Research Article

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Nicotinamide as a biostimulant improves soybean growth and yield

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Abstract: Modern agriculture seeks, through technology, to increase agricultural productivity, aiming at greater sustainability during food production. The application of vitamins meets this objective, acting as a biostimulant for different cultivation conditions. In this sense, the aim of this research is to evaluate the effect of the foliar application of nicotinamide on the soybean crop in two growing environments. The treatments were composed of the nicotinamide concentrations of 0, 100, 200, 300, 400, 500, and 600 mg L^{-1} of water applied when the plants were at the R3 stage (beginning of pod formation), exogenously, by foliar spraying. The application of nicotinamide resulted in increases in the vegetative traits of plant height and the number of branches per plant by 9.7 and 7.8%, respectively. Regarding the productive characteristics, considering the average of the two cultivation sites, the application of the vitamin resulted in increments of about 15.62, 18.57, 20.53, 4.32, and 19.19% on the number of pods, number of grains, mass of grains per plant, mass of 1,000 grains, and yield, respectively. In this way, we conclude that nicotinamide, applied at concentrations ranging from 237.8 to 373.8 mg L^{-1}, promotes benefits to soybean growth and yield in different environments, and can be considered as a biostimulant.

Keywords: vitamins, niacin, bioinputs, *Glycine max*

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1 Introduction

One of the great challenges in agriculture is to increase crop production, using the minimum of inputs to promote the plant's capacity to expand its yield potential. This goal can be achieved by using products applied exogenously to plant seeds before sowing or to plants at different growth stages to overcome or ameliorate unfavorable conditions, reducing negative effects on crop yield and quality [1,2]. Thus, substances with a protective or biostimulant character that promote the proper development of the plant are sought [3,4]. Among the biostimulant products are B-complex vitamins, also known as nicotinamide, vitamin B3, or niacin [5,6].

Plants require vitamins in small quantities [7], acting as coenzymes, regulating metabolism, and increasing plant activities without toxic or mutagenic effects [8]. Nicotinamide is a water-soluble vitamin, a constituent of the coenzymes NADH and NADPH that participate in many enzymatic oxidation–reduction reactions (redox reactions) in cells [5] and is considered essential [9] for participating in the metabolization of carbohydrates, fats, and proteins involved in photosynthesis and cellular respiration [10].

In addition, nicotinamide is associated with stress signaling, inducing or regulating secondary metabolite accumulation and plant defense metabolism action [11–14]. Thus, plants can increase nicotinamide concentration after situations that cause oxidative stress and induce defensive metabolism [12].

Nicotinamide is considered a growth regulator substance, which, when applied in small amounts, can cause physiological changes in the plant, such as the biosynthesis of enzymes, nucleic acids, and proteins [1]. The stimulatory effects of nicotinamide on plant growth are shown to correlate with increased content and activity levels of endogenous promoters such as gibberellic acid and indoleacetic acid that promote plant organ growth [15]. In addition, it indirectly contributes to plant growth by performing energy transport in the plant cell [16].

Some research links the exogenous use of nicotinamide in leaf application with plant growth and yield characteristics [17]. The effects of nicotinamide are observed on vegetative growth through cell expansion, reserve accumulation, and increase in crop yield and its components, and this can occur in different growing environments, some of which are adverse, such as water deficit and soil salinity [4,6,13].

Several studies with the exogenous application of nicotinamide have shown the potential of this vitamin in different crops, with positive effects on growth, yield, metabolism, and/or physiological traits. Favorable results have been verified for *Triticum aestivum* [18], *Zea mays* [4,19], *Vicia faba* [1,6,20,21], *Pisum sativum* [22], *Helianthus annuus* [23], *Oryza sativa* [24], *Beta vulgaris* var. *saccharifera* [3], and *Phaseolus vulgaris* [25].

This research is based on the hypothesis that the exogenous use of nicotinamide via the foliar application can promote improvements related to the agronomic characteristics of growth and yield in soybean plants. Therefore, the aim of this research is to evaluate the effect of the foliar application of nicotinamide on the soybean crop in two growing environments.

2 Materials and methods

2.1 Characteristics of the experiment site

Two experiments were conducted in the 2021/2022 harvest in two cultivation sites with distinct edaphic and environmental characteristics. One site was in the area of the Federal University of Mato Grosso do Sul, the campus of Chapadão do Sul-MS (CHS), at 18°48′45.9″S, 52°36′00.3″W, and an altitude of 820 m. The other site was in the area of the State University of Mato Grosso do Sul, the unit of Cassilândia-MS (CAS), at 19°06′48″S, 51°44′03″W, and an altitude of 510 m.

In both locations, the climate is classified as humid tropical, with a rainy summer and dry winter extending from May to September (Köppen classification Aw). In CHS, the annual temperature is between 13 and 28°C, and the average rainfall is 1,850 mm [26]. In CAS, the average annual temperature is 24.1°C, and the average rainfall is 1,520 mm. During the experiment, rainfall and air temperature data were obtained at both sites (Figure 1a and b).

