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Research article

Use of systemic biofertilizers in sugarcane results in highly reproducible increments in yield and quality of harvests

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ABSTRACT

The utilization of a novel (systemic) biofertilizer containing *Pseudomonas fluorescens, Azospirillum brasilense*, and *Bacillus subtilis* and possessing the technology to facilitate the entry of bacteria through the stomata, was evaluated at three localities in Mexico (Potrero Nuevo, Veracruz; Ameca, Jalisco; and Champotón, Campeche) in two sugarcane varieties (NCO-310 and Mex 57–473) at different time scales. Inoculation of the systemic biofertilizer was imposed over the local agricultural management of the sugarcane; chemical fertilization of the experimental parcels at Potrero Nuevo was done using 70-20-20 and 120-80-80 at Ameca and Champotón. Three doses of the biofertilizer per hectare were applied during the annual productive cycle of sugarcane at each site; one year at Potrero Nuevo and Champotón; and six years at Ameca. The annual sugarcane yield was evaluated at each site. Additionally, sugar quality (°Brix or sucrose content) was evaluated at the three localities, while different variables of stalk performance were also measured at Ameca and Champotón. Our data provide evidence that this systemic biofertilizer consistently and reliably increased the sugarcane yield at all localities during the time of evaluation, ranging from 73.7 tons ha^{-1} at Potrero Nuevo (2.5 times increase; $P < 0.05$) and 77.7 tons ha^{-1} at Ameca (1.9 times increase; $P < 0.05$) to 23.8 tons ha^{-1} at Champotón (1.4 times increase; $P < 0.05$). This increase in sugarcane biomass was related to increased tillering rather than increased stalk height or diameter. This novel biological product improved the sugarcane quality in terms of °Brix ($P < 0.05$, 2.6° difference) and sucrose content ($P < 0.5$, 0.7% difference).

1. Introduction

Approximately 80% of global sugar production is sourced from sugarcane (*Saccharum* spp.), which grows in tropical and subtropical climates. The remaining 20% is derived from sugar beets, which are grown mostly in the temperate zones of the Northern Hemisphere. Sugarcane is a valuable crop because of its adaptation to different types of soil and environments and its potential to

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produce sugar, ethanol, biodegradable products, energy, and food for animal production. At 1.9 billion tons, sugarcane was the most produced crop worldwide in 2020, accounting for 20% of the global production of primary crops [1]. In the same year, America was the leading region in the production of sugarcane (54% of the global production), with Brazil as the principal producer (757,117,000 tons; 40.5% of global production), followed by Mexico (53,953,000 tons; 2.8% of total production). Globally, sugarcane biomass levels are subject to different factors; however, climate (particularly rainfall and temperature) and climate change are the key factors driving sugarcane production, especially in many developing countries [2]. In tropical areas, a high biomass of sugarcane production in the range of 150–300 tons ha^{-1} $year^{-1}$ can be achieved depending on the management and production systems employed [3]. However, a record sugar production of 516,529 tons ha^{-1} $year^{-1}$ in Fiji was recorded in 1994 because of favorable weather conditions [4]. World sugarcane yields increased from 52 tons ha^{-1} $year^{-1}$ in 1972 to 70 tons ha^{-1} $year^{-1}$ in 2021, displaying a 0.63% average annual growth rate [5]. Simulation models based on climate change data suggest that the sugarcane yield is expected to increase over the next few years. For example, the cane yield in Brazil is expected to be 15–59% higher than the current yield by 2050 [6]. Sugarcane genotype also plays a central role in obtaining high yields in the field. For example, in Mexico, the varieties CP 72–2086, Mex 69–290, and Mex 79–431 represent more than 70% of the land cultivated for this crop because of their productive potential. CP 72–2086 has a potential yield of 115 tons ha^{-1}, while Mex 69–290 yield can reach up to 200 tons ha^{-1} under optimal agronomic conditions [7]. Due to its high capacity for biomass production, sugarcane requires elevated quantities of nitrogen (180–250 kg ha^{-1} $year^{-1}$). Although part of the demand for this element is satisfied by the mineralization of soil organic matter, this input is not sufficient to achieve high yields; therefore, the complementary nitrogen must be supplied either by chemical or by organic fertilization [8]. Due to the increase in chemical input costs and the growing public concerns about the impact of agrochemicals on the environment and human health, modern agriculture has increasingly focused on the use of biological products as an alternative to completely or partially substitute agrochemicals (particularly, fertilizers), alleviate abiotic environmental stress in crops [9–11], or even increase the yield of harvests beyond the conventional limits [12]. In the case of sugarcane, chemical supplies (mainly fertilizers) account for 22–25% of the total production cost [13]; therefore, biological products have drawn attention as a sustainable alternative to mineral fertilizers in sugarcane [14,15]. Efforts have been made to reduce nitrogen [16] and phosphate fertilization [17]. Beneficial interactions between plant growth-promoting microorganisms and sugarcane have been reported by different researchers worldwide [12,15,18,19]. *Azospirillum, Azotobacter, Bacillus, Burkholderia, Gluconacetobacter, Herbaspirillum, Pseudomonas*, and *Rhizobium* are some of the most common genera of diazotrophic plant growth-promoting bacteria associated with sugarcane and have been studied under both greenhouse and field conditions [20–22]. Part of the interest in the use of diazotrophic microorganisms stems from information derived from diverse studies showing that biological nitrogen fixation can satisfy up to 70% of sugarcane nutritional requirements [23]. Velasco–Velasco [20] mentioned that different studies in Brazil have shown the feasibility of obtaining high yields of sugarcane using efficient microorganisms and 50 kg N ha^{-1}. According to Singh et al. [15], different investigations have demonstrated that Brazilian sugarcane cultivars can acquire up to 40–100 kg N ha^{-1} $year^{-1}$ from biological nitrogen fixation. Similar results for *Paraburkholderia* sp., a nitrogen-fixing bacterium, were reported in Australia by Qiu et al. [24]. When these authors supplied sugarcane with 120 kg N ha^{-1} (as conventional urea; 120 N treatment), the experimental plots yielded approximately 87.5 tons ha^{-1}, while those supplied with the same chemical dose and biofertilized with *Paraburkholderia* sp. (120NB treatment) had a cane yield of 96 tons ha^{-1} (a 10% increment); sugar yield was slightly and significantly higher at the 120NB treatment (approximately 14 tons ha^{-1}) than at the 120 N treatment (13 tons ha^{-1}). On the other hand, Ahmed [25] in Egypt, observed that increasing levels of a phosphate solubilizing biofertilizer, based on *Bacillus megatherium,* significantly improved the number of millable cane per m^2, millable cane height, diameter, °Brix, sucrose (%), sugar recovery (%), and cane and sugar yields in plant cane and its ratoon crops. Thus, the evidence provided by these and other studies distinctly indicates that the inoculation of crop plants with plant growth-promoting microorganisms results in increased productivity and quality [26]. However, field studies have shown that this technology has some important drawbacks, such as high variability (0–38% increase in yield) and low reproducibility in several crops [13,17,27–31] as genetic and environmental factors play a central role in plant and growth-promoting microorganism interactions [18].

