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Nicotinamide and *azospirillum brasilense* enhance grain yield and economic return in sorghum cultivation

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ABSTRACT

Sorghum has become an important crop option for the second growing season, a period characterized by high instability in water availability. Although sorghum is considered drought-tolerant, studies have been conducted to improve grain yield through the application of biostimulants. Therefore, this study aimed to evaluate the effects of nicotinamide and *Azospirillum brasilense*, applied individually or in combination, at two sorghum growth stages. The treatments consisted of applying *A. brasilense* at 10 mL L^{-1} and nicotinamide at 300 mg L^{-1}, either individually or in combination, at two phenological stages (2 and 6), in addition to a control without biostimulant application. Biometric and yield-related traits, as well as the economic performance of the treatments, were evaluated. Among the plant traits assessed, only grain yield was affected by nicotinamide and *A. brasilense* application. Moreover, all treatments involving biostimulant application increased economic return. For both grain yield and economic return, application at growth stage 6 produced the greatest response. Therefore, nicotinamide and *A. brasilense*, applied individually or in combination, increased sorghum grain yield and economic return, with the greatest numerical and economic responses observed at growth stage 6.

Keywords: Biostimulants*; Sorghum bicolor* (L.) Moench; Endophytic diazotrophic bacterium; Vitamin B3.

Nicotinamida e *azospirillum brasilense* promovem a produtividade e a lucratividade no cultivo de sorgo

RESUMO

O sorgo tem se tornado uma opção importante para a segunda safra, período com elevada instabilidade hídrica. Apesar de ser considerado resistente à seca, estudos visando promover a produtividade do sorgo através da aplicação de produtos bioestimulantes vêm sendo desenvolvidos. Desta maneira, o objetivo deste trabalho foi avaliar o manejo da aplicação de nicotinamida e *Azospirillum brasilense* na cultura do sorgo em dois estádios de desenvolvimento da planta. Os tratamentos foram constituídos pela aplicação de A. brasilense a 10 mL L^{-1} e nicotinamida a 300 mg L^{-1}, isoladamente ou em conjunto, em dois estádios fenológicos (2 e 6), além de um controle sem aplicação dos bioestimulantes. Foram avaliadas as características biométricas e produtivas, além dos dados econômicos referentes aos tratamentos. Constatou-se que, entre as características das plantas, apenas a produtividade foi afetada pelo manejo de nicotinamida e *A. brasilense* e, em complemento, o retorno econômico foi incrementado por todos os tratamentos compostos pela aplicação dos bioestimulantes. Em ambos os casos, destacou-se a aplicação em estádio 6, a qual elevou o retorno em até 29,75%. Dessa maneira, concluiu-se que a aplicação de nicotinamida e *A. brasilense*, de maneira conjunta ou isolada, é eficaz para incrementar a produtividade e o retorno econômico da cultura do sorgo, com as maiores respostas numéricas e econômicas observadas no estádio 6.

Palavras-chave: Bioinsumos; *Sorghum bicolor* (L.) Moench; Bactéria diazotrófica endofítica; Vitamina B3.

1. Introduction

Global sorghum production reached 57.3 million metric tons in 2023, from a harvested area of 39.8 million hectares, with an average yield of 1.4 Mg ha^{-1} (FAO, 2025). In Brazil, sorghum production for the 2024/25 growing season is estimated at 4.7 million metric tons, from a cultivated area of 1.5 million hectares, with an estimated grain yield of 3,121 kg ha^{-1} (CONAB, 2025).

Sorghum (*Sorghum bicolor* (L.) Moench) is an important cereal crop with a wide range of uses, including animal feed, silage production, feed manufacturing, human consumption, flour production, use as a cover crop in no-tillage systems, and even biodiesel production. This crop is adapted to a wide range of environments and climatic conditions and is recognized for its tolerance to abiotic stresses, particularly drought and salinity (Bakari et al., 2023). In Brazil, grain sorghum is grown almost exclusively during the second growing season, following soybean harvest, when precipitation begins to decline and water deficiency may occur during crop development.

