Azospirillum brasilense and jasmonic acid as mitigators of water stress in creole corn plants

Azospirillum brasilense y ácido jasmónico como atenuadores del estrés hídrico en plantas de maíz criollo

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ABSTRACT

The aim of this research was to evaluate the action of *Azospirillum brasilense* and jasmonic acid as mitigators of water stress caused by various irrigation levels in Creole corn crops. The experiment was conducted in a greenhouse using a completely randomized factorial design consisting of 2 levels of *A. brasilense* (absence and presence) x 3 doses of jasmonic acid x 3 irrigation depths determined by three evapotranspiration (ET0) multipliers (0.6, 0.8, and 1.0 of the evapotranspiration measured in the crop area). The design included 4 replicates, totaling 72 experimental units. Evaluations were carried out at the R6 physiological maturity stage for number of tassels, tassel branch number, number of leaves on the corn cob, and number of ears per plant. Analysis of variance showed significant difference among the variables studied. The irrigation depth of 0.8 of the evapotranspiration showed significant interactions between *A. brasilense* and jasmonic acid in reducing water stress in creole corn plants. The results for the irrigation depth 0.6 of the evapotranspiration, with the use of mitigators, showed smaller reductions in stress (11.91% reduction compared to 0.8 of the ET0). The treatment of 10 µmol L^{-1} jasmonic acid was the most favorable for reducing stress and consequently increasing the productivity of creole corn plants, coming closest to the estimated optimal dosage.

Key words: production, resistance, signaling, water stress, growth regulator.

RESUMEN

El objetivo de esta investigación fue evaluar la acción de *Azospirillum brasilense* y ácido jasmónico como atenuadores del estrés hídrico provocado por diferentes niveles de riego en el cultivo de maíz criollo. El experimento se llevó a cabo en un invernadero, el diseño experimental fue completamente al azar en un esquema factorial con dos niveles de *A. brasilense* (ausencia y presencia) x 3 dosis de ácido jasmónico x 3 profundidades de riego determinadas por tres coeficientes multiplicadores de la evapotranspiración (ET0) (0,6, 0,8 y 1,0 de la evapotranspiración medida en el área del cultivo), con 4 repeticiones, para un total de 72 unidades experimentales. Las variables estudiadas fueron: número de panículas, número de ramas de la panoja, número de hojas en la mazorca de maíz y número de mazorcas por planta. Se realizó un análisis de varianza, en el cual se demostró que hubo diferencias significativas entre los tratamientos para las variables estudiadas. La lámina de riego de 0,8 de la evapotranspiración mostró interacciones significativas entre *A. brasilense* y ácido jasmónico en la reducción del estrés hídrico en plantas de maíz criollo. Los resultados para la lámina de riego de 0,6 de la evapotranspiración, con el uso de atenuadores, mostraron reducciones del estrés (reducción del 11,91% respecto al 0,8 de la ET0). El tratamiento de 10 µmol L^{-1} de ácido jasmónico fue el que más se acercó a las dosis estimadas y resultó ser el más favorable para la reducción del estrés y consecuente aumento de la productividad de las plantas de maíz criollo.

Palabras clave: producción, resistencia, señalización, estrés hídrico, regulador de crecimiento.

Introduction

Corn (*Zea mays* L.) stands out internationally as one of the most relevant agricultural products and is indispensable in animal feed as it is the main source of energy. It is also used in the production of renewable fuel. In 2024, Brazil will be the second largest corn producer in the world, behind the United States. Production is estimated at 115.72 million t, including the three harvests, which is 12.3% or 16.17 million t below that produced in 2022/23 (Companhia Nacional de Abastecimento – Conab, 2024).

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Oliveira *et al*. (2021) pointed out that creole corn can be an alternative to hybrid cultivars, which are dependent on chemical inputs and more advanced technologies. Another peculiar characteristic of native plants, resulting from genetic factors, is their resistance to biotic and abiotic stresses (Modesto *et al*., 2021).

The cultural role of Creole maize is of great relevance, as well as its economic influence in certain countries around the world, specifically those in America, especially Latin America, where the first maize cultivars originated (Jasper & Swiech, 2019). Generally, native plantations are inferior in terms of productivity when compared to commercial ones; however, they are essential for establishing a source of genetic variability (Araújo *et al*., 2018).

