Article

Potential of *Bacillus* native to the Comarca Lagunera as a biofertilizer in the production of forage corn

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Abstract

Conventional agriculture is based on a production system dependent on a high use of synthetic inputs and today faces difficulties in maintaining acceptable levels in agricultural production due to the increase in costs and environmental pollution. A sustainable alternative to this problem is the use of plant growth-promoting bacteria, being a potential alternative when using them as biofertilizers. The objective of the work was to evaluate strains of *Bacillus* (*Bacillus subtilis* and *Bacillus amyloquefaciens*) native to the Lagunera region, testing their potential as biofertilizers in the cultivation of forage corn and evaluating the yield of dry matter. In the treatments, bacteria were applied individually, in combination with each other and with 50% of the recommended dose of chemical fertilizer, the variables evaluated were: leaf area, leaf weight, green corncob weight, cane weight, green corncob diameter, green corncob length, dry matter yield, root volume displacement, fresh root weight and dry root weight. Dry matter yield in *Bacillus* treatments did not show statistically significant differences compared to the chemical control, but bacterial treatments were numerically better. In the same way, an increase in root mass was observed in these treatments, so their use is considered as a viable alternative to replace chemical fertilizer.

Keywords: dry matter, PGPB, root mass.

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Introduction

The conventional agriculture model is based on a production system dependent on a high use of synthetic inputs, where monoculture predominates, which is justified as a fundamental tool to achieve greater efficiency in the production process; however, this production system has shown serious sustainability problems and has caused the deterioration of soils and water (Gómez *et al*., 2018). The limited availability of nutrients for crop production has led to an excess in the application of synthetic fertilizers, which in turn has generated a progressive deterioration of agricultural soils (Pimentel *et al*., 2005).

A sustainable alternative to this problem is the use of plant growth-promoting bacteria (PGPB) (Vejan *et al*., 2016). The interactions of PGPB with plants and microorganisms are very complex and use different mechanisms of action to promote plant growth, which are grouped into: 1) biofertilization; 2) phyto-stimulation; and 3) biocontrol (Moreno *et al*., 2018).

PGPB have beneficial effects on plants through direct and indirect mechanisms or a combination of both. Among the direct mechanisms, the following stand out: nitrogen (N) fixation, synthesis of phytohormones, vitamins and enzymes, solubilization of inorganic phosphorus (P) and mineralization of organic phosphate, oxidation of sulfides, increase in root permeability, nitrite production, nitrate accumulation, reduction of heavy metal toxicity and activity of the ACC deaminase enzyme, the secretion of siderophores, the reduction of ethylene levels in soils, and the increase in root permeability (Moreno *et al*., 2018).

Corn (*Zea mays* L.) is widely used in the feeding of cattle destined for the production of meat and milk in countries of America and Europe. It is considered the 'king of silages' due to the contribution in the content of dry matter, soluble sugars, starch and buffer capacity (Sánchez and Hidalgo, 2018). In the Comarca Lagunera of Mexico, the production of bovine milk is the main agricultural activity and requires a large amount of quality fodder (García *et al*., 2019). In 2020, around 68 000 ha of forage corn (*Zea mays* L.) and a similar area for grain production were established (SIAP, 2020).

The objective of the work was to evaluate strains of *Bacillus subtilis* and *Bacillus amyloquefaciens* native to the Lagunera region, testing their potential as biofertilizers in the cultivation of forage corn, evaluating the dry yield and the root mass.

Materials and methods

The experiment was established during the spring agricultural cycles of 2020 and 2021, in the experimental agricultural field of the Faculty of Agriculture and Zootechnics-UJED, which is located at the parallel 25° 46' 50" north latitude and at the meridian 103° 21' 02" west longitude and at an altitude of 1 110 m. Two corn genotypes were used (G1: Galáctico Hybrid and G2: San Lorenzo Variety), whose seeds were soaked (Martínez *et al*., 2020) in a liquid medium that contained strains of *Bacillus subtilis* and *Bacillus amyloquefaciens*, which were isolated from corn and sorghum in lands of the Comarca Lagunera and identified in the GenBank of the National Center for Biotechnology Information (NCBI) through the BLAST tool. The treatments evaluated are detailed in Table 1.

Table 1. Treatments evaluated.

