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Agronomic efficiency of foliar nanozinc applied at reproductive stages of soybean

Eficiência agronômica do nanozinco aplicado via foliar em estádios reprodutivos da soja

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Highlights

Foliar application of Zn at reproductive stages improves soybean yield components.
The R5.1 stage showed greater responsiveness to Zn than the R1 stage.
Optimal Zn doses ranged from 0.75 to 1.06 L ha^{-1} for maximizing grain yield.
Grain yield and pod/grain number or grain weight were strongly correlated.
Nanoformulated Zn with amino acids effectively enhanced agronomic performance.

Abstract

Zinc (Zn) is an essential micronutrient in plant metabolism and is involved in the enzyme activation and synthesis of growth hormones such as indole-3-acetic acid. Although required in small amounts, the low availability of Zn in Brazilian soils limits soybean productivity. The aim of this study was to evaluate the effects of different foliar Zn doses (0, 0.5, 1.0, 1.5, and 2.0 L ha^{-1}) applied at two reproductive stages (R1 and R5.1) on morpho-agronomic traits, yield components, and grain yield of soybean. Experiments were conducted during two growing seasons (2022/23 and 2024/25) using a randomized complete block design with four replicates. The evaluated variables included the number of pods (NP), number of grains per plant (NG), thousand-grain weight (TGW), and grain yield (YLD). Significant effects of various Zn doses were observed for NG, TGW, and YLD, with quadratic responses indicating an optimal range of 0.75–1.06 L ha^{-1} for yield maximization. Although application timing alone was not statistically significant, foliar Zn application at the R5.1 stage showed greater biological responsiveness than at R1, likely because of higher Zn demand and utilization during grain filling. Correlation analysis revealed that in the 2022/23 season, yield was strongly associated with NP and NG ($r > 0.85$), whereas in 2024/25, TGW

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contributed more to yield formation. These findings highlight the importance of foliar Zn application during advanced reproductive stages, particularly R5.1, as a strategy to optimize Zn-use efficiency and enhance soybean yield in high-performance production systems.
Key words: Nano Zinc. Foliar fertilization. Soybean (*Glycine max* (L.)). Plant nutrition.

Resumo

O zinco (Zn) é um micronutriente essencial no metabolismo vegetal, estando envolvido na ativação enzimática e na síntese de hormônios de crescimento, como o ácido indol-3-acético. Embora requerido em pequenas quantidades, a baixa disponibilidade de Zn nos solos brasileiros pode limitar a produtividade da soja. Este estudo teve como objetivo avaliar os efeitos de diferentes doses de Zn via aplicação foliar (0, 0,5, 1,0, 1,5 e 2,0 L ha^{-1}), aplicadas em dois estádios reprodutivos (R1 e R5.1), sobre características morfoagronômicas, componentes de rendimento e produtividade de grãos da soja. Os experimentos foram conduzidos durante duas safras agrícolas (2022/23 e 2024/25), utilizando o delineamento em blocos casualizados, com quatro repetições. As variáveis avaliadas incluíram o número de vagens (NP), o número de grãos por planta (NG), a massa de mil grãos (MMG) e a produtividade de grãos (YLD). Efeitos significativos das doses de Zn foram observados para NG, MMG e YLD, com respostas quadráticas indicando uma faixa ótima entre 0,75 e 1,06 L ha^{-1} para a maximização da produtividade. Embora o estádio de aplicação isoladamente não tenha sido estatisticamente significativo, a aplicação foliar de Zn no estádio R5.1 apresentou maior responsividade biológica em comparação ao R1, possivelmente em função da maior demanda e utilização de Zn durante o enchimento de grãos. A análise de correlação revelou que, na safra 2022/23, a produtividade esteve fortemente associada ao NP e ao NG ($r > 0,85$), enquanto na safra 2024/25 a MMG apresentou contribuição relativamente maior para a formação do rendimento. Esses resultados destacam a importância da aplicação foliar de Zn em estádios reprodutivos mais avançados, especialmente em R5.1, como estratégia para otimizar a eficiência de uso do Zn e aumentar a produtividade da soja em sistemas de produção de alto desempenho.
Palavras-chave: Nano Zinco. Adubação foliar. Soja (*Glycine max* (L.)). Nutrição de plantas.