Creole varieties tend to be resistant to environmental changes and phytopathologies, as they adapt better to unfavorable circumstances, such as high temperatures and pest attacks (Eicholz *et al*., 2018). One of the most effective techniques to conserve Creole varieties is the use of local seed banks (Mateus *et al*., 2020). An indication of the superiority of Creole varieties over commercial ones is their productivity, whether in grains or in volume of straw for feeding dairy cattle (Sah *et al*., 2020).

According to Pandit *et al*. (2018), the choice of corn cultivation period takes into account the rainfall patterns in different Brazilian regions to obtain better results in productivity. Sah *et al*. (2020) reported that water management techniques are essential to increase crop production and, above all, to improve the efficient use of water, and should focus on irrigated agriculture planning.

The use of Plant Growth-Promoting Bacteria (PGPB) can favor the cultivation of corn (Silva *et al*., 2022). According to Schaefer *et al*. (2019), PGPB act as a defense mechanism against adverse factors such as water deficit. With the use of *Azospirillum brasilense*, the synthesis of plant hormones that signal stress such as jasmonic acid and abscisic acid, as well as growth promoters, has been observed, especially for the increase of the root system, which provides greater water absorption (Fukami *et al*., 2018).

Jasmonic acid is a phytohormone that plays a role in the growth, development, and response to various stress states in plants (Wang *et al*., 2020). According to Kerbauy (2019), some studies have suggested that jasmonic acid may be associated with the expression of resistance genes and involved in the signaling response to biotic and abiotic stress conditions.

Oliveira *et al*. (2020) report that irrigation of plantations is not always carried out to fully meet the crop's water needs, and management is guided by economic objectives rather than by maximum physiological productivity. It is essential to study the action of mitigators of low water conditions in the soil, which can also provide greater plant performance and enhance the productivity of Creole corn.

According to Crisóstomo *et al*. (2018), there is a lack of information on Creole corn cultivars in Brazil, considering the limited availability of production technology and distinctions of size and arrangement of leaves in the plants, making further studies regarding the population management of Creole corn plants indispensable. Given the above, the aim of this study was to evaluate the action of *Azospirillum brasilense* and jasmonic acid as mitigators of water stress caused by various irrigation levels in Creole corn crops.

Materials and methods

Description of the experimental area

The experiment was conducted from November 2020 to February 2021 in a greenhouse belonging to the Integrated Management of Weeds of the Amazon (Mipdam) group, located at the Institute of Agrarian Sciences (ICA) of the Federal Rural University of the Amazon (UFRA), in the municipality of Belém (PA). This site is geographically located at 48°30'16" W and 1°27'21" S, at an average altitude of 10 m a.s.l. and temperature and average relative humidity of 36.5°C and 66% RH, respectively, measured with a digital thermo-hygrometer model K29-5070H (Kasvi®).

Experimental design

The experimental design was completely randomized in a factorial scheme consisting of 2 levels of *A. brasilense* (absence and presence) x 3 irrigation depths determined by three evapotranspiration multiplier coefficients (0.6, 0.8, and 1.0 of the evapotranspiration measured in the crop area) (Deaquiz *et al*., 2014) x 3 doses of jasmonic acid (Sigma Chemical Co., USA) (0, 10, and 20 μmol L^{-1}), with 4 replicates, totaling 72 experimental units. Five seeds were sown per pot, with a capacity of 11.7 L, containing a dystrophic yellow Latosol soil substrate, which was sent for analysis (Tab. 1) after being previously sieved in a 2 mm sieve. The first thinning was performed at 7 d, leaving only two plants per pot, and the second was performed at 14 d, leaving one plant per pot (which served as each of the experimental units). Nitrogen fertilization with urea was carried out at planting and covering was done as recommended by the liming and fertilization recommendation book for the State of Pará (Brasil *et al*., 2020).

TABLE 1. Results of the soil chemical analysis.

pH		TOC	OM	P	N	K	Ca	Mg	Al (KCl)	H+Al	SB
$CaCl_2$	Buffer solution (SMP)	g dm^{-3}		mg dm^{-3}	mg L^{-1}	mmolc dm^{-3}					
5.9	6.5	40.0	94.0	87.0	2688.0	2.9	31.0	19.0	0.0	25.0	65.1

TOC: Total organic carbon, OM: soil organic matter, P: phosphorus, N: nitrogen, K: potassium, Ca: calcium, Mg: magnesium, Al: aluminum, H: hydrogen, SB: sum of bases, SMP: Shoemaker-MacLean-Pratt buffer.