For second-season cultivation, rapid crop establishment during the initial developmental phase, from germination to root system establishment, is essential due to the limited soil moisture availability during this period. To achieve this, the use of early-maturing, stress-tolerant genotypes, together with inputs that promote rapid establishment by enhancing root development, is of paramount importance (Buchelt et al., 2019; Gaiotto et al., 2023). Among these inputs, biostimulants have been highlighted for their potential to promote plant growth.

The effects of biostimulants are diverse and may vary among plant species and environmental conditions. Therefore, further studies are needed under different growing environments, particularly those subjected to stress conditions (Kolling et al., 2016). In Brazil, scientific reports on the use of biostimulants in sorghum remain limited, with existing studies primarily focusing on mitigating salinity stress (Desoky et al., 2018; Desoky et al., 2019) and nutritional stress (Ferreira et al., 2020).

Among the various biostimulants available, the use of bacteria and vitamins appears promising for sorghum. Species of the genus *Azospirillum* are diazotrophic plant growth-promoting bacteria capable of biological nitrogen fixation, particularly in association with grasses (Galeano et al., 2019; Vega et al., 2020). In sorghum, this bacterium warrants further investigation because hybrids differ from one another and exhibit distinct root exudate characteristics (Cadore, 2016), which may affect bacterial effectiveness.

Another compound with potential use as a biostimulant is nicotinamide, the amide form of the water-soluble vitamin B3, or niacin (Taiz et al., 2021). Nicotinamide has potential as a biostimulant because of its involvement in cellular redox metabolism, plant growth regulation (Mohamed et al., 2020), and mitigation of oxidative damage (El-Lethy et al., 2019), resulting in improved plant performance following exogenous application, as reported for maize (Araújo et al., 2024; Vendruscolo et al., 2024) and soybean (Lima et al., 2024).

Therefore, based on the hypothesis that *A. brasilense* and nicotinamide, applied individually or in combination, may promote sorghum growth and grain yield, this study aimed to evaluate their effects at two sorghum growth stages.

2. Material and Methods

The experiment was established and conducted at the Experimental Farm of the Mato Grosso do Sul State University, Cassilândia Unit (19°05′46″ S, 51°48′50″ W; 521 m above sea level). According to the Köppen climate classification, the region has a tropical savanna climate (Aw), characterized by a rainy summer and a dry winter (winter precipitation < 60 mm), with a mean annual precipitation of 1,520 mm and a mean annual temperature of 24.1 °C. During the experimental period, climatic data, including air temperature, relative humidity, and precipitation, were recorded (Figure 1).

The soil in the experimental area was classified as Neossolo Quartzarênico according to the Brazilian Soil Classification System. During the two growing seasons preceding the experiment, the area was cultivated with maize and soybean, respectively, and had received liming, fertilization, and other standard crop management practices. Before the sorghum experiment was established, the soil had the following chemical properties: pH, 5.3; Mehlich^{-1} P, 4.6 mg dm^{-3}; K, 0.39 cmolc dm^{-3}; Ca, 2.30 cmolc dm^{-3}; Mg, 0.8 cmolc dm^{-3}; cation exchange capacity, 5.0 cmolc dm^{-3}; base saturation, 69.9%; and organic matter, 18.1 g dm^{-3}. Before sorghum sowing, the area was desiccated with a glyphosate-based herbicide (720 g kg^{-1}) at a rate of 3.5 kg ha^{-1}. In addition, 50 kg ha^{-1} of N and 25 kg ha^{-1} of K_2O were surface-applied as topdressing in the form of urea and potassium chloride when the plants reached growth stage 2 (collar of the fifth leaf visible).

