

Brazilian Journal of Agricultural and Environmental Engineering v.27, n.7, p.550-558, 2023 Campina Grande, PB – http://www.agriambi.com.br – http://www.scielo.br/rbeaa

DOI: http://dx.doi.org/10.1590/1807-1929/agriambi.v27n7p550-558

Biostimulants increase growth and yield of second-crop maize¹

Bioestimulantes aumentam o crescimento e a produtividade do milho de segunda safra

Silvia E. N. Thomé², Sebastião F. Lima², Izabela C. de Oliveira³, Lucymara M. Contardi², Eduardo P. Vendruscolo⁴, Maria G. de O. Andrade⁵, Meire A. S. Cordeiro², Jeysielli C. Arguelho², Janaina J. de Oliveira²

¹ Research developed at Universidade Federal de Mato Grosso do Sul, Campus de Chapadão do Sul, Chapadão do Sul, MS, Brazil

² Universidade Federal de Mato Grosso do Sul/Faculdade de Agronomia, Chapadão do Sul, MS, Brazil

³ Universidade Estadual Paulista/Programa de Pós-Graduação em Agronomia, Ilha Solteira, SP, Brazil

⁴ Universidade Estadual de Mato Grosso do Sul/Faculdade de Agronomia, Cassilândia, MS, Brazil

⁵ Universidade Estadual Paulista/Programa de Pós-Graduação em Agronomia, Botucatu, SP, Brazil

HIGHLIGHTS:

The use of phytohormones and nicotinamide promotes growth and yield in maize crop when used together or alone. Phytohormones increase maize grain yield by 6.0%. Nicotinamide increases maize grain yield by 11.6%.

ABSTRACT: The use of biostimulants in second-crop maize (*Zea mays*) can promote higher physiological activity in plants, resulting in higher grain yield. This study aimed to evaluate the effects of phytohormones and nicotinamide on growth and yield traits of second-crop maize. The statistical design used was a randomized block design arranged in a factorial scheme with two doses of phytohormones (0 and 500 mL ha⁻¹) × five concentrations of nicotinamide (0, 200, 400, 600, and 800 mg L⁻¹) with four replications. The use of biostimulants favored all variables evaluated for the maize crop. The isolated use of phytohormones and nicotinamide promoted grain yields of 6089.6 and 6242.5 kg ha⁻¹, respectively, representing gains of 6.0 and 11.6%, respectively, compared with the control. The application of 800 mg L⁻¹ nicotinamide resulted in the highest maize grain yield. The use of phytohormones and nicotinamide, isolated or associated, was favorable for the growth characteristics and grain yield of second-crop maize. A synergistic effect was noted between phytohormones and nicotinamide on the first ear insertion height and number of rows per ear.

Key words: off-season maize, growth regulator, nicotinamide, phytohormones, Zea mays L.

RESUMO: O uso de bioestimulantes no milho segunda safra (*Zea mays*) pode promover maior atividade fisiológica das plantas, resultando em maior produtividade de grãos. Assim, este trabalho teve como objetivo avaliar as características de crescimento e produtividade do milho segunda safra sob aplicação de fitormônios e nicotinamida. O delineamento estatístico utilizado foi em blocos casualizados dispostos em esquema fatorial com 2 doses de fitohormônios (0 e 500 mL ha⁻¹) × 5 concentrações de nicotinamida (0, 200, 400, 600 e 800 mg L⁻¹) com quatro repetições. O uso dos dois bioestimulantes favoreceu todas as variáveis avaliadas para a cultura do milho. O uso isolado de fitormônios e nicotinamida promoveu produtividades de grãos de 6.089,6 e 6.242,5 kg ha⁻¹, representando respectivamente, ganhos de 6,0 e 11,6%, em relação à testemunha. A aplicação de 800 mg L⁻¹ de nicotinamida proporcionou maior produtividade de grãos de fitohormônios e nicotinamida, isolados ou associados, foram favoráveis às características de crescimento e produtividade do milho segunda safra. Houve efeito sinérgico de fitormônios e nicotinamida para altura de inserção da primeira espiga e o número de fileiras por espiga.

Palavras-chave: milho safrinha, regulador de crescimento, fitohormônios, nicotinamida, Zea mays L.

This is an open-access article distributed under the Creative Commons Attribution 4.0 International License.



INTRODUCTION

Maize (*Zea mays*) is one of the most important grains produced and consumed in Brazil and worldwide (Pinheiro et al., 2021), with a production of 113.1 million tons for the 2021/2022 harvest, of which 85.9 million tons resulted from the second harvest (CONAB, 2023). Using the latest large-scale technologies, the second harvest is no longer a subsistence crop. Therefore, in the search for sustainability, plant biostimulants can be used as growth promoters for crops (Panfili et al., 2019).

Biostimulants are defined as mixtures of natural or synthetic plant regulators, chemical compounds (vitamins and nutrients), algal extracts, microorganisms, and amino acids (Frasca et al., 2020; Araújo et al., 2021). These products can improve crop yield and quality (Colla et al., 2021; Vendruscolo & Lima, 2021).

