

Original Article

Vitamin application affects gas exchange, growth, and yield of soybean plants

Aplicação de vitaminas afeta a troca gasosa, o crescimento e a produtividade de plantas de soja

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Abstract

The application of biostimulants in agriculture has been used to increase crop yield. This study evaluated the effects of exogenous application of thiamine and nicotinamide on soybean plants. The experiment was conducted in Cassilândia, MS. The randomized blocks design with 5 treatments and 8 replications was used. The treatments consisted of concentrations of nicotinamide and thiamine at 0, 50, and 100 mg L⁻¹ of water, applied exogenously when the plants were at the V3 stage. Gas exchange, number of grains per pod, number of pods, and grain yield were assessed. Plant height was increased by applying vitamins, with all treatments outperforming the control. The application of nicotinamide or thiamine at doses between 50 and 100 mg L⁻¹ favors the development and grain yield of soybean plants, making it possible to use them as a biostimulant.

Keywords: *Glycine max*, agricultural sustainability, vitamin B.

Resumo

A aplicação de bioestimulantes na agricultura tem sido empregado para aumentar a produtividade agrícola. Assim, este estudo teve como objetivo avaliar os efeitos da aplicação exógena de tiamina e nicotinamida em plantas de soja. O experimento foi conduzido em Cassilândia-MS. O delineamento utilizado foi de blocos casualizados, com 5 tratamentos e 8 repetições. Os tratamentos foram compostos pelas concentrações de nicotinamida e tiamina nas doses de 0, 50, 100 mg L⁻¹ de água, aplicadas quando as plantas estavam no estágio V3 de forma exógena. Foi avaliado as trocas gasosas, número de grãos por vagem, número de vagens e produtividade. A altura de plantas foi incrementada pela aplicação das vitaminas, sendo que todos os tratamentos superaram o controle. A aplicação de nicotinamida ou tiamina favorece o desenvolvimento e a produtividade das plantas de soja nas doses entre 50 e 100 mg L⁻¹, possibilitando a sua utilização em caráter bioestimulante.

Palavras-chave: *Glycine max*, sustentabilidade agrícola, vitamina B.

1. Introduction

Soybeans (*Glycine max* (L.) Merrill) are one of the world's main agricultural commodities grown in approximately 60 countries. This crop is planted in 46.02 million hectares in Brazil and is the main export product (CONAB, 2024). It is the most economically important oilseed in the world due to the high oil and protein content in the seed, with a balanced amino acid composition, and is also an important source of food in animal and human nutrition (Alaswad et al., 2021). Although Brazil has suitable soil and climate conditions for high yields, soybeans have been affected by climate change, resulting in reduced yield and profit margins (Souza Batista et al., 2023).

Abiotic and biotic adversities, such as salinity, drought, extreme temperatures, nutrient deficiencies, diseases, and pest attacks, have been exacerbated globally by the complex interaction of changing climatic conditions (Chaudhry and Sidhu, 2022). In addition, dependence on factors of high production and the use of chemicals has further damaged ecosystems and soil microbiota, necessitating sustainable measures for agricultural development (Yang et al., 2020).

Biostimulants can mitigate these adverse conditions on soybean crops, an effective strategy for improving plant adaptability and resistance to abiotic stresses. These products can reduce the use of fertilizers and pesticides, minimizing the impact of agricultural activities on the

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environment (Cuadrado et al., 2019; Del Buono, 2021). Biostimulant products include B vitamins, nicotinamide (vitamin B3), and thiamine (vitamin B1).

B complex vitamins have a growth promoting function but are still little exploited commercially (Lima et al., 2023). Among these, nicotinamide is considered a growth regulating substance that, when applied in small quantities, can cause physiological changes in the plant, such as the biosynthesis of enzymes, nucleic acids, and proteins (Dawood et al., 2019). It also contributes indirectly to plant growth by transporting energy in the plant cell (Dong et. al., 2015).