Introduction

Zinc (Zn) is an essential micronutrient for plant metabolism. It acts as an enzymatic cofactor in several biochemical reactions and participates in the synthesis of tryptophan, a precursor of indole-3-acetic acid, which is a key hormone for plant growth (Marschner, 2012). Although required in small amounts (from 3 to 150 mg kg^{-1}) its availability in Brazilian soils is naturally low (Sulino & Buso, 2021), which compromises crop development, including that of soybean. Soybeans extract approximately 47 g of Zn from the soil, of which approximately 30 g is exported through harvesting, to produce one ton of grain (Pauletti & Motta, 2017; Alloway et al., 2009; Das & Green, 2016).

The absorption of Zn by plants is slow in the first weeks after sowing and intensifies between 60 and 90 d (Bataglia & Mascarenhas, 1977). In this context, soil fertilization may be inefficient during reproductive stages, making foliar application a promising alternative, as it supplies Zn^{2+} directly during periods of high physiological demand (Broadley et al., 2007).

Several studies have demonstrated that foliar Zn application is effective in meeting soybean demand, even when soils have adequate levels of micronutrients (Inocêncio et al., 2012). Positive results have been reported across different phenological stages, including V9, R1, and R3, with increases in foliar Zn content, leaf area index, and grain yield (Parmoon et al., 2015; Sadeghzadeh, 2013; Cakmak & Kutman, 2018). Oliveira et al. (2019) found that foliar application at stage R4 was the most efficient for biofortification, increasing Zn accumulation in the grains and protein content.

These findings indicate that foliar spraying is a promising practice; however, the literature still presents divergences regarding the timing of application and optimal doses to maximize nutrient efficiency. While some studies reported better results at the vegetative stages, others demonstrated superior gains when Zn was supplied at later reproductive stages, such as R4 and R5.1 (Lončarić et al., 2024; Oliveira et al., 2019). Moreover, quadratic responses to Zn doses have been observed, indicating that both Zn deficiency and excess can compromise yield and grain quality (Rashid et al., 2023; Hui et al., 2025).

Recently, nanoparticulate Zn (ZnO NP) formulations have gained attention for their high absorption and translocation efficiencies, expanding their potential response to foliar sprays (García-López et al., 2019; Wang et al., 2023). However, studies are still ongoing regarding the definition of adequate doses and the most suitable timing of application, especially in soybeans, which highlights the need for further investigation.

Therefore, the objective of this study was to evaluate the effects of foliar application of different doses of Zn in the nanoparticulate form at two soybean reproductive stages (R1 and R5.1) on yield components and morphoagronomic variables, to contribute to the improvement of technical recommendations and practical strategies for foliar fertilization with micronutrients.

Materials and Methods

The study was conducted during two soybean growing seasons, October 2022 to March 2023 (2022/2023 season) and October 2024 to March 2025 (2024/2025 season), at the Experimental Farm of the State University of Londrina (FAZESC–UEL), located in Londrina, Paraná State, Brazil. The soil in the experimental area was classified as Rhodic Hapludox, with a clayey texture (Santos et al., 2018).

Before the establishment of the experiment in each growing season, soil samples were collected from the 0 to 20 cm layer for chemical characterization, which revealed the following attributes: 10.3 g dm^{-3} of organic matter; pH ($CaCl_2$) of 4.8; 11.6 mg dm^{-3} of P; 0.53 cmolc dm^{-3} of K^+; 6.33 cmolc dm^{-3} of Ca^{2+}; 1.50 cmolc dm^{-3} of Mg^{2+}; base saturation of 67.9%; and 1.8 mg dm^{-3} of Zn. Weed control was performed by chemical desiccation using glyphosate (1,000 g ha^{-1} a.i.) combined with 2,4-D (1.5 L ha^{-1} a.i.) 15 days prior to sowing.

The experimental design was a randomized complete block arranged in a 2 × 5 factorial scheme with four replications. Treatments comprised foliar application of five zinc doses (0, 0.5, 1.0, 1.5, and 2.0 L ha^{-1}) combined with two soybean reproductive stages, according to Fehr and Caviness (1977): R1 (beginning of flowering), characterized by the presence of the first open flower on the main stem; and R5.1 (beginning of grain filling), defined by the presence of visibly formed grains (approximately 3 mm in diameter) in pods located in the middle third of the plant.