The soils of CHS and CAS were classified as Latossolo Vermelho Distrofico and Neossolo Quartzarênico [27], respectively. Soil sampling for chemical analysis was performed in the 0.00–0.20 m layer and had the following results for CHS: pH ($CaCl_2$) = 5.0, P (Melich I), K, S, B, Cu, Fe, Mn, and Zn = 13.0, 101, 2.8, 0.21, 0.8, 59, 15.2, and 5.1 mg dm^{-3}, respectively; Ca, Mg, H + Al, and cation exchange capacity (CEC) = 3. 40, 1.30, 4.5, and 9.5 $cmol_c$ dm^{-3}, respectively; base saturation (V%) = 52.4, and organic matter (OM) = 24.5 g dm^{-3}. The texture presented: clay = 495 g dm^{-3}, silt = 50 g dm^{-3}, and sand = 455 g dm^{-3}. For CAS the values were: pH ($CaCl_2$) = 6.3, P (Melich I) and K = 9.0 and 257.4 mg dm^{-3}, respectively; Ca, Mg, H + Al, and CEC = 4.2, 1.0, 0.9, and 6.8 $cmol_c$ dm^{-3}, respectively; base saturation (V%) = 87, and organic matter (OM) = 13.0 g dm^{-3}.

2.2 Statistical design, treatments, and plots

The experimental design was a randomized block design with seven treatments and four repetitions, totaling 28 plots. The treatments were composed of the nicotinamide concentrations of 0, 100, 200, 300, 400, 500, and 600 mg L^{-1} of water, applied when the plant was at the R3 stage (beginning of pod formation), exogenously, by foliar spraying, using an electric pump with constant pressure and flow rate of 136 L ha^{-1}.

The experimental plots comprised five rows 5 m long, spaced 0.50 m apart, with a distribution of 15 seeds per meter. The three central rows were used for the evaluations. The cultivar NS6601 IPRO, with an early maturity, indeterminate growth, stable yield, and adaptability to various environments, was used.

2.3 Conducting the experiment

In the CHS area, 30 days before sowing, the soil was corrected with dolomitic limestone (860 kg ha^{-1}), calculated according to soil analysis to raise the base saturation to 60%. In CAS, there was no need for soil correction. Five days before the direct sowing of soybeans, weeds were desiccated with glyphosate at a dose of 1.11 kg acid equivalent (a.e.) ha^{-1}. Sowing was conducted with a tractor-drawn mechanical vacuum seeder in CHS and CAS on October 28 and November 22, 2021, respectively. The seeds were inoculated with *Bradyrhizobium japonicum* using the commercial liquid inoculant Total Nitro Max® containing the SEMIA 5,079 strain (5 × 10^9 viable cells per mL) at a dose of 300 mL per 100 kg of seeds.

In the CHS area, 80 kg ha^{-1} of P_2O_5 was applied in the sowing furrow using simple superphosphate. The topdressing fertilization was performed at the V5 stage (fourth

