

Defoliation and inoculation with *Bacillus aryabhattai* on maize development and productivity in Triângulo Mineiro

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ABSTRACT

Maize is a globally significant cereal, with Brazil standing out as one of the leading producers and exporters. This study aimed to evaluate the potential of *Bacillus aryabhattai* in maize cultivation under different stress levels. The experiment was conducted at the Federal Institute of Education, Science and Technology of Triângulo Mineiro – Campus Uberlândia. It used a randomized block design with five replications and treatments in a 3x2 factorial scheme (three different levels of leaf removal and the presence or absence of *Bacillus aryabhattai*). Agronomic traits evaluated included ear length (EL), ear diameter (ED), thousand-grain weight (TGW), and productivity (PROD). Results showed statistical significance only for thousand-grain weight (TGW), with leaf removal affecting this trait. Leaf removal reduced TGW, with the no-leaf-removal treatment (D3) showing the highest average. No significant differences were observed in the interaction between leaf removal and *Bacillus aryabhattai* for TGW or other agronomic traits. The coefficient of variation was low for EL, ED, and TGW, indicating good experimental precision. Water deficit during the productive phase negatively impacted crop characteristics. Inoculation, under the studied conditions, did not show a significant effect on mitigating the impacts of leaf removal or promoting plant growth.

Keywords: Microorganism. Symbiosis. *Zea Mays L.*

Desfolha e inoculação com *Bacillus aryabhattai* no desenvolvimento e produtividade do milho no Triângulo Mineiro

RESUMO

O milho é um cereal de importância econômica global, com o Brasil se destacando como um dos principais produtores e exportadores. Este estudo objetivou avaliar o potencial do *Bacillus aryabhattai* na cultura do milho e em diferentes níveis de estresse. O experimento foi conduzido no Instituto Federal de Educação, Ciência e Tecnologia do Triângulo Mineiro – Campus Uberlândia. O ensaio foi conduzido em delineamento de blocos casualizados, com cinco repetições, e tratamentos em esquema fatorial 3x2 (três diferentes níveis de desfolha, e ausência ou presença do *Bacillus aryabhattai*). Foram avaliados os caracteres agrônômicos: comprimento de espiga (CE), diâmetro de espiga (DE), peso de mil grãos (PMG), e produtividade (PROD). Os resultados indicaram uma significância estatística apenas para o peso de mil grãos (PMG), com o nível de desfolha influenciando essa característica. A desfolha reduziu o PMG, sendo que o tratamento sem desfolha (D3) apresentou a maior média. Não foram observadas diferenças significativas na interação entre desfolha e *Bacillus aryabhattai* para PMG ou para outros caracteres agrônômicos. O coeficiente de variação

experimental foi baixo para CE, DE e PG, sugerindo boa precisão experimental. O déficit hídrico durante a fase produtiva impactou negativamente as características da cultura. A inoculação, nas condições estudadas, não demonstrou efeito significativo na mitigação dos impactos da desfolha ou na promoção do crescimento das plantas.

Palavras-chave: Microrganismo. Simbiose. *Zea Mays L.*

INTRODUCTION

Maize (*Zea mays L.*) is one of the most economically significant crops globally, widely cultivated for human consumption, animal feed, and biofuels. In Brazil, the area planted with maize exceeded 22.1 million hectares in 2023, establishing it as one of the country's most crucial crops. According to the United States Department of Agriculture (USDA, 2024), Brazil emerged as the third-largest maize producer and the top global exporter in the 2022/23 crop season, highlighting the strategic importance of this crop for both national and international economies.

In response to the challenges faced by modern agriculture, innovative management techniques have been developed to enhance productivity and sustainability. Among these practices, the use of bioinputs, particularly beneficial rhizobacteria, has gained prominence. The rhizosphere, the zone extending 1 to 4 mm from plant roots, harbors a diverse microbial community that includes both phytopathogens and plant growth-promoting microorganisms (PGPM) (GOUDA et al., 2018; TIWARI; LATA, 2018). These microorganisms, including bacteria, fungi, and algae, positively influence plants under various environmental stresses (TIWARI et al., 2019).