Biostimulants containing auxin, cytokinin, and gibberellin may favor an increase in the absorption of water and nutrients by plants, thus allowing resistance to water stress, which second-crop maize is subject to, besides the less light availability. Vitamins such as nicotinamide can alleviate biotic and abiotic stresses by acting on electron transport and cellular and respiratory metabolism (Kirkland & Meyer-Ficca, 2018), indirectly assisting in vegetative growth and development through cell elongation, leading to an increase in yield traits, especially under adverse conditions, as it acts directly on plant tissues (Berglund et al., 2017).

Considering the potential of biostimulants, this study hypothesizes that using these products in second-crop maize can improve production components and yield. This study aimed to evaluate the growth and yield traits of secondcrop maize under the application of phytohormones and nicotinamide.

MATERIAL AND METHODS

The study was performed from 21/02/2020 to 08/08/2020 in an experimental area of the Universidade Federal de Mato

Grosso do Sul, Campus of Chapadão do Sul, MS, at latitude 18° 48' 459" S and longitude 52° 36' 003" W, and at an altitude of 820 m. According to Köppen's classification, the climate of the region is defined as humid tropical (Aw), where rain occurs during the summer and dry winter. The average annual temperature ranges from 13 to 28 °C, and the average annual rainfall is 1,850 mm (Cunha et al., 2013). The temperature and precipitation data obtained during the experimental period are shown in Figure 1.

The soil where the experiment was installed was classified as Oxisol (United States, 2014) which corresponds to that of Latossolo Vermelho distrófico in the Brazilian soil classification system (EMBRAPA, 2018). Prior to the installation of the experiment, sampling was performed in the 0-0.20 m layer, collecting 20 simple samples with the help of an auger, which were then homogenized to form the composite sample, whose chemical analysis results were as follows: pH (CaCl₂) = 4.6; K = 0.28; Ca = 2.40; Mg = 0.40; Al = 0.17; and H + Al = 5.00 (all in cmol_c dm⁻³); P (Mehlich) = 11.0; Fe = 37.0; Mn = 11.8; Zn = 7.2, and B = 0.26 (all in mg dm⁻³), V% (base saturation) = 43.0, and CEC (cation exchange capacity) = 7.1 cmol_c dm⁻³. The values for clay, sand, and silt were 44, 38, and 18 dag kg⁻¹, respectively.

The treatments consisted of two biostimulants, defined from now on as phytohormones and nicotinamide. The statistical design used was a randomized block design arranged in a factorial scheme with two doses of phytohormones (0 and 500 mL ha⁻¹) × five concentrations of nicotinamide (0, 200, 400, 600, and 800 mg L⁻¹, purity of 98.5%, Dinâmica) with four replicates. The phytohormones used was Stimulate^{*} (cytokinin 0.09 g L⁻¹ + gibberellic acid 0.05 g L⁻¹ + 4-indole-3-butyric acid 0.05 g L⁻¹), applied in the commercial dose (250-500 mL ha⁻¹).

To reach the nicotinamide concentrations, dilutions were prepared in distilled water without the addition of any product during application. The definition of nicotinamide concentration considered the extrapolation of values commonly found in the literature, which in most cases is



Source: INMET

Figure 1. Average maximum and minimum temperature, and rainfall in the experimental area of the Federal University of Mato Grosso do Sul, Campus Chapadão do Sul-MS, from 21/02/2020 to 05/08/2020

approximately 100 mg L⁻¹. Some studies have already expanded these values, as in the one reported by Abdelhamid et al. (2013), to 400 mg L⁻¹.

The treatments were applied via foliar application, in the morning, using an 8 L backpack sprayer with constant pressure and a spray volume of 200 L ha⁻¹, only at the V5 stage (beginning of ear formation). To avoid drift and contamination of nearby plots, a plastic sheet was stretched across the edges of the plots during application. To maintain the application pattern and standardize the amount of product on the plants, the procedure was performed by only one previously trained applicator.

The experimental plots consisted of five rows, each 5 m long, with a space of 0.45 m between them. Only three central rows were considered for observation. The hybrid used was FS450 PW, which is characterized by high yield potential, super-early grain health, long husk leaves, and stalk quality.

The experiment was performed under a no-tillage system, in succession with soybean crops. Soil correction was performed with dolomitic limestone with an ECCE of 90%, applying 2.1 t ha⁻¹ to raise the base saturation to 70% according to the soil analysis. Before sowing, the area was desiccated with glyphosate (1.0 kg a.e. ha⁻¹). Sowing was performed on February 21, 2020, using a seven-row seed drill. Three seeds per meter were used in this study. In the sowing furrow, 230 kg ha⁻¹ of MAP fertilizer (10% N and 46% P_2O_5) was applied. For topdressing fertilization, at the V4 stage, 80 kg of K₂O ha⁻¹ and 140 kg of N ha⁻¹ were applied using potassium chloride and urea as sources, respectively.

