International Journal of Agriculture and Biosciences 2025 14(6): 1246-1254.
https://doi.org/10.47278/journal.ijab/2025.109

RESEARCH ARTICLE eISSN: 2306-3599; pISSN: 2305-6622

Rhizobacteria-Vitamin-Plant Synergism Interaction and its Biostimulant Properties on Gas Exchange and Growth of Sorghum Plants Subjected to Water Deficit

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Article History
Article # 25-141
Received: 28-Mar-25
Revised: 10-May-25
Accepted: 23-May-25
Online First: 18-July-25

ABSTRACT

Changes in climatic conditions in agricultural environments have significantly altered how we produce food. The introduction of more resilient species and technologies that enhance their productive potential has been continually studied. In this context, sorghum is a promising crop for cultivating areas less favorable for corn. When combined with growth-promoting and bioprotective bioinputs, it can support the establishment of the crop and mitigate the effects of adverse environmental conditions. This study evaluated the effect of applying *Azospirillum brasilense* and nicotinamide on the gas exchange and growth characteristics of sorghum plants under water deficit stress. The experiment was conducted in a greenhouse using a randomized block design with four treatments and five replications. The treatments were: T1 (control), T2 (foliar application of nicotinamide at 200mg L^{-1}), T3 (foliar application of *A. brasilense* at 2mL L^{-1}), and T4 (combined application of nicotinamide and *A. brasilense*). It was found that the application of both products, either together or separately, increased the gas exchange capacity of the plants after water stress, along with a gain in root volume ranging from 86% to 127%, which was accompanied by increased dry weight accumulation in both the shoot and the root system. We conclude that foliar application of *A. brasilense* and nicotinamide, alone or in combination, promotes improvements in gas exchange and growth in sorghum plants subjected to water stress, particularly in the root organs.

Keywords: *Sorghum bicolor* L., *Azospirillum brasilense*, Nicotinamide, Regenerative agriculture.

INTRODUCTION

Climate change has been central to many discussions about food security (Leisner 2020). Changes in water availability and rising temperatures are among the most critical factors for agriculture, especially grain production (Ingrao et al., 2023), which could suffer a total loss. In this context, the agricultural sector must seek alternatives to minimize these effects, enhance safety for farmers and consumers, and adapt to new environmental conditions.

Abiotic stresses such as drought and temperature excesses can lead to physiological and hormonal changes in plants, culminating in reduced crop growth and yield (Farooq et al., 2009). The first changes occur at the cellular level, including an increase in reactive oxygen species (ROS), membrane degradation, and a reduction in leaf water content, which leads to reduced stomatal opening and net CO_2 assimilation rate (Farooq et al., 2009; Asadi et al., 2021), and consequently a reduction in height, dry mass, and grain yield.

In many regions of Brazil, prolonged rainfall during the summer has enabled two consecutive cropping seasons, with soybean typically cultivated first, followed by corn in the second season, commonly referred to as the "safrinha". However, the increased inconsistency of rainfall in the latter half of the rainy season and the intensification of temperature rise have caused significant losses in corn production. Therefore, farmers in Brazil's Central region

Cite this Article as: Oliveira DCSBD, Souza IF, Santana TD, Vendruscolo EP, Silva GPVD, Binotti FFDS, Lima SFD and Bortolheiro FPDAP, 2025. Rhizobacteria-vitamin-plant synergism interaction and its biostimulant properties on gas exchange and growth of sorghum plants subjected to water deficit. International Journal of Agriculture and Biosciences 14(6): 1246-1254. https://doi.org/10.47278/journal.ijab/2025.109

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have increasingly adopted more resilient species, such as sorghum, and have intensified efforts to develop genotypes adapted to the specific soil and climatic conditions of each area, aiming at grain production, animal feed, or even bioenergy (Von Pinho et al., 2022).

Sorghum production in Brazil has been growing annually and is estimated at 4.9 million tons for the 2023/2024 cropping season, an increase of 4% compared to the 2022/2023 cropping season (4.7 million t). The 2022/2023 cropping season increased by 51% compared to the 2021/2022 cropping season (3.1 million t). The planted area also follows this growth, with an estimated 1.5 million hectares planted in the 2023/2024 cropping season, an increase of 7% (1.4 million ha) compared to the 2022/2023 cropping season, which increased by 27% compared to the 2021/2022 cropping season (1.1 million ha) (Conab, 2024). World sorghum production in 2022 was 57.6 million tons on 40.8 million hectares (FAO, 2024).

With compositional characteristics similar to corn, sorghum has been an attractive alternative for Brazilian farmers, as it is better adapted to low soil water content and high temperatures than corn (Carmo et al., 2020; Bernardino et al., 2021). This characteristic of sorghum can also be enhanced by the use of technologies such as bioinputs, some of which have been widely studied for crops of the Poaceae genus, such as *A. brasilense* (Barbosa et al., 2022; Gaiotto et al., 2023; Hernández et al., 2024) and others with the potential to be used in commercial crops due to the promising responses found by the scientific community, such as nicotinamide (Thomé et al., 2023; Vendruscolo et al., 2024a). Soares et al. (2022) observed a 14% increase in sorghum grain yield under water deficit when inoculated with *A. brasilense*.