Plant growth-promoting rhizobacteria (PGPR) colonize roots, inducing both root and shoot growth, increasing lateral root formation, and enhancing nodulation through the production of siderophores, exopolysaccharides, metabolites, and phytohormones like indole acetic acid (IAA) and cytokinins (CK) (TIWARI et al., 2019). PGPR modify root functioning and improve plant nutrition by fixing nitrogen and solubilizing phosphates, thereby maintaining soil fertility and health and resulting in increased crop productivity (TSOTESI et al., 2022).

Among beneficial microorganisms, *Bacillus* species, such as *Bacillus (Priestia) aryabhatai*, stand out for their significant potential in mitigating abiotic stresses, such as water stress, which is a major limiting factor for maize productivity (SILVA, 2023). Studies show that inoculation with these rhizobacteria can alleviate the effects of water stress, promoting more robust root development and efficient water and nutrient absorption. In addition to water stress, defoliation, which can result from management

practices or adverse climatic events, can negatively impact maize productivity by compromising the plant's photosynthetic capacity (Oliveira et al., 2013). Various defoliation experiments have shown a pronounced decline in yield, underscoring the importance of apical leaves for final yield. Among the production components most affected by defoliation during this period are ear number, size, weight, and grain number and weight.

The genus *Bacillus*, now reclassified as *Priestia*, includes species with diverse applications; some are pathogenic to humans, while others possess plant growth-promoting characteristics that enhance agricultural production directly or indirectly. *Bacillus aryabhatai*, first isolated and identified by Shivaji et al. (2009), has been used as a bio-stimulant in various crops, including maize in Brazil. Projects led by Embrapa, such as the isolation of *B. aryabhatai* from the rhizosphere of the mandacaru (*Cereus jamacaru*) in the Caatinga and licensed to the company NOOA, have demonstrated its potential as a bio-stimulant (NOOA, 2024).

Therefore, the objective of this study aims to evaluate, under field conditions, the effects of inoculation with *Bacillus aryabhatai* on the development and productivity of maize plants in the Triangulo Mineiro region under different levels of defoliation.

MATERIALS AND METHODS

The experiment was conducted at the Federal Institute of Education, Science, and Technology of Triangulo Mineiro – Campus Uberlandia, Sobradinho farm, located in the municipality of Uberlandia – MG, at coordinates 18°46'25"S and 48°17'48"W. The climate classification, according to Köppen, is Aw, tropical with two well-defined seasons: one hot and rainy, and the other cool and dry. The soil was classified as Dystrophic red latosol (Oxisol) (Dos SANTOS et al., 2018) with a very clayey texture. Meteorological data were collected from an automatic weather station installed at the Sobradinho farm, 1303.89 meters from the experimental area.

On February 5, 2024, the hybrid BM990 Vip3, developed by Biomatrix, was sown. The seeds were industrially treated with Fortenza Cruizer® for control of caterpillars and early pests of the crop. During the experiment, no irrigation, insecticide, or fungicide applications were performed.

Planting fertilization was done with the application of 300 kg/ha of NPK 08:28:16 fertilizer. The top-dressing, applied in a single application at the V4 stage of the crop,

consisted of urea and potassium chloride in a 3:1 ratio, totaling a dose of 200 kg/ha. This application provided a total of 150 kg/ha of nitrogen and 50 kg/ha of potassium chloride. Additionally, 38 days after planting, a directed application of glyphosate herbicide was made for the control of weed infestations.

The experimental plots consisted of four rows, each five meters long, with the two central rows defined as the useful area. The spacing adopted was 0.60 meters between rows and 0.20 meters between plants, totaling 100 plants per plot.

The experiment was conducted in a randomized block design, in a 3x2 factorial arrangement, with three levels of defoliation and two levels of *Bacillus aryabhatai* application. The defoliation levels were: D1 - defoliation at flowering, removing the two leaves below the ear and one leaf above the ear; D2 - defoliation of all leaves below the ear and one leaf above the ear; and D3 - control, with no defoliation. The levels of *Bacillus aryabhatai* application were: with and without bioinput application. Thus, six treatments were evaluated: (T1) with *Bacillus* and defoliation D1; (T2) without *Bacillus* and defoliation D1; (T3) with *Bacillus* and defoliation D2; (T4) without *Bacillus* and defoliation D2; (T5) with *Bacillus* and defoliation D3; and (T6) without *Bacillus* and defoliation D3.