Weed control in maize was performed by glyphosate application at a dose of 1.0 kg a.e. ha^{-1} at the V4 and V7 stages. Regarding pests, only fall armyworm (*Spodoptera frugiperda*) attack was observed, which was controlled by the application of chlorfenapyr (120 g a.i. ha^{-1}), applied alone at the V4 stage, and together with methyl (126 g a.i. ha^{-1}) at the VT stage (tasseling). For diseases, epoxiconazole (50 g a.i. ha^{-1}) + fluxapyroxad (50 g a.i. ha^{-1}) + pyraclostrobin (81 g a.i. ha^{-1}) and picoxystrobin (90 g a.i. ha^{-1}) + cyproconazole (36 g a.i. ha^{-1}) were applied to control rust at the V7 stage.

At the VT stage (tasseling), ten plants were evaluated for plant height (PH), first-ear insertion height (EH), and stalk diameter (SD). To determine the PH, the distance from the base of the plant to the end of the tassel was measured, and EH was evaluated by measuring the distance from the base of the plant to the height of the first ear. A digital caliper was positioned above the adventitious roots to measure SD. Data of these evaluations were obtained from ten plants in the observation area of each plot.

Destructive analyses were performed in the tacking stage (60 days after seed emergence), and the leaf area (LA) of the maize crop was obtained by measuring the length and width of all leaves of three plants in the center of each leaf (Mondo et al., 2009). Leaf area data were determined according to Eq. 1.

$$LA = a \times (L \times W) \tag{1}$$

where:

a - 0.75 - rectangle area correction factor $L \times W$;

- L length of leave; and
- W width of leave.

In the tracking stage, leaf dry mass (LDM) and stalk dry mass (SDM) were evaluated. To obtain LDM, all leaves of the three plants used to determine the leaf area were cut and placed in a paper bag for drying in an oven at 65°C until constant mass; the same procedure was followed to obtain SDM.

The Falker Chlorophyll Index (FCI) was obtained using a Clorofilog^{*} chlorophyll meter with the plants in the silking stage (R1). Measurements were taken on five leaves of five different plants per plot using the insertion leaf of the last ear.

After maize maturation, all ears of the useful plot were harvested. Three ears were removed to determine the length (EL) and diameter of the ear (ED), measured using a digital caliper. The number of rows per ear (NR) and the number of grains per row (NGR) were also recorded. All remaining ears were threshed to determine the 1000-grain weight (1000 W) and grain yield (GY), with the grain mass adjusted to 13% moisture.

Before proceeding with the analysis of variance, the homogeneity of variance and normality of errors were verified using Cochran and Lilliefors tests (Possatto Junior et al., 2019), respectively. The data were subjected to analysis of variance with the means of the qualitative factors compared by the Tukey test at a probability of 0.05, and the means of the quantitative factors evaluated by the regression analysis using the Sisvar software (Ferreira, 2019). The correlation network and canonical variables were analyzed using the Rbio program (Bhering, 2017).

RESULTS AND DISCUSSION

The interaction between phytohormones and nicotinamide significantly influenced plant height, ear insertion height, ear diameter, and 1000-grain weight. The use of phytohormones and nicotinamide separately influenced all variables studied, except the Falker chlorophyll index and number of rows per ear, which were affected only by the application of nicotinamide (Table 1).

The isolated effect of the use of phytohormones resulted in gains of 1.8, 12.3, 4.5, 6.3, 1.9, 3.7, and 6.0% for the variables SD, LA, LDM, SDM, EL, NR, and GY, respectively, when compared to the absence of application of this biostimulant (Table 1).

The gibberellic acid in the phytohormones, which was applied in this experiment when in low concentrations, is capable of activating the production of the enzyme xyloglucan endotransglycolysade (EXE); this makes the cell wall more flexible and thus promotes the expansion of plant tissues (Taiz et al., 2017), indicating the possibility of participating in the increase in the diameter of the maize stalk and, consequently, in its dry mass (Table 1).

Auxins and cytokinins are responsible for leaf growth and expansion, with young leaves being active centers of auxin synthesis (Neumann et al., 2017). This fact, combined with the possible presence of EXE activated from the gibberellic acid present in the biostimulant, could provide greater leaf area and dry mass of leaves, thus explaining the results verified in this study (Table 1).