Foliar applications of NanoZn, a liquid fertilizer containing 40% (w/w) ZnO in nanometric form enriched with nitrogen (1% w/w), total organic carbon (2% w/w), and amino acids with biostimulant properties, were carried out. Applications were performed using a CO_2-pressurized backpack sprayer calibrated to deliver 150 L ha^{-1} with a non-ionic surfactant at 0.05% (v/v) added to improve droplet spreading and leaf coverage.

Each experimental plot measured 5.0 m × 3.0 m, totaling 15.0 m². The soybean cultivar used was BMX Fibra (64i61RSF IPRO), which has an indeterminate growth habit. Basal fertilization comprised 200 kg ha^{-1} of NPK (04-20-20) applied at sowing. At the V4 stage, topdressing fertilization was performed using potassium chloride (KCl) at a rate of 100 kg ha^{-1}, equivalent to 60 kg ha^{-1} of K_2O. Seeds were inoculated with *Bradyrhizobium japonicum* strains SEMIA 5079 and SEMIA 5080, at a minimum concentration of 1 × 10^9 viable cells mL^{-1}, applying 100 mL of inoculant per 50 kg of seeds.

Sowing was carried out in late October in both growing seasons, with a target plant population of 220,000 plants ha^{-1}. Crop management practices related to pest, disease, and weed control followed technical recommendations for soybean cultivation in the region and were kept consistent across growing seasons.

At physiological maturity, the following variables were evaluated: plant density (DP; plants m^{-2}), plant height (PH; m), number of pods per plant (NP), number of grains per plant (NG), thousand-grain weight (TGW; g), and grain yield (YLD, kg ha^{-1}), with yield values corrected to standard moisture content.

The data were subjected to an analysis of variance (ANOVA) after verifying the assumptions of the statistical model. The normality of residuals was assessed using the Shapiro–Wilk test, and the homogeneity of variances was evaluated using Bartlett's test, both at a 5% significance level. Means across application stages were compared using Tukey's test ($p \leq 0.05$), and the effect of Zn doses was evaluated using regression analysis. All statistical analyses were performed using the R software using the AgroR package (Castillo-Mateo et al., 2023).

Results

Analysis of variance for the 2022/2023 growing season (Table 1) showed that the timing of zinc application (A) did not significantly affect any of the evaluated variables, including plant density (DP), PH, TGW, NG, NP, and grain yield (YLD). In contrast, Zn doses significantly influenced TGW, NG, NP, and YLD ($p \leq 0.05$), whereas DP and PH were not affected. No significant interaction between application timing and Zn dose (A × Zn) was detected for any trait, indicating that the crop response was driven primarily by the Zn application rate rather than by the reproductive stage at which the nutrient was applied.

Table 1

Analysis of variance for plant density (DP), plant height (PH), number of pods per plant (NP), number of grains per plant (NG), thousand-grain weight (TGW), and grain yield (YLD) of soybean as affected by zinc doses and application timing during the 2022/2023 growing season

Causes of variation	P-VALUE						
	L.D.	DP	PH	TGW	NG	NP	YLD
Application timing (A)	1	0.1966	0.979	0.8891	0.6495	0.6495	0.4569
Zn Doses (Zn)	4	0.1764	0.3904	**0.0131***	**0.0245***	**0.0245***	**0.0421***
A x Zn	4	0.1577	0.166	0.7835	0.3648	0.3648	0.7893
Block	3	0.1227	0.2276	0.8671	0.7765	0.7765	0.3893
Residue	27	-	-	-	-	-	-
C.V. (5%)		5.3	8.75	6.14	22.49	21.72	24.31
Overall Average		30.7	1.23	146.57	168.71	68.84	3,591.88

*: Significant at 5%. Number of plants per m^2 (DP), plant height (PH), thousand-grain weight (TGW, g), number of grains (NG), number of pods (NP), and yield (YLD, kg ha^{-1}).

The coefficients of variation ranged from 5.30% for NP to 24.31% for YLD, indicating an acceptable experimental precision. Mean values across treatments were 30.7 plants m^{-2} for DP, 1.23 m for PH, 146.57 g for TGW, 168.71 grains per plant for NG, 68.84 pods per plant for NP, and 3,591.88 kg ha^{-1} for YLD.