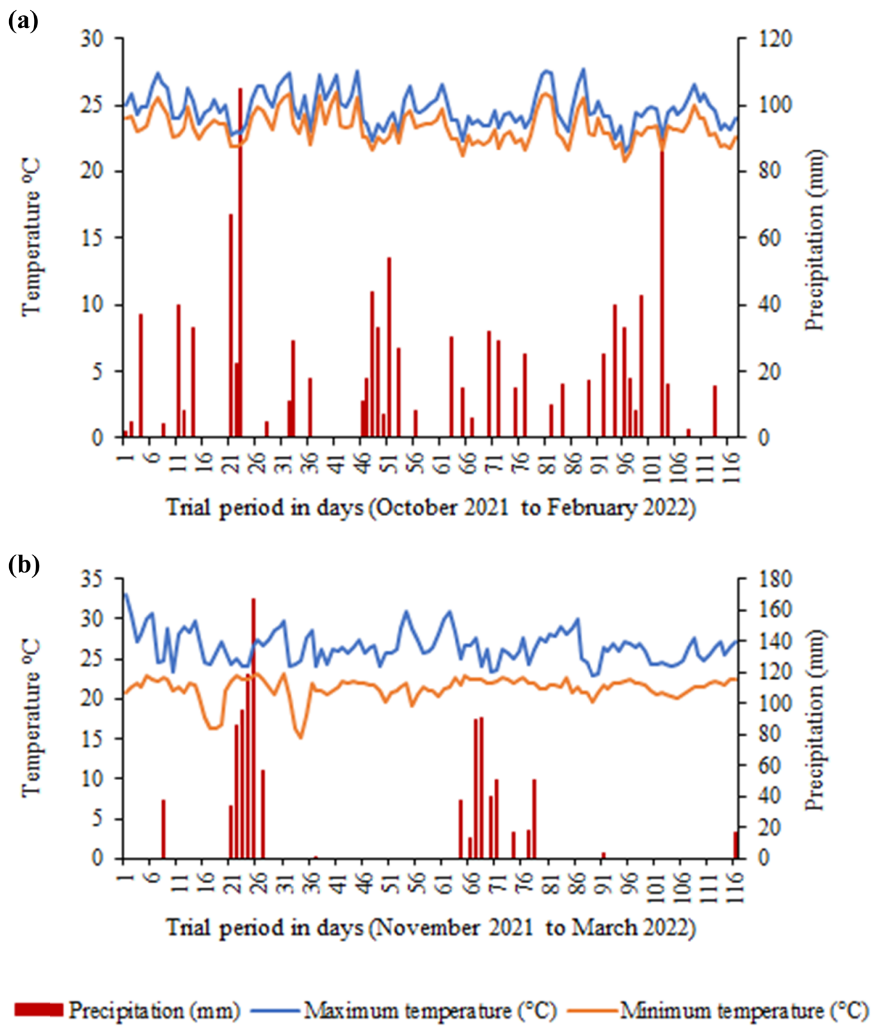

Figure 1: Maximum and minimum temperatures and precipitation at the Chapadão do Sul (a) and Cassilândia (b) sites during the experimental period.

trifoliate leaf completely expanded), with 100 kg ha^{-1} of K_2O, using potassium chloride. In the CAS area, sowing was done on top of the residual fertilizer from the previous harvest.

To control weeds during cultivation in the CHS area, the herbicide Glyphosate potassium was used, at a dose of 0.5 kg a.e. ha^{-1}, in two applications, at 24 and 33 days after sowing (DAS). A fungicide application was also performed at 53 DAS to prevent the occurrence of Asian soybean rust (*Phakopsora pachyrhizi*) using Difenoconazole (87.5 g a.i. ha^{-1}) + Cyproconazole (52.5 g a.i. ha^{-1}), plus the adjuvant Ethylenexy (6.0 g a.i. 100 L^{-1} of water). In the CAS area, a single application of the herbicide Glyphosate potassium was performed to control weeds at a dose of 0.5 kg a.e. ha^{-1}, at 14 DAS. At 11 DAS, the insecticide Methoxyfenozide (48 g a.i. ha^{-1}) was applied to control caterpillars. At 28 DAS, the application of Methoxyfenozide (48 g a.i. ha^{-1}) was repeated along with the fungicide Azoxystrobin (60 g a.i. ha^{-1}) + Benzovindiflupyr (30 g a.i. ha^{-1}). At 74 DAS, the insecticide Methoxyfenozide was repeated at the same dose, along with the fungicide Pyraclostrobin (66.5 g a.i. ha^{-1}) + Epoxiconazole (25 g a.i. ha^{-1}). Finally, at 105 days, the insecticide Beta-Cypermethrin (30 g a.i. ha^{-1}) was applied to control stink bugs.

2.4 Characteristics evaluated

Harvesting was performed for the CHS and CAS sites on February 21 and March 18, 2022, corresponding to 116 DAS for both locations, respectively. At harvest time, five plants per plot were collected to determine the plant height (PH), the first pod insertion height (FP), the number of branches per plant (NB), the number of pods per plant (NP), the number of grains per plant (NG), and grain mass per plant (GM). Subsequently, the whole plot was harvested and threshed to determine the 1,000-grain weight (1,000 W) and yield (GY). All grain mass was adjusted to 13% moisture. The 1,000 W was obtained from 8 lots of 100 seeds. PH, FP, and NB were obtained only on the CHS site.

2.5 Statistical analysis

The assumptions of normality distribution and homogeneity of variances were verified for the data. The data were submitted for analysis of variance with the means of the quantitative factors evaluated by the regression analysis using the Sisvar software [28]. The correlation network and canonical variables were also analyzed using the Rbio program [29].

3 Results

The variables PH, FP, and NB were evaluated only for the CHS area, and it was found that nicotinamide affected PH and NB without interfering with FP. The other variables were evaluated in both areas. It was found that the use of nicotinamide influenced all variables except 1,000 W in the CAS site (Table 1).

The nicotinamide concentration that provided the highest PH was 364.2 mg L^{-1}, representing a 9.7% gain over the control treatment (Figure 2). For NB, the best nicotinamide concentration was 237.8 mg L^{-1}. High concentrations of nicotinamide are observed to cause a reduction in the NB per plant, which can reach a reduction of 7.8% compared to the control.

In CHS, the concentration of 321.3 mg L^{-1} of nicotinamide resulted in 54.7 pods per plant (Figure 3a). In CAS, the highest pod production per plant was achieved with the concentration of 313.3 mg of nicotinamide, resulting in 66.8 pods (Figure 3b). The highest NG was achieved with concentrations of 373.8 and 318.8 mg L^{-1} of nicotinamide, which resulted in 162.0 and 145.8 grains per plant in CHS and CAS, respectively (Figure 3c and d).