553

Table 1. Plant height (PH), ear insertion height (EH), stalk diameter (SD), leaf area (LA), leaf dry mass (LDM), stalk dry mass (SDM), Falker chlorophyll index (FCI), ear length (EL), ear diameter (ED), number of rows per ear (NR), number of beans per row (NGR), 1000-grain weight (1000 W), and yield (GY) of second-crop maize according to the application of phytohormones and nicotinamide

SV	DF	Mean squares							
		PH	EH	SD	LA	LDM	SDM	FCI	
Block	3	0.0010	0.0024	0.0533	54.5353	1.2538	5.8992	3.8341	
Phy	1	0.1514**	0.1763**	1.3838**	3188.5654**	23.1699**	163.7281**	4.7197 ^{ns}	
Nic	4	0.1181**	0.0205**	1.2745**	311.4942**	25.31300**	44.7579**	7.6050**	
Phy x Nic	4	0.0298**	0.0031**	0.0173 ^{ns}	7.7394 ^{ns}	0.2124 ^{ns}	1.3634 ^{ns}	0.0983 ^{ns}	
Residue	27	0.0026	0.0007	0.0776	14.0884	1.3578	5.6618	1.7476	
CV%		1.81	1.90	1.34	2.44	3.39	3.60	2.28	
		EL	ED	NR	NGR	1000 W	GY		
Block	3	0.0188	0.0062	1.223	0.9739	8.6572	27578.9		
Phy	1	0.6300*	0.0407**	3.3062**	2.6249 ^{ns}	1463.3741**	1190940.1**		
Nic	4	0.9127**	0.0521**	1.9000**	4.6733*	233.5134**	551359.8**		
Phy x Nic	4	0.0752 ^{ns}	0.0053**	0.3375 ^{ns}	0.1704 ^{ns}	58.1243**	14189.5 ^{ns}		
Residue	27	0.0906	0.0018	0.3896	1.1940	7.4740	13625.2		
CV%		2.29	0.96	3.92	3.59	1.16	1.97		
Phy		SD (mm)		LA (cm ²)		LDM (g)		M (g)	
Presence		21.05 a		162.70 a		35.10 a		68.12 a	
Absence		20.67 b		144.84 b		33.58 b		64.08 b	
		EL (cm)		NR	G	GY (kg ha⁻¹)			
Presence		13.30 a		16.20 a	(5089.60 a			
Absence		13.05 b		15.62 b	Ę	5744.50 b			

^{ns} - Not significant (p > 0.05); * - Significant at $p \le 0.05$; ** - Significant at $p \le 0.01$; SV - Source of variation; DF - Degree of freedom; Phy - Phytohormones; Nic - Nicotinamide; CV - Coefficient of variation. Means followed by the same lowercase letter in the column do not indicate a significant difference between them by Tukey's test at $p \le 0.05$

For EL, NR, and GY, the higher values obtained using phytohormones were probably due to the positive effects on root growth and photosynthesis. Indolebutyric acid, present at low concentrations in this biostimulant, stimulates the growth of the root system (Taiz et al., 2017; Lima et al., 2018) proven by positive results in the growth of the root system of other species, as observed in studies by Azevedo et al. (2021) and Singh et al. (2021). The cytokinins present in the phytohormones also act on the root development of plants by regulating cell division, increasing the size of the apical meristem of the root and playing an important role in the proliferation of the initial cells and vascularization of the root (Taiz et al., 2017).

Therefore, the use of phytohormones, owing to the presence of cytokinin in their composition, can indirectly promote increased leaf area (Neumann et al., 2017) and root growth (Taiz et al., 2017; Lima et al., 2018), thereby intensifying photosynthesis and nutrient absorption from the soil, mitigating the adverse effects of abiotic stresses, and increasing the values of production components and grain yield (Yakhin et al., 2017). Lima et al. (2020) achieved higher yields of sweet maize ears using the same biostimulant.

PH and EH (Figures 2A and B) showed the greatest increase in their means with increasing concentrations of nicotinamide in the presence of phytohormones. The gain in plant height and first ear insertion height was 17.0 and 12.3%, respectively, with a concentration of 800 mg L⁻¹ compared to that in the absence of this biostimulant. For PH, only from the concentration of 400 mg L⁻¹ of nicotinamide, there was an effect of the use of phytohormones, whereas for EH, the values were always higher associated with the use of phytohormones, regardless of the nicotinamide concentration.

For the SD and LA variables (Figures 2C and D), the greatest gains were obtained with the highest concentration of

nicotinamide, reaching 4.9 and 10.8%, respectively, compared to that in the absence of this vitamin.

Nicotinamide acts on the activity of the photosynthetic system, increasing reserve energy (Abdelhamid et al., 2013), which is essential for the development of new organs such as leaves, stems, and roots (Berglund et al., 2017); this probably favored the highest values for PH, EH, and SD (Figures 2A, B, and C). The use of this vitamin can also promote the accumulation of carbohydrates in the leaves, which favors their expansion (Taiz et al., 2017) in addition to promoting an increase in chlorophyll and carotenoids (El-Bassiouny et al., 2014), which would explain the greater leaf area found in this study (Figure 2D). Colla et al. (2021) had also achieved greater leaf area in maize with the use of nicotinamide.

The variables LDM, SDM, and FCI (Figures 3A, B, and C) had an increase in their averages of 13.8, 9.5, and 4.3%, respectively, with the use of the highest concentration of nicotinamide, when compared to the absence of this vitamin. In contrast, the variable EL (Figure 3D) reached the highest value, 13.43 cm, with a concentration of 360.6 mL L^{-1} , representing a gain of 4.0% compared to the absence of this vitamin.

