



Agronomic characteristics and yield of corn after inoculation with *Bacillus amyloliquefaciens*

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Abstract

Corn is one of the most important crops in Brazil and, due to its physiological characteristics, it has a high productive potential and high nutritional quality, for animal and human nutrition. Therefore, this work sought to evaluate *Bacillus amyloliquefaciens* subsp. *plantarum* FZB42 on agronomic characteristics and yield of corn crop. A randomized block design was used, with six doses of *B. amyloliquefaciens* subsp. *Plantarum* FZB42 (0.0; 2.5; 5.0; 7.5; 10.0, and 12.5 mL kg⁻¹ seeds) and four repetitions, totaling 24 experimental units. The study was conducted in the experimental area of annual crops of the Instituto Federal de Educação, Ciência e Tecnologia do Sul de Minas Gerais (Federal Institute of Education, Science and Technology of the South of Minas Gerais — IFSULDEMINAS), Muzambinho Campus, in the 2017/18 agricultural year. The agronomic evaluations were: plant height, upper ear insertion height, culm diameter, leaf nitrogen content, forage fresh mass, incidence of *Fusarium* in the ear, number of rows per ear, number of grains per row, mass of 1000 grains, and yield. The nutritional evaluations performed were: crude protein, ash, ether extract, crude fiber, fiber in acid detergent, and fiber in neutral detergent. In summary, the inoculation of *B. amyloliquefaciens* subsp. *Plantarum* FZB42 in corn seeds, regardless of the dose applied, did not interfere with the agronomic characteristics and nutritional quality of corn silage.

Keywords: *Bacillus amyloliquefaciens*. Inoculation. *Zea mays* L. Nutrition.

Introduction

Among the cereals grown in Brazil, corn (*Zea mays* L.) is the most expressive in production. According to Conab (2022), the 2021/22 harvest was 20.2% higher in production than the previous one, totaling 113,272.1 thousand tons, with an area of 21,581.9 thousand hectares, referring to the sum of the first, second, and third harvests.

Furthermore, the culture of fodder corn has great importance for micro-regions prominent in bovine farming, especially for milk production, because of its excellent characteristics for silage and for having great digestibility and palatability index (PONTES, 2014).

In the agricultural context, reducing the use of chemicals or using them better within the production system has gained prominence. Thus, bacteria with biotechnological abilities have been attracting attention for fitting into a model of sustainable agriculture, which, besides yield, is also concerned with environmental conservation (FIGUEIREDO et al., 2010).

The use of root colonizing bacteria with plant growth-promoting activity showed to be an efficient and environmentally friendly alternative to pesticides and chemical fertilizers (QIAO et al., 2014). *B. amyloliquefaciens* strains associated with those belonging to subsp. *plantarum* stand out for their ability to colonize the plant rhizosphere, stimulate plant growth, and

suppress competitive phytopathogenic bacteria and fungi (QIAO et al., 2014).

Bacteria of the genus *Bacillus* are gram-positive and can be aerobic, facultative, or anaerobic, in addition to being heat resistant. Most of them have simple nutritional needs, requiring some amino acids and B-complex vitamins as growth factors (STANIER; DUODOROFF; ADELBERG, 1969). They form endospores (resistance structures) and have the ability to produce antibiotics (FREITAS; PIZZINATTO, 1997). The formation of endospores increases resistance to adverse factors. Thus, they can be stored as inoculants for a longer period and have longer permanence in the soil (PETRAS; CASIDA, 1985).

The species of *B. amyloliquefaciens* FZB42 is type *B. amyloliquefaciens* subsp. *plantarum* (BORRISS et al., 2011). This strain has some well-characterized agronomic abilities, such as the production of indolic compounds (SZILAGYI-ZECCHIN et al., 2015; IDRIS et al., 2007), siderophores (SZILAGYI-ZECCHIN et al., 2015; CHEN et al., 2007), and phytase (IDRIS et al., 2002). However, it does not produce 1-aminocyclopropane-1-carboxylase (ACC) deaminase (CUARTAS, 2010) and does not fix nitrogen, as it lacks the genes referring to this characteristic (CHEN et al., 2007).