Inoculation with liquid *Bacillus aryabhatai*, industrially produced with a minimum concentration of 1.0×10^9 colony-forming units per mL (CFU/mL), was performed during the vegetative stage of maize (V6) (approximately 30 days after sowing / March 8, 2024), applied at the base of the stem using a backpack sprayer. A concentration of 10 mL of the commercial product was mixed with 15 liters of water, applying 20 mL of the solution per plant, resulting in 1.11 liters of commercial product per hectare.

Harvesting was performed manually at physiological maturity, 113 days after planting. Ten central ears from the useful area of each plot were collected randomly, and the following parameters were assessed: ear length (EL, in cm), ear diameter (ED, in mm), ear weight without straw (EW, in g), thousand-grain weight (TGW, in g), and yield (YLD, in t/ha). For the yield, humidity was corrected to 14% using the formula: $YLD_{14\%} = W_f(1 - H)/0.86$ where $YLD_{14\%}$ is the weight corrected to 14% humidity; W_f is the field weight of the trait per plot; and H is the grain humidity in each plot, expressed in decimals. The same formula was used to correct the thousand-grain weight trait. Data were subjected to analysis of variance using the F test, and means were compared using the

Tukey test at a 5% significance level, with the aid of R Core Team (2024) and Jamovi (2022).

Climatological data used for the discussion of this study were collected from the Climatological Station at Campus Uberlândia, located 1500 meters from the experimental area.