The maximum GM was obtained at 349.0 and 319.6 mg L^{-1} of nicotinamide concentrations, reaching 27.6 and 18.3 g, respectively, for CHS and CAS (Figure 4c and d). For 1,000 W, there was no equation adjustment for CAS. The highest value of 1,000 W in CHS, 170.8 g, was reached with a concentration of 314.9 mg L^{-1} of nicotinamide.

The highest GY in CHS was 4538.9 kg ha^{-1}, achieved with 315.7 mg L^{-1} of nicotinamide, while in CAS, the yield was 4393.1 kg ha^{-1}, obtained at the 323.8 mg L^{-1} of nicotinamide (Figure 5). Note that the use of the vitamin narrowed the difference in GY since without vitamin application, CHS produced 14.5% more grain than CAS.

Table 1: PH, FP, NB, NP, NG, GM, 1,000 W, and grain yield (GY) for CHS and CAS of soybean plants according to the concentrations of nicotinamide applied via foliar spraying

SV	DF	Mean square – CHS				
		PH	FP	NB		
Block	3	1.10	1.53	0.04		
Nic	6	31.49**	1.48ns	0.14*		
Error	18	3.99	1.74	0.04		
CV (%)		2.46	8.84	5.37		
Average		81.24	14.89	3.91		
		Mean square – CHS				
		NP	NG	GM	1,000 W	GY
Block	3	4.51	12.78	0.54	1.25	2882.54
Nic	6	12.53**	184.80**	9.80**	43.95**	165451.85**
Error	18	2.66	11.28	0.42	4.48	28655.38
CV (%)		3.08	2.15	2.47	1.26	3.89
Average		52.93	156.09	26.21	167.80	4346.67
		Mean square – CAS				
		NP	NG	GM	1,000 W	GY
Block	3	6.82	6.87	0.76	28.48	43746.93
Nic	6	185.54**	968.69**	8.82**	179.35ns	508117.65**
Error	18	16.79	27.61	0.94	69.20	54387.73
CV (%)		6.83	3.90	5.74	6.59	5.74
Average		61.80	134.85	16.94	126.17	4066.29

** and ns – Significant at $p \leq 0.01$ and, not significant by *t*-test, respectively.

The correlation network was drawn up from the Pearson matrix, where positive correlations are expressed in green lines and negative correlations in red lines, the magnitude of the correlation being proportional to the thickness of the lines (Figures 6 and 7). For CHS, all correlations were positive. It can be seen that 1,000 W, NG, and NP were strongly correlated with MG, indicating that these variables individually contributed the most to the increase in GM. On the other hand, no single variable stood out in correlation with GY. However, all correlations were positive, indicating that the sum of the contributions of each variable was important in increasing GY (Figure 6).

For CAS, there was also a positive correlation between all variables. A cluster with strong correlations occurred between the variables GY, GM, NP, and NG, indicating that the increase in one of these variables reflects an increase in the others. The variable 1,000 W was far from the cluster and with correlations of weak magnitude with the other variables, indicating that it contributes little to the increase in the other variables (Figure 7).

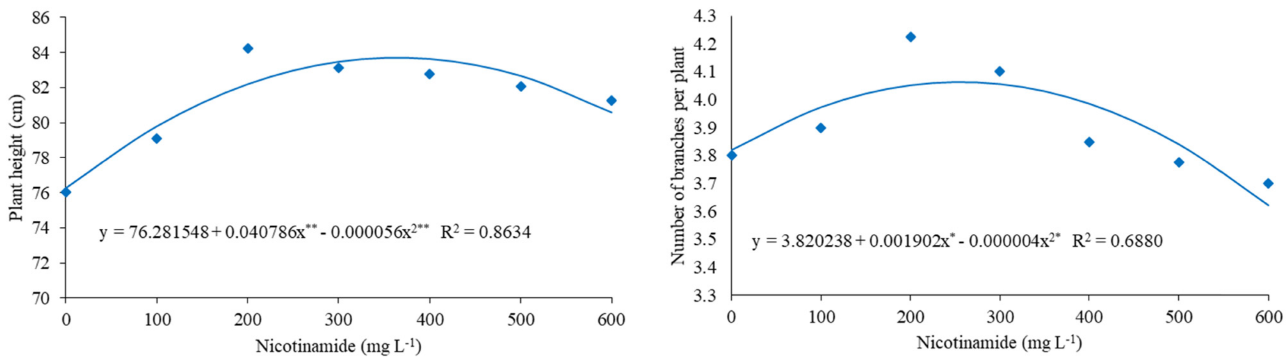

Figure 2: PH and NB per plant of soybean plants according to the concentrations of nicotinamide applied via foliar spraying.