Therefore, the objective was to evaluate *Bacillus amyloliquefaciens* subsp. *plantarum* FZB42 on agronomic characteristics and yield of the corn crop.

Material and methods

The study was conducted at the Instituto Federal de Educação, Ciência e Tecnologia do Sul de Minas Gerais (Federal Institute of Education, Science, and Technology of the South of Minas Gerais — IFSULDEMINAS), Muzambinho Campus, in the 2017/2018 agricultural year. The experimental area has typical Dystrophic

Red Yellow Latosol (SANTOS et al., 2018) and is located at an altitude of 1020 m, latitude of 21°22'33" South, and longitude of 46°31'32" West. The predominant climatic classification of the region is a tropical climate of altitude. According to Köppen (1948), this climate is characterized by its rainy summer and more or less dry winter. The average temperature and the average annual rainfall are 21.37°C and 1,600 mm, respectively (APARECIDO et al., 2014).

The experimental design used was a randomized block design, with six doses of *B. amyloliquefaciens* subsp. *plantarum* FZB42 (0.0; 2.5; 5.0; 7.5; 10.0, and 12.5 mL kg⁻¹ seeds) with four repetitions, totaling 24 experimental units. For this purpose, we selected the transgenic corn simple hybrid 2B587RR, double aptitude, precocious, with semi-double yellow-orange grains, tolerant to the herbicide molecule glyphosate, and with a population of 80 thousand plants ha⁻¹.

The experimental plots had six lines 5.0 m long by 3.6 m wide, spaced at 0.6 m from each other, considering that the total area of each experimental plot was 18.0 m² and, of the four central lines, two were used for silage evaluation and two for grain.

Initially, in September 2017, soil sampling of the experimental field was conducted to characterize its fertility (Table 1). In October, the soil was prepared in a conventional way to receive the seeds. In November, forage corn seeds were inoculated with the bacterium *B. amyloliquefaciens* subsp. *plantarum* FZB42 at the time of sowing.

At seeding, 500 kg ha⁻¹ of the 04-14-08 formulation and 138 kg ha⁻¹ of KCl were used after the recommendation. In cover, 26 days after seeding (DAS), at the V4 stage, when plants with four visible and expanded leaves were identified, 700 kg ha⁻¹ of ammonium sulfate (20%) was used.

Table 1 - Chemical attributes of the soil, at a depth of 0-20 cm, before the sowing of the experiment. Muzambinho – MG, agricultural year 2017/18.

Depth	pH	P	K	Al	Ca	Mg	H+Al	SB	T	P-rem	V	M	M.O.
	water	mg/dm ³		-----		cmolc/dm ³	-----			mg/L	---- %	---	dag/kg
0-20 cm	6.32	42.9	157	0.00	5.55	1.25	1.84	7.2	9.0	17.5	79.6	0	2.23

Extraction methods: pH: water; M.O.: S. Sulfurous; P, K, Cu, Fe, Mn, Zn: Mehlich-I; P-rem: CaCl₂; Ca, Mg, Al: KCl; H+Al: SMP buffer; B: hot water.

Weed control was performed with weeding at 15 DAS and with the post-emergence herbicide nicosulfuron at a dose of 1.25 L ha⁻¹ of the commercial product, at 28 DAS. Phytosanitary management was performed with two applications of insecticides, the first at 16 DAS with the insecticide thiamethoxam + lambda-cyhalothrin at a dose of 250 mL ha⁻¹ of the commercial product, and the second at 37 DAS with chlorpyrifos at a dose of 1 L ha⁻¹ of the commercial product.

At the time of male flowering of corn plants, VT phase (FANCELLI, 2015), ten plants were marked in the useful area of each experimental plot for the following evaluations: plant height (HEI; cm), measured with a graduated ruler from the neck of the plant to the insertion of the flag leaf; upper ear insertion height (INSHEI; cm), measured with a graduated ruler from the neck of the plant to its insertion; culm diameter (CD; mm), measured with a digital pachymeter on the second internode from bottom to top; leaf nitrogen (N) content (LNC; g kg⁻¹), determined by collecting the whole leaf, opposite and below the upper ear, removing its central vein, drying it in an oven and grounding in a Willey mill, and subsequently chemically analyzing to determine the leaf N content (MALAVOLTA; VITTI; OLIVEIRA, 1989).