Some research has linked the exogenous use of nicotinamide in foliar application with plant growth and yield characteristics in soybean crops (Lima et al., 2024a). The effects of nicotinamide are observed in vegetative growth through cell expansion, accumulation of reserves, and increased yield of crops and their components (Lima et al., 2024b), and this can occur in different growing environments, some of which are adverse, such as water deficit and soil salinity (Colla et al., 2021; Abdelhamid et al., 2013; El Bassiouny et al., 2014).

Thiamine is another growth biostimulant vitamin cofactor in many plant physiological mechanisms (Colinas and Fitzpatrick, 2015). It acts in the antioxidant system to alleviate reactive oxygen species (ROS) (Subki et al., 2018). Respiration and enzyme activity are also related to thiamine levels (Fitzpatrick and Chapman, 2020). Exogenous application of thiamine increased stress tolerance by activating stress responsive genes and calcium signal transduction (Li et al., 2022). In addition, studies have reported that the use of thiamine increases the content of amino acids, total free amino acids, proline, soluble sugars, photosynthesis, polypeptides, polyenol oxidase isoenzymes, peroxidase, growth, and yield components in plants under water stress (El-Bassiouny et al., 2023). Given the benefits pointed out by previous studies and under

the hypothesis that vitamins can benefit crops, the study aimed to evaluate the effects of exogenous application of thiamine and nicotinamide on soybean plants.

2. Material and Methods

The experiment was conducted under a protected environment (greenhouse) with dimensions of 18.0 x 8.0 x 4.0 m (144 m²), covered with light diffusing low-density polyethylene (LDPE) film, with a zenithal opening sealed with a 30% white screen, with a 30% black side and front shading screen closed at 90° and a movable aluminized thermo reflective screen (LuxiNet®) under the LDPE film. The protected environment, belonging to the Mato Grosso do Sul State University, in Cassilândia, Mato Grosso do Sul, Brazil, 19°06'48"S, 51°44'03"W, and 510 m of altitude. During the experiment, rainfall and air temperature data were obtained at the experiment site (Figure 1).

The experimental design was a randomized block design with 5 treatments and 8 replicates. The treatments tested were: T1 = control; T2 = 50 mg nicotinamide L⁻¹ water; T3 = 50 mg thiamine L⁻¹ water; T4 = 100 mg nicotinamide L⁻¹ water; T5 = 100 mg thiamine L⁻¹ water. Each experimental plot consisted of an 8 L black polyethylene pot filled with soil containing three soybean plants.

Sowing of the soybean cultivar "DM 75174 RSF IPRO" was carried out in previously corrected soil at a depth of 3 cm. Ten seeds were sown per pot in the second half of October 2021, and thinning was carried out after the cotyledon leaves had emerged, leaving two plants per pot. Potassium fertilizer was applied at a dose equivalent to 80 kg ha⁻¹, divided into 50% before sowing and 30 days after plant emergence. To control diseases and pests, 20 days after sowing, fungicide Mancozeb was applied at a dose of 1.5 kg i.a. ha⁻¹ and insecticide Methoxyfenozide at a dose of 21.6 g i.a. ha⁻¹. Azoxystrobin + Benzovindiflupyr was

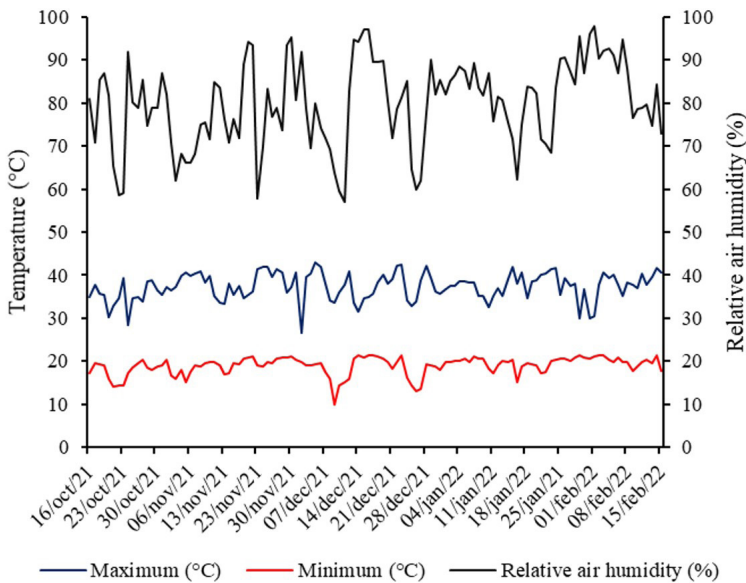


Figure 1. Maximum and minimum temperatures and rainfall during the experimental period.