Internal plant tissues have been suggested to provide a more suitable habitat for (diazotrophic) plant growth-promoting bacteria than the rhizosphere because of the greater availability of nutrients and the low 0_2 environment required for optimum nitrogenase functioning [12]. Consequently, multiple efforts have been devoted to isolating endophytic microorganisms to improve the biological nitrogen contribution to the N economy of crops [30].

In 2010, our research group developed a novel technology called “Micro In” by which it is possible to introduce beneficial microorganisms through the stomata, making the employment of microorganisms a more efficient and repeatable tool for increasing the productivity and quality of crops. Products derived from this technology are called systemic biological products [12]. As defined by us, a systemic biological product is characterized by introducing beneficial microorganisms inside plants via plant stomata [32]. Compared to conventional biological products, several advantages of systemic biologicals have been delineated by Aguado-Santacruz et al. [12]. The interior of plant tissues has a more favorable environment for the survival and proliferation of microorganisms. Additionally, all of their beneficial activities are more efficient because the active compounds that promote growth do not suffer losses (as happens, for example, in the case of nitrogen fixation in the rhizosphere) and act more directly on plant metabolism (for example, hormones, volatile compounds, ACC deaminase, antibiotics, and HCN). Additional advantages of systemic (endophytic) microorganisms have been mentioned by Vaishnav et al. [9], Aguado-Santacruz et al. [12], and Singh et al. [15].

Thus, we hypothesized that using a systemic biofertilizer would result in more consistent and reliable sugarcane yield and quality. In this study, we present changes in the yield and quality of sugarcane treated with a systemic biofertilizer. Data from various years and localities in Mexico are presented. Our data support the highly efficient and reproducible functioning of the systemic biofertilizer for sugarcane yield and quality improvement, both on temporal and spatial scales. This novel disruptive technology is expected to make the utilization of beneficial microorganisms a more reliable tool for achieving high harvest yields and enhancing the quality of

agricultural products.

2. Materials and methods

2.1. Study sites

This study was conducted for nine years at three sugarcane plantations located in three different localities in Mexico: Ameca, Jalisco State; Potrero, Veracruz State; and Champotón, Campeche State. These sites are located between 10 and 1230 masl, with annual mean temperatures ranging from 22 °C in Ameca to 25.9 °C in Champotón (Table 1). The hottest climate as indicated by the annual temperatures prevails at Champotón and all three sites are characterized by high precipitation regimes (≥1300 mm) [33,34]. The soil prevailing at Ameca is classified as Phaeozem, while those predominating at Potrero Nuevo and Champotón are of the Vertisol type (Table 1).