Quadratic regression analysis revealed significant responses of NP, NG, TGW, and YLD to increasing Zn doses during the 2022/2023 season (Figure 1). For all variables, the fitted models indicated an initial increase, followed by a decline at higher Zn concentrations. Maximum values were estimated at Zn doses close to 1.0 L ha^{-1}, at which NP reached approximately 55 pods per plant, NG exceeded 120 grains per plant, TGW approached 110 g, and grain yield surpassed 4,000 kg ha^{-1}. These responses indicated that moderate Zn supply enhanced pod formation, grain set, seed mass accumulation, and overall productivity, whereas excessive Zn application reduced crop performance.

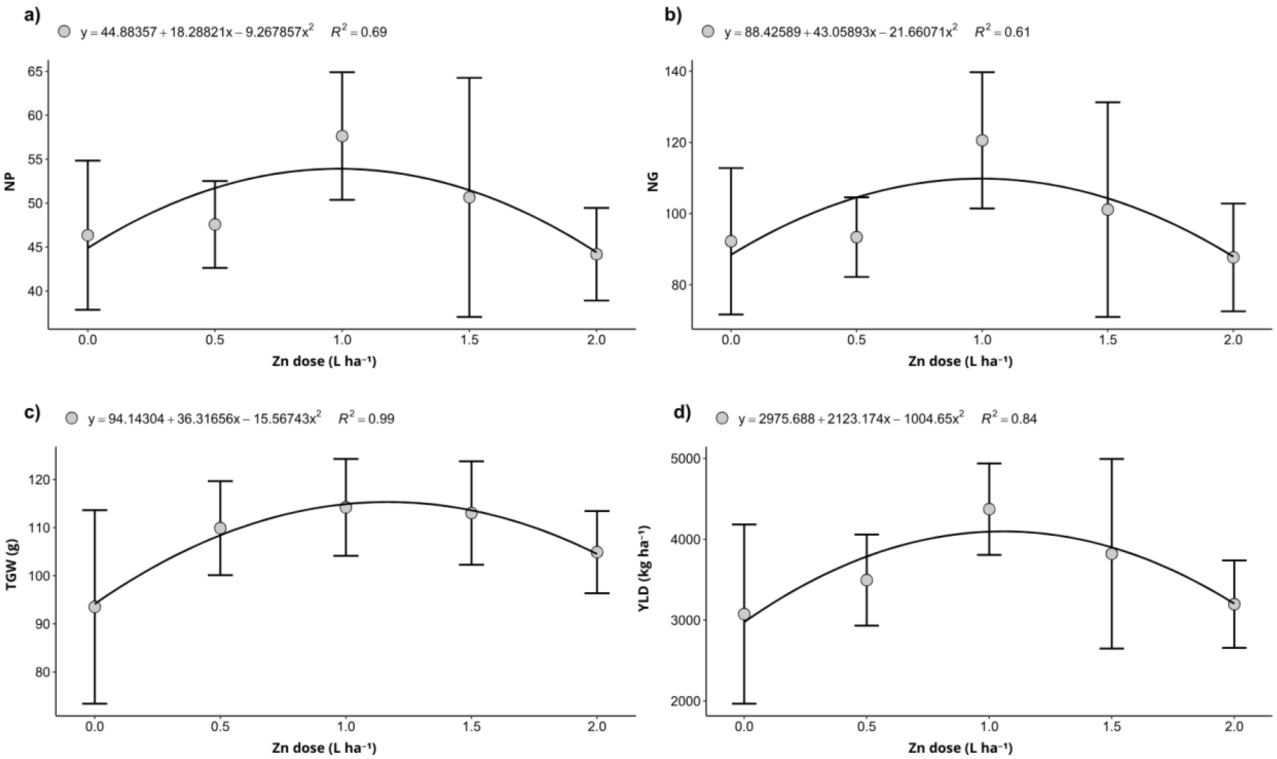

Figure 1. Quadratic regression analysis of the effects of zinc doses on the number of pods per plant (NP), number of grains per plant (NG), thousand-grain weight (TGW), and grain yield (YLD) of soybean during the 2022/2023 growing season.