In the canonical variable analysis, the accumulated variance in the first two variables was 92.4% in CHS and 99.6% in CAS (Figures 8 and 9), giving credibility to representation in a two-dimensional plot in both experiments since the percentages of accumulated variance in the first two canonical variables should be greater than 80% [30].

In CHS, it is observed that the 300 mg L^{-1} nicotinamide concentration stood out for the increment of the 1,000 W variable. In contrast, the vectors representing the NP, GM, GY, and NG variables were similar. The contributing treatments were the 200 and 400 mg L^{-1} nicotinamide concentrations (Figure 8). This indicates that increased production component values and soybean yields occur at nicotinamide concentrations between 200 and 400 mg L^{-1}. Other treatments did not influence any of the variables analyzed.

In the CAS site (Figure 9), the NP and NG vectors were similar, and the treatment that contributed the most was the 400 mg L^{-1} of nicotinamide. The GM and GY vectors were

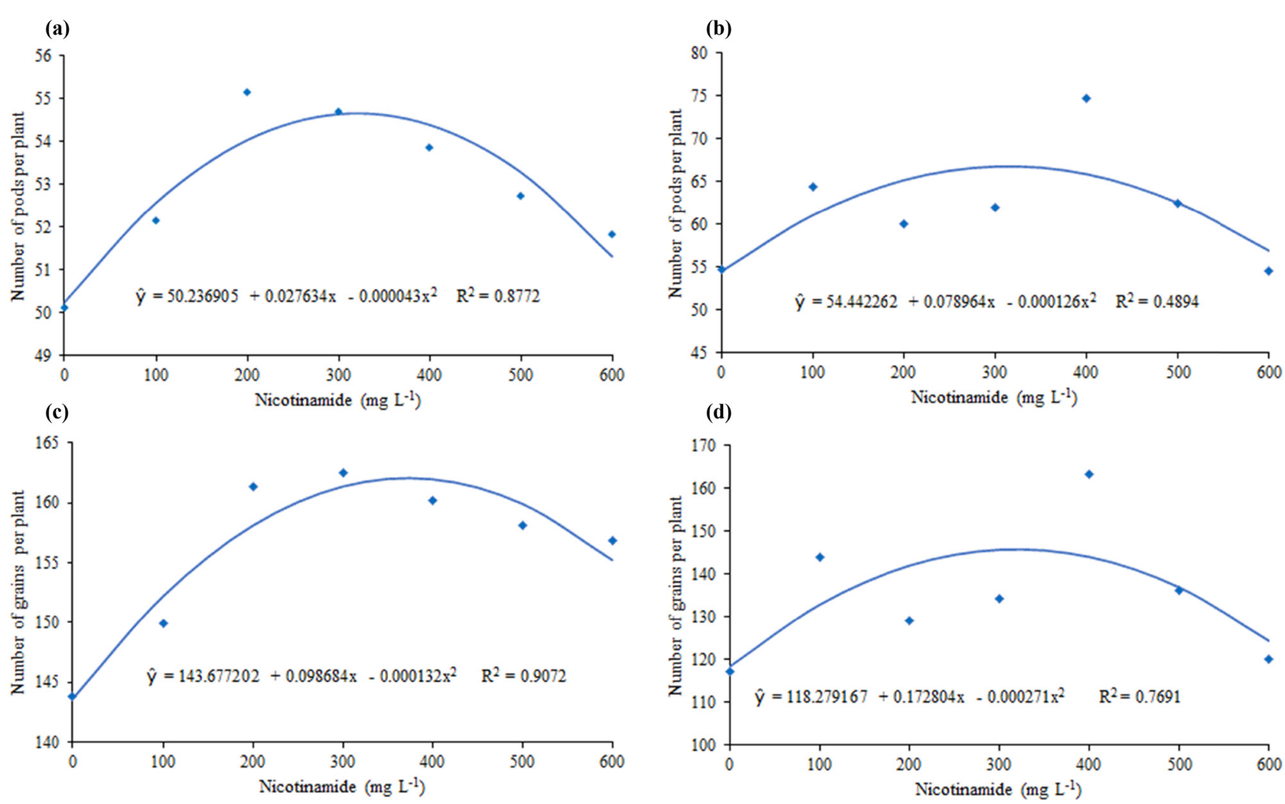

Figure 3: Number of pods per plant in CHS (a) and CAS (b) and number of grains per plant in CHS (c) and CAS (d) of soybean plants according to the concentrations of nicotinamide applied via foliar spraying.