2.2. Experimental treatments

At each site, the chemical fertilization dosage normally used by local sugarcane farmers was considered. At Potrero Nuevo the fertilization dosage for the sugarcane parcels was 70-20-20, while at Ameca and Champotón the fertilization formula was 120-80-80. In this study, we evaluated a commercial systemic biofertilizer named BactoCROP® (distributed by the company BIOqualitum, www.bioqualitum.com) that contains *Pseudomonas fluorescens*, *Azospirillum brasilense*, and *Bacillus subtilis* as active ingredients. This biofertilizer also contained minimal concentrations of some nutrients because of the medium used in its formulation (Table 2) and was applied to the plots in addition to the aforementioned chemical fertilization dosage considered at each site.

The systemic biofertilizer was dissolved in water and immediately sprayed over the crop. The amount of water used in each application to dissolve the biofertilizer varied between 250 and 600 L according to the development of the plants, ensuring that all plants were well sprinkled on the foliage and/or on the base of the tufts. At each site, three applications (1.5 kg each per hectare) were performed during the sugarcane cycle. The first was performed from March–April, when the meristematic activity of the sugarcane tufts (ratoons) started or when a novel plantation was established, employing a tractor sprayer with 80-04 nozzles attached or a backpack sprayer. The second dose was applied for one month (May), and the third for two months (June) after the initial biofertilization of plants. In these subsequent applications, two-third of the product solution was applied to the base of the plants and one-third was directed to the leaves. Water was applied to the control plants instead of the biofertilizer solution, considering the same amount, timing, and distribution.

Two hectares were sown with the sugarcane variety NCO-310 at Potrero Nuevo in 2009; this material is employed by 2% of the sugarcane producers of México [35]. Systemic biofertilizer was first applied on 1 ha of land (biofertilized parcel) at the time of planting cuttings using a backpack sprayer. The second and third applications were performed on the biologically treated parcel, as previously stated. Another hectare was subjected to conventional agronomic management by a producer (control parcel).

At Ameca, three parcels of 0.5 ha, 1.0 ha, and 1.5 ha, and two parcels of 3.0 ha, were sown with the sugarcane variety Mex 57–473 (9 ha in total) in 2010. This sugarcane variety accounts for 3% of the Mexican territory cultivated for this crop [35]. The first application of systemic biofertilizer on every parcel was carried out on half of the sugarcane surface (ratoons) in 2014, so these

Table 1
Description of study sites in México.

	Sites		
	Potrero Nuevo	Ameca	Champotón
Coordinates			
North latitude	18°53′39''	20°32′52″	19°21′00''
West longitude	96°47′02''	104°02′50''	90°43′30''
Height (masl)	515	1230	10
Annual mean temperature (°C)	22.3	22.0	25.9
Annual mean maximum temperature (°C)	25.1	32.9	32.0
Annual mean minimum temperature (°C)	13.4	8.5	19.9
Annual mean precipitation (mm)	1651	1458	1300
Soil type	Vertisol	Phaeozem	Vertisol
Sugarcane variety sown	NCO-310	Mex 57-473	NCO-310
Years of evaluation	2009	2014–2019	2018
Condition of the sugarcane crop	New planting	Ratoons	Ratoons
Number of experimental parcels	1	5	4
Variables analyzed per site	Cane yield	Cane yield	Cane yield
	°Brix	Stalk weight	Stalk height
		Stalk height	No. tillers m^{-2}
		Stalk diameter	Total chlorophyll
		No. tillers per tuft No. internodes	Sucrose content
		°Brix	

Table 2
Complete composition of the biofertilizer employed in this study.

Component	%
Protein	9.3
Polysaccharides	8.2
Carbohydrates	9.3
Phosphorous	0.7
Potassium	1.2
Iron	1.9
Calcium	0.5
Magnesium	0.5
Pseudomonas fluorescens (1×10^8 CFU)	1.0
Azospirillum brasilense (1×10^8 CFU)	1.0
Bacillus subtilis (1×10^8 CFU)	1.0

applications were carried out in four-year-old ratoons. All the applications followed the instructions aforementioned. Subsequently, applications were repeated annually from 2014 to 2019.

Finally, at Champotón, four experimental sugarcane 1-ha parcels were established with the variety NCO-310 in 2016. The one-year evaluation scheme considered at Potrero Nuevo was followed at this site in 2018. However, at Champotón the experimental applications were performed on two-year-old ratoons.

After harvesting, the sugarcane trunk height was uniformized at all study sites by cutting stumps 5–10 cm above the soil surface. Additionally, soil compaction in the parcels was broken every year by plowing the land at 10–20 cm.