In the 2024/2025 growing season, analysis of variance (Table 2) indicated no significant effect of application timing (A) on any of the evaluated variables. The zinc dose significantly affected TGW, NG, and YLD ($p < 0.05$), whereas DP, PH, and NP were not affected. No significant interaction between the application timing and Zn dose (A × Zn) was observed, confirming the consistency of the dose-dependent response across seasons.

Table 2

Analysis of variance for plant density (DP), plant height (PH), number of pods per plant (NP), number of grains per plant (NG), thousand-grain weight (TGW), and grain yield (YLD) of soybean as affected by zinc doses and application timing during the 2024/2025 growing season

Causes of variation	G.L.	P-VALUE					
		DP	PH	TGW	NG	NP	YLD
Application timing (A)	1	0.2976	0.5087	0.8896	0.6495	0.48845	0.8896
Zn Doses (Zn)	4	0.2865	0.1370	**0.0131***	**0.0245***	0.2556	**0.0131***
A x Zn	4	0.3517	0.8993	0.7835	0.3648	0.4158	0.7835
Block	3	0.1239	0.4215	0.8671	0.7765	0.9161	0.8671
Residue	27	-	-	-	-	-	-
C.V. (5%)		6.7	4.7	6.14	22.59	27.15	6.14
Overall Average		24.3	1.00	146.57	168.70	71.19	4,299.39

*: significant at 5%. Number of plants per m^2 (DP), plant height (PH), thousand-grain weight (TGW, g), number of grains (NG), number of pods (NP), and yield (YLD, kg ha^{-1}).

The coefficients of variation were low for PH (4.70%), TGW (6.14%), and YLD (6.14%) but higher for NG (22.59%) and NP (27.15%). Mean values were 24.3 plants m^{-2} for DP, 1.00 m for PH, 146.57 g for TGW, 168.70 grains per plant for NG, 71.19 pods per plant for NP, and 4,299.39 kg ha^{-1} for YLD.

Quadratic regression models demonstrated significant responses of NG, TGW, and YLD to Zn doses during the 2024/2025 season (Figure 2). NG exhibited a strong quadratic response (R^2 = 0.96), with maximum values of approximately 200 grains per plant at Zn rates near 1.0 L ha^{-1}. TGW also increased with Zn application, reaching peak values close to 155 g at intermediate doses, before declining at higher rates (R^2 = 0.61). Grain yield followed a similar pattern, with maximum values around 4,600 kg ha^{-1} at Zn doses between 0.8 and 1.0 L ha^{-1} (R^2 = 0.61).