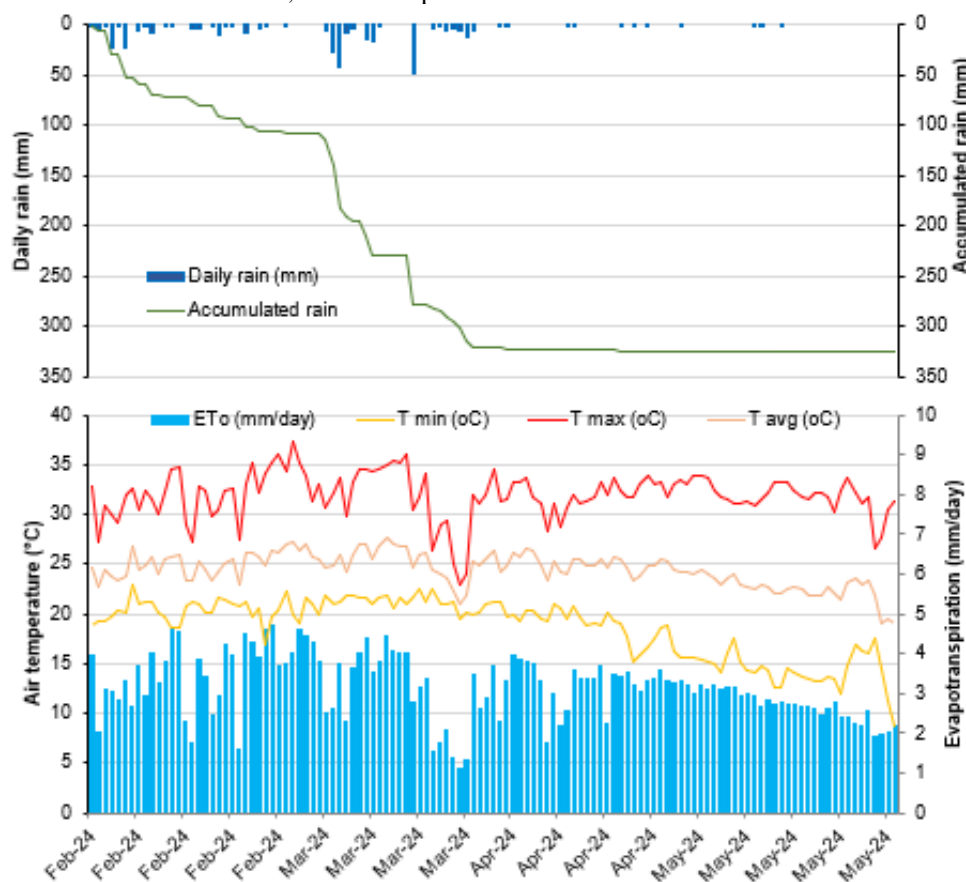
RESULTS AND DISCUSSION

During the development of the experiment (from February 5 to May 27, 2024), higher concentrations of rainfall were observed only up to 54 days after planting (March 29, 2024), with 321 mm of the total 325.4 mm of rainfall throughout the growing period concentrated during this time (Figure 1). Notably, male flowering occurred around March 26, with no significant rainfall observed after the reproductive phase.

According to the daily sequential water balance, the water deficit for the studied cycle was 147 mm. Consequently, there was a marked reduction in water availability for the crop throughout the reproductive phase, which is critical for determining maize productivity. Due to the cultivation as a second crop and the lack of irrigation in the experimental area, the water regime significantly impacted the results obtained for the agronomic characteristics and productivity of the crop. It was also observed that during the initial two-thirds of the crop cycle, the temperature range varied from around 20°C for the minimum temperature to 32°C for the maximum temperature. Towards the end of the cycle, minimum temperatures dropped below 15°C while maximum temperatures remained above 30°C.

Os títulos das tabelas e ilustrações (desenhos, esquemas, fluxogramas, fotografias, gráficos, mapas, organogramas, plantas, quadros e outras) devem ser digitados em Times New Roman, tamanho 10, justificados, com espaçamento simples, mencionados logo acima das respectivas tabelas e ilustrações, citadas em ordem numérica de acordo com a apresentação no texto, como no exemplo a seguir:

Figure 1 – Air temperature, rainfall, and reference potential evapotranspiration from February to May 2024 at the Sobradinho Farm, IFTM Campus Uberlândia.



ETo = Evapotranspiration; T min = minimum temperature; T max = maximum temperature; T avg = temperature average.

It is noteworthy that different strains of the plant growth-promoting rhizobacteria *Bacillus aryabhattai* isolated in Brazil have been studied for their ability to reduce climatic stresses, particularly water stress. These strains originated from isolations of cactus species found in the Caatinga (KAVAMURA et al., 2013). From these initial studies, a strain was selected that showed the best results in promoting plant growth and is now commercially available with the aim of mitigating the impact of water deficit on maize crops. However, more studies are needed to ensure these results, as producers expect maintenance of productivity levels.

In our study, based on the analysis of variance results (Table 1), significance was found only for the defoliation factor in the thousand-grain weight (TGW) at 5% significance level using the F test. For the other characteristics, there were no statistically significant differences regarding the interactions of the two study factors. The coefficients of variation were low for most traits, indicating good precision and control of experimental variability.

Table 1 – Mean squares and coefficients of variation of ANOVA for agronomic traits.

Source of variation	Ear length	Ear diameter	Thousand-grain weight	Productivity
Block	0.7095	1.67	79.2	0.0677
Defoliation	0.1756 ^{NS}	2.29 ^{NS}	1457.4*	0.1506 ^{NS}
Bacillus	0.2306 ^{NS}	1.5 ^{NS}	42.8 ^{NS}	0.0126 ^{NS}
Defoliation* Bacillus	0.0967 ^{NS}	13.37 ^{NS}	16.6 ^{NS}	0.1386 ^{NS}
Error	0.6124	10.87	116.9	0.0692
CV%	5.76	9.2	8.69	12.89
Total	1.8248	29.7	1712.9	0.4387

^{NS} not significant; * significant at 5% probability by F test, respectively.

According to the analysis of variance, the defoliation factor had a significant effect on thousand-grain weight at a 5% significance level using the F test. This result suggests that different levels of defoliation significantly influence this characteristic, indicating that leaf removal can affect the plant's ability to allocate resources for grain development. The defoliation level D3 (control with no defoliation) had the highest mean compared to multiple means, according to the Tukey test at a 5% significance level. In this case, defoliation (D1 and D2) caused stress in the plants, where greater defoliation naturally reduces the area available for photosynthesis, resulting in decreased photosynthates and nutrient translocation to the grains. Thus, the more extensive the defoliation, the greater the reduction in ear quality and productivity.