Treatment	Fertilization
1	*B. subtillis* (BS)
2	*B. subtillis* + 50% chemical fertilization (BS50)
3	*B. amyloquefaciens* (BA)
4	*B. amyloquefaciens* + 50% chemical fertilization (BA50)
5	*B. subtillis* + *B. amyloquefaciens* (BS + BA)
6	100% chemical fertilization (220-180-00) (Q100)
7	50% chemical fertilization (110-90-00) (Q50)
8	Blank (without fertilizer) (B)

Native strains of *Bacillus* isolated in the Lagunera region were used in the experiment and a concentration of $1x10^7$ was used in the inoculated treatments (Bashan, 1998; Canto *et al*., 2004; Lara *et al*., 2013). For treatments that required chemical fertilization, map commercial fertilizers (11-52-00) and urea (46-00-00) were used. The application of the chemical fertilizer was carried out in two parts, one at the time of sowing and another at the beginning of flowering, in band. The seeds were deposited at a distance of 15 centimeters between seeds and distance of 75 cm between furrows, to obtain a population density of 88 888 plants ha^{-1}.

The eight treatments were established in experimental plots of 2.7 m by 2.5 m long, in four furrows and having an average of 64 plants per experimental unit. Each treatment was established with three repetitions and a linear meter (seven plants) was taken to record the variables. Furrow irrigation was used, applying a pre-sowing irrigation and three more supplemental irrigations for an irrigation sheet of 60 cm (INIFAP, 2014).

The experiment was kept in the field until 115 days after sowing. The vegetative variables that were evaluated were: leaf area (LA), leaf weight (LW), green corncob weight (GW), cane weight (CW), green corncob diameter (GD), green corncob length (GL), dry matter yield (DMY), root volume displacement (RVD), fresh root weight (FRW) and dry root weight (DRW). The statistical analysis of the collected data was carried out in the statistical program SAS (Statistical Analysis System, Version 9.2). The analyses performed were analysis of variance, comparison of means by Tukey (α= 0.05) and Pearson’s correlation coefficients. The means of the two cultivation cycles were used for the analysis.

Results and discussion

In the analysis of variance, highly significant differences ($p\leq 0.05$) were observed in treatments for the variables of root volume displacement (RVD), fresh root weight (FRW) and dry root weight (DRW). No significant differences were observed for the rest of the variables. Regarding the genotypes that were used, highly significant differences were observed in all the variables evaluated. Likewise, the comparisons of means show that the treatments where bacteria were applied had numerically superior results in all other variables (Table 2).

Table 2. Table of Tukey's mean comparison (α= 0.05) for the different treatments.

Treatments	LA	LW	GW	CW	GD	GL
	(cm^2)	(kg)			(cm)	
T1 BS	3 266.9 a	0.13 a	0.259 a	0.274 a	4.89 a	16.86 a
T2 BS50	3 393.7 a	0.128 a	0.272 a	0.28 a	4.87 a	17.13 a
T3 BA	3 104 a	0.129 a	0.322 a	0.27 a	4.88 a	16.62 a
T4 BA50	3 154.4 a	0.151 a	0.258 a	0.265 a	4.9 a	16.48 a
T5 BS + BA	3 388.1 a	0.152 a	0.247 a	0.248 a	4.79 a	16.52 a
T6 Q100	3 197.5 a	0.125 a	0.269 a	0.359 a	4.9 a	17.15 a
T7 Q50	3 108.8 a	0.123 a	0.249 a	0.312 a	4.86 a	16.49 a
T8 B	3 003.3 a	0.12 a	0.234 a	0.239 a	4.77 a	16.4 a
MSD	670.3	0.0532	0.0654	0.1374	0.3632	1.8171

Means with different letters are statistically different. LA= leaf area; LW= leaf weight; GW= green corncob weight; CW= cane weight, GD= green corncob diameter; GL= green corncob length; MSD= minimum significant difference.

Table 2. Table of Tukey's mean comparison (α= 0.05) for the different treatments (continued).