A. brasilense is widely used to produce important grain species such as corn. The use of this bacterium can promote an increase in auxin levels, which stimulate root development and provide better conditions for the absorption of water and nutrients by plants, mitigating the deleterious effects of low rainfall periods (Avila et al., 2020; Marques et al., 2021; Espindula et al., 2023). For sorghum grown under water restriction conditions in the second crop, inoculating seeds with *A. brasilense* did not result in better development or production responses (Soares et al., 2021). However, Andrade et al. (2019) observed that inoculation with *A. brasilense* resulted in gains in terms of mass accumulation in the root system of the species.

Nicotinamide, like *A. brasilense*, stimulates mechanisms related to increased auxin levels in plants and the expression of genes that activate important defensive mechanisms (Ghosh et al., 2020; Li et al., 2024). This vitamin is also related to improved gas exchange conditions and recovery of activities after periods of stress (Vendruscolo et al., 2024a), increasing the crop's yield potential in the field under second-crop conditions (Thomé et al., 2023). In part, the improvement in crop conditions is also related to the stimulation in the production of coenzymes such as NADH and NADPH, which have an oxidoreductive action, enhancing the plant's ability to maintain energy transfer within the cells (Ferreira et al., 2024).

Sorghum is considered a tolerant species to abiotic stress conditions (Hadebe et al., 2020; Asadi et al., 2021); however, the stress response depends on the genotype used, the phenological stage of the crop, and the duration and intensity of the stress. On average, sorghum experiences significant growth and leaf expansion 20 to 30 days after emergence, during which water deficiency can harm the crop. Assefa et al. (2010) observed a 36%-55% reduction in sorghum yield due to water deficit during the vegetative and reproductive stages, respectively. It is, therefore, necessary to use technologies that can help crops in times of stress, such as vitamins and biostimulants.

For the combined application of *A. brasilense* and nicotinamide, Vendruscolo et al. (2024b) found that under hydroponic cultivation conditions, without the occurrence of environmental stresses, there was a positive interaction of these bioinputs, increasing the growth capacity of pumpkin plants. This growth was especially marked in the root system, which could imply greater resistance of these plants if they were stressed due to water deficit. In this sense, it is observed that the increase in root volume is essential to increase the resilience of plants grown in systems without irrigation, especially in regions with irregular precipitation or in second-crop periods, as occurs in Brazil (Thomé et al., 2023).

Based on the potential of these two bioinputs, it was hypothesized that a synergistic effect could occur with their simultaneous application to sorghum plants subjected to water deficit, increasing the capacity of these plants to resist deleterious effects through physiological and morphological changes, such as the increase in the root system. This study aimed to understand the behavior of sorghum plants exposed to water deficit and the application of bioinputs (*Azospirillum brasilense* and nicotinamide), evaluating the gas exchange characteristics and growth parameters.

MATERIALS & METHODS

Characterization of the Experimental Area

The experiment was conducted in a protected environment at the Experimental Station of the Mato Grosso do Sul State University, Cassilândia University Unit, at 19º05'46" S and 51º48'50" W and an altitude of 521m above sea level. According to the Köppen classification, the regional climate is tropical rainy (Aw-type), with rainy summers and dry winters (winter rainfall of less than 60mm) and average annual rainfall and temperature of 1,520mm and 24.1°C, respectively. The experiments were conducted in a greenhouse with a galvanized steel structure, measuring 8.00m wide, 18.00m long, and 3.50m high, closed at a 45° angle, with monofilament mesh throughout, providing 18% shading, and metal cultivation benches 0.90m high, on which the containers were placed. The climatic conditions in the environment were obtained from a weather station installed in the central part of the screen (Fig. 1).

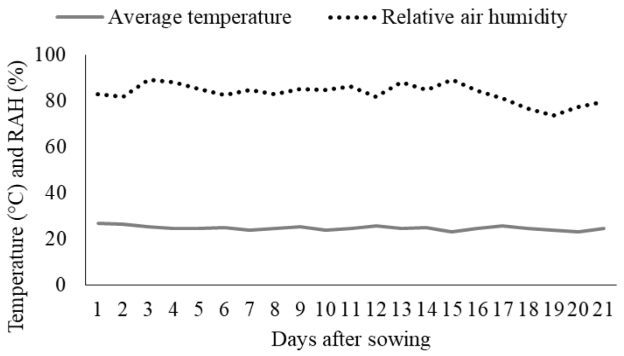

Fig. 1: Average temperature and relative humidity during the experimental period.

Experimental Design and Conduct

The experimental design was randomized blocks, with four treatments and five replications. The treatments consisted of T1 (control), T2 (foliar application of nicotinamide, 200mg L^{-1}), T3 (foliar application of *Azospirillum brasilense*, 2mL L^{-1}), and T4 (combined application of nicotinamide, 200mg L^{-1} and *A. brasilense*, 2mL L^{-1}).