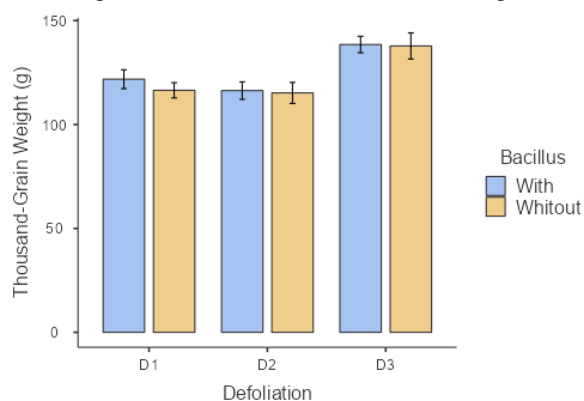
However, when comparing TGW with and without the application of *B. aryabhattai*, and the interaction between defoliation and its application, no statistical difference was observed. This suggests that the application of *Bacillus aryabhattai* alone did not have a detectable impact on TGW, and the interaction between defoliation and Bacillus also showed no significant effects. This may indicate that Bacillus's ability to promote growth or protect against stresses was insufficient to counterbalance or modify the effects of defoliation on TGW (Table 2, Figure 2).

Table 2 – Mean table of the interaction between the factors Defoliation x Bacillus for the agronomic characteristic of thousand-grain weight (g).

Thousand-grain weight (g)		
Defoliation	Bacillus	
	With	Without
D1	121.800 ABa	116.450 Ba
D2	116.325 Ba	115.200 Ba
D3	138.495 Aa	137.805 Aa

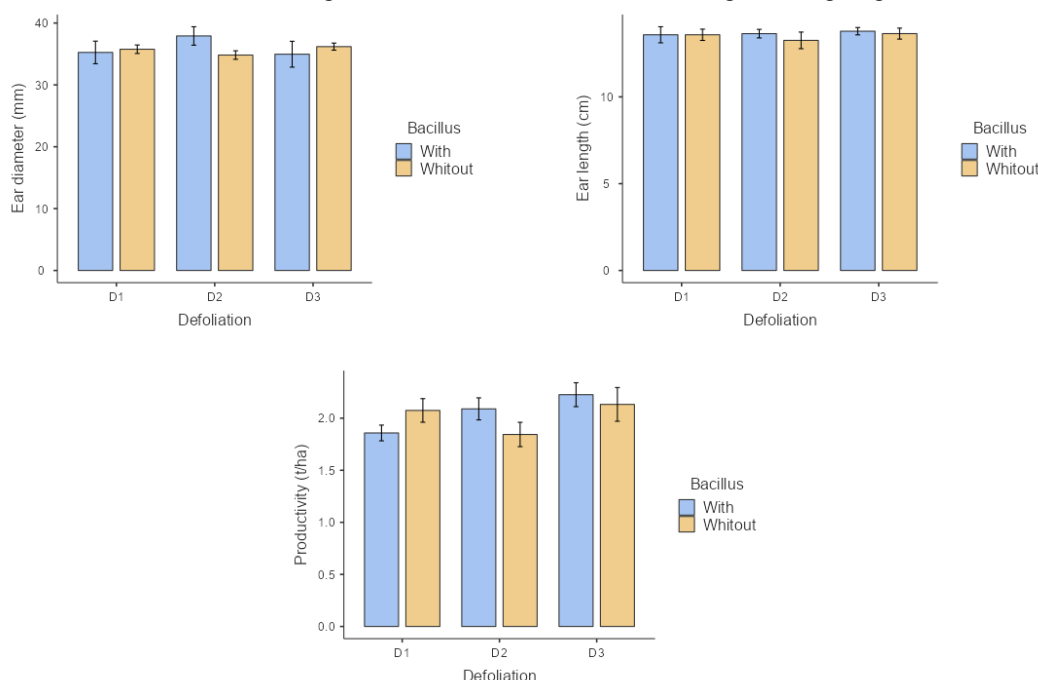
Means followed by the same uppercase letter vertically and lowercase letters horizontally do not differ from each other according to the Tukey test at a 5% probability level.