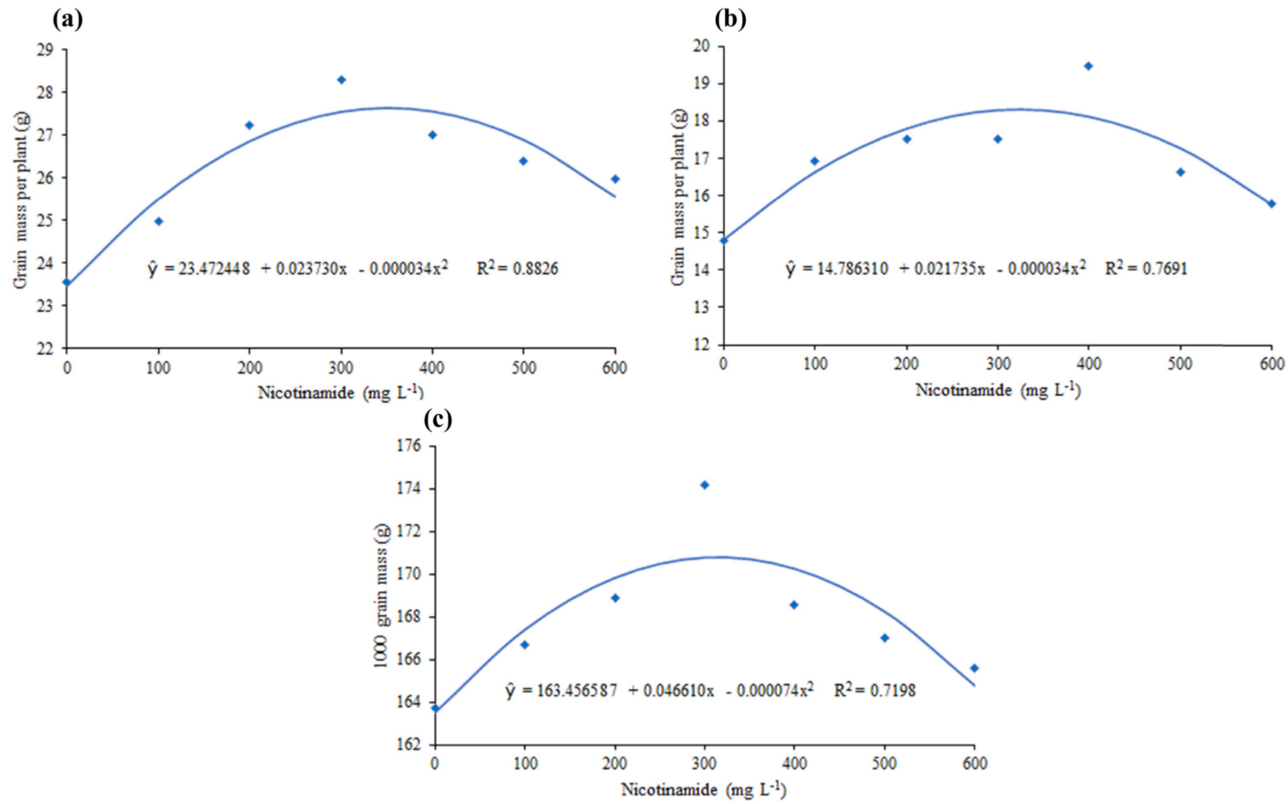

Figure 4: Grain mass per plant in CHS (a) and CAS (b) and 1,000-grain weight in CHS (c) of soybean plants according to the concentrations of nicotinamide applied via foliar spraying.

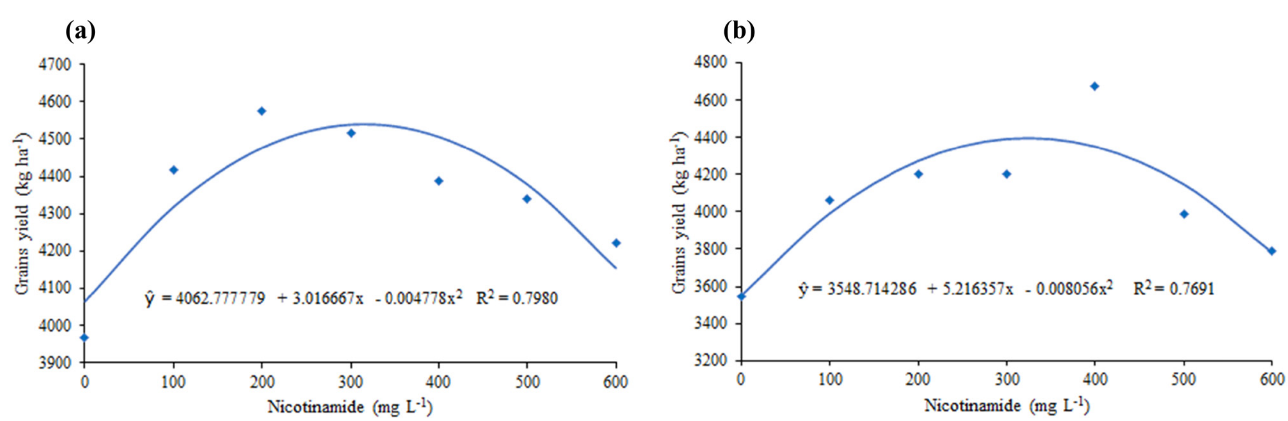

Figure 5: Grain yield in CHS (a) and CAS (b) of soybean plants according to the concentrations of nicotinamide applied via foliar spraying.

also similar at 300 mg L^{-1} of nicotinamide. The 200 mg L^{-1} of nicotinamide influenced the 1,000 W vector to a lesser extent, which was distant from the other variables.