The corn hybrid was harvested as forage in two useful rows of 5.0 m at 114 DAS, cut at 20 cm from the soil, at the stage between R4 and R5 (FANCELLI, 2015), i.e., when the grains were between mealy and mealy-hard. The total whole plants of each plot were weighed to

determine the forage fresh mass (FFM) in t ha⁻¹, then ground in a tractor-driven silage machine into particles 5 to 6 cm long. After homogenization of the ground total, each plot was ensiled on the same day of harvest in mini silos made from PVC tubes 50 cm long and 100 mm in diameter. The ensiled samples were compacted, the tubes sealed, and stored in the shade for 45 days for the silage fermentation process to take place.

The physical-chemical analyses were performed in triplicate in the Bromatology and Water Laboratory of the IFSULDEMINAS, Muzambinho Campus. The 105°C moisture (U105) was determined according to the gravimetric technique, using heat in a ventilated oven at a temperature of 105°C, with sporadic checks until a constant mass was obtained, according to AOAC (2016); crude protein (CP), to determine the nitrogen content by distillation in a Mikrokjedahl apparatus (AOAC, 2016), using the factor 6.25 to calculate the crude protein content; ash (ASH), fixed mineral material or ash fraction, determined gravimetrically by evaluating the mass loss of the material subjected to heating at 550°C in a muffle furnace (AOAC, 2016); ether extract (EE) extracted by the Goldfish method (AOAC, 2016); crude fiber (CF) by the gravimetric method after acid hydrolysis (KAMER; GINKEL, 1952); fiber in acid detergent (FAD) and fiber in neutral detergent (FND) determined by the gravimetric method by SILVA (1990).

At the R6 stage, characterized as physiological maturity (FANCELLI, 2015), the ears of six plants in each of the two useful rows were harvested, i.e., the ears of 12 plants per

plot, when the grains contained approximately 18 to 21% moisture for the following evaluations: number of rows per ear (NROW); the number of grains per ear (NGRAINS); the mass of 1000 grains in g (M1000), obtained from the total of grains originating from the threshing of all ears from the 12 plants in each plot, with four samples taken at random, which were submitted to weighing; and grain yield (PROD; t ha⁻¹).

When harvesting the ears, the rot severity was evaluated. The severity was evaluated by a diagrammatic scale that estimates the percentage of the ear area with symptoms of rot (*Fusarium*), characterized by coverage with white to pinkish mycelium and the presence of darkened grains and/or white streaks on the pericarp. This scale comprises seven grades, being 1 = 0% — the area of the ear without symptoms —, 2 = 1 to 3%, 3 = 4 to 10%, 4 = 11 to 25%, 5 = 26 to 50%, 6 = 51 to 75%, and 7 = 76 to 100% of the ear area exhibiting visible infection symptoms (AFOLABI et al., 2007).

The data collected were submitted to variance analysis using an *F*-test. The means were compared to each other using the Tukey's test (5%) using the program SISVAR® version 5.3 (FERREIRA, 2011).

Results and discussion

Table 2 shows that there was no significant response to the different doses used of *B. amyloliquefaciens* subsp. *plantarum* FZB42 for the parameters evaluated: plant height, the height of insertion of the upper ear, stem diameter, leaf N content, and fresh silage mass.

For all doses, the corn hybrid used grew in height above its characterization, which is 205 cm (Table 2), presented by Pereira Filho and Borghi (2020). In the study conducted by Szilagyi-Zecchin et al. (2015), in an *in vitro* assay with tomato seedlings inoculated with *B. amyloliquefaciens* subsp. *plantarum* FZB42, plant growth was verified. Gaspareto (2018) shows that *B. amyloliquefaciens* increases calcium extraction, which is the chemical element responsible for cell wall formation.

The result of the upper ear insertion height is directly related to growth, which is defined by the optimal levels of acetic indole acid (auxins) produced by the plant and by the ability to absorb water and nutrients. However, Pereira Filho and Borghi (2020) showed the opposite for the upper ear insertion height, which is characterized as 105 cm, but our values were lower, even with higher plant heights (Table 2).