Figure 2 – Graphical representation of the means related to the interaction between the factors Defoliation and Bacillus for the agronomic characteristic of Thousand-grain weight (g).



For the other agronomic traits evaluated, neither of the factors (defoliation or Bacillus) nor their interactions showed statistically significant differences (Figure 3). The coefficients of variation (CV%) for the agronomic traits ranged from 5.76% to 18.79%, which is relatively acceptable and indicates that the experimental variability is under control.

Figure 3 – Graphical representation of the means related to the interaction between the factors Defoliation and *Bacillus* for the agronomic characteristic of Thousand-grain weight (g).



According to the study by May et al. (2019), the application of *Bacillus aryabhattai* bacteria can mitigate the effects of water stress in sugarcane seedlings when associated with irrigation, suggesting that the presence of water enhances the beneficial effects of the bacteria. This effect was not observed in the present study due to the lack of irrigation and low rainfall in the region during the experiment.

In another laboratory study, the application of *B. aryabhattai* at doses ranging from 20 to 22 ml/kg of inoculant resulted in increased growth of both shoot and root and an increase in dry matter production when added to the treatment of maize seeds (hybrid 20A38 VIP3) 18 days after sowing in sand. This increase was detectable up to doses of around 400 to 450 mL/ha in seed treatment. Beyond this dose, there was a reduction in the increment, equaling the control without inoculation; however, there was no negative impact on germination (STEINER et al., 2024).

According to Silva (2023), who investigated the impact of water regimes and the application of *Bacillus aryabhattai* on agronomic performance in maize, water availability for plants resulted in higher crop productivity. However, this effect was more pronounced in plants that did not receive inoculation. According to this author, the plants were inoculated via seed, whereas in the present work, inoculation was performed

approximately 30 days after plant emergence. This difference in inoculation method may have influenced the observed results.

The application of *Bacillus aryabhattai* CMMA 1363 is typically done either through seed treatment or via drench at sowing (NOOA, 2024; FUGA et al., 2023). In this study, an attempt was made to achieve results with a new method of application and positioning for this bioinput.

It is observed that there is still considerable variation in maize responses to the application of *B. aryabhattai*. At low doses in seed treatment, less than 2 mL/kg of seed, no significant difference in productivity was observed between inoculated seeds and the control in three edaphoclimatic regions (Paranapanema, Rio Verde, and Uberlandia) (FUGA et al., 2023). In the Paranapanema and Uberlandia conditions, at the highest studied dose of 6 mL/kg of seed, the productivity achieved was similar to the control without inoculation, while in Rio Verde and Planaltina, this dose was higher than the control and similar to the lowest studied dose of 1 mL/kg of seed. This study highlights that different hybrids were used in each region, and productivity varied, with the minimum observed in the control being 42.8 sc/ha in Rio Verde (GO) and the maximum in the control being 207.1 sc/ha in Uberlandia (MG). Despite such variation in the results, the recommended dose was 4 mL/kg of maize seed.

In the present study, an increase in productivity was observed with the presence of *B. aryabhattai* in the control without defoliation (D3) and with defoliation of only 3 leaves (D2), although no statistically significant difference was found, suggesting that the application may result in gains under certain conditions.

CONCLUSION

Defoliation results in a reduction in the thousand-grain weight (TGW) of maize, regardless of the application of *Bacillus aryabhattai* via drench at the V6-7 stage at a dose of 1.1 L/ha.

Inoculation with *Bacillus aryabhattai* did not show significant effects on thousand-grain weight, ear length, ear diameter, or productivity, indicating that, under the experimental conditions, the application of *Bacillus* was not sufficient to mitigate the negative effects of defoliation or to promote growth in a detectable manner.

The interaction between defoliation and *Bacillus* application did not result in significant differences in the evaluated traits, suggesting that the combination of these factors did not provide additional benefits in terms of productivity or plant development.

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