4 Discussion

The positive results verified with the application of nicotinamide are related to the biostimulant characteristic of this compound, which is capable of stimulating the physiological system of plants when applied exogenously [4]. In this sense, the gains in vegetative and reproductive tissue development obtained in the present study (Figures 2–5) can be associated with higher energy transfer activity in the photosystem, which is responsible for transforming light energy into photoassimilates used in the processes of carboxylation and cell division [16], which results in a biostimulant action in plants submitted to different environmental conditions (Figure 1).

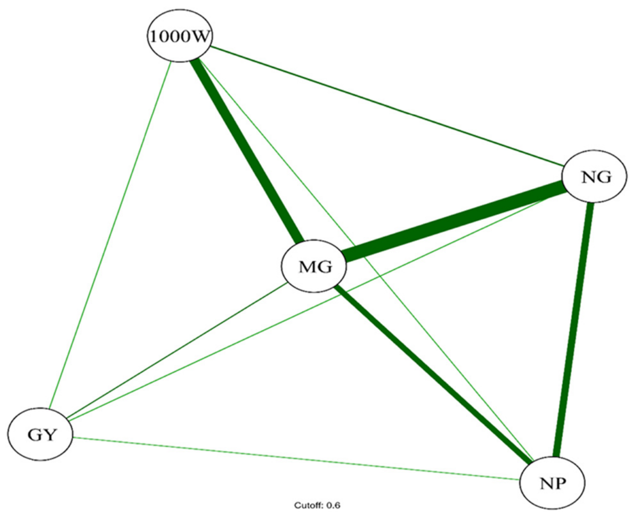

Figure 6: Correlation network between CHS variables as a function of different nicotinamide concentrations in soybean.

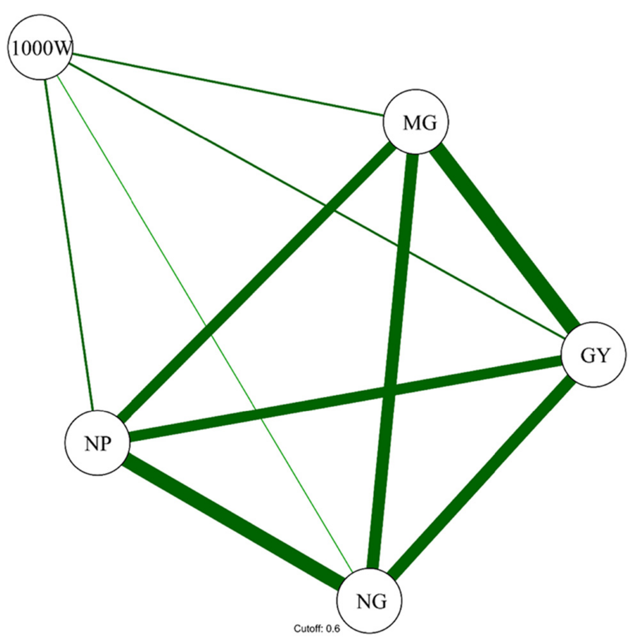

Figure 7: Correlation network between CAS variables as a function of different nicotinamide concentrations in soybean.

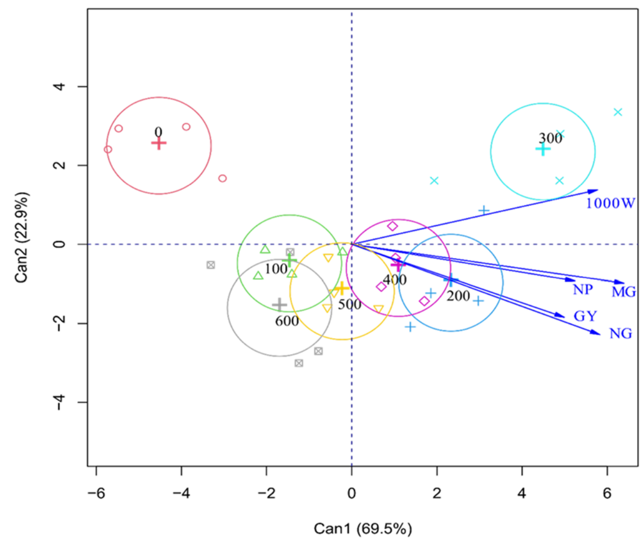

Figure 8: Canonical variables for CHS as a function of different nicotinamide concentrations in soybean. Different colors represent treatments with nicotinamide concentrations.