2.3. Verification of the presence of the bacteria within the internal tissues of sugar cane. Re-isolation of beneficial microorganisms

At the end of the sugarcane production cycle, 25 stalks from each parcel were randomly collected from all study sites to re-isolate the bacteria previously applied to the sugarcane plants. Sugarcane stalk pieces were washed under running water and cut into 4 cm fragments. Subsequently, under a laminar flow hood, these pieces were disinfected with 70% ethanol for 1 min and 3% sodium hypochlorite for 2 min and subsequently washed thrice with sterile distilled water. The barks of these fragments were removed using a sterilized scalpel and forceps and smaller pieces (1×1 cm) were cut and placed on sterile paper napkins to eliminate excess moisture. Finally, the sugarcane pieces were placed on Petri dishes containing selective media for the isolation of *Bacillus subtilis* (BS medium) [36], diazotrophic bacteria (Congo Red and Elmarc media) [37], and *Pseudomonas* (Gould S1 medium) [38]. The Petri dishes containing the sugarcane pieces were incubated for 24 h at 28–30 °C. Morphologically distinct colonies were selected and purified.

2.4. DNA extraction, PCR, and restriction analysis

Briefly, the re-isolated microorganisms were grown in 10 mL of their respective media for 24 h. Then, the bacterial samples were centrifuged at 5000 rpm for 10 min. The supernatant was discarded and the remaining pellets were used for genomic DNA extraction using the sarcosine method [39]. To verify DNA quality, electrophoresis was performed on a 1% agarose gel. Using the extracted DNA as a template, fragments of the internal transcribed spacer (ITS) regions were amplified using the primers G1 (SEC ID NO: 7) and L1 (SEC ID NO:8) [40], in mixtures containing (in 50 μl final volumes): genomic DNA (150 ng), Buffer 1X, $MgCl_2$ (2 mM), nucleotides (50 μM each), primers (0.2 μM each) and Taq polymerase (1 U per reaction). Then, the mixtures were placed in a Thermo Scientific thermocycler, considering the following conditions for amplifications: initial phase, 5 min at 94 °C for one cycle; denaturation at 94 °C for 1 min; annealing at 55 °C for 2 min; and extension at 72 °C for 2 min for 35 cycles. A 7-min final extension at 72 °C for 1 cycle was performed at the end of the cycling steps. After amplification, samples were maintained at 4 °C. The amplicons obtained were purified using a QIAEX II kit (Qiagen) and subsequently restricted using the enzyme *Dde* 1 (Table 3). Previously, we generated DNA fingerprints of three bacteria based on the restriction patterns of ITS fragments amplified using the restriction enzymes *Hae*III, *Dde*I, and *Hha*I.

Table 3
Expected band sizes (bp) by the amplification of the ITS region and its restriction patterns (bp) generated with the enzyme *Dde*I in the three bacteria.

	Bacterial species		
	P. fluorescens	***A. brasilense***	***B. subtilis***
Amplified fragments from ITS region	692	668	455
	654		265
	614		
Restriction fragments generated with *DdeI*	195	190	208
	138	122	
	105	88	

3. Experimental design

3.1. Cane yield

This variable was determined for all sites. Ten randomly replicated plots were harvested per treatment (biofertilized and non-biofertilized) at each site. Each plot consisted of five rows of sugarcane (10 m long and 1.8 m row spacing). Cane yield was determined in 2009 at Potrero Nuevo, from 2014 to 2019 in the five parcels located at Ameca and finally, in 2018 at the four parcels located at Champotón.

3.2. Cane yield components and quality

At Potrero Nuevo, the quality of the harvested canes was analyzed using °Brix (in 2009), while at Ameca, six variables were studied in 2013, namely, stalk weight, stalk height, stalk diameter, number of canes per tuft, number of internodes, and °Brix. Finally, at Champotón, stalk height, number of canes per tuft, total foliar chlorophyll concentration, and sucrose content (%) were analyzed in the 1-ha four parcels located at this site. For all these additional variables, 25 replicates (tufts) per treatment site were analyzed.

Chlorophyll content was determined following the method described in Aguado-Santacruz et al. [41], while the sucrose content (%) and °Brix were determined following the methodology of the Official Mexican Standards established for the Sugar Industry [42].

3.3. Statistical analysis

At Potrero Nuevo, one-way ANOVA was used to analyze differences among the variables of the biofertilized and non-biofertilized (conventional) parcels. At Ameca and Champotón, where several parcels were analyzed per site (five and four, respectively), treatments were established in a randomized block design. In these cases, two-way ANOVA was employed to examine the data of all parcels established at each site, considering the same treatments (biofertilized and non-biofertilized). Significant differences ($P < 0.05$) among the analyzed variables were determined using Tukey's test [43].