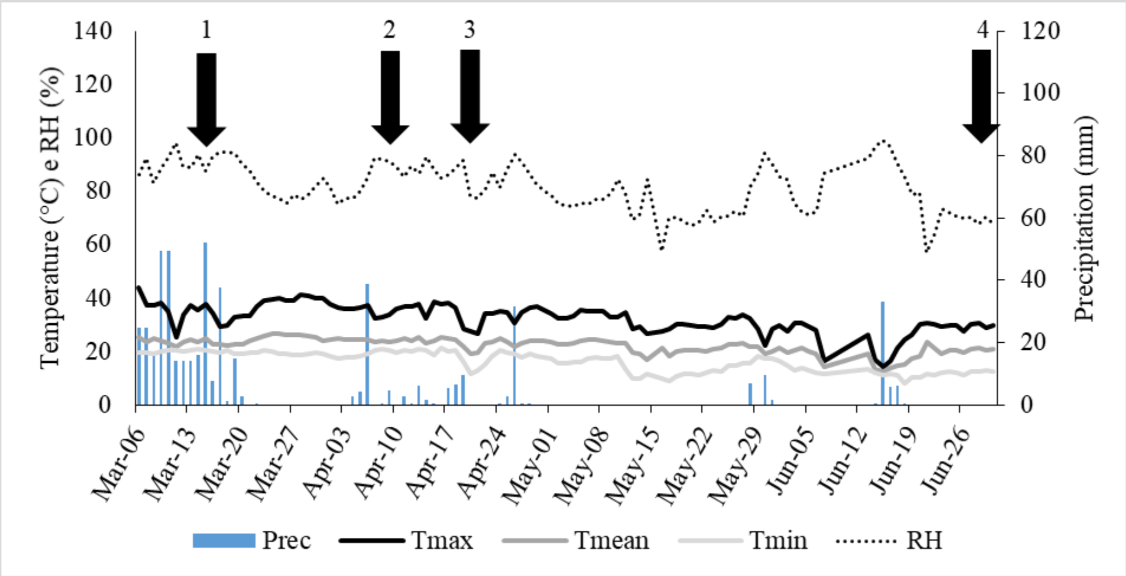

Figure 1. Maximum (Tmax), mean (Tmean), and minimum (Tmin) air temperatures, relative humidity (RH), and precipitation (Prec) during the experimental period (March 16, 2023, to July 5, 2023). 1 – sowing; 2 – topdressing fertilization; 3 – treatment application; 4 – harvest.

The experiment was conducted using a randomized complete block design with seven treatments and four replications: T1 – untreated control; T2 – *A. brasilense* at 10 mL L^{-1} applied at growth stage 2; T3 – nicotinamide at 300 mg L^{-1} applied at growth stage 2; T4 – nicotinamide at 300 mg L^{-1} applied at growth stage 6 (50% flowering); T5 – *A. brasilense* at 10 mL L^{-1} + nicotinamide at 300 mg L^{-1} applied at growth stage 2; T6 – *A. brasilense* at 10 mL L^{-1} + nicotinamide at 300 mg L^{-1} applied at growth stage 6; and T7 – *A. brasilense* at 10 mL L^{-1} applied at growth stage 6.

Each experimental plot consisted of five 4-m-long rows. The two outer rows and 0.5 m from each end of the three central rows were excluded, and the remaining area was considered the harvest area. Certified seeds of the B1G211 hybrid were mechanically sown using a five-row mounted planter adjusted to distribute 15 seeds m^{-1}, with a row spacing of 0.50 m adjusted to distribute 15 seeds per meter, with 0.50 m row spacing.

Two weeks after sowing, an atrazine-based herbicide (500 g L^{-1}) was applied at a rate of 5 L ha^{-1}. The treatments were applied by foliar spraying when the plants reached the growth stage specified for each treatment. Nicotinamide and *A. brasilense* were diluted in a spray solution containing 7.5 mL L^{-1} of a fungicide formulation composed of carbendazim (250 g L^{-1}) and tebuconazole (125 g L^{-1}), together with 1 mL L^{-1} Tween® 80. The applications were performed using a hand-held sprayer at a spray volume equivalent to 200 L ha^{-1}.

Harvest was performed when the plants reached growth stage 9. Ten plants were sampled from each plot within a 2.0 m section of the two central rows to evaluate plant height, peduncle length, panicle length, number of leaves, stem diameter, grain mass per plant, number of grains per plant, and thousand-grain weight. Grain yield was estimated by extrapolating the plot data to 1.0 hectare using the plant population within the harvest area, with grain moisture corrected to 13%.