The higher leaf and stalk dry mass observed using nicotinamide (Figures 3A and B) was possibly due to the greater plant height, stalk diameter, and leaf area (Figures 2A, B, and C) obtained with the use of this vitamin. The higher FCI value obtained using nicotinamide (Figure 3C) is probably because this vitamin increases photosynthetic pigments in the leaves (El-Bassiouny et al., 2014). Vendruscolo & Seleguini (2020) has proved this fact by finding an increase in the Falker chlorophyll index in maize with the application of this vitamin. As nicotinamide acts on the photosynthetic system, resulting in greater photosynthesis, it indirectly stimulates the production of chlorophyll, which is necessary for the functioning of the entire complex (Abdelhamid et al., 2013).



** - Significant at $p \le 0.01$ by F-test; W/PHY - phytohormones presence; and WO/PHY - phytohormone absence. Means followed by different letters at the same nicotinamide concentration indicate significant differences by the F test ($p \le 0.05$)

Figure 2. Plant height (A) and ear insertion height (B) under different nicotinamide concentration and phytohormones; and stalk diameter (C) and leaf area (D) according to the nicotinamide application in second-crop maize



^{** -} Significant at $p \le 0.01$ by F-test

Figure 3. Dry mass of leaves (A), dry mass of stalk (B), Falker chlorophyll index (C), and ear length (D) as a function of nicotinamide application in second-crop maize

The ED (Figure 4A) and 1000 W (Figure 4B) variables had their highest mean values obtained in the presence of phytohormones, reaching 4.7 mm and 248.7 g, with concentrations of 489.3 and 789.2 mg L⁻¹ of nicotinamide, respectively. These values represent a gain of 2.5 and 7.5% compared with that in the absence of nicotinamide use, respectively, for ED and 1000 W. ED was favored by the presence of phytohormones only in the absence of nicotinamide and at concentrations of 200 and 600 mg L⁻¹, whereas 1000 W showed the highest values in the presence of phytohormones, regardless of nicotinamide concentration.

For NR (Figure 4C) and NGR (Figure 4D), the highest values (16.3 and 31.0) were obtained with concentrations of 527.9 and 394.8 mg L^{-1} of nicotinamide, representing a gain of 7.3 and 4.7%, without the use of this vitamin, respectively.

B-complex vitamins can regulate carbon metabolism and protein synthesis, increasing the transport of photoassimilates during grain filling (Kaya et al., 2015) in addition to harmonizing these biochemical processes due to the uptake of nutrients such as Na and K, which are essential for regulating cell activities (Goyer, 2010). This could explain the higher values obtained for NR, NGR, and 1000 W using nicotinamide. Furthermore, this vitamin stimulates the accumulation of nitrogenous constituents by acting directly on the chloroplast and increasing its mass during physiological maturation (Bassuony et al., 2008). The higher values of EL (Figure 3D) and ED (Figure 4A) may eb attributed to the increase in grain production per ear, which requires a larger support structure.

А. B. 4.7 255 W/PHY a W/PHY ²⁵⁰ 3245 245 240 4.6 а Ear diameter (cm) а 4.5 ug 235 b a 4.4 b b b h 4.3 Thousand 225 h =4.377142+0.000685**x - 0.0000007**x2 $R^2 = 0.5990$ $\hat{\mathbf{y}}_{(W/PHY)} = 231.244286 + 0.044195^{**}x - 0.000028^{**}x^2$ $R^2 = 0.9080$ 220 4.2 $(OPHY) = 4.287929 + 0.000704^{**}x - 0.000001^{**}x^2$ $R^2 = 0.9334$ WO(PHY) = 224.939857 + 0.036361**x - 0.000039**x² $R^2 = 0.7807$ 215 4.1 210 0 200 400 600 800 200 400 600 800 0 Nicotinamide concentration (mg L-1) Nicotinamide concentration (mg L-1) C. D. 17.0 32.0 31.5 16.5 Number of grains per row 31.0 Number of rows 16.0 30.5 P 15.5 30.0 15.0 = 29.631215 + 0.007107**x - 0.000009 R2=0 7174 29.5 = 15.133929 + 0.004223**x - 0.000004*x² R² = 0.9333 14.5 29.0 14.0 28.5 200 0 400 600 800 0 200 400 600 800 Nicotinamide concentration (mg L-1) Nicotinamide concentration (mg L-1)

The highest maize grain yield was achieved with the highest concentration of nicotinamide, reaching 6242.5 kg ha⁻¹, representing a gain of 11.6% compared with that in the absence of this vitamin (Figure 5).