Each experimental plot consisted of a 0.8 L black polyethylene pot, previously filled with commercial substrate, containing one sorghum plant. Sowing was conducted at a depth of 3 cm, using two seeds of the B1G211 hybrid and thinning the plants after emergence. Every week, 100mL of the nutrient solution was applied, consisting of 18% N, 8% P, 30% K, 3% S, 3% Mg, 0.14% Fe, 0.04% B, 0.04% Mn, 0.03% Cu, 0.019% Mo, 0.006% Ni, 0.002% Co, and 15% Ca. The biostimulants used were *A. brasilense*, obtained from the commercial product AzoTotal, which contains at least 2×10^{11} CFU L^{-1}, and nicotinamide, which has a 100% guarantee of the active ingredient (a.i.) and comes from a specialized laboratory (Acs Cientifica®).

The solutions for the treatments were prepared in fresh water, diluting the components by stirring and adding Tween 80. The solutions were applied using a hand sprayer, with the application directed at the pots containing the plants in sufficient volume to obtain leaf wetness 15 days after sowing. After the application, irrigation was suspended, and the plants were monitored daily to check transpiration until they reached a value 90% lower than that initially observed (Heinemann et al., 2011). At that point, water stress was identified, and irrigation resumed. All treatments also underwent the stress period, which lasted six days, with no plants under adequate irrigation.

Irrigation was conducted automatically using a micro-sprinkler system installed inside the screen. Due to the characteristics of the irrigation system, the application intensity was 13mm h^{-1}, equivalent to 0.21mm min^{-1}. Two 15-min pulses of irrigation were programmed daily, with a 12-hour interval.

Evaluations

Evaluations began 24 hours after irrigation was resumed. Measurements of net photosynthesis (A), stomatal conductance (gs), intercellular CO_2 concentration (Ci), and transpiration rate (E) were carried out in the morning, between 8:00 and 10:00 a.m., when plants exhibited peak gas exchange activity. Data were obtained using a portable infrared gas analyzer (LCi, ADC Bioscientific, Hertfordshire, UK). Based on these measurements, water use efficiency (A/E) and instantaneous carboxylation efficiency (A/Ci) were calculated.

The following variables were assessed 21 days after sowing: plant height (from the surface of the substrate to the apex of the plant), stem diameter, number of leaves, root volume, root dry weight, and shoot dry weight. Plant height was measured using a tape graduated in centimeters, while stem diameter was assessed with a digital caliper. Root volume was obtained after washing the roots by inserting them into a beaker containing a known volume of water and measuring the volume displaced after total submersion. The plants were sectioned into root and shoot, placed in paper bags, labeled, and taken to a forced-air oven at 65ºC until they reached a constant weight to determine the dry weight. The weight was then determined using a precision scale.

Statistical Analysis

The data were subjected to analysis of variance (ANOVA), and the means were compared using the t-test (LSD) at a defined probability level, performed with Sisvar 5.6 software (Ferreira 2019). Pearson's correlation analysis was also conducted (R software version 4.3.3, *qgraph* package), along with multivariate analysis, including canonical variable analysis (*Candisc* package) and principal component analysis (*Ggfortify* and *Factoextra* packages), all performed in R (R Core Team 2024).

RESULTS

The application of the treatments resulted in a reduced intercellular CO_2 concentration (Ci), particularly under the combined application of *A. brasilense* and nicotinamide, which yielded the lowest value (Fig. 2A). For stomatal conductance (*gs*), transpiration (*E*), and net assimilation rate (*A*), the three treatments in which the biostimulants were applied were superior (Fig. 2B, C and D).

The water use efficiency values did not differ between treatments. Still, for carboxylation efficiency, the combined application of *A. brasilense* and nicotinamide resulted in the highest average observed, followed by the isolated application of both biostimulants (Fig. 3B).

The plant height and root volume of plants treated with nicotinamide alone stood out, but there was no difference when applied together with *A. brasilense* or when treated with the bacterium alone, respectively. For root volume, all the treatments with biostimulants were superior to the control treatment (Fig. 4A and B). There was no difference between the treatments regarding stem diameter and number of leaves (Fig. 4C and D).

For the accumulation of dry weight in the plant organs, it was found that the treatments resulted in different behaviors. The application of nicotinamide alone increased the accumulation of dry weight in the stem of the plants. The application of *A. brasilense* resulted in the highest values observed for accumulating dry weight in the roots, followed by nicotinamide. The combined application