applied 40 days after sowing at a 75.0 + 37.5 g a.i dose. ha⁻¹. The treatments were applied when the plants reached the V3 stage using a hand pump with a flow rate of 10 mL s⁻¹.

When flowering began (50% of the plants with at least one flower open), the characteristics of net photosynthesis (*A*), stomatal conductance (*gS*), intracellular CO₂ concentration (*Ci*), and transpiration (*E*) were assessed in the morning, when the plants were in full gas exchange activity. The instantaneous carboxylation efficiency (*EICI*) was calculated using the *A/Ci* ratio and the water use efficiency (*WUE*) using the *A/E* ratio. The relative chlorophyll index was also measured using a digital meter, and the number of leaves was counted.

At the end of the cycle, when the soya plants were at phenological stage R8, they were harvested and assessed for height, stem diameter, number of branches, number of pods, number of seeds per pod, and mass of seeds per plant. To do this, a graduated ruler was used to measure height, a digital caliper for stem diameter, and a semi analytical scale to obtain the mass of seeds per plant.

The data was subjected to analysis of variance (ANOVA), and the means were compared using the t test (LSD) at the 5% probability level. The analyses were conducted using SISVAR statistical software (Ferreira, 2019). Pearson correlation analysis was also carried out using Microsoft Excel.

3. Results

The exogenous application of vitamins influenced all the agronomic characteristics except the relative chlorophyll index (Table 1).

There was a decrease in intracellular CO₂ content (*Ci*) with the application of vitamin solutions containing thiamine at 50 and 100 mg L⁻¹ and nicotinamide at 100 mg L⁻¹ (Figure 2A). For the characteristics of stomatal

conductance (*gS*), transpiration (*E*), and net photosynthesis (*A*), it was observed that the application of treatments containing nicotinamide at 50 and 100 mg L⁻¹, and thiamine at 50 mg L⁻¹, resulted in significant increases in the variables (Figure 2B, 2C, 2D).

There was a significant increase in water use efficiency (Figure 3A) and carboxylation efficiency (Figure 3B) when the treatments containing nicotinamide at 50 and 100 mg L⁻¹ and thiamine at 50 mg L⁻¹ were applied. In terms of carboxylation efficiency, the treatment containing 100 mg L⁻¹ of thiamine was also found to be superior when compared to the control, but this same treatment obtained a lower result than the other vitamin solutions.

No significant differences were observed between the treatments for relative chlorophyll content (Figure 4A). However, for the number of leaves, the treatment composed of thiamine at 50 mg L⁻¹ was superior, followed by the treatment composed of nicotinamide at a concentration of 100 mg L⁻¹, which differed significantly from the control treatment (Figure 4B).

Plant height was increased by applying vitamins, with all treatments outperforming the control (Figure 5A). The treatments with nicotinamide at 50 and 100 mg L⁻¹ and thiamine at 50 mg L⁻¹ were superior in terms of stem diameter and number of branches, while the treatments with nicotinamide at 100 mg L⁻¹ and thiamine at 50 and 100 mg L⁻¹ were superior in terms of height of insertion of the first pod.

All the vitamin treatments increased the characteristics of the number of pods, number of grains, and grain yield (Figure 6). However, for grain yield, nicotinamide treatment at 100 mg L⁻¹ was superior, with no significant difference between the treatments with nicotinamide at 50 mg L⁻¹ and thiamine at 100 mg L⁻¹ (Figure 6C).