Based on grain yield, the gross revenue and percentage economic gain for each treatment were also determined. For these calculations, only the variable costs specific to each treatment were considered, corresponding to the purchase of *A. brasilense* and nicotinamide, using the average prices recorded during the experimental period (*A. brasilense* = US$ 17.70 L^{-1}; nicotinamide = US$ 110.65 kg^{-1}). All other production costs, such as labor, agricultural inputs, and machinery operation, which were common to all treatments, were excluded from the analysis. A baseline total operating cost of US$ 232.84 was adopted.

To evaluate the economic performance of each treatment, gross revenue and break-even yield were calculated according to Martin et al. (1998): (a) gross revenue was calculated by multiplying grain yield, expressed as 60 kg bags ha^{-1}, by the market price of US$ 7.08 per 60 kg bag of sorghum; and (b) break-even yield, defined as the minimum grain yield required to cover the total operating cost.

The biometric and yield-related data were analyzed using analysis of variance (ANOVA), and treatment means were compared using the Tukey test at the 5% significance level. Gross revenue was not analyzed separately because it was calculated directly from grain yield using a fixed market price.

3. Results and Discussion

It was found that, among the sorghum plant traits evaluated, only grain yield was affected by the application management of nicotinamide and *Azospirillum brasilense* (Table 1).

Table 1. Summary of the analysis of variance for plant height (PH), peduncle length (PL), panicle length (PanL), number of leaves (NL), stem diameter (SD), thousand-grain weight (TGW), grain mass per plant (GMP), number of grains per plant (NGP), and grain yield (GY) of sorghum subjected to the application of *Azospirillum brasilense* and nicotinamide.

SV	Mean squares								
	PH	PL	PanL	NL	SD	TGW	GMP	NGP	GY
Block	0.0042	0.22	26.95	13.67	5.31	0.52	316.37	751706.58	4.28
Treatment	0.0061ns	6.14ns	2.21ns	0.42ns	5.22ns	4.93ns	310.43ns	497631.22ns	34.10*
Error	0.0056	11.59	3.86	0.28	3.21	7.15	158.04	504351.04	7.01
HSD	0.18	7.96	4.59	1.24	4.19	6.25	29.38	1659.92	6.19
CV (%)	6.18	28.35	6.13	10.61	10.90	12.32	10.71	13.02	10.76

SV – Source of variation; CV – Coefficient of variation; * and ns – significant and not significant, respectively, according to the F-test at the 1% probability level.

For grain yield, superior performance was observed for the treatments consisting of nicotinamide alone, *A. brasilense* alone, and nicotinamide combined with *A. brasilense*, all applied at growth stage 6 (Figure 2). However, these treatments did not differ significantly from those applied at growth stage 2 and differed only from the control.

Application of nicotinamide at growth stage 6 increased grain yield by 29.7% compared with the untreated control. Likewise, the application of *A. brasilense* at growth stage 6 increased grain yield by 28.0%, while the combined application of nicotinamide and *A. brasilense* at the same growth stage resulted in a 24.7% increase in grain yield relative to the untreated control (Figure 2).

Regarding the economic return of the treatments, the greatest increases in gross revenue were obtained with the application of nicotinamide alone (29.75%), *A. brasilense* alone (23.86%), and nicotinamide combined with *A. brasilense* (27.33%), all applied at growth stage 6 (Table 2).

The maximum cost associated with product application was US$ 39.83 when nicotinamide and *A. brasilense* were applied in combination, corresponding to a break-even yield of 5.63 bags ha^{-1} required to offset the application costs. Accordingly, although the combined application at growth stage 6 resulted in an increase of 12.8 bags ha^{-1}, which was sufficient to cover the application costs, the same was not observed for the application at growth stage 2, where the increase was only 4.91 bags ha^{-1}, insufficient to offset the cost of the products. All remaining biostimulant treatments generated revenue gains greater than their respective product acquisition costs (Table 2).