4. Results

Evaluation of the effect of systemic biofertilizer in sugarcane was first performed in Potrero Nuevo, Veracruz, in 2009. That year the climatic conditions (precipitation = 1651 mm, average ambient temperature = 23.7 °C) were favorable for sugarcane growth and production (Fig. 1).

The cane yield obtained at Potrero Nuevo on the biofertilized parcel (119.8 ton•ha^{-1}) was significantly higher ($P < 0.05$) than the production registered at the conventionally managed parcel (46.1 ton^{-1}; Table 4). Although this great difference between the biofertilized parcel and the conventionally managed parcel can be partially explained by the systemic biofertilizer functioning, the conventional management method followed by Potrero Nuevo farmers was suboptimal because the yield obtained at this site was low, even for the historic regional average because yields of 70–80 tons ha^{-1} are very common at this area. Owing to the low yield achieved with conventional management, systemic biofertilizers were able to increase the sugarcane yield 2.5-fold at this site. Additionally, the quality of the juice was improved by 2.6°Brix by the application of biological products (Table 4).

The results obtained at Potrero Nuevo encouraged the validation of the biofertilizer at a larger scale. Thus, we initiated a long-term study at Ameca, Jalisco, considering different local micro-edaphic conditions. The precipitation that occurred during this study (2014–2019; Table 5) was favorable for sugarcane growth.

Five experimental parcels, varying between 0.5 and 3 ha, were studied at this site. These parcels were biofertilized, whereas their counterparts were conventionally managed. Our data indicated a highly consistent effect of biofertilizer on sugarcane production on both spatial and temporal scales (Table 6). Increases in sugarcane yield fluctuated between 92.8% in 2018 (*i.e.*, 161.4 *vs* 83.7 ton•ha^{-1}) to 42.3% in 2017 (*i.e.*, 117.9 *vs* 75.8 ton•ha^{-1}); with the exception of the results obtained in parcel 3 in 2016, and parcels 2 and 3 in 2017, the sugarcane yield was always significantly higher ($P < 0.05$) in the systematically biofertilized plots (Table 6).

To better understand our initial results, a study was conducted in 2014 at Ameca to analyze some of the yield components in the

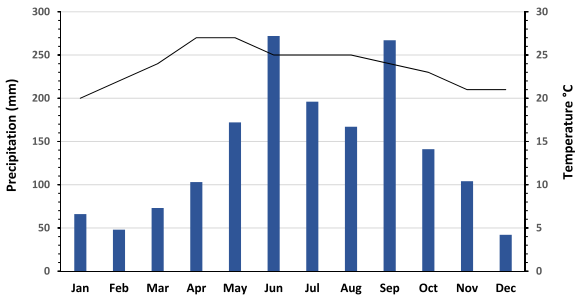

Fig. 1. Climograph of Potrero Nuevo, Veracruz for the year of the study (2009).

Table 4
Cane yield and °Brix at Potrero Nuevo, Veracruz, México, in 2009.

Variables	Treatment	
	Biofertilized	Conventional
Cane yield (ton ha^{-1})	119.8a[a]	46.1b
°Brix	18.8a	16.2b

[a] Average values from 10 replicate plots (yield) and 25 replicates (°Brix). Columns with the same letter are not significantly different, as determined by Tukey's mean separation test ($P > 0.05$).

Table 5
Precipitation during the study period at Ameca, Jalisco, México.

	Year					
	2014	**2015**	**2016**	**2017**	**2018**	**2019**
Precipitation (mm)	853	1027	1328	1.030	893	1134

biofertilized sugarcane plants (Table 7). Stalk weight, stalk height, and number of canes per tuft were significantly higher ($P < 0.05$) in the biofertilized plots than in the conventionally managed plots; stalk diameter and number of internodes per cane were similar between treatments ($P > 0.05$). Although differences in stalk weight and height were evident between the biofertilized and non-biofertilized plants, the greatest contribution to the increase in yield was mainly caused by the increased number of canes per plant, *i.e.*, an increased tillering rate. Additionally, the quality of the sugarcane juice was significantly ($P < 0.05$) improved by 2.6 °Brix (Table 7).

Finally, validation of the systemic biological product was carried out at Champotón, Campeche, in 2019, when climatic conditions were optimal for sugarcane growth (Fig. 2). Higher sugarcane yields were achieved in the biofertilized parcels established at this site ($P < 0.05$). The average increase in the sugarcane yield at the four parcels of Champotón was 36.5% (*i.e.*, 88.9 *vs* 65.1 ton•ha^{-1}; Table 8).

We noted an apparent increase in chlorophyll in the leaves after spraying the sugarcane plants with the systemic biofertilizer, therefore, we analyzed the content of this pigment in the plants (Table 8). This analysis confirmed our initial suppositions as the content of this pigment was always higher in the parcels sprayed with the biofertilizer (2.6 vs 2.0 mg•g^{-1} FW; *i.e.*, 29.1% increase).