Pearson correlation analysis showed a negative correlation between the *Ci* trait, *WUE*, *EICI*, and the number of grains. It was also found that the physiological

Table 1. Summary of the analysis of variance for internal CO₂ concentration, transpiration rate, stomatal conductance, net photosynthetic rate, water use efficiency, carboxylation efficiency, relative chlorophyll index, number of leaves, plant height, stem diameter, number of branches, first pod insertion, number of pods, number of grains and grain weight of soybean plants grown under vitamin application.

DF	Mean square							
	Ci	E	gS	A	WUE	EICI	RCI	NL
7	691.52	0.24	0	1.3	0.1	0.00005	7.29	1.56
4	5216.18*	3.79*	0.0085*	46.36*	1.44*	0.001*	16.12 ^{ns}	14.59*
28	1083.88	0.3	0.0008	2.38	0.27	0.00007	8.82	3.2
CV%	14.37	25.82	42.9	27.14	19.74	31.71	6.8	9.39
DF	PH	SD	NB	FPI	NP	NG	GW	
5	8.75	0.05	0.32	2.61	92.75	359.97	6.83	
4	53.48*	0.28*	0.60**	9.37*	519.83*	1927.92**	34.58*	
20	11.64	0.05	0.17	1.64	71.15	599.36	5.58	
CV%	4.98	3.92	12.73	6.61	10.39	12.86	11.16	

DF = degrees of freedom; CV = coefficient of variation; Ci = intracellular CO₂; E = transpiration; gS = stomatal conductance; A = net photosynthesis; WUE = water use efficiency; EICI = carboxylation efficiency; RCI = relative chlorophyll index; NL = number of leaves; NP = number of pods; NG = number of grains; PH = plant height; SD = stem diameter; FPI = first pod insertion; NB = number of branches; GW = grain weight. *, ** and ns – Significant at $p \leq 0.05$, 0.01 and, not significant by t-test, respectively.

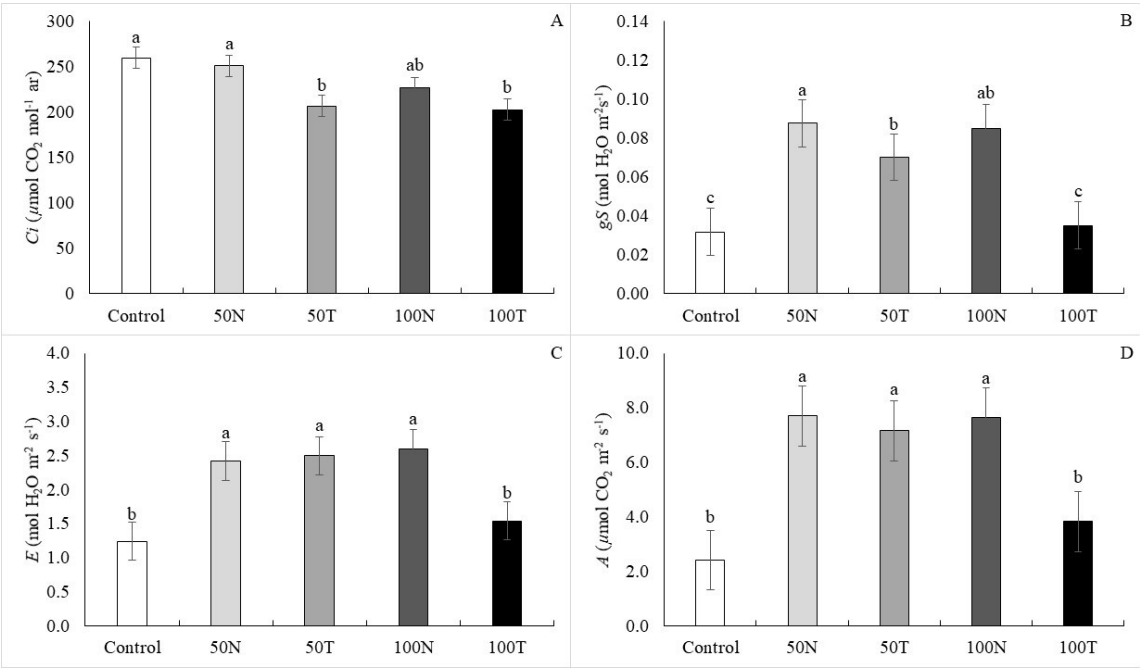


Figure 2. Internal CO₂ concentration (A), stomatal conductance (B), transpiration (C), and net photosynthesis (D) of soybean plants treated with vitamin solutions. Different letters on the bars indicate a significant difference between the means (P < 0.05). The bars represent the mean values (n = 8). 50N = 50 mg nicotinamide L-1 water; 50T = 50 mg thiamine L-1 water; 100N = 100 mg nicotinamide L-1 water; 100T = 100 mg thiamine L-1 water.