Treatments	DMY	RVD	FRW	DRW
	(kg)	(Ml)	(g)	
T1 BS	22 994 a	158.33 a	133.67 a	73.33 a
T2 BS50	23 190 a	124.83 ab	128 ab	63 a
T3 BA	22 138 a	77.17 ab	68.33 ab	31.67 ab
T4 BA50	21 844 a	98.67 ab	86.33 ab	40.33 ab
T5 BS + BA	21 938 a	118.17 ab	102 ab	47.83 ab
T6 Q100	22 873 a	91 ab	102 ab	46.67 ab
T7 Q50	22 024 a	89.67 ab	93.67 ab	44.33 ab
T8 B	20 406 a	40.5 b	40.67 b	16.33 b
MSD	5 604.3	87.097	89.36	46.337

Means with different letters are statistically different. DMY= dry matter yield; RVD= root volume displacement; FRW= fresh root weight; DRW= dry root weight; MSD= minimum significant difference.

In corn, when plant growth-promoting bacteria are associated with plant roots, they help in the production and productivity of the crop, acting in the increase of the aerial part and root system (Domínguez *et al*., 2020). This assertion is consistent with the results shown in the research, since the vegetative variables showed similarities with those corresponding to chemical treatments, which confirms that inoculation with PGPR can reduce the application of nitrogen fertilizer (Cheng *et al*., 2011). Using *Bacillus* as a biofertilizer in combination with half of nitrogen fertilizer allows the latter to be saved without affecting crop yield (Fangying *et al*., 2021).

Dry yield

In grasses, inoculation with PGPR favors the production of dry matter in the roots and aerial part (Reis *et al*., 2000). Inoculations of bacteria in Marandú grass have shown increases in dry matter production, indicating that this practice as a sustainable alternative to increase forage production (Oliveira *et al*., 2007). Although the yield values did not show statistical differences, in the present research it is observed that the dry yield of treatments that include *B. amyloquefaciens* is similar to that of the chemical treatment, and even those of *B. subtilis* are superior to the latter.

Biofertilizers mixed with chemical fertilizers can be a viable alternative to maintain or increase yield, while reducing the use of chemical fertilizers in agricultural production systems (González *et al*., 2021), this is because inoculation with biofertilizer improves the growing and development conditions of corn plants, mainly in the root (Rentería *et al*., 2018; Cantaro *et al*., 2019).

Volume displacement, fresh and dry weight of the root

The treatments T1 *B. subtillis* and T2 *B. subtilis* + 50 demonstrated superiority in the root variables, producing greater mass. Sánchez *et al*. (2012) reported that, in an experiment where they inoculated tomato plants with various bacteria, *Bacillus* showed a positive response in terms of root elongation and increased dry weight of the plant, being very similar in relation to the control. Abraham *et al*. (2018) mentions that the fresh and dry weights of the root had a constant trend in the higher values and statistically significant differences when inoculating the culture with strains of *B. subtilis* in the melon culture. The application of biofertilizers that include *B. subtilis* improves root growth (Bo *et al*., 2020; Fangying *et al*., 2021). This is possibly due to the fact that greater root growth represents a greater area for the absorption of nutrients in the soil (Pilatuña, 2018), giving the plant the opportunity to obtain more nutrients.

Genotypes

Table 3 shows the comparison of means for the genotypes used, the San Lorenzo variety stands out in most of the variables.

Table 3. Tukey's mean comparison (α= 0.05) for the genotypes used.

Genotype	LA	LW	GW	CW	GD	GL
San Lorenzo	3580.8 a	0.136 a	0.291 a	0.333 a	4.7 a	17.12 a
Galáctico	2823.4 b	0.129 a	0.225 a	0.229 b	4.6 a	16.29 b
MSD	213.59	0.017	0.025	.043	0.115	0.579

Means with different letters are statistically different. LA= leaf area; LW= leaf weight; GW= green corncob weight; CW= cane weight, GD= green corncob diameter; GL= green corncob length; MSD= minimum significant difference.

Table 3. Tukey's mean comparison (α= 0.05) for the genotypes used (continued).

Genotype	DMY	RVD	FRW	DRW
San Lorenzo	24 050.8 a	131.089 a	132.5 a	61.25 a
Galáctico	20 301.1 b	68.5 b	56.17 b	29.62 b
MSD	1 780	27.33	28.04	14.54

Means with different letters are statistically different. DMY= dry matter yield; RVD= root volume displacement; FRW= fresh root weight; DRW= dry root weight; MSD= minimum significant difference.