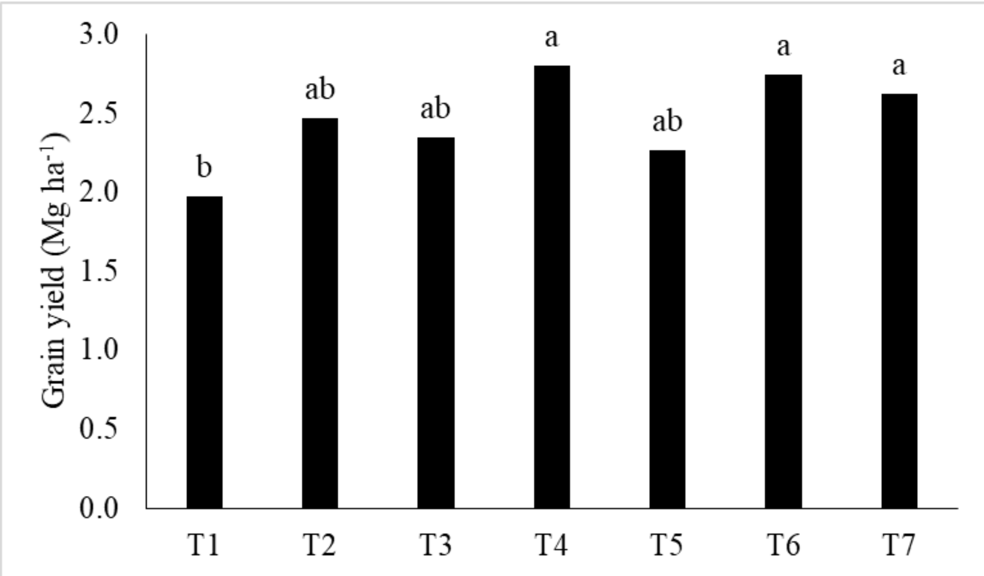

Figure 2. Grain yield of sorghum subjected to the application of *Azospirillum brasilense* (Azos) and nicotinamide (Nico). T1 – untreated control; T2 – Azos at 10 mL L^{-1} applied at growth stage 2; T3 – Nico at 300 mg L^{-1} applied at growth stage 2; T4 – Nico at 300 mg L^{-1} applied at growth stage 6 (50% flowering); T5 – Azos at 10 mL L^{-1} + Nico at 300 mg L^{-1} applied at growth stage 2; T6 – Azos at 10 mL L^{-1} + Nico at 300 mg L^{-1} applied at growth stage 6; and T7 – Azos at 10 mL L^{-1} applied at growth stage 6. Means followed by the same letter do not differ according to Tukey's test at $p \leq 0.05$.

Table 2. Analysis of product acquisition cost, grain yield (60-kg bags), break-even yield, gross revenue, and increase in gross revenue of sorghum subjected to the application of *A. brasilense* and nicotinamide.

Treatment	Cost (US\$ ha^{-1})	Grain yield (60-kg bags ha^{-1})	Break-even yield (60 kg bags ha^{-1})	Gross revenue (US\$ ha^{-1})	Increase in gross revenue (%)
T1	0	32.88	32.88	232.81	0
T2	35.41	41.13	37.88	255.85	19.08
T3	4.43	39.06	33.51	281.01	15.96
T4	4.43	46.74	33.51	335.38	29.75
T5	39.83	37.79	38.51	236.62	11.98
T6	39.83	45.68	38.51	292.51	27.33
T7	35.41	43.68	37.88	273.92	23.86

T1 – untreated control; T2 – *A. brasilense* at 10 mL L^{-1} applied at growth stage 2; T3 – nicotinamide (Nico) at 300 mg L^{-1} applied at growth stage 2; T4 – nicotinamide (Nico) at 300 mg L^{-1} applied at growth stage 6 (50% flowering); T5 – *A. brasilense* at 10 mL L^{-1} + nicotinamide (Nico) at 300 mg L^{-1} applied at growth stage 2; T6 – *A. brasilense* at 10 mL L^{-1} + nicotinamide (Nico) at 300 mg L^{-1} applied at growth stage 6; T7 – *A. brasilense* at 10 mL L^{-1} applied at growth stage 6.

The application of nicotinamide increased both grain yield and gross revenue (Table 2). This response may be attributed to the physiological effects associated with this vitamin, including enhanced tissue resistance and improved cellular energy transport, which indirectly promote plant growth and development (Dong, Stockwell and Goyer, 2015). In addition, nicotinamide is a precursor and structural component of NAD and NADP, coenzymes involved in cellular redox reactions and metabolic processes (Taiz et al., 2021), and also contributes to plant defense against certain insect pests (Berglund et al., 2016).