Table 2 – Height (HEI; cm), ear insertion height (INSHEI; cm), culm diameter (CD; mm), leaf N content (LNC; g kg⁻¹), and forage fresh mass (FFM; t ha⁻¹) of corn as a function of different doses of *Bacillus amyloliquefaciens* subsp. *plantarum* FZB42. Muzambinho – MG, 2017/18.

Dose (mL kg ⁻¹ seed)	HEI (cm)	INSHEI (cm)	CD (mm)	LNC (g kg ⁻¹)	FFM (t ha ⁻¹)
0.0	231.95	92.30	27.38	34.40	26.50
2.5	226.50	92.40	27.45	33.83	24.05
5.0	233.03	94.65	28.18	33.63	27.95
7.5	241.58	95.38	28.25	34.80	27.85
10.0	233.23	96.08	28.45	34.53	28.33
12.5	231.33	97.63	28.88	34.48	27.20
F (%)	0.5245 ^{ns}	0.7289 ^{ns}	0.2703 ^{ns}	0.5172 ^{ns}	0.9362 ^{ns}
CV (%)	4.51	5.91	3.47	2.81	23.59

Means followed by the same letter do not differ among themselves by Tukey test at 5% probability; ^{ns} Not significant.

Studies by Balbinot (2018) using *Bacillus* spp., showed that this genus can directly influence culm diameter; however, when associated with split nitrogen fertilization, which was not the case in our study, it results in similar culm diameters regardless of the dose used (Table 2).

The values found for leaf nitrogen content were similar (Table 2) and within the adequate range (27–35 g kg⁻¹) according to Cantarella, Raij, and Camargo (1997). Gaspareto (2018) also found no difference in leaf nitrogen content between inoculated and non-inoculated treatments with different *Bacillus* species. Forage fresh mass (Table 2) showed low values, because, according to Valente et al. (1991), this should be above 30 t ha⁻¹.

The data in Table 3, such as *Fusarium* severity on the ears, number of rows on the ears, number of grains per row, 1000 grain mass, and grain yield, shows no statistical difference between the doses evaluated.

Regardless of the *B. amyloliquefaciens* dose used, *Fusarium* failed to show differentiation for the control of rot, persisting in all doses in the evaluated grains (Table 3), disagreeing with that observed by Ferreira et al. (2021), which affirms that the use of *Bacillus* spp. is promising for the corn crop in *Fusarium* control.

Table 3 shows no difference in the number of grain rows in relation to the doses of *B. amyloliquefaciens* subsp. *plantarum* FZB42 used. Oliveira Junior (2021) observed no gain in the number of corn grain rows when using *B. amyloliquefaciens*, which was also verified by Ferreira and Lange (2018), evaluating the efficiency of *B. amyloliquefaciens* application via foliar and in the planting furrow.

The number of grains per row had no interference with the *B. amyloliquefaciens* subsp. *plantarum* FZB42 used, differently from that observed by Oliveira Júnior (2021) and Ferreira and Lange (2018). Andreotti et al. (2008) found that seed inoculation with other bacteria of the genus *Bacillus* increased the thousand-grain mass. In contrast, Ferreira and Lange (2018) observed an increase, but with *B. amyloliquefaciens* applied via foliar and in the planting furrow, as in our study (Table 3).

As for grain yield, the treatments did not differ from each other (Table 3). Studies by Lima et al. (2011) concluded that inoculating corn seeds with bacteria of the genus *Bacillus* improves plant development and increases grain yield, which was not found in the application of *B. amyloliquefaciens* via foliar and in the planting furrow by Ferreira and Lange (2018).

Table 3 – Notes for the severity of *Fusarium* on the ear (FUSARIUM), number of rows per ear (NROW), number of grains per ear (NGRAIN), the mass of 1000 grains (M1000G; g), and grain yield (PROD; t ha⁻¹) of corn as a function of the different doses of *Bacillus amyloliquefaciens* subsp. *plantarum* FZB42. Muzambinho – MG, 2017/18.