Puente *et al.* (2016) mention that, when different genotypes are used with biofertilizers, some of them respond more favorably. This is consistent with the present work, where the San Lorenzo variety showed higher values in most of the variables. Similarly, Aguilar *et al.* (2015), in an experiment using biofertilizers in combination with lower doses of nitrogen chemical fertilizer, highlight that they had significant increases in yield and dry matter, which is attributed to the genotype used. In grasses, the microorganisms present in the rhizosphere vary between species and even between genotypes of the same species, which is mainly attributed to the variations of each plant, due to the qualitative and quantitative characteristics of the root exudates (Loredo *et al*., 2004). Pearson's correlations (Table 4) show the coefficients of correlation between the variables, with the root variables standing out.

Table 4. Pearson's correlation coefficients.

	LA	CW	GW	GD	GL	DMY	RVD	FRW	DRW
LW	0.01163	0.04875	0.12354	0.03002	-0.02568	-0.15652	-0.01467	-0.12634	-0.10572
LA		0.35795*	0.33838*	0.26216	0.60134**	0.64013**	0.44916**	0.47007**	0.4239**
CW			0.10066	0.10204*	0.43311	0.59546**	0.29556*	0.31477*	0.30483*
GW				0.28251	0.2227	0.20886	0.03372	-0.00051	-0.05064
GD					0.18599	0.47093**	-0.08864	-0.03319	-0.0146
GL						0.55977**	0.28395	0.33218*	0.28932*
DMY							0.35669**	0.43108**	0.38727**
RVD								0.90981**	0.92053**
FRW									0.95954**

Significance level of Pearson: *= 0.05> *p*> 0.01; **= *p*> 0.01; LW= leaf weight; LA= leaf area; GW= green corncob weight; GD= green corncob diameter; GL= green corncob length; DMY= dry matter yield; RVD= root volume displacement; FRW= fresh root weight; DRW= dry root weight.

Dry yield

It can be observed that there is a highly significant correlation of dry yield with the variables of leaf (LA), cane weight (CW) and green corncob (GD and GL). Montemayor *et al*. (2006) indicated that the increase in dry matter production is mainly due to the higher plant height and green corncob weight, which shows a positive correlation between the variables.

Leaf area

As for leaf area (LA), (Li *et al*., 2020) mention that, when the interception of active photosynthetic radiation by the canopy is increased, the accumulation of dry matter is promoted in a general way, which could explain the positive and highly significant correlations of this variable with all the others evaluated. From the point of view of the study factor, which is the PGPB, the root growth stimulated by the treatments where the bacteria were applied could have led to greater absorption of nutrients and water. According to Campillo *et al*. (2012), the productivity of a crop depends on the ability of the vegetation cover to intercept incident radiation, which is a function of the available leaf area, the architecture of the vegetation cover and the efficiency of conversion of the energy captured by the plant into biomass, however, deficiencies in the contributions of water and nutrients can reduce the growth rate of the leaves, reducing yield below optimal levels due to insufficient energy capture.

Volume displacement, fresh and dry weight of the root

Root variables (RVD, FRW and DRW) show a highly significant correlation for most variables. This can be explained because *Bacillus* has the ability to produce phytohormones (such as indole-3-acetic acid (IAA), auxins, cytokinins and gibberellins) in addition to participating in the asymbiotic fixation of nitrogen (N_2), siderophores, solubilizing mineral phosphate and other nutrients, which increases the number of lateral roots and root hairs, this translates into an increase in the area available for the absorption of water and nutrients (González and Fuentes, 2017; Fernandes *et al*., 2020). In these variables we can highlight the correlation with dry yield (DMY), which indicates that as there is a greater root area, there is a greater absorption of nutrients, and therefore a higher yield (Syed and Tollamadugu, 2019; Martínez *et al*., 2020; Bo *et al*., 2020).

Conclusions

In the present work, strains of *Bacillus subtilis* and *Bacillus amyloquefaciens* and their potential as biofertilizers applied to forage corn were evaluated. The treatments where bacteria were applied proved to be superior in the agronomic variables evaluated, especially those where *Bacillus subtilis* was used as a source of biofertilization. Likewise, an increase in root mass was observed in all treatments where bacteria were used and the root mass correlated positively and significantly with the production of dry matter, demonstrating that they are a viable alternative for use as biofertilizers.

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