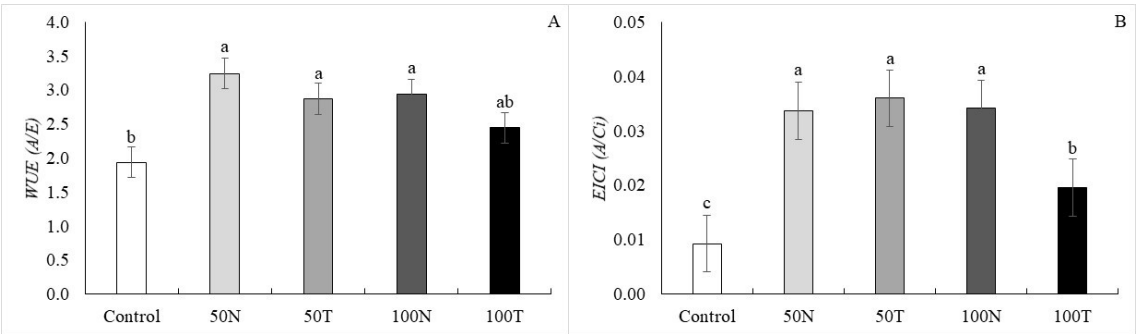


Figure 3. Water use efficiency (A) and intrinsic carboxylation efficiency (B) of soybean plants treated with vitamin solutions. Different letters on the bars indicate a significant difference between the means (P < 0.05). The bars represent the mean values (n = 8). 50N = 50 mg nicotinamide L-1 water; 50T = 50 mg thiamine L-1 water; 100N = 100 mg nicotinamide L-1 water; 100T = 100 mg thiamine L-1 water.

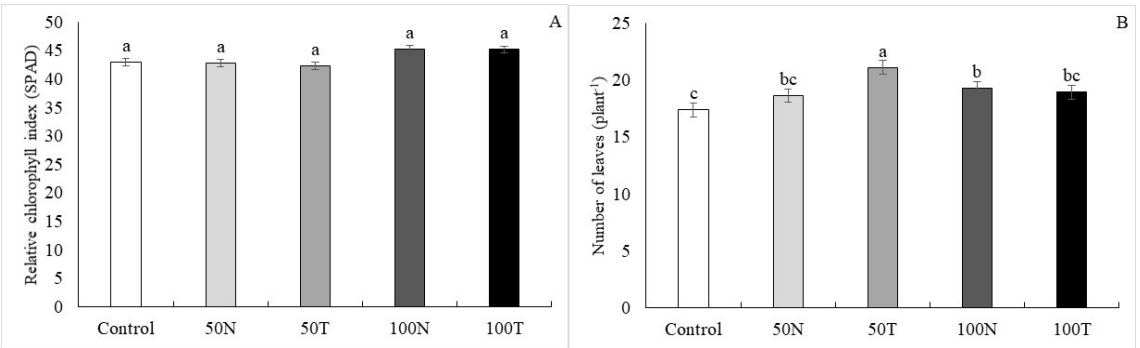


Figure 4. Relative chlorophyll content (A) and number of leaves (B) of soybean plants treated with vitamin solutions. Different letters on the bars indicate a significant difference between the means (P < 0.05). The bars represent the mean values (n = 6). 50N = 50 mg nicotinamide L-1 water; 50T = 50 mg thiamine L-1 water; 100N = 100 mg nicotinamide L-1 water; 100T = 100 mg thiamine L-1 water.