Dose (mL kg ⁻¹ seed)	FUSARIUM (%)	NROW	NGRAINS	M1000G (g)	PROD (t ha ⁻¹)
0.0	1.32	16.63	30.10	370.50	12.16
2.5	2.41	16.45	26.50	335.15	9.68
5.0	3.47	17.25	30.50	352.98	12.54
7.5	1.02	16.38	29.93	422.18	14.00
10.0	3.78	17.10	30.53	365.45	12.87
12.5	1.05	16.25	28.15	344.93	10.59
F (%)	0.3187 ^{ns}	0.4879 ^{ns}	0.8156 ^{ns}	0.2916 ^{ns}	0.2751 ^{ns}
CV (%)	100.09	5.08	16.74	14.42	22.17

Means followed by the same letter do not differ among themselves by Tukey test at 5% probability; ^{ns} Not significant.

After analyzing the bromatological characteristics, we found no statistical difference between the doses of *B. amyloliquefaciens* subsp. *plantarum* FZB42 (Table 4).

According to Marcondes et al. (2012), the appropriate values for crude protein are between 7 and 10%, thus in all treatments the values are not appropriate, being lower and statistically equal regardless of the used dose of *B. amyloliquefaciens* subsp. *plantarum* FZB42 (Table 4).

The ash contents obtained in our study were similar among the different doses employed (Table 4), and are not within the desired, according to studies conducted by Assis et al. (2014), which range from 4.02 to 4.62%.

According to the NRC (2001), the ether extract contents in ruminant feed should not exceed 7%, as a decrease in rumen fermentation, fiber digestibility, and feed passage rate may occur. Thus, the contents of ether extract obtained in our study are adequate for all doses that did not differ (Table 4). But the studies of Marcondes et al. (2012), show that the appropriate values for the ether extract are from 2 to 5%, which was obtained in the control, and the doses of 10.0 and 12.5 mL kg⁻¹ seed of *B. amyloliquefaciens* subsp. *plantarum* FZB42 used.

Ensminger, Oldfield, and Heinemann (1990) indicate average values of 24.5% for crude fiber (CF), which were higher than those of our study, which were statistically equal (Table 4). Values above 60% of FND, according to Van Soest (1994), correlate negatively with consumption, whereas values of FAD above 35% reduce digestibility. Thus, the values of FAD and FND obtained in this study are suitable for animal consumption (Table 4), as they were lower than these in all treatments, which did not differ from each other (Table 4). According to data from Pereira (2016), the values considered ideal for this FND should be from 45 to 52% of dry matter, only verified in the dose of 2.5 mL kg⁻¹ seed of *B. amyloliquefaciens* subsp. *plantarum* FZB42 used (Table 4).

Conclusions

The inoculation of *Bacillus amyloliquefaciens* subsp. *plantarum* FZB42 on corn seed regardless of the dose applied did not interfere with agronomic characteristics, grain yield, or nutritional quality of corn silage.

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Table 4 – Crude protein (CP; %), ash (ASH; %), ether extract (EE; %), crude fiber (CF; %), the fiber in neutral detergent (FND; %), and fiber in acid detergent (FAD; %) of corn as a function of the different doses of *Bacillus amyloliquefaciens* subsp. *plantarum* FZB42. Muzambinho – MG, 2017/18.

Dose (mL kg ⁻¹ seed)	CP (%)	ASH (%)	EE (%)	CF (%)	FND (%)	FDA (%)
0.0	4.99	3.46	2.03	21.26	39.59	22.16
2.5	5.19	3.6	1.79	19.04	47.65	21.38
5.0	4.67	3.86	1.79	21.59	41.79	22.20
7.5	5.07	3.46	1.85	18.73	38.29	19.78
10.0	5.13	3.45	2.10	18.45	40.77	19.16
12.5	5.20	3.39	2.24	18.60	40.18	25.37
F (%)	0.4952 ^{ns}	0.8560 ^{ns}	0.6100 ^{ns}	0.3433 ^{ns}	0.6798 ^{ns}	0.3675 ^{ns}
CV (%)	8.18	16.66	22.09	13.18	20.04	18.87

Means followed by the same letter do not differ among themselves by Tukey test at 5% probability; ^{ns} Not significant.

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