

The effects of foliar application of selenium sources at different soybean development stages, focusing on leaf morphology, development, and grain yield

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The effects of foliar application of selenium sources at different soybean development stages, focusing on leaf morphology, development, and grain yield

Abstract: This study aimed to understand the effects of foliar application of selenium sources at different soybean development stages, focusing on leaf morphology, development, and grain yield. The experiment was carried out in December 2024, at the “Capão da Onça” School Farm of the State University of Ponta Grossa (UEPG), in the South-Central region of the State of Paraná, Brazil. The experimental design was a completely randomized block (RBC) design in a 2 x 3 factorial arrangement, where the first factor was application at the Vegetative7 and Reproductive1 development stages, interacting with the selenium sources: selenate, selenite, and absence of the nutrient, totaling 6 treatments with four replicates, totalling 24 plots. The selenium influences soybean plant development. The use of 10 µM selenate improves soybean crop development, grain yield, palisade parenchyma thickness, xylem diameter, phloem diameter, stomatal density and stomatal functionality. Selenium application at the Vegetative7 development stage can result in increased plant height, palisade parenchyma thickness, and phloem diameter.

Key words: *Glycine max* L., micronutrient, plant growth, grain production

Učinki foliarne uporabe virov selena v različnih razvojnih fazah soje, s poudarkom na morfologiji listov, razvoju in pridelku zrn

Izveček: Namen te raziskave je bil razumeti učinke foliarne uporabe selena v različnih razvojnih fazah soje, pri čemer se je osredotočila na morfologijo listov, razvoj in pridelek zrnja. Poskus je bil izveden decembra 2024 na šolski kmetiji »Capão da Onça« državne univerze Ponta Grossa (UEPG) v južni osrednji regiji zvezne države Paraná v Braziliji. Zasnova poskusa je bil popolni naključni bločni poskus (RBC) v faktorski razporeditvi 2 x 3, kjer je bil prvi dejavnik uporaba na razvojnih stopnjah “Vegetative7” in “Reproductive1”, v interakciji z viri selena: selenat, selenit in odsotnost hranila, skupaj 6 obravnavanj s štirimi ponovitvami, skupaj 24 ploskev. Selen je vplival na razvoj rastlin soje. Uporaba 10 µM selenata je izboljšala razvoj rastlin soje, pridelka zrn, debeline parenhima palisad, premera ksilema, premera floema, gostote in funkcionalnosti rež. Uporaba selena v razvojni fazi “Vegetative7” lahko povzroči povečanje višine rastlin, debeline palisadnega parenhima in premera floema.

Gljučne besede: *Glycine max* L., mikrohranila, rast rastlin, tvorba zrnja

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1 INTRODUCTION

Soybeans (*Glycine max* L.) are important to the Brazilian economy, as they are the most widely produced crop in the country, leading in both cultivated area and volume of grains harvested. This makes the country one of the world's largest exporters of soybeans, both in the form of raw grains and processed products such as meal and oil, with the main destinations being China, the European Union, and other Asian countries. In the 2024/25 harvest, production is estimated at approximately 169.49 million tons (Oliveira *et al.*, 2024), with a planted area of 47.61 million hectares (Fabião *et al.*, 2025) and an average productivity of 3,200 kg ha⁻¹ (Nakatani *et al.*, 2024).

Soybeans, when exposed to unfavorable environmental conditions, may exhibit restricted growth and low productivity due to physiological responses occurring through the expression of antioxidant enzymes and morphological changes in the conformation of the internal structures of the plant's organ tissues (Cunha *et al.*, 2023). It is important to look for alternatives to minimize these negative effects. Good management is necessary from sowing to harvesting, where the application of micronutrients via foliar application can favor the action of enzymes such as zinc (Zn), nickel (Ni), molybdenum (Mo) and even selenium (Se) (Lisboa *et al.*, 2024).