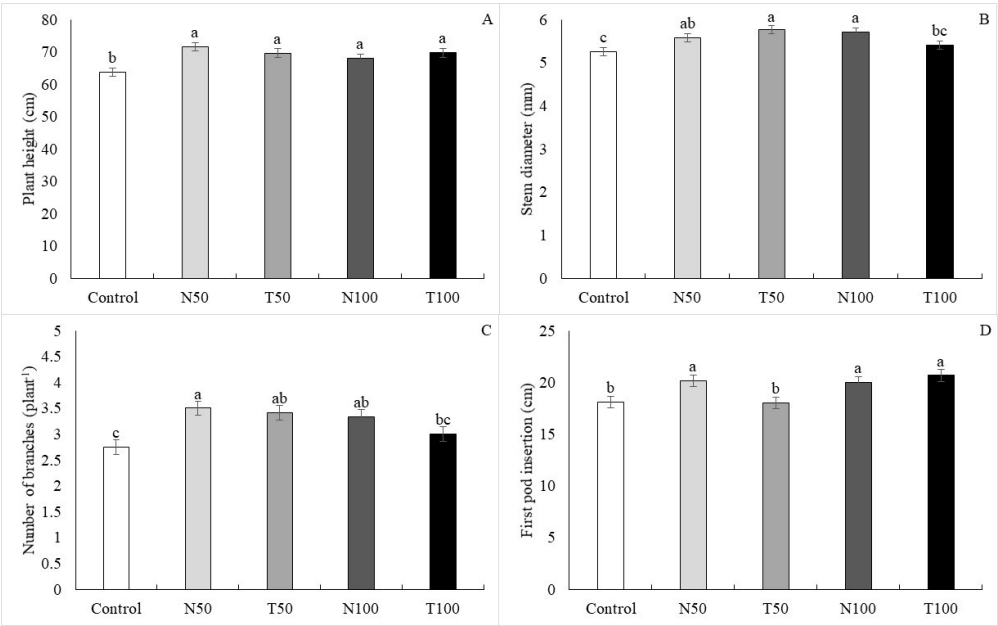


Figure 5. Plant height (A), stem diameter (B), number of branches (C), and first pod insertion (D) of soybean plants treated with vitamin solutions. Different letters on the bars indicate a significant difference between the means ($P < 0.05$). The bars represent the mean values ($n = 6$). 50N = 50 mg nicotinamide L-1 water; 50T = 50 mg thiamine L-1 water; 100N = 100 mg nicotinamide L-1 water; 100T = 100 mg thiamine L-1 water.