Contrarily, stalk height was significantly higher ($P < 0.05$) in only two of the four parcels (Table 8), while the number of canes per m^2 was always statistically higher in the biofertilized parcels than those in the conventionally managed parcels ($P < 0.05$); this represents almost a 50% increase in the number of tillers per m^2 in the biofertilized plots.

Finally, as it occurred at Potrero Nuevo and Ameca, we could confirm that the systemic biofertilizer not only benefited sugarcane yield but also quality, as the sucrose content was significantly higher ($P < 0.05$) in three of the four parcels established at Champotón (5.7-fold increase; Table 8).

5. Discussion

In this study, we found that the use of a systemic biological product in sugarcane resulted in consistent increases not only in production but also in quality, both on a temporal and spatial scale. The technology incorporated in the biofertilizer, permitting the entry of the microorganisms into the vascular system of the plants, was able to increase consistently the sugarcane yield up to 2.5-fold at Potrero Nuevo, 1.9-fold at Ameca, and 1.7-fold at Champotón (Tables 4, 6 and 8).

The grass family (Poaceae) includes different plant species that are important for human consumption, such as rice, wheat, maize, oats, barley, and sugarcane. This plant family is characterized by modular growth called tillering, which is important for understanding grass growth and regrowth. Tillers are novel grass shoots made up of consecutive segments called phytomers, which are composed of a growing point (an apical meristem that may turn into a seed head), stem, leaves, root nodes, and latent buds. The process by which novel aerial shoots emerge through lateral growth is called tillering [44]. Tillering is one of the most important agronomic traits of grasses as the number of tillers per plant determines not only panicle number, a key component of grain yield but also the total plant biomass.

From the data presented here, we can conclude that increased production in grass sugarcane by the inoculation of plant growth-promoting microorganisms was linked to an increased tillering rate rather than to an increased stalk diameter or height. Some biofertilizers seem to stimulate tillering in grasses. For example, Agake et al. [45] found an increased tillering rate in two of three varieties of rice inoculated with *Bacillus pumilus* (approximately 15–30%). In addition to the increased tiller number, these authors were able to detect a statistically higher plant height in biofertilized plants than in control plants. They also noticed an increase in nitrogen content (as measured by spad technology) during the early growth of rice in the field, which we detected (and measured as chlorophyll content) in our sugarcane parcels. Studies on wheat biofertilized with *Azotobacter* spp. and other nutrient-solubilizing bacteria have also reported augmented yields related to increased tillering [46].

In India, Shanthy and Venkatesaperumal [47] found that 81.7% of surveyed farmers noticed that tillering increased in biofertilized sugarcane fields, which in turn led to increased cane yield. These authors also emphasized the necessity of using effective and

Table 6
Cane yield (tons ha^{-1}) obtained at Ameca, Jalisco, México, from 2014 to 2019.

Parcels	Years											
	2014		**2015**		**2016**		**2017**		**2018**		**2019**	
	Biofertilized	***Conventional***	***Biofertilized***	***Conventional***	***Biofertilized***	***Conventional***	***Biofertilized***	***Conventional***	***Biofertilized***	***Conventional***	***Biofertilized***	***Conventional***
1	116.1a[a]	80.1b	174.2a	82.0b	161.3a	82.0b	116.1a	74.0b	161.1a	84.0b	151.4a	80.8b
2	125.0a	77.3b	107.1a	84.0b	100.0a	80.0b	73.6a	76.4a	163.4a	82.8b	150.5a	80.7b
3	125.0a	80.0b	109.4a	78.2b	82.5a	81.0a	76.9a	77.9a	163.5a	83.4b	150.3a	79.2b
4	163.6a	76.5b	172.7a	82.1b	172.7a	77.8b	172.7a	76.8b	164.3a	83.6b	149.9a	81.2b
5	150.0a	81.7b	125.0a	81.8b	150.0a	81.1b	150.0a	73.8b	159.7a	84.7b	147.9a	81.7b
Mean	**135.9a**	**79.1b**	**137.7a**	**81.6b**	**133.3a**	**80.4b**	**117.9a**	**75.8b**	**161.4a**	**83.7b**	**150.0a**	**80.7b**
Difference	**56.8**		**56.1**		**48.9**		**32.1**		**77.7**		**69.3**	
%	**71.8**		**68.7**		**60.9**		**42.3**		**92.8**		**85.9**	

[a] Average values from ten replicates. Columns with the same letter are not significantly different, as determined by Tukey's mean separation test ($P > 0.05$).

Table 7
Productive variables analyzed in biofertilized and conventionally managed sugarcane ratoons located at Ameca, Jalisco, México, during 2014.