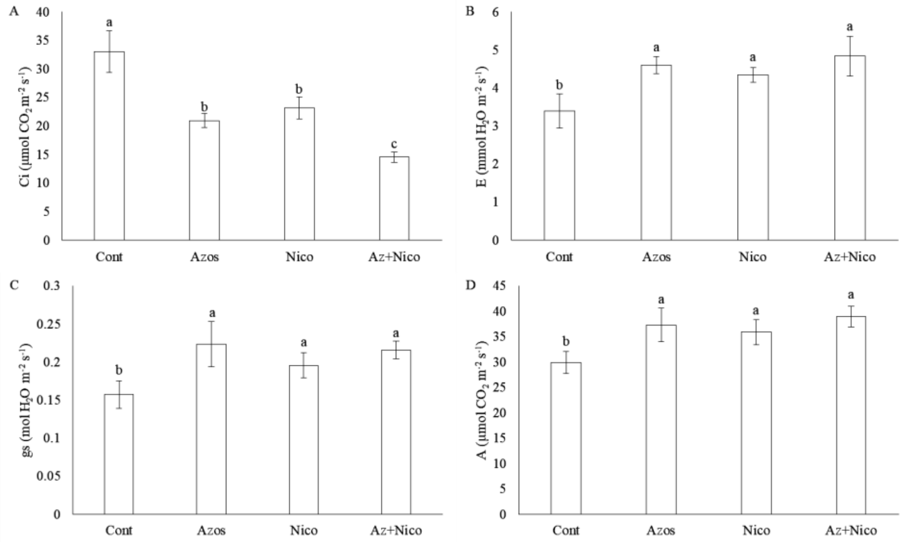

Fig. 2: Leaf mesophyll CO_2 content (*A*), transpiration (*B*), stomatal conductance (*C*), and net assimilation rate (*D*) in sorghum plants subjected to water restriction and application of biostimulants. Different letters indicate a significant difference between the means using the t-test (LSD) at 5% probability. Bars indicate the mean value of the treatments (n = 5), and the lines above the bars indicate the standard deviation.

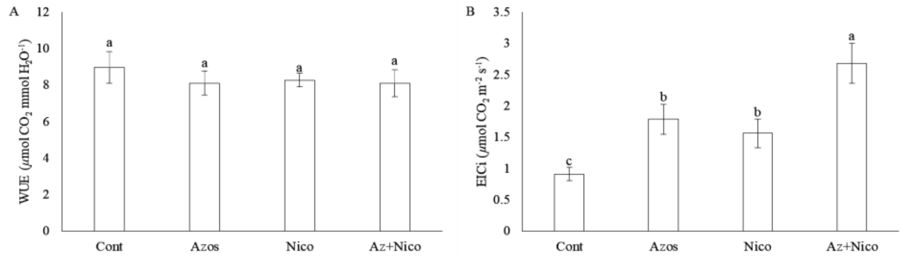

Fig. 3: Water use efficiency (A) and carboxylation efficiency (B) in sorghum plants subjected to water restriction and application of biostimulants. Different letters indicate a significant difference between the means using the t-test (LSD) at 5% probability. Bars indicate the mean value of treatments (n = 5), and the lines above the bars indicate the standard deviation.

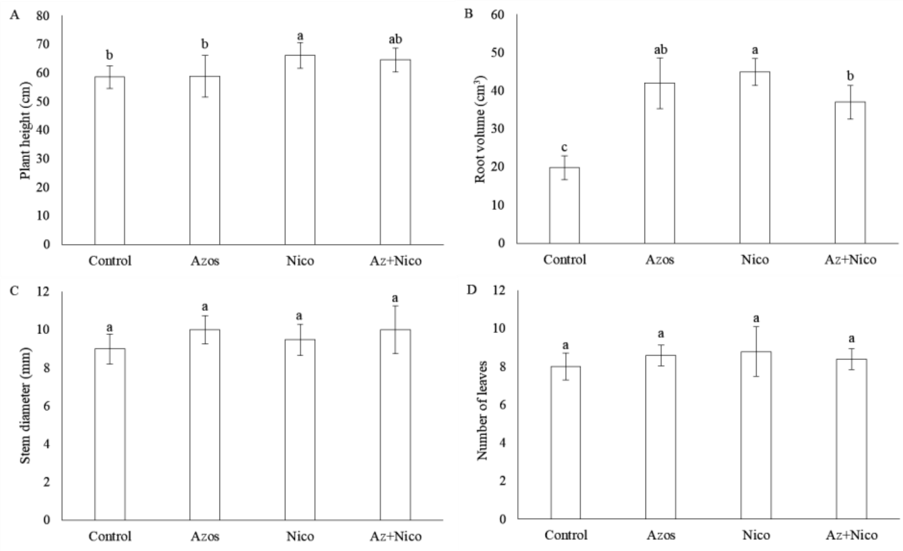

Fig. 4: Plant height (*A*), root volume (*B*), stem diameter (*C*), and number of leaves (*D*) in sorghum plants subjected to water restriction and application of biostimulants. Different letters indicate a significant difference between the means using the t-test (LSD) at 5% probability. Bars indicate the mean value of treatments (n = 5), and the lines above the bars indicate the standard deviation.

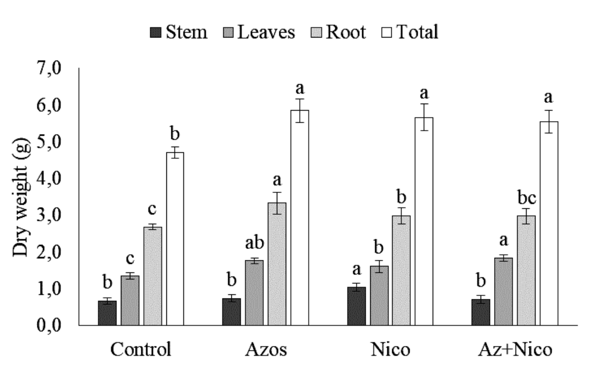

Fig. 5: Dry weight of stalk, leaves, root, and total in sorghum plants subjected to water restriction and application of biostimulants. Different letters indicate a significant difference between the means of the same characteristics using the t-test (LSD) at 5% probability. Bars indicate the mean value of treatments (n = 5), and the lines above the bars indicate the standard deviation.

allowed the greatest dry weight accumulation in the leaves, followed by the isolated applications of *A. brasilense* and nicotinamide, respectively. Even with variable responses concerning the growth stimuli of the different organs, all the treatments with biostimulants were superior to the control in terms of total dry weight (Fig. 5).