The content of each mineral nutrient was determined in the Soil laboratory of the Brazilian Institute of Analysis (IBRA) according to the methods described by IAC (2001) and EMBRAPA (2009).

Creole corn seeds of the variety BRS 4157 Sol-da-morning Nitroflint, provided by the Popular Peasant Movement (MCP) of the municipality of Igarapé-açú were used. The seeds underwent asepsis with 1% sodium hypochlorite for 3 min and then were washed with distilled water and placed on paper towels to dry (Cicero & Silva, 2003).

Subsequently, the corn seeds were inoculated (Fig. 1) with *A. brasilense,* strains AbV5 and AbV6, in a peat-based vehicle, about 2 h before sowing, applying a 10% glucose solution to moisten the seeds and then the inoculant (50 g of inoculant/10 kg of seeds), covering all the seeds. The seeds were left in the shade and in an airy place for drying and adherence of the inoculant, according to the manufacturer's instructions (Ministério da Agricultura, Pecuária e Abastecimento, 2009).

FIGURE 1. Seed inoculation.

All treatments were irrigated daily for 7 d until total evapotranspiration (1.0) was restored for seedling establishment (Ministério da Agricultura, Pecuária e Abastecimento, 2009). From that point on, no irrigation was carried out in the treatments with 0.6, and 0.8 of the evapotranspiration. Only the treatment with 1.0 of the evapotranspiration was irrigated every day. Once all treatments reached their respective percentages, daily irrigation was done according to the treatments.

The application of jasmonic acid (Sigma Chemical Co., USA) was carried out in three treatments – control (without application), 10 μmol L^{-1}, and 20 μmol L^{-1} – via foliar spray with a manual sprayer (Fig. 2B) using 5 ml per plant, applied adaxially and abaxially to all leaves. The solution was diluted in distilled water and Tween 20 (0.05%) to enhance adhesion to the leaves (Lopes *et al*., 2009). The applications took place at 20 and 40 d after sowing.

Biometric variables

When the plants reached the R6 stage, between 90 and 105 d after sowing, which characterizes physiological maturity, the following evaluations were performed: number of tassels (NT): counting all tassels per plant, according to the methodology described by Vital *et al*. (2015); tassel branch number (TBN); number of leaves on the corn cob (NLC): counting all leaves with on the corn cob, according to the methodology described by Vital *et al*. (2015); and number of ears of corn (NE): counting all the ears , according to the methodology described by Martins *et al.* (2016).

Statistical analysis

The experimental data were submitted to the Shapiro-Wilk and Levene tests at 5% of significance to verify the normality and homoscedasticity of the data, respectively. Subsequently, analysis of variance was carried out, in which the developments that proved to be significant were evaluated. The effects of jasmonic acid doses on the presence and absence of *A. brasilense* at different irrigation depths were studied by polynomial regression analysis, observing the results of the F test (P<0.05) from the analysis of variance with the Sisvar statistical software (Ferreira, 2019).

Results and discussion

The number of tassels (NT) did not show adjustment to the linear or quadratic models (Tab. 2), indicating that the plants maintained a pattern in this variable that did not change according to the treatments used. At 58 d after sowing more than 90% of the plants already had tassels, normally following their phenological stage. Modesto *et al*. (2021) observed the phenological stages in corn plants, noting that about 90% of the plants had tassels at 60 d after sowing.

For Singh *et al*. (2021), Creole varieties, as they are genotypes with a broad genetic base, are able to respond better to abiotic and biotic stresses, which may present productive potential to match or exceed the production of hybrid cultivars, thus making them an alternative for sustainable production, reducing production costs with inputs and minimizing the use of technological packages.

Jasmonic acid (JA) is a phytohormone that acts as a plant growth regulator and plays a role in the growth and development of plants under environmental stress. Higher levels of jasmonates accumulate in actively growing tissues, such as hypocotyls, flowers, and pods (Yang *et al*., 2019). JA also regulates such processes of plant development and growth as fruit ripening and maturation and production of viable pollen, seed germination, and development of anthers and pollen grains (Ruan *et al*., 2019). JA can regulate various aspects of plant development, including root growth, stamen development, flowering, and leaf senescence (Wang *et al*., 2020).