To achieve high yields, plants must maintain a high energy level. Thus, the application of nicotinamide can reduce energy homeostasis by providing a precursor of NAD⁺ (Berglund et al., 2017). In addition, group B vitamins can function as antioxidants, promote singlet oxygen extinction, and reduce stress factors (Zhang et al., 2020). As a result, the increase in the production of carbohydrates and the accumulation of energy reserves contributed to the increase in yield, as





* or ** - Significant at $p \le 0.05$ and ≤ 0.01 by F-test, respectively; W/PHY - phytohormones presence; and WO/PHY - phytohormone absence. Means followed by different letters at the same nicotinamide concentration indicate significant differences by the F test ($p \le 0.05$) **Figure 4.** Ear diameter (A) and 1000-grain weight (B) under different nicotinamide concentration and phytohormones; and number of rows per ear (C) and number of grains per row (D) as a function of nicotinamide application in second-crop maize verified for maize in this study (Figure 4). Colla et al. (2021) also achieved higher maize productivity after nicotinamide treatment.

In the correlation network generated by the Pearson matrix, the correlation magnitude was proportional to the thickness of the lines. In this study, most of the correlations between the analyzed variables were positive (Figure 6). It is possible to observe the formation of a cohesive group with a strong positive correlation between EH, LA, FCI, SD, SDM, LDM, 1000 W, PH, GY, NR, and ED. The correlation network allows the visualization and identification of pairs of variables that present strong correlations and determines which groups of variables most significantly influence the traits. Apparently, grain yield (GY) had positive correlations with all variables analyzed, meaning that all variables contributed to the increase in grain yield.

The variable NGR was the most distant, having a weak correlation with most variables, except for ED and EL, with which it had a mean positive correlation, indicating that ears with a greater number of grains per row tend to have greater length and diameter of the ear.

The magnitude of the correlation was proportional to the thickness of the lines. The highlighted lines have correlation coefficients greater than 0.6 (Figure 6).

Canonical variables were analyzed to verify the association between treatments and variables (Figure 7). The accumulated variance in the first two canonical variables was 92.4%, giving credibility to the representation in a two-dimensional graph, because the percentages of accumulated variance in the first two canonical variables were greater than 80%.



Figure 6. Network of phenotypic correlations between the variables plant height (PH), ear insertion height (EH), stalk diameter (SD), leaf area (LA), leaf dry mass (LDM), stalk dry mass (SDM), Falker chlorophyll index (FCI), ear length (EL), ear diameter (ED), number of rows per ear (NR), number of grains per row (NGR), 1000-grain weight (1000W), and grain yield (GY) according to the phytohormones and nicotinamide application in second-crop maize



Green lines represent positive corrections, and red lines represent negative correlations. A and P - Absence and presence of phytohormones; 0, 200, 400, 600, and 800 - nicotinamide concentrations in mg L^{-1}

Figure 7. Analysis of canonical variables for plant height (PH), ear insertion height (EH), stalk diameter (SD), leaf area (LA), leaf dry mass (LDM), stalk dry mass (SDM), Falker chlorophyll index (FCI), ear length (EL), ear diameter (ED), number of rows per ear (NR), number of grains per row (NGR), 1000-grain weight (1000W), and grain yield (GY) according to the application of phytohormones and nicotinamide in second-crop maize

Treatments P0, A0, A200, A400, A600, and A800 (presence of biostimulant + absence of nicotinamide and absence of biostimulant in the five concentrations of nicotinamide) did not contribute to any variable analyzed.

The results of the canonical analysis indicated that none of the treatments that received phytohormones or associated with a low concentration of nicotinamide influenced any variable. The presence of the phytohormone with a concentration of 400 and 600 mg L^{-1} of nicotinamide stands out for the NR vector and at a dosage of 800 mg L^{-1} of nicotinamide for the EH vector, indicating that when the phytohormone is used in combination with higher concentrations of nicotinamide; the height of insertion of the first ear and the number of rows per ear tend to be greater, indicating a possible synergistic effect between the two biostimulants.

The positive effects of auxin, cytokinin, and gibberellic acid, present in phytohormones, on plant tissue expansion, cell division, early cell proliferation, and increased photosynthesis (Neumann et al., 2017; Taiz et al., 2017; Yakhin et al., 2017), could be maximized when combined with nicotinamide. This vitamin participates in the activation of the photosynthetic system and accumulation of energy reserves, resulting in new structures in the plant (Abdelhamid et al., 2013; Berglund et al., 2017). The physiological actions of these two biostimulants may explain their effects on NR and EH (Figure 7).

For the other variables (Figure 7), the action of nicotinamide, regardless of the phytohormones, was sufficient to reach the highest values. As it is considered a growth-regulating substance, nicotinamide can cause physiological changes in plants, such as the biosynthesis of enzymes, nucleic acids, and proteins, which can result in greater plant growth and productivity (Bassuony et al., 2008; Goyer, 2010; Kaya et al, 2015).

CONCLUSIONS

1. The use of phytohormones and nicotinamide, isolated or associated, were favorable for growth characteristics and grain yield of second-crop maize.

2. A synergistic effect of phytohormones and nicotinamide was noted, for first ear insertion height and number of rows per ear.