It was found that the characteristics of transpiration, stomatal conductance, net assimilation rate, leaf dry weight accumulation, and plant stem diameter are positively correlated with each other. In contrast, the increase in these characteristics is negatively correlated with increased leaf mesophyll CO_2 content and water use efficiency. In addition, root volume is positively correlated with the number of leaves and the accumulation of total dry weight, the latter being negatively correlated with water use efficiency (Fig. 6).

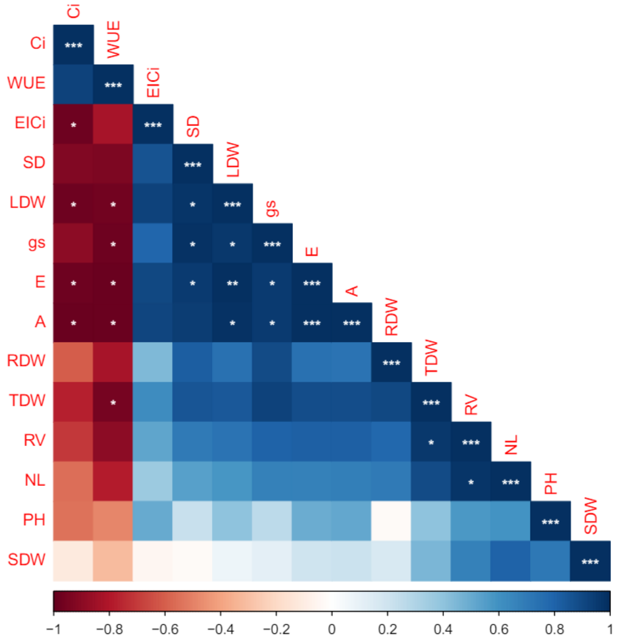

Fig. 6: Estimate of Pearson linear correlation coefficient between the characteristics of CO_2 content in the leaf mesophyll (Ci), transpiration (E), stomatal conductance (gs), net assimilation rate (A), water use efficiency (WUE), carboxylation efficiency (EICi), plant height (PH), root volume (RV), stalk diameter (SD), number of leaves (NL), stalk dry weight (SDW), leaf dry weight (LDW), root dry weight (RDW), and total dry weight (TDW) of sorghum plants subjected to water restriction and the application of biostimulants.

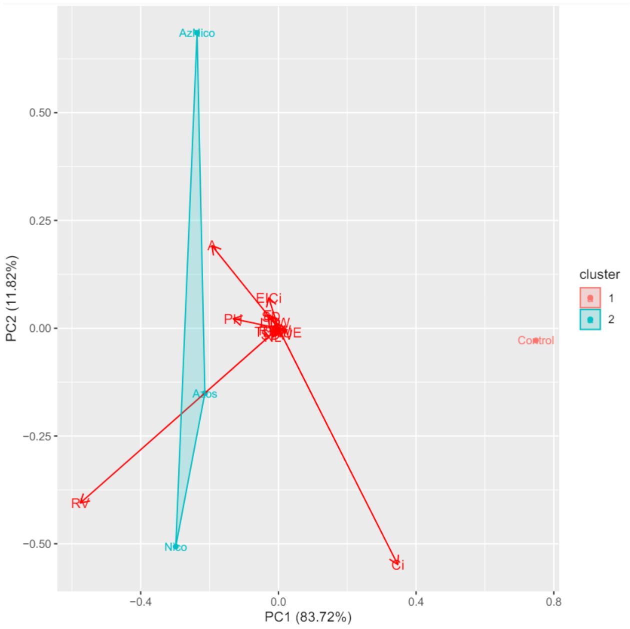

Fig. 7: Analysis of canonical variables among growth variables: leaf mesophyll CO_2 content (*Ci*), transpiration (*E*), stomatal conductance (*gs*), net assimilation rate (*A*), water use efficiency (*WUE*), carboxylation efficiency (*EICi*), plant height (PH), root volume (RV), stalk diameter (SD), number of leaves (NL), stalk dry weight (SDW), leaf dry weight (LDW), root dry weight (RDW), and total dry weight (TDW) of sorghum plants subjected to water restriction and the application of biostimulants. Treatments = Control, Nico (Nicotinamide), Azos (*A. brasilense*), and AzNico (Nicotinamide + *A. brasilense*).

The analysis of canonical variables allows for evaluating the effectiveness of the applied treatments. In this sense, the isolated application of nicotinamide or *A. brasilense* had a greater impact on the root volume. In addition, the combined application of *A. brasilense* and nicotinamide had a greater effect on the net assimilation rate. At the same time, all the treatments with the biostimulants tended to have a lower CO_2 concentration in the leaf mesophyll (Fig. 7).