It can be seen from Table 3 that there was an adjustment to the quadratic model in tassel branch number (TBN) in the irrigation conditions of 0.6, and 0.8 of the ET0 with the presence of *A. brasilense*, obtaining the maximum technical efficiency (Ymte) at 0.8 of evapotranspiration (ET0), with an increase of 11.91% compared to 0.6 of the ET0. In the absence of *A. brasilense*, an adjustment to the increasing linear model is observed, indicating that the higher the dose of jasmonic acid, the higher the TBN. In the condition of 1.0 of the ET0 and absence of *A. brasilense*, there was an adjustment to the decreasing linear model, showing that the JA doses had a negative effect on these treatments. In the presence of *A. brasilense,* the TBN did not present adjustments to the quadratic and linear model, showing that the results were similar despite the doses of JA. JA can stimulate flowering and raise tolerance to low water conditions (Kerbauy, 2019). According to El Sabagh *et al*. (2018), minimizing the effects of water deficit is one of the benefits of inoculation with *A. brasilense* in forages.

Bacteria of the genus *Azospirillum* are free-living microorganisms capable of proliferating in the roots of plants, promoting benefits such as stimulating root growth. This results in different effects including an increase in the absorption surface of the roots, which leads to an increase in the volume of the exploited soil, an increase in water

TABLE 2. Number of tassels (NT) of creole corn plants subjected to three irrigation depths with *A. brasilense* and jasmonic acid as mitigators.

ET0 (irrigation)	*A. brasilense* (Absence/Presence)	JA (μmol L^{-1})			Equation	R^2	JA
		0	10	20			
0.6	Absence	1.00	1.00	1.00	ns	-	-
	Presence	1.00	1.00	1.00	ns	-	-
0.8	Absence	1.00	1.00	1.00	ns	-	-
	Presence	1.00	1.00	1.00	ns	-	-
1.0	Absence	1.00	1.00	1.00	ns	-	-
	Presence	1.00	1.00	1.00	ns	-	-

ET0 – evapotranspiration. The irrigation depths were determined by three evapotranspiration multiplier coefficients. ns: not significant according to the F test (P<0.05), R^2 – determination coefficient, JA – jasmonic acid.

TABLE 3. Tassel branch number (TBN) of creole corn plants cultivated at three irrigation depths with *A. brasilense* and jasmonic acid as mitigators.

ET0 (irrigation)	*A. brasilense* (Absence/Presence)	JA (μmol L^{-1})			Equation	R^2	JA
		0	10	20			
0.6	Absence	7.00	7.50	7.75	$Y=7.04+0.037X$	0.96	-
	Presence	8.50	9.00	5.75	$Y=8.50+0.24X-0.019X^2$	0.99	6.33
0.8	Absence	8.75	9.75	11.25	$Y=8.67+0.12X$	0.98	-
	Presence	9.25	10.50	9.50	$Y=9.25+0.24X-0.011X^2$	0.99	10.56
1.0	Absence	10.75	9.75	9.25	$Y=10.67-0.075X$	0.96	-
	Presence	11.50	10.50	10.50	ns	-	-

ET0 – evapotranspiration. The irrigation depths were determined by three evapotranspiration multiplier coefficients. ns: not significant according to the F test (P<0.05), R^2 – determination coefficient, JA – jasmonic acid.

absorption and mineral nutrient acquisition, and greater tolerance to stress. These factors contribute to plants with more vigor and productivity (Fukami *et al*., 2018). Inoculation makes it possible, in some cases, to obtain good yields or gains in crop growth (Leite *et al*., 2018; Modesto *et al*., 2021).

Inoculation of corn with diazotrophic bacteria reduces the need for nitrogen fertilization and mitigates environmental contamination risks due to the bacteria's biological nitrogen-fixation capacity. *A. brasilense* and nitrogen fertilization resulted in increased corn biomass, production and yield in an integrated crop-livestock system (Shaefer *et al*., 2019).