ACKNOWLEDGEMENTS

Support from the Universidade Federal de Mato Grosso do Sul is acknowledged. This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brazil (CAPES) - Finance Code 001.

LITERATURE CITED

- Abdelhamid, M. A.; Sadak mervat, S. H.; Schmidhalter, U.; El-saady, A. M. Interactive effects of salinity stress and nicotinamide on physiological and biochemical parameters of faba bean plant. Acta Biológica Colombiana, v.18, p.499-510, 2013.
- Araújo, L. L. M. de; Ramos, D.; Brachtvogel, E.; Kovalski, A. Ação de bioestimulantes em cultivares comerciais de soja na região Norte do Vale Araguaia - MT. Revista Pesquisagro, v.4, p.3-21, 2021. https://doi.org/10.33912/pagro.v4i1.1146
- Azevedo, M. L. de; Titon, M.; Machado, E. L. M.; Assis Júnior, S. L. de; Freitas, E. C. S. de. Influência do ácido indolbutírico no enraizamento de miniestacas caulinar e foliar de mogno-africano (*Khaya grandifoliola* C. DC.). Ciência Florestal, v.31, p.898-919, 2021. https://doi.org/10.5902/1980509837225
- Bassuony, F. M.; Hassanein, R. A.; Baraka, D. M.; Khalil, R. R. Physiological effects of nicotinamide and ascorbic acid on *Zea* mays plant grown under salinity stress. II-Changes in nitrogen constituents, protein profiles, protease enzyme and certain inorganic cations. Australian Journal of Basic and Applied Sciences, v.2, p.350-359, 2008.
- Bhering, L. L. Rbio: A tool for biometric and statistical analysis using the R platform. Crop Breeding and Applied Biotechnology, v.17, p.187-190, 2017. <u>https://doi.org/10.1590/1984-70332017v17n2s29</u>
- Berglund, T.; Wallstrom, A.; Nguyen, T. V.; Laurell, C.; Ohlsson, A. B. Nicotinamide; antioxidative and DNA hypomethylation effects in plant cells. Plant Physiology Biochemistry, v.118, p.551-560, 2017. https://doi.org/10.1016/j.plaphy.2017.07.023
- Colla, R. L. da S.; Lima, S. F. de; Vendruscolo, E. P.; Secco, V. A.; Piati, G. L.; Santos, O. F. dos; Abreu, M. S. Does foliar nicotinamide application affect second-crop corn (*Zea mays*)? Revista de la Facultad de Ciencias Agrarias - UNCuyo, v.53, p.64-70, 2021. https://doi.org/10.48162/rev.39.040
- CONAB Companhia Nacional de Abastecimento. Acompanhamento da safra brasileira de grãos, Brasília: CONAB, 2023. 83p.

- Cunha, F. F. da; Magalhães, F. F.; Castro, M. A. de. Métodos para estimativa da evapotranspiração de referência para Chapadão do Sul-MS. Revista Engenharia na Agricultura, v.21, p.159-172. 2013. <u>https://doi.org/10.13083/reveng.v21i2.346</u>
- El-Bassiouny, H. S. M.; Bakry, B. A.; Attia, A. A. E. M.; Allah, M. M. A. Physiological role of humic acid and nicotinamide on improving plant growth, yield, and mineral nutrient of wheat (*Triticum durum*) grown under newly reclaimed sandy soil. Agricultural Sciences, v.5, p.687-700, 2014. <u>https://doi. org/10.4236/as.2014.58072</u>
- EMBRAPA Empresa Brasileira de Pesquisa Agropecuária. Sistema brasileiro de classificação de solos. 5.ed. Rio de Janeiro: Embrapa, 2018, 356p.
- Ferreira, D. F. Sisvar: a computer analysis system to fixed effects split plot type designs: Sisvar. Brazilian Journal of Biometrics, v.37, p.529-535, 2019. <u>https://doi.org/10.28951/rbb.v37i4.450</u>
- Frasca, L. L. de M.; Nascente, A. S.; Lanna, A. C.; Carvalho, M. C. S.; Costa, G. G. Bioestimulantes no crescimento vegetal e desempenho agronômico do feijão comum de ciclo superprecoce. Revista Agrarian, v.13, p.27-41, 2020. <u>https://doi.org/10.30612/agrarian.v13i47.8571</u>
- Goyer, A. Thiamine in plants: aspects of its metabolism and functions. Phytochemistry, v.71, p.1615-1624. 2010. <u>https://doi.org/10.1016/j.phytochem.2010.06.022</u>
- Kaya, C.; Ashraf, M.; Sonmez, O.; Tuna, A. L.; Polat, T.; Aydemir, S. Exogenous application of thiamin promotes growth and antioxidative defense system at initial phases of development in salt-stressed plants of two maize cultivars differing in salinity tolerance. Acta Physiologiae Plantarum, v.37, p.1741-1753. 2015. https://doi.org/10.1007/s11738-014-1741-3
- Kirkland, J. B.; Meyer-Ficca, M. L. Niacin. Advances in Food and Nutrition Research. v.83, p.83-149, 2018. <u>https://doi.org/10.1016/ bs.afnr.2017.11.003</u>
- Lima, C. C.; Ohashi, T. S.; Silveira, A. S. Efeito de diferentes concentrações de AIB e procedências geográficas no enraizamento de estacas de Paricá. Ciência Florestal, v.28, p.1282-1292, 2018. <u>https://doi.org/10.5902/1980509833380</u>
- Lima, S. F.; Jesus, A. A.; Vendruscolo, E. P.; Oliveira, T. R.; Andrade, M. G. O.; Simon, C. A. Development and production of sweet corn applied with biostimulant as seed treatment. Horticultura Brasileira v.38, p.94-100, 2020. <u>https://doi.org/10.1590/S0102-053620200115</u>
- Mondo, V. H. V; Carvalho, S. J. P; Labonia, V. D. S.; Dourado Neto, D.; Cicero, S. M. Comparação de métodos para estimativa de área foliar em plantas de milho. Revista Brasileira de Milho e Sorgo. v.8, p.233-246, 2009. <u>https://doi.org/10.18512/1980-6477/rbms. v8n3p233-246</u>
- Neumann, E. R.; Resende, J. T. V.; Camargo, L. K. P.; Chagas, R. R.; Lima Filho, R. B. Produção de mudas de batata doce em ambiente protegido com aplicação de extrato de Ascophyllum nodosum. Horticultura Brasileira, v.35, p.490-498, 2017. <u>https://doi.org/10.1590/S0102-053620170404</u>
- Panfili, I.; Bartucca, M. L.; Marrolo, G.; Povero, G.; Buono, D. D. Application of a plant biostimulant to improve maize (*Zea mays*) tolerance to Merolachlor. Journal of Agricultural Food Chemistry, v.67 p.12164-12171, 2019. <u>https://doi.org/10.1021/acs.jafc.9b04949</u>