Treatment	Variables					
	Stalk weight (Kg)	Stalk height (m)	Stalk diameter (cm)	No. tillers/tuft	No. internodes	°Brix
Biofertilized	2.46a[a]	2.47a	2.02b	18a	15.2a	19.30a
Conventional	1.79b	1.95b	1.78b	11b	15.5a	16.69b

[a] Average values of 25 replicates. Rows with the same letter are not significantly different between biofertilized and conventionally managed parcels, as determined by Tukey's mean separation ($P > 0.05$).

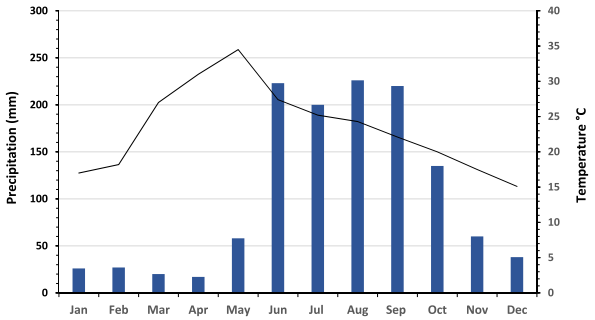

Fig. 2. Climograph of Champotón, Campeche for the year of the study (2019).

high-quality biofertilizers for sugarcane production.

Schultz et al. [16] studied the effects of inoculating a consortium of five diazotrophic bacteria, *Gluconacetobacter diazotrophicus, Herbaspirillum seropedicae*, *Herbaspirillum rubrisubalbicans*, *Nitrospirillum amazonense*, and *Paraburkholderia tropica,* on novel plantations of two sugarcane varieties in two locations in Brazil. In comparison to absolute control, they found that biological inoculation and N chemical fertilization on the "Sapucaia" plantation promoted increased stem yield equivalent to 22.3 and 26.5 tons ha^{-1} in the RB867515 variety, respectively. Conversely, as compared to absolute control, inoculation with the bacterial consortium and application of the N fertilizer in the RB867515 variety on the "Coruripe" plantation increased stem yield to 38.0 and 42.4 tons ha^{-1}, respectively. At this same location, the RB72454 variety showed respective increases of 16.7 and 37.5 tons ha^{-1} in the inoculated and the N fertilized treatments compared to those of the absolute control. Statistical differences between the biological and the chemical treatments were only found in the RB72454 variety at the 'Coruripe' site, where chemical fertilization increased stem yield to 20.8 tons ha^{-1} more than that of plants treated with the bacterial consortium. In addition, both treatments increased the total recoverable sugar yield.

It is important to define the extent to which an increase in yield and quality can be expected in sugarcane using beneficial microorganisms. This is not an easy task because the extent of the yield increase obtained in this crop to date is linked not only to crop variety, the efficiency of the biofertilizer, or the influence of environmental factors, but also to the technological level and supplies employed by the farmer for sugarcane cultivation [12,18,48].

Antunes et al. [30] studied the effects of inoculating the sugarcane variety RB 92579 with *Azospirillum amazonense, Herbaspirillum seropedicae, Herbaspirillum rubrisubalbicans*, *Gluconacetobacter diazotrophicus*, and *Burkholderia tropica*. These authors showed that these strains did not improve the yield or biological nitrogen fixation in sugarcane. However, the industrial characteristics during the three cycles of sugarcane analysis showed positive or negative alterations in the production of sugars and fibers, without clear patterns.

Ramesh et al. [13] reported that increases in sugarcane production in the field from 5 to 10% have been reported when using nitrogen-fixing biofertilizers such as *Azospirillum* sp. or *Azotobacter* sp. However, the numbers provided by Schultz et al. [49] were higher because they found a 13.5% net increase in sugarcane stalk yield in comparison to an absolute control (no chemical or biological fertilization) in a study conducted in Brazil with two sugarcane varieties, which corresponded to the average of the first planted cane and the following two consecutive ratoons in the two analyzed varieties. Finally, Ortega et al. [50] reported that increases in sugarcane yield, ranging from 25 to 35%, were possible when biofertilizers were added to this crop.

Leonel et al. [17] conducted an experiment in Brazil to determine the optimal combinations of microorganisms and phosphate fertilization in terms of the yield and quality of sugarcane. They reported that low (45 kg ha^{-1}) and average (90 and 135 kg ha^{-1}) P_2O_5 rates along with single and/or combined inoculations of *Azospirillum brasilense, Bacillus subtilis*, and *Pseudomonas fluorescens* (except for the combination of the three bacteria) provided greater cane sugar yields than the control treatment. The highest yields were obtained with *A. brasilense* + *B. subtilis* inoculation associated with 45 kg P_2O_5 kg ha^{-1} (211 ton ha^{-1}) and with *B. subtilis* + *P. fluorescens* associated with 135 kg P_2O_5 kg ha^{-1} (218 ton ha^{-1}), which reflected respective increases of 38% and 31% in stalk yield compared to the control treatments. Similarly, the highest sugar yields (32.2 ton ha^{-1}) was obtained with *P. fluorescens* inoculation +135 kg P_2O_5 ha^{-1} followed by the inoculation of *A. brasilense* + *B. subtilis* associated with 45 kg P_2O_5 ha^{-1} (30.3 t ha^{-1}).