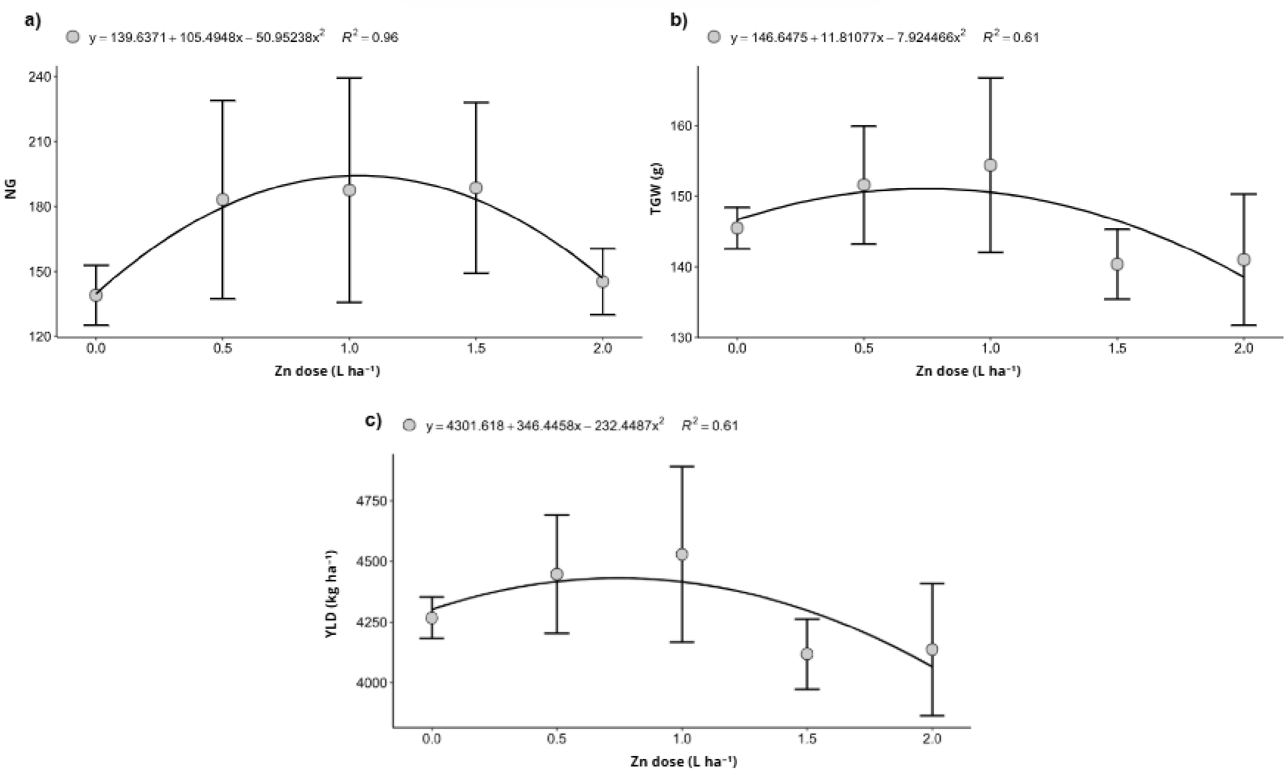

Figure 2. Quadratic regression analysis of the effects of zinc doses on the number of grains per plant (NG), thousand-grain weight (TGW), and grain yield (YLD) of soybean during the 2024/2025 growing season.

Correlation analysis revealed contrasting yield formation patterns between the growing seasons (Figure 3). In 2022/2023 (Figure 3a), the grain yield was strongly and positively correlated with NP (*r* = 0.88), NG (*r* = 0.85), and TGW (*r* = 0.70), indicating that productivity was largely determined by sink establishment, particularly pod and grain numbers. NP and NG were almost perfectly correlated (*r* = 0.99), whereas PH showed no significant association with the yield or other yield components.

In contrast, in the 2024/2025 season (Figure 3b), although NP and NG remained strongly correlated (*r* = 0.96), their association with grain yield was weak (*r* = 0.21). TGW also showed a positive but low correlation with yield (*r* = 0.21), whereas PH exhibited weak correlations with all variables. These results indicate reduced sink limitation under the environmental conditions of this season, with yield formation less dependent on the individual yield components.

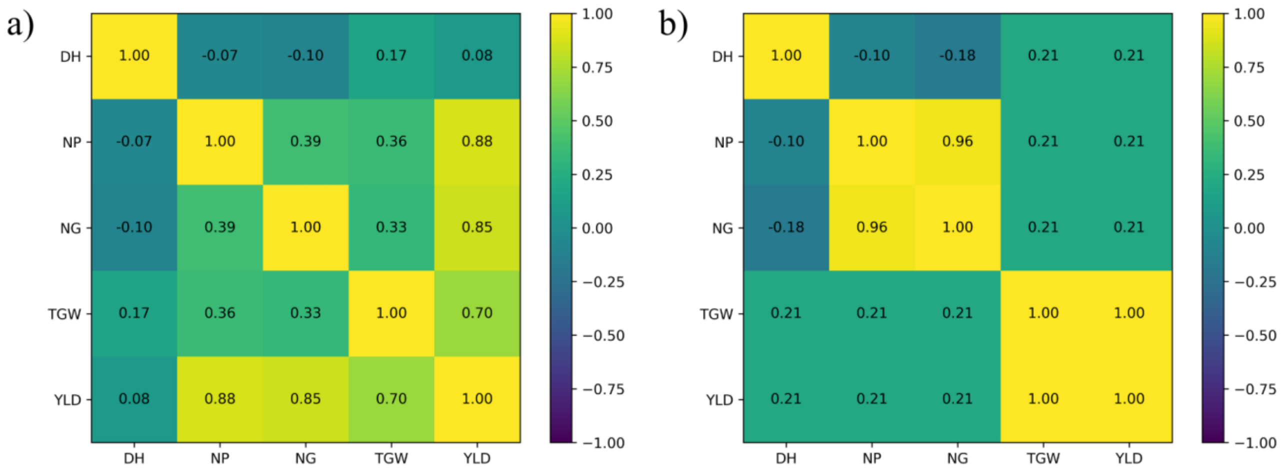

Figure 3. Pearson correlation matrices among plant height (PH), number of pods per plant (NP), number of grains per plant (NG), thousand-grain weight (TGW), and grain yield (YLD) of soybean in the 2022/2023 (a) and 2024/2025 (b) growing seasons.