DISCUSSION

The application of nicotinamide and *A. brasilense* stimulated the net assimilation rate, stomatal conductance, and carboxylation efficiency, which were reflected in the height, root volume, and dry weight of the sorghum plants after water deficiency (Fig. 2, 3, and 4). The beneficial effects of the interaction between the bacteria and the vitamin are attributed to their biostimulant properties, which enhance the plants' efficiency in utilizing available resources. This can be seen in the lower CO_2 levels in the leaf mesophylls of plants subjected to the application of biostimulants (Fig. 2 and 8) and the negative correlation that this characteristic has with the other gas exchange parameters quantified (*E, gs,* and *A*), as well as carboxylation efficiency and the growth of leaf organs (Fig. 6).

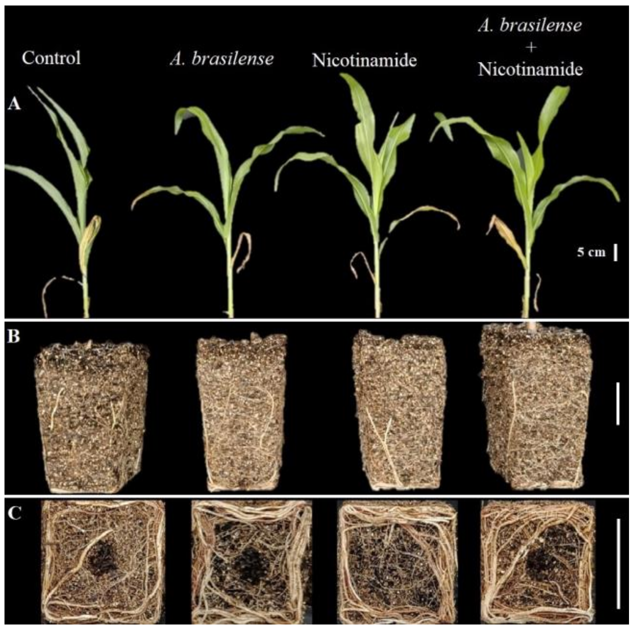

Fig. 8: Visual comparison of the shoot (A) and root system from the side (B) and bottom (C) views of sorghum plants treated with biostimulants 24 hours after irrigation was resumed.

The greater carboxylation efficiency obtained by applying biostimulants (Fig. 3B) is intrinsically linked to the better performance of sorghum plants subjected to periods of water stress, indicating that even under adverse conditions, there is greater CO_2 consumption, while photosynthetic activity is maintained or even increased. These responses are also associated with enhanced root development, which increases the plant's ability to withstand periods of stress (Avila et al., 2020). Similar results were obtained for corn plants subjected to water deficit, in which the *A. brasilense* application resulted in increased gas exchange activity, accompanied by increased root volume (Marques et al., 2021).

Also, in this study, the stimulus to root development was directly impacted by the application of the biostimulants, an effect evidenced by the greater volume of the root system in the treatments composed of bacteria and/or the vitamin (Fig. 4B). In this sense, it is worth noting that the increase in volume was also accompanied by a greater accumulation of dry weight in the root and shoot organs (Fig. 5), culminating in more vigorous plants, especially after water conditions were re-established (Fig. 8).

In plants of the Poaceae family, the application of *A. brasilense* effectively promotes root growth, increasing both root volume and length (Avila et al., 2020; Marques et al., 2021; Pii et al., 2019). In part, this effect is related to the stimulation of endogenous auxin production, especially indole acetic acid (IAA) (Silva et al., 2017), which involves changes in the expression of genes related to responses to abiotic stresses and enables plants to develop mechanisms aimed at maintaining their metabolic activities (Espindula et al., 2023). Transcriptomics has shown that *A. brasilense* induces significant transcriptional changes in carbon and nitrogen metabolism in roots, which can lead to increases in root biomass (Zhao et al., 2023).

The expression of genes associated with abiotic stress responses, triggered by the presence of *A. brasilense*, also leads to increased levels of photosynthetic pigments, soluble sugars, proteins, and free proline. Additionally, it enhances the activity of antioxidant enzymes such as peroxidase (POD), superoxide dismutase (SOD), and ascorbate peroxidase (APX) while reducing the accumulation of reactive oxygen species involved in tissue oxidation, including hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) (Peralta et al., 2021; Omer et al., 2022).

Similarly to what was observed for the reactions triggered by the application of *A. brasilense*, nicotinamide resulted in morphophysiological changes that allow the metabolism and growth of plant organs to be maintained (Fig. 2, 3, and 4). In part, these effects are related to the stimulation of the production of the coenzymes NADH and NADPH, which influences the maintenance of the redox state (oxidoreductase), being a plant growth biostimulant (Ferreira et al., 2024), allowing plants to maintain the production of photoassimilates, which are essential for the maintenance of metabolism during periods of environmental stress (Taiz et al., 2017).