The increase in grain yield may also be associated with the ability of nicotinamide to modulate plant growth, cellular metabolism, and antioxidant defense mechanisms (Berglund et al., 2017). Foliar nicotinamide application has been reported to increase leaf area in maize (Colla et al., 2021), an effect that may contribute to greater photosynthetic capacity and improved tolerance to abiotic stress (Vendruscolo et al., 2024). These responses may favor assimilate accumulation and subsequent mobilization during periods of stress or organ development, according to the plant's physiological requirements (Berglund et al., 2017; Dawood et al., 2019; El-Lethy et al., 2019).

The grain-yield gains observed with *A. brasilense* application (Figure 2) may be associated with several plant growth-promoting mechanisms. *Azospirillum brasilense* is a diazotrophic bacterium capable of biological nitrogen fixation (Cadore, 2016). In addition, *A. brasilense* has been shown to regulate plant gene expression, promote plant development, and enhance signaling pathways involved in responses to both abiotic and biotic stresses (Fukami et al., 2018; Galindo et al., 2020).

Azospirillum brasilense is also an important plant growth-promoting bacterium that can produce or modulate phytohormones such as auxins, cytokinins, and gibberellins, while enhancing dry matter accumulation and nitrogen uptake (Galeano et al., 2019; Vega et al., 2020). Gibberellins, in particular, promote cell elongation, thereby contributing to plant growth (Dawood et al., 2019; Mohamed et al., 2020).

Results regarding the application of this bacterium in sorghum remain inconsistent. Ubert and Soligo (2015), for example, reported no effect of *Azospirillum brasilense* application on sorghum grain yield. In contrast, Andrade et al. (2019) observed positive effects of inoculation on sorghum, improving seedling emergence and reducing the mean emergence time. The authors also reported that seed inoculation and in-furrow application reduced the mean emergence time of seedlings of the 1G 282 and IG 1090 sorghum hybrids.

Bossolani et al. (2014) reported that inoculation with *A. brasilense* increased the number of tillers per plant in sorghum and, when combined with a nitrogen rate of 60 kg ha^{-1}, resulted in greater total fresh biomass and total dry biomass in sweet sorghum. Similarly, Silva et al. (2020) reported that the application of *A. brasilense* to coffee (*Coffea arabica*) seedlings promoted greater taproot growth and increased root fresh mass.

These results highlight the potential of foliar *A. brasilense* application in sorghum, an approach that has received limited attention because most studies have evaluated seed inoculation. In addition, nicotinamide, applied alone or in combination with *A. brasilense*, increased sorghum grain yield, particularly when applied at growth stage 6.

4. Conclusions

Nicotinamide and *A. brasilense*, applied individually or in combination, increased sorghum grain yield and economic return. The greatest numerical and economic responses were observed when the treatments were applied at growth stage 6.

Authors' Contribution

Conceptualization and methodology: Matheus Rodrigues Oliveira, Eduardo Pradi Vendruscolo; data collection and curation: Matheus Rodrigues Oliveira, Brenda Luciana Queiroz Ribeiro, Cássio de Castro Seron, Gabriela da Silva da Costa; formal analysis: Eduardo Pradi Vendruscolo, Fernanda Pacheco de Almeida Prado Bortolheiro; data interpretation: Matheus Rodrigues Oliveira, Eduardo Pradi Vendruscolo; project administration: Matheus Rodrigues Oliveira, Eduardo Pradi Vendruscolo; supervision: Eduardo Pradi Vendruscolo, Cássio de Castro Seron, Fernanda Pacheco de Almeida Prado Bortolheiro; preparation of the original draft: Matheus Rodrigues Oliveira, Eduardo Pradi Vendruscolo; writing -revision and editing: Cássio de Castro Seron, Fernanda Pacheco de Almeida Prado Bortolheiro; Gabriela da Silva da Costa. All authors read and approved the final version of the manuscript.

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