Since the first isolation of endophytic microorganisms in the 50s [51] different studies have focused on their beneficial activities in plants, particularly concerning biological nitrogen fixation [52,53]. Some studies have shown that biological nitrogen fixation can

Table 8
Productive variables analyzed in biofertilized and conventionally managed sugarcane ratoons located at Champotón, Campeche, México, during 2018.

Parcels	Variables									
	Cane yield (ton•ha^{-1})		**Stalk height (m)**		**No. tillers•m^{-2}**		**Total chlorophyll (mg•g^{-1} FW)**		**Sucrose (%)**	
	Biofertilized	*Conventional*	*Biofertilized*	*Conventional*	*Biofertilized*	*Conventional*	*Biofertilized*	*Conventional*	*Biofertilized*	*Conventional*
1	73.6a[a]	55.4b	2.29a	1.78b	11.0a	7.0b	2.3a	1.8b	14.30a	13.53b
2	99.3a	77.7b	1.53a	1.51a	22.0a	17.0b	2.7a	2.1b	14.00a	13.47a
3	108.1a	78.3b	1.46a	1.11a	13.0a	8.0b	2.8a	2.2b	14.41a	13.45b
4	74.5a	49.1b	0.63a	0.34b	12.0a	7.0b	2.4a	1.8b	13.77a	12.98b
Mean	**88.9a**	**65.1b**	**1.5a**	**1.2b**	**14.5a**	**9.7b**	**2.6a**	**2.0b**	**14.1a**	**13.4b**
Difference	**23.8**		**0.3**		**4.8**		**0.6**		**0.8**	
%	**36.5**		**24.7**		**49.6**		**29.1**		**5.7**	

[a] Average values from ten replicates (cane yield and no. tillers m^{-2})) or 25 replicates (the remaining variables). Columns with the same letter are not significantly different between biofertilized and conventionally managed parcels, as determined by Tukey's mean separation ($P > 0.05$).

contribute up to 40 kg ha^{-1} of N; however, this subsidy can vary depending on different factors such as plant genotype, soil, and climatic and agronomical management conditions [30]. Furthermore, studies conducted in sugarcane using diazotrophic bacterial strains show that although increases in yield and quality of sugarcane are expected when these microorganisms are inoculated, these improvements are not always related to a change in the natural contribution of biological nitrogen fixation to the plant nitrogen economy, but probably to other plant growth-promoting activities of the microorganisms, such as the production of hormones [16,29,49].

In this study, we validated the use of a systemic biofertilizer that efficiently and reliably stimulates the production and quality of sugarcane. Differences between the biofertilized and non-biofertilized parcels at each site can be attributed to the biological products employed because the parcels were located next to each other in areas with similar climate, soil properties, and historic land management. Additionally, all agronomic practices were identical in both parcels; the only difference between them was the biofertilizer application. Finally, the analyses conducted on the bacteria re-isolated from sugarcane plant stalks (Table 3) yielded positive results showing the presence of the three bacteria in all the sugarcane plantations.

Although it has been stated that beneficial interactions between plants and microorganisms are controlled by a great variety of environmental factors [18,30], from the data presented here, we can conclude that by using the systemic biological technology proposed in this study, these drawbacks can be circumvented in sugarcane and other crops.

Currently, systemic biofertilizers are being evaluated in other localities and sugarcane varieties with results similar to those presented in this study. We believe that this disruptive technology must be extended to other environments, crops, and countries to improve the productivity and quality of agricultural harvests. A novel research avenue has emerged in which the interests of the economy, environmental safeguarding, and human health concur.

As the first approach to facilitate the adoption of this technology (and conversely to other studies carried out in sugarcane), we explored the possibility of increasing the production and quality of sugarcane by imposing our technological framework over the traditional management of farmers (*i.e.*, considering all their traditional agricultural practices, including fertilization dosage and timing) because the adoption of new technologies by farmers is not easy. We confirmed that once sugarcane producers adopted the technology, they confidently reduced the traditional fertilization dosage normally at the second or third application within the fertilization program by at least 20–30%.

Data availability statement

Data related to this research have not been deposited in a publicly available repository. However, upon request, they will be available to anyone who requires them.

CRediT authorship contribution statement

Gerardo Armando Aguado-Santacruz: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Jesús Manuel Arreola Tostado:** Methodology, Investigation, Conceptualization. **César Aguirre-Mancilla:** Methodology, Formal analysis, Conceptualization. **Edmundo García Moya:** Methodology, Investigation, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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