Nicotinamide positively influenced root development (Fig. 4 and 8). This action was also verified for pumpkin, for which the exogenous application of nicotinamide also induces gains in root growth (Vendruscolo et al., 2024b), an effect related to the increase in endogenous auxin levels triggered by the vitamin's action (Ahmad et al., 2021; Laurell et al., 2022). This increase in root volume is also involved in improving the conditions for plant development under stressful conditions, as seen in sugarcane subjected to water deficit (Ramos et al., 2023) and in soybean and corn crops grown in field conditions, where interaction with the environment implies the constant occurrence of biotic and abiotic stresses (Thomé et al., 2023; de Lima et al., 2024).

The presence of extra nicotinamide implies an increase in free proline in plant organs (Mohamed et al., 2020). This compound plays a crucial role as an osmoprotectant in various tissues where it is synthesized, including organelles such as chloroplasts, helping to restore the photosynthetic apparatus (Ghosh et al., 2020). In addition, exogenously applied nicotinamide attenuates the deleterious effects of abiotic stresses, maintaining or even reducing the action of reactive oxygen species, such as hydrogen peroxide and malondialdehyde, which act in lipid oxidation, and increasing the activity of enzymes such as superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) (Li et al., 2024). The increase in POD and CAT activity reflects the high potential of applying nicotinamide as a stress attenuator since these enzymes have an important action in the decomposition of hydrogen peroxide (Hasanuzzaman et al., 2020). These factors imply better performance regarding plant gas exchange (Fig. 2 and 3).

Although little studied, the interaction between *A. brasilense* and nicotinamide is effective in increasing gas exchange activities, as seen in this study (Fig. 2 and 3) and the growth of vegetative organs, as seen in pumpkin crops grown in a hydroponic system (Vendruscolo et al., 2024a) and coffee seedlings (Lima et al., 2023), indicating a synergistic effect between the technologies.

To understand the interaction between plant growth-promoting rhizobacteria (*Azospirillum brasilense*) and the NADH/NADPH (nicotinamide) coenzyme pathway and its biostimulant properties on gas exchange and plant growth (Fig. 9), we should also point out that plant growth and development occur from meristematic tissue, forming new cells that differentiate to form various plant structures.

Nicotinamide is absorbed by the plant either through the root system or through foliar application and is converted in the plant to NAD+/NADH and NADP+/NADPH (Hori 1979) (Fig. 9). For growth to occur in the meristematic region, the proper formation of the coenzymes NAD^+/NADH and $NADP^+$/NADPH is essential. These coenzymes act as activated electron carriers: NAD^+/NADH is primarily involved in oxidative catabolic reactions, while $NADP^+$/NADPH participates in reductive biosynthetic (anabolic) processes, functioning as electron acceptors or donors depending on the pathway. This compound is necessary for biosynthetic reactions and bioconversions, and low NADPH availability limits these physiological processes in plants (Poulsen et al., 2005), with nicotinamide being a growth biostimulant (Fig. 9; Fig. 4A, B; Fig. 5; Fig. 7; and Fig. 8).

In plants, water stress causes the formation of reactive oxygen species, which leads to oxidative stress and is harmful to the plant if the antioxidant system does not act adequately to neutralize this oxidative stress (redox homeostasis). Thus, the action of nicotinamide or vitamin B3 comes into play, maintaining the oxidative balance (Berglund et al., 2017), making it possible to maintain the redox state of the cell (Maria and Moreira 2011; Kirkland and Meyer-Ficca 2018; Ferreira et al., 2023) under conditions of environmental stress (Ramos et al., 2023; Hussein et al., 2014).