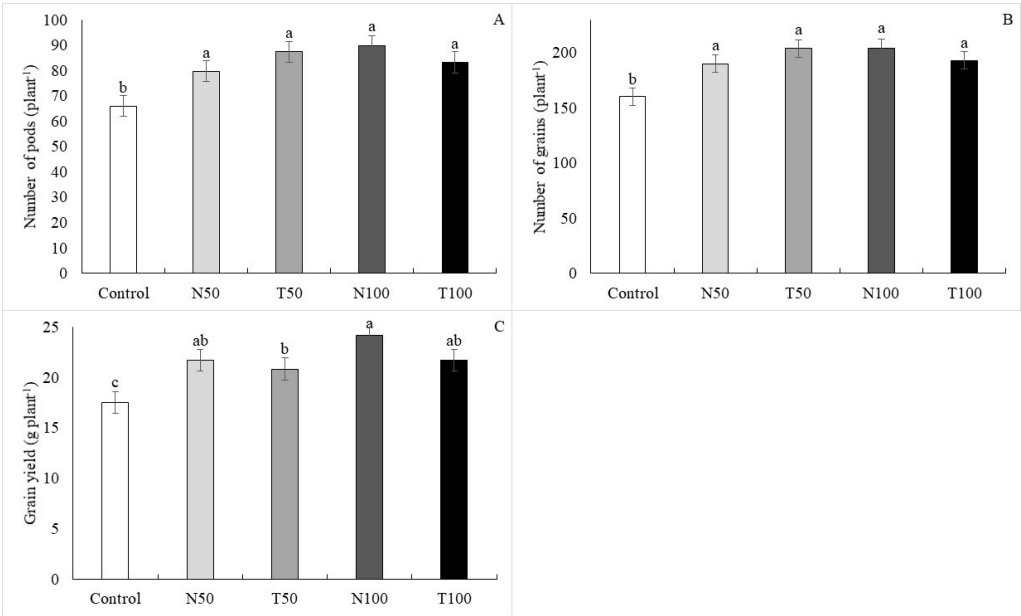


Figure 6. Number of pods (A), number of grains (B), and grain yield (C) of soybean plants treated with vitamin solutions. Different letters on the bars indicate a significant difference between the means ($P < 0.05$). The bars represent the mean values ($n = 6$). 50N = 50 mg nicotinamide L-1 water; 50T = 50 mg thiamine L-1 water; 100N = 100 mg nicotinamide L-1 water; 100T = 100 mg thiamine L-1 water.

characteristics of E, gS, A, WUE, and EICI were positively correlated with the biometric and production characteristics of plant height, stem diameter, number of branches, number of pods, number of grains, and grain mass. In addition, gains in vegetative development also increased variables related to grain production (Table 2).

4. Discussion

The application of vitamins to the soybean crop positively influenced the agronomic characteristics of the crop since these vitamins are biostimulants, which are capable of stimulating the physiological system of plants

Table 2. Estimate of Pearson linear correlation coefficient between the characteristics of soybean plants grown under vitamin application.

	Ci	E	gS	A	WUE	EICI	RCI	NL	NP	NG	PH	SD	FPI	NB	GW
Ci		ns													
E	0.20														
gS	0.38 **	0.94 *													
A	-0.15	0.88 *	0.81 *												
WUE	-0.65 *	0.30	0.25	0.69 *											
EICI	-0.48 *	0.70 *	0.56 *	0.93 *	0.82 *										
RCI	0.15	-0.02	0.09	-0.03	-0.05	-0.13									
NL	-0.14	0.04	-0.08	-0.01	0.03	0.04	-0.03								
NP	-0.28	0.44 *	0.34 **	0.53 *	0.42 *	0.54 *	0.09	0.11							
NG	-0.34 **	0.43 *	0.28	0.53 *	0.38	0.56 *	0.12	0.01	0.73 *						
PH	-0.26	0.50 *	0.44 *	0.54 *	0.44 *	0.53 *	0.15	0.06	0.46 *	0.50 *					
SD	-0.04	0.64 *	0.51 *	0.55 *	0.22	0.50 *	-0.02	0.39 **	0.57 *	0.45 *	0.45 *				
FPI	-0.20	0.20	0.24	0.23	0.25	0.20	0.32 **	-0.29	0.12	0.18	0.39 **	0.04			
NB	-0.07	0.55 *	0.48 *	0.57 *	0.38 **	0.52 *	-0.29	0.13	0.44 *	0.33 **	0.34 **	0.42 *	0.17		
GW	-0.19	0.41 *	0.37 **	0.51 *	0.45 *	0.47 *	0.20	-0.07	0.68 *	0.58 *	0.46 *	0.39 **	0.45 *	0.38 **	
			* ($p \leq 0.01$)			** ($p \leq 0.05$)			non significant		** ($p \leq 0.05$)		* ($p \leq 0.01$)		

Ci = intracellular CO₂; E = transpiration; gS = stomatal conductance; A = net photosynthesis; WUE = water use efficiency; EICI = carboxylation efficiency; RCI = relative chlorophyll index; NL = number of leaves; NP = number of pods; NG = number of grains; PH = plant height; SD = stem diameter; FPI = first pod insertion; NB = number of branches; GW = grain weight.

* , ** – Significant at $p \leq 0.05, 0.01$ by t-test, respectively.

when applied exogenously (Lima et al., 2024a; Kausar et al., 2023). In this sense, the gains in the development of vegetative and reproductive tissues observed in this study (Figures 5 and 6) may be associated with the greater metabolic activity of the plants. Nicotinamide plays a leading role in energy transport activities in the photosystem, participating in the constitution of $\text{NADP}^+/\text{NADPH}$, acting as an electron donor in anabolic reactions, and as an electron acceptor in catabolic reactions (Ferreira et al., 2023).