Climatic conditions differed markedly between the growing seasons (Figure 4). The 2022/2023 season was characterized by an irregular rainfall distribution and greater thermal variability, whereas the 2024/2025 season exhibited more evenly distributed precipitation and more stable temperatures. These contrasting conditions coincided with differences in yield determination: productivity was more strongly associated with pod and grain numbers under the more restrictive conditions of 2022/2023, whereas in 2024/2025, grain yield was influenced by a more balanced contribution of grain number and TGW.

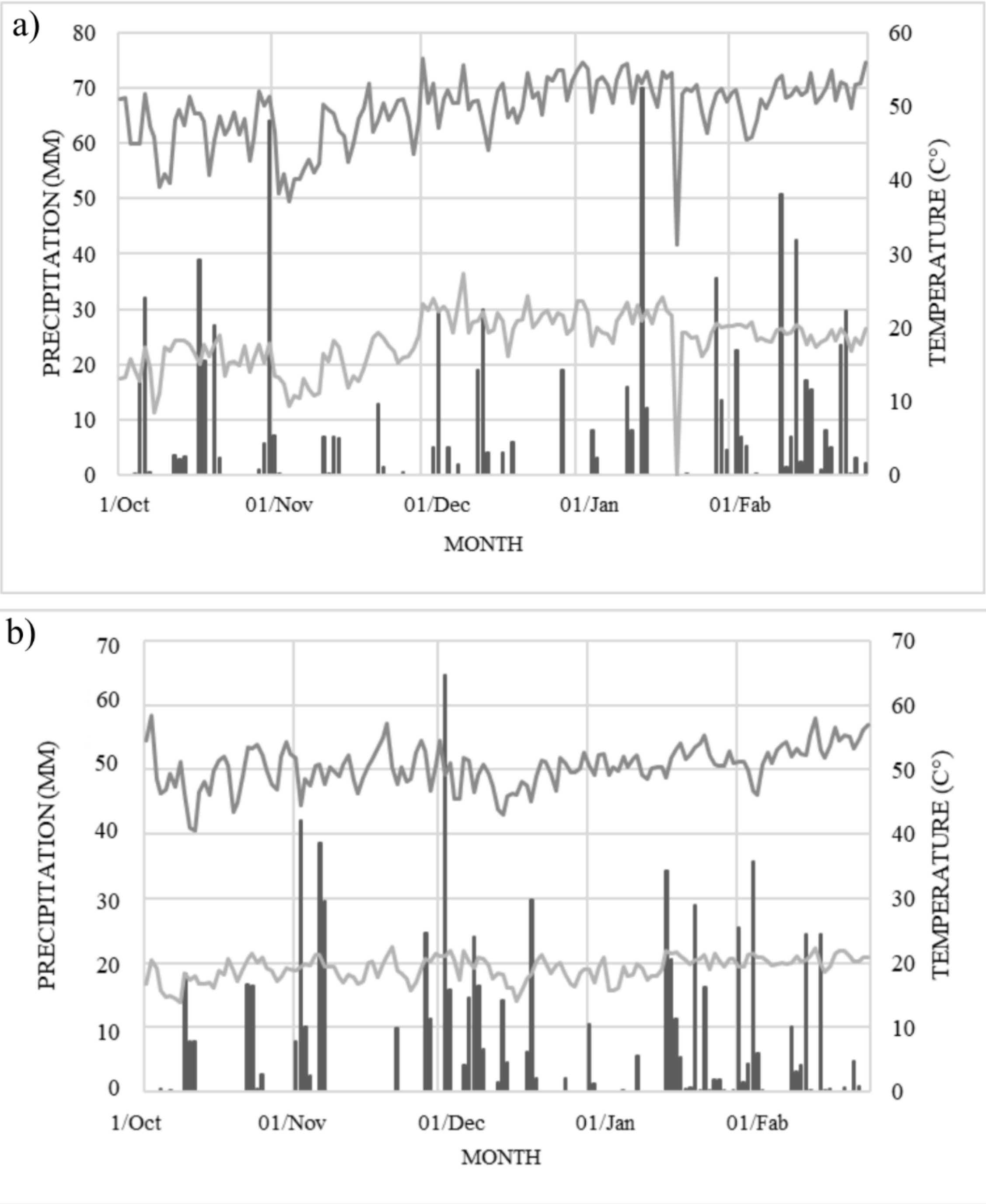

Figure 4. Rainfall distribution and mean air temperature during the soybean growing cycles in the 2022/2023 and 2024/2025 seasons.
Climate data were obtained from a weather station at the Experimental Farm of the State University of Londrina (FAZESC/UEL).

Discussion

The results demonstrated that the response of soybean to foliar Zn application was consistently driven by Zn dose rather than by application timing, across growing seasons. The absence of significant effects of the application stage (R1 vs. R5.1) and the lack of interaction between timing and Zn dose indicated that under the conditions of this study, the soybean crop exhibited a similar responsiveness to foliar Zn supply across the evaluated reproductive stages. This suggests that Zn demand during reproductive development is not restricted to a narrow phenological window but rather reflects a broader period of physiological requirements.

During the 2022/2023 growing season, Zn doses will significantly affect all yield components directly related to sink establishment, including the NP per plant, the NG per plant, TGW, and grain yield. The quadratic response observed for these variables indicated that a moderate Zn supply enhanced reproductive differentiation and grain set, whereas excessive doses negatively affected crop performance. The strong correlations between grain yield and NP, NG, and TGW reinforce the idea that yield formation in this season was predominantly determined by sink size, particularly pod and grain number.

In contrast, during the 2024/2025 growing season, Zn dose influenced NG, TGW, and YLD, whereas NP was not significantly affected. Although NP and NG remained strongly correlated, their association with grain yield was markedly weaker than that observed in the previous season. This indicates a shift in the yield formation dynamics, in which the grain-filling efficiency, expressed by TGW, played a relatively greater role in determining the final yield. The consistent quadratic responses of NG, TGW, and YLD to Zn doses further confirmed that intermediate Zn levels optimized the physiological processes associated with grain development, whereas higher doses resulted in diminishing returns.