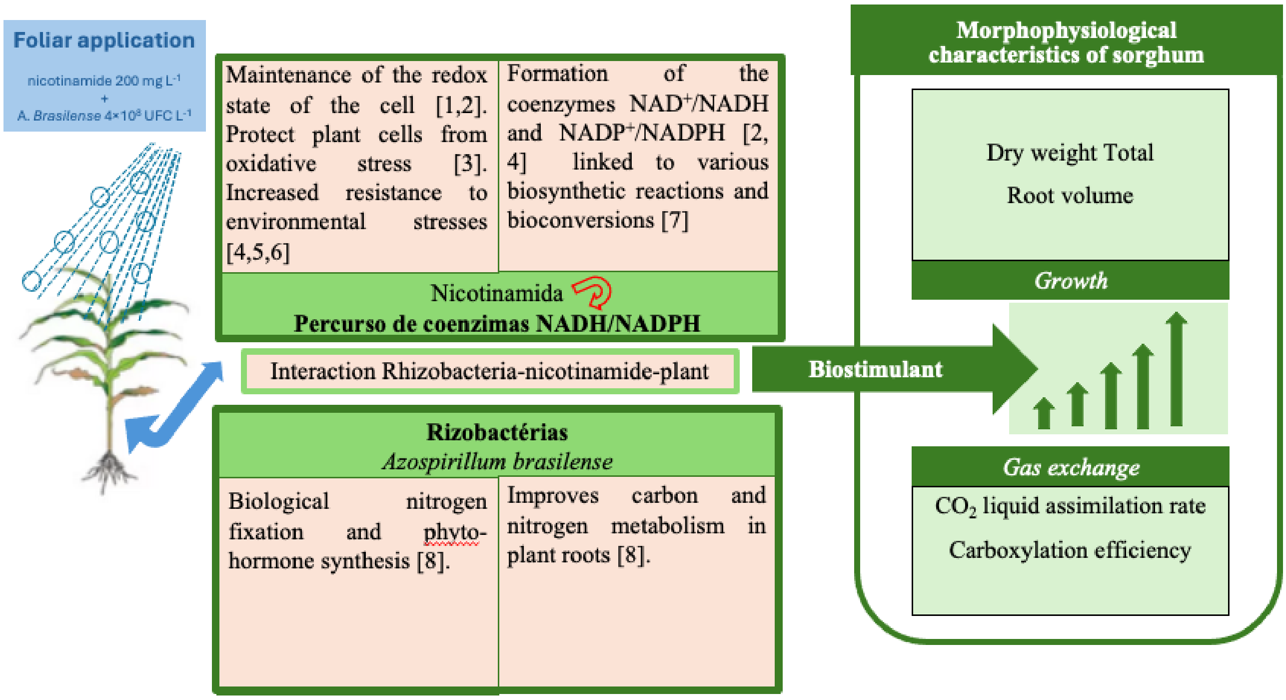

Fig. 9: Interaction between plant growth-promoting rhizobacteria (*Azospirillum brasilense*) and the NADH/NADPH (nicotinamide) coenzyme pathway: biostimulant effects on gas exchange and plant growth. Adapted from: [1] Maria and Moreira (2011), [2] Kirkland & Meyer-Ficca (2018), [3] Berglund et al. (2017), [4] Zhu et al. (2018), [5] Ramos et al. (2023), [6] Hussein et al. (2014), [7] Poulsen et al. (2005), [8] Silva et al. (2017), [9] Zhao et al. (2023).

Plant growth and development (Fig. 4A, B; Fig. 5; and Fig. 8) depend on intracellular concentrations of NADH, which influence ATP production. The ATP and NADPH generated in the photochemical phase of photosynthesis are necessary for the plant carboxylation process (Fig. 2A, C; Fig. 3B). The NAD+/NADH and NADP+/NADPH coenzymes produced from nicotinamide influence the biochemical routes of the plant secondary metabolism (Taiz et al., 2017).

Plant growth-promoting rhizobacteria, in this case, *A. brasilense*, are non-symbiotic diazotrophic bacteria that can fix atmospheric nitrogen and be present both in the rhizosphere and endophytically. They can biosynthesize indoleacetic acid (IAA) (Crozier et al., 1988; Silva et al., 2017), influencing the architecture of the root system (Fig. 8) and increasing the volume of roots (Pii et al., 2019) (Fig. 4B, 7 and 8).

A. brasilense causes a reduction in cell elongation (inhibited primary root and elongated lateral roots and root hairs) but does not affect the general organization or cell division of the primary root meristem, enabling a root system with greater production of secondary roots and root hairs (dense and shallow root system). This leads to significant changes in the characteristics of root architecture (Fig. 8) and shows a biostimulant effect on growth (Fig. 9) and increased root volume (Fig. 4B, 7, and 8) (Zhao et al., 2023). Root volume (RV) is directly related to leaf formation (NL), and leaf dry weight (LDW) positively influences the CO_2 assimilation rate (A) (Fig. 6), thus showing that the increase in root volume caused by *A. brasilense* (Fig. 4B and 7) has a biostimulant effect on gas exchange (Fig. 2D and 3B) and sorghum growth (Fig. 9).

Exploring new technologies for the agricultural sector is a valuable tool for maintaining food security, given that sudden changes in climatic conditions can, even in a short time, compromise yield. Thus, by compiling our findings and the information generated by previous studies, we can infer that both *A. brasilense* and nicotinamide have biostimulant and bioprotective characteristics, which result in potential application together or in isolation, benefiting crops in regions where the occurrence of stress has been observed continuously.

Conclusion

The foliar application of *Azospirillum brasilense* and nicotinamide, either individually or in combination, acts as a biostimulant that enhances gas exchange and promotes shoot and root growth in sorghum plants under water deficit conditions. These findings suggest that such technologies can be used preventively to improve crop performance under environmental stress.

DECLARATIONS

Funding: This research was partially funded by Coordination for the Improvement of Higher Education Personnel - Brazil (CAPES) - Financing Code 001.

Acknowledgement: We are grateful to the State University of Mato Grosso do Sul and to the Research Group for Innovation and Advancement of Agriculture – INNOVA - for the availability of structure and technical personnel.

Conflicts of Interest: None.

Data Availability: Data will be available at request.

Ethics Statement: This study did not require ethical review, as it did not involve sensitive human data or animal subjects.

Author's Contributions: Débora Cristina Sousa Barros de Oliveira, Iasmin Freitas Souza, Tamiris Dias Santana, Eduardo Pradi Vendruscolo, and Giovana Pinheiro Viana da Silva contributed to the execution of the experiment, data collection, writing, interpretation and review of the manuscript. Flávio Ferreira da Silva Binotti, Sebastião Ferreira de Lima, and Fernanda Pacheco de Almeida Prado Bortolheiro contributed to the writing, statistical analyses and interpretation, and revision of the manuscript.

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