- Pinheiro, L. da S.; Gatti, V. C. do M.; Oliveira, J. T. de; Silva, J. N. da; Silva, V. F. A.; Silva, P. A. Características agro econômicas do milho: uma revisão. Natural Resources, v.11, p.13-21, 2021. <u>http:// doi.org/10.6008/CBPC2237-9290.2021.002.0003</u>
- Possatto Junior, O.; Bertgna, F. A. B.; Peterlini, E.; Baleroni, A. G.; Rossi, R. M.; Zeni Neto, H. Survey of statistical methods applied in articles published in Acta Scientiarum. Agronomy from 1998 to 2016. Acta Scientiarum. Agronomy, v.41, p.e42641, 2019. http://doi.org/10.4025/actasciagron.v41i.42641
- Singh, B.; Rawat, J. S.; Gusain, Y. S.; Khanduri, V. P.; Riyal, M. K.; Kumar, P. Shoot position, cutting types and auxin treatments influence rooting response on *Tecoma stans*. Ornamental Horticulture, v.27, p.213-220, 2021. <u>https://doi.org/10.1590/2447-536X.v27i2.2262</u>
- Taiz, L.; Zeiger, E.; Møller, I. M.; Murphy, A. Fisiologia e desenvolvimento vegetal. Porto Alegre: Artmed, 2017.
- United States. Soil Survey Staff. Keys to soil taxonomy (12th ed.) Washington: USDA NRCS. 2014. Available on: <<u>http://www.nrcs.usda.gov/wps/portal/nrcs/main/soils/survey/</u>>. Accessed on: Jan. 2023.

- Yakhin, O. I.; Lubyanov, A. A.; Yakhin, I. A.; Brown, P. H. Biostimulants in plant science: A global perspective. Frontiers in Plant Science, v.7, p.1-32, 2017. <u>https://doi.org/10.3389/ fpls.2016.02049</u>
- Vendruscolo, E. P.; Lima, S. F. de. The Azospirillum genus and the cultivation of vegetables. A review. Biotechnology, Agronomy and Society and Environment, v.24, p.236-246, 2021. <u>https:// doi.org/10.25518/1780-4507.19175</u>
- Vendruscolo, E. P.; Seleguini, A. Effects of vitamin pré-sowing treatment on sweet maize seedlings irrigated with saline water. Acta Agronômica, v.69, p.20-25, 2020. <u>https://doi.org/10.15446/ acag.v69n1.67528</u>
- Zhang, H.; Xiang, Y.; He, N; Liu, X.; Liu, H.; Fang, L.; Zhang, F.; Sun, X.; Zhang, D.; Li, X.; Terzaghi, W.; Yan, J.; Dai, M. Enhanced vitamin C production mediated by an ABA-Induced PTP-like nucleotidase improves plant drought tolerance in arabidopsis and maize. Molecular Plant, v.5, p.760-776. 2020. <u>https://doi. org/10.1016/j.molp.2020.02.005</u>