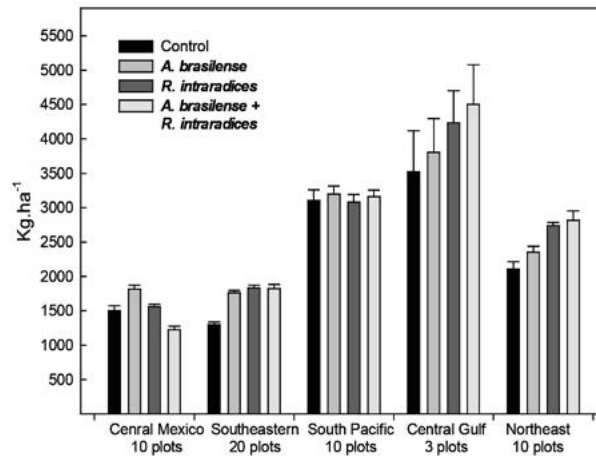


Figure 5. Yield of native maize from different Mexican regions, using microbial biofertilizers. The vertical line indicates a standard error (\pm).

area of effective absorption of the roots through the formation of a wide extraradical hyphae network, improving nutrient absorption efficiency (George, 2000). Currently, the use of microorganisms that promote plant growth, consequently improving the yield of different crops, has been widely spread (Radha and Rao, 2014). Figure 6 includes the response of maize to different combinations of microbial biofertilizers and high doses of fertilizers.

Using a combination of chemical fertilizers and biofertilizers can improve nutrient availability in the plant-soil system. The efficient application of several biofertilizers in the plots can greatly reduce the use of mineral fertilizers (Berg, 2009). In addition, the offer of these products is increasing, both for horticulture and for soil restoration (Gianinazzi and Vosátka, 2004).

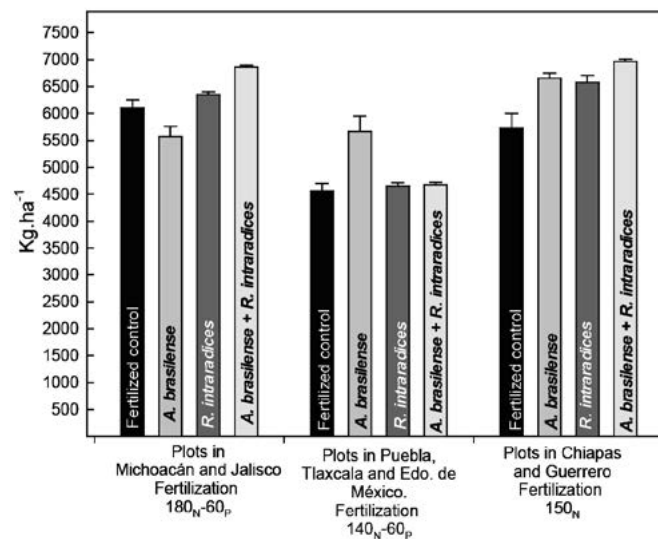


Figure 6. Yield of maize varieties from different regions of Mexico, using microbial biofertilizers and different doses of chemical fertilizers. The vertical line indicates a standard error (\pm).

The content of soil nutrients is fundamental to successfully grow sustainable and productive crops. Soil nutrients allow to obtain higher yields (Brady and Weil, 2002) and the use of rhizobacteria can reduce the doses of chemical fertilizers applied to crops (Prisa *et al.*, 2023). Ekinici *et al.* (2021) have identified several plant growth-promoting rhizobacteria (PGPR) which can significantly impact plant growth.

CONCLUSIONS

In Mexico, microbial biofertilizers (individually or in co-inoculation treatments) can increase crop yield under different environmental and management conditions. In most of the cases, the bacteria/mycorrhizal fungi co-inoculation promoted growth and yield in host plants. Rhizobia established effective symbiosis, promoting bean growth, decreasing the application of nitrogen fertilizers, and reducing production costs. Biofertilizers are a more acceptable ecological alternative for sustainable agriculture than the agrochemical fertilizers used in other production systems. The excessive use of agrochemicals has seriously damaged the environment. Currently, research about biofertilizers is abundant. In addition, it proves that PGPRs can improve crop nutrition, achieve higher yields, and/or reduce production costs (chemical fertilization).

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