The distinct correlation patterns observed between seasons were closely associated with the contrasting climatic conditions. In 2022/2023, irregular rainfall and greater thermal variability likely constrained assimilate availability, making the yield more dependent on pod and grain establishment. Under these conditions, Zn-induced improvements in reproductive differentiation and grain sets directly translated into higher yields. Conversely, in the more climatically stable 2024/2025 season, reduced environmental stress likely alleviated sink limitation, diminishing the relative influence of pod and grain numbers on yield, and increasing the importance of grain filling, as reflected by TGW.

Across both seasons, the regression analyses consistently identified Zn doses close to 1.0 L ha^{-1} as optimal for maximizing grain yield. At these rates, increases in NG, TGW, and YLD were observed, whereas higher doses led to a decline in performance, confirming the narrow threshold between Zn sufficiency and excess. This pattern highlights the importance of precise dose management in foliar Zn fertilization, as both Zn deficiency and oversupply can negatively affect yield components.

Overall, these findings indicate that foliar Zn application enhances soybean yield, primarily through dose-dependent effects on reproductive development and grain filling, with seasonal environmental conditions modulating the relative contributions of individual yield components. The consistency of optimal Zn doses across contrasting seasons reinforces the robustness of intermediate application rates. In contrast, the lack of timing effects suggests flexibility in the choice of reproductive stage for foliar Zn application under similar edaphoclimatic conditions.

Conclusion

Foliar Zn application in soybeans at reproductive stages R1 and R5 proved to be an agronomically effective strategy to optimize yield components, particularly TGW and overall productivity. The analyses indicated a significant and consistent response across the evaluated growing seasons, with the range of 0.75 to 1.06 L ha^{-1} identified as optimal.

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