Nicotinamide also influences the photosynthetic capacity of plants (Figure 2 D) since many of the responses related to the transformation of light energy into chemical energy by the plant are dependent on the oxidized form of NADPH, NADP, which acts as an electron accepting enzyme (Waskell and Kim, 2015). As a result, the plants have a higher chlorophyll content, number of leaves (Figure 4), and increased production components (Figure 6). In field cultivation, Lima et al., (2024b) found that the application of nicotinamide also made it possible to increase soybean yields in locations with different soil and climate characteristics, which was related to the biostimulus caused by the vitamin.

Thiamine application also increased plant height when evaluated on turnip plants (Jabeen et al., 2021). Nicotinamide improved the growth and apical development of barley under heavy metal stress conditions (Sedzik et al., 2019) and increased the height and dry weight of broad bean plants (Abdelhamid et al., 2013). These results were compatible with this study, in which the treatments with doses of 50 and 100 mg L⁻¹ showed average height and diameter increases compared to the control (Figures 5 and 6).

Applying thiamine at 100 mg L⁻¹ improved yield attributes in wheat cultivars. Similar results were reported by Aminifard et al., (2018) in coriander, fenugreek, and pea by Kausar et al. (2023). The application of thiamine to maize seeds before emergence under arsenic stress conditions increased height, fresh and dry weight of the shoot and roots, chlorophyll and carotenoid content, net assimilation rate, transpiration rate, and stomatal conductance (Atif et al., 2022).

In addition, photosynthetic pigments, membrane integrity, enzymatic antioxidants, total phenolic content, and ascorbic acid are significantly improved when thiamine is applied (El Metwally and Sadak, 2019). The application of thiamine can cause a reduction in potassium (K^+) leakage and increase the content of glycine, betaine, and proline, thus decreasing hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) at the same time (Khalil et al., 2022). Stress tolerance is also improved by initiating stress responsive genes and calcium signaling in the cells (Li et al., 2022), an important characteristic given that crops are exposed to constant biotic and abiotic stresses of varying intensities.

It has been observed that during abiotic stresses, a series of internal events occur in plants, which signal the need to activate resistance systems. In this respect, producing reactive oxygen species (ROS) is important in activating these defenses at the molecular level, but with adverse effects on the plant system when in large quantities (El-Bassiouny et al., 2023; Ragaey et al., 2022). The production of ROS may be linked to proteins homologous to respiratory oxidase, also known as nicotinamide adenine dinucleotide phosphate (NADPH) oxidases (Liu and He, 2016), resulting in a decrease in the concentration of this coenzyme. However, additional amounts of nicotinamide can decrease the

deleterious effects of oxidation by increasing the activity of enzymes that promote DNA recovery, which results in efficient energy homeostasis (Berglund et al., 2017).

The attenuation of the effects of water stress by the application of thiamine has also been observed in rice (Vendruscolo et al., 2020), wheat (Amjad et al., 2021), and sugarcane (Ramos et al., 2023). These effects are related to the exogenous introduction of thiamine, which plays a fundamental role as a coenzyme to regulate many metabolic processes in cells (Kamarudin et al., 2017), due to which plants have a greater efficiency of water use, managing to develop in adverse conditions (Figure 3). Due to its protective nature, this vitamin allows for a rapid reestablishment of metabolic processes in plants after the stress has ceased, which implies greater growth potential when compared to plants without application (Vendruscolo et al., 2024).

In addition, the set of benefits seen in this study as a result of the application of vitamins enabled a set of reactions to be verified aimed at improving the physiological capacity of the plants, which was reflected in their development and production characteristics (Table 2). This fact and the results previously verified in the literature show the high potential for using these vitamins in commercial cultivation environments.

5. Conclusion

The exogenous application of nicotinamide or thiamine favors the development and productivity of soybean plants at doses of 50 and 100 mg L⁻¹, making it possible to use it as a biostimulant in soybean cultivation.

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