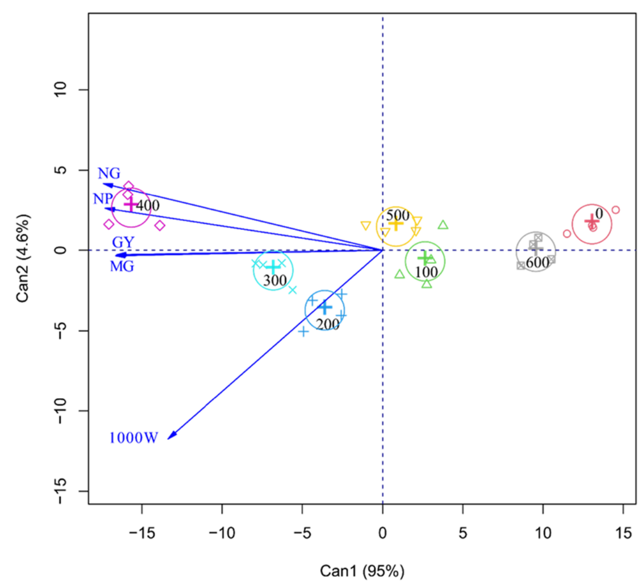

Figure 9: Canonical variables for CAS as a function of different nicotinamide concentrations in soybean. Different colors represent treatments with nicotinamide concentrations.

Studies that evaluated the physiological effect of nicotinamide indicated that changes in plant metabolic activity allow plants to more efficiently perform activities related to growth and development, given that nicotinamide is a precursor to NAD+ and a promoter of indole-acetic acid levels, which is directly related to cell division [9,13,21,31]. This set of responses has also been found for other species of agricultural interest, such as beans [32] and corn [4]. This increase in metabolic activities is also involved in the production of energy reserves, which are demanded during the grain production and filling phase [33], a stage that the plants in the present study were at the time of the vitamin application, as well as being related to better plant performance [34] since reproductive characteristics are correlated with the productive capacity of plants (Figures 6 and 7).

The effects of exogenous nicotinamide application on the increment of energy reserve contents have also been found for the fava bean crop, where the application of nicotinamide concentrations of up to 50 mg L^{-1} provided significant increments in the number of carbohydrates present in plants and seeds [1,21]. This extra accumulation is essential for the plant in uncontrolled growing environments since the activation and maintenance of the protective responses to the different stresses depend on the presence of sugars, which are consumed during the period in which the plant is under the effect of the stresses [35], as those observed in the CAS site, where two periods of intense water deficit occurred, accompanied by temperatures near 30°C (Figure 1b).

It can be seen that during abiotic stresses, a series of internal events occur in plants, which signal the need to activate resistance systems. In this aspect, 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 high quantities [36,37]. The production of ROS may be linked to respiratory oxidase homologous proteins, also known as nicotinamide adenine dinucleotide phosphate (NADPH) oxidases [38], resulting in decreased concentration of this coenzyme. However, additional amounts of nicotinamide can decrease the deleterious effects of oxidation by increasing the activity of enzymes promoting DNA recovery, which results in efficient energy homeostasis [31].

In complement, the amelioration of the adverse effects of the ROS action is also due to the involvement of nicotinamide against the oxidation of membranes and organelles perform the capture of light energy, in addition to the participation in the activation of enzymes related to the reduction in atmospheric carbon, resulting in maintenance or even an increase in pigments such as chlorophyll and anthocyanins [1,21,39]. Also, an increase of proline was observed in plant tissues in other studies that used nicotinamide [21], one of the most studied osmoprotectants, and it can be produced in several plant organs, including chloroplasts, where it contributes to the protection of the photosynthetic system [40].

These attributes that give nicotinamide the ability to act as a mitigator of abiotic stresses [41] are highly relevant, considering that the cultivation environments are mostly located in tropical and subtropical regions, with a predisposition to periods of water restriction, high solar radiation, and increased air temperature [42], which affects soybean cultivation [43]. In addition, high temperatures can also be observed in temperate regions, affecting the production of important grain-producing crops such as wheat [44]. These facts imply the need for significant changes in production systems, with the implementation of practices allowing greater food safety [45].

The changes caused by the presence of extra nicotinamide and its results regarding the promotion of metabolic and physiological activities and field performance of soybean make this compound a promising tool to help the development of more efficient production systems with greater adaptability to climate change. The application of nicotinamide, as well as other vitamins studied, has an important aspect of interest for farmers, the cost-benefit [46], and can be incorporated into the agricultural management of soybean since the performance responses are shown to be similar (Figures 8 and 9) for different conditions regarding soil and climate characteristics.

5 Conclusion

Nicotinamide applied at concentrations ranging from 237.8 to 373.8 mg L^{-1} promotes soybean growth and yield in different environments, and can be being considered as a biostimulant.

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Conflict of interest: The authors state no conflict of interest.

Data availability statement: The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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