From Table 4, it can be seen that NLS did not show adjustment to the linear or quadratic models, indicating that there was no maximum technical efficiency in this variable. Despite the lack of differences in NLS, stigmas can be observed 58 d after sowing, reaching more than 90% of the plants at 63 d after sowing, matching the period of the usual phenological stage of the culture. Sah *et al*. (2020), when evaluating the impact of water deficit stress in maize, observed that the interval from cultivation to flowering of maize plants increased from 80 to 120 d, on average, after the sowing of different maize genotypes in India, which resulted in losses of 30 to 60% in grain yield.

In the variable of number of ears of corn (NE) (Tab. 5), an adjustment to the decreasing linear model was observed under the condition of irrigation of 0.8 of the ET0 and in the presence of A. *brasilense,* indicating that the doses of jasmonic acid promoted an inverse effect for these circumstances. The other treatments showed no adjustment to the linear or quadratic models, suggesting a consistent pattern of NE in each plant subjected to the aforementioned treatments.

According to Modesto *et al.* (2021), jasmonic acid provokes a signal transduction network that leads to a cascade of events responsible for the physiological adaptation to the state of abiotic stress. The inoculant A. *brasilense* mitigated the deleterious effects caused by drought and promoted better growth of the root system, enhancing the tolerance of maize plants to water deficit (Marques *et al*., 2021).

Conclusions

The irrigation depth of 0.8 of the evapotranspiration showed the most favorable interactions between

TABLE 4. Number of leaves on the corn cob (NLC) of creole maize plants submitted to three irrigation depths with *A. brasilense* and jasmonic acid as mitigators.

ET0 (irrigation)	*A. brasilense* (Absence/Presence)	JA (μmol L^{-1})			Equation	R^2	JA
		0	10	20			
0.6	Absence	1.25	1.25	1.75	ns	-	-
	Presence	1.25	1.50	1.25	ns	-	-
0.8	Absence	1.25	1.00	1.00	ns	-	-
	Presence	1.00	1.00	1.00	ns	-	-
1.0	Absence	1.00	1.00	1.00	ns	-	-
	Presence	1.50	1.00	1.00	ns	-	-

ET0 – evapotranspiration. The irrigation depths were determined by three evapotranspiration multiplier coefficients. ns: not significant according to the F test (P<0.05), R^2 – determination coefficient, JA – jasmonic acid.

TABLE 5. Number of ears per plant (NE) of creole corn plants subjected to three irrigation depths with *A. brasilense* and jasmonic acid as mitigators.

ET0 (irrigation)	*A. brasilense* (Absence/Presence)	JA (μmol L^{-1})			Equation	R^2	JA
		0	10	20			
0.6	Absence	0.75	1.00	0.75	ns	-	-
	Presence	0.75	0.75	1.00	ns	-	-
0.8	Absence	1.00	0.75	1.00	ns	-	-
	Presence	1.25	1.00	0.75	Y=1.25-0.025X	0.99	-
1.0	Absence	0.25	0.75	0.75	ns	-	-
	Presence	1.50	0.75	0.75	ns	-	-

ET0 – evapotranspiration. The irrigation depths were determined by three evapotranspiration multiplier coefficients. ns: not significant according to the F test (P<0.05), R^2 – determination coefficient, JA – jasmonic acid.

Azospirillum brasilense and jasmonic acid in reducing water stress in creole corn plants. The results for the irrigation depth of 0.6 of the evapotranspiration, with the use of mitigators, showed smaller reductions in stress (11.91% reduction in relation to 0.8 of the ET0). The treatment with 10 µmol L^{-1} of jasmonic acid was the closest to the estimated dosages that proved to be the most favorable for reducing stress, consequently increasing the productivity of creole corn plants.

Conflict of interest statement

The authors declare that there is no conflict of interests regarding the publication of this article.

Author's contributions

EFLS: conceptualization, methodology, validation, formal analysis, research, and writing – original draft; KBST: designed the experiment, analyzed the data, wrote, and edited the manuscript; GGTNM: resources, writing, and review & editing; ACS: validation, resources, writing, and review & editing; SCSO: validation, writing – review & editing; BFG: conceptualization, resources, writing – review & editing, supervision; PAS: validation, writing – review & editing; JTO: validation, writing – review & editing; GMRP: validation, writing – review & editing; CFON: validation, analysis and interpretation of data, writing the draft of the manuscript. All authors have read and approved the final version of the manuscript.

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