Selenium is not considered an essential nutrient for plants, but it has beneficial effects in small amounts (Cunha *et al.*, 2023). This element can promote greater vegetative and root development. It also plays a role in resistance to abiotic stresses such as drought, salinity, and heavy metals, through its antioxidant defense action in increasing the concentration of the enzyme catalase (CAT), superoxide dismutase (SOD), and peroxidase (POD), which combat reactive oxygen species (ROS) (Huang *et al.*, 2022). This element also helps in the absorption of nitrogen (Cunha *et al.*, 2023) and phosphorus (Mora *et al.*, 2008), and helps in the process of resistance to fungal or bacterial infections (Perfileva & Krutovsky, 2024; Zian *et al.*, 2024). Selenium can be used for different purposes in different crops; it can be applied to soybean, wheat and rice plants in biofortification programs (Yang *et al.*, 2003; Desouky *et al.*, 2024) and in forage crops for indirect animal supplementation (Mrltina *et al.*, 2024).

Choosing the best method of selenium application can help the producer, and it can be applied directly to deficient soils, generally with sources of sodium selenate or selenite, at a concentration of 1.0 to 10 g ha⁻¹, which are then absorbed by the roots (Burmistrov *et al.*, 2025). It can also be applied in seed treatment before sowing in the form of a liquid solution, it can improve germination and initial vigor of plants (Zeeshan *et al.*, 2024). Another way to apply selenium is via foliar application through

the cuticle or via stomata. In very low concentrations, its absorption is rapid, being up to eight times more efficient than absorption via soil (Borbély *et al.*, 2021). In soybean crops, small doses of between 10 and 50 g ha⁻¹ in the form of elemental Se are generally recommended, through foliar application between the Vegetative7 and Vegetative8 development stages. It is important to emphasize that an excess of this element in plants can cause toxicity (Martinez *et al.*, 2009).

Selenium, when applied in adequate doses, can cause several positive changes in the leaf tissues of plants, reducing lipid peroxidation (Cunha *et al.*, 2022; Cunha *et al.*, 2023), preserving leaf structures for longer, and maintaining efficiency in physiological processes in cellular organelles, especially in chloroplasts, which can increase chlorophyll a and b levels, favor photosynthesis and making leaves greener and more active (Hu *et al.*, 2024). It can increase the thickness of the palisade and spongy parenchyma, as Se induces thickening of the leaf tissue, which may be linked to greater photosynthetic capacity (Desouky *et al.*, 2024). In plants such as safflower (*Carthamus tinctorius* L.), use with selenium resulted in increased stem diameter and thickening of the cortical parenchyma, even under salt stress (Fatahiyan *et al.*, 2025).

In *Atractylodes macrocephala* Koidz., foliar application of selenium significantly increased leaf area and chlorophyll content, indicating improvements in the photosynthetic capacity of the crop, as pointed out by Zhou *et al.*, (2021). In tomato plants subjected to water stress, foliar application of selenium reduced the malondialdehyde (MDA) content in the leaves, indicating lower lipid peroxidation and, therefore, greater integrity of cell membranes as observed by Song *et al.*, (2024). Many changes in the internal tissues of plant organs are not visible to the naked eye, which makes it necessary to understand these characteristics for informed decision-making when choosing the timing of foliar application and the source of selenium.

This study aimed to understand the leaf morphology of soybeans cultivated after foliar application of selenium sources at different development stages.

2 MATERIAL AND METHODS

2.1 CHARACTERISTICS OF THE EXPERIMENTAL AREA

The experiment was carried out in December 2024, at the "Capão da Onça" School Farm of the State University of Ponta Grossa (UEPG), in the South-Central region of the State of Paraná, with geographic coordinates

Table 1: Chemical attributes of the soil at the time of experiment installation.

pH	OM	P	K	Ca	Mg	H+Al	Al ³⁺	SB	CEC	V %	M %
CaCl ₂	gdm ⁻³	mgdm ⁻³									
4.7	21	43	2.5	19	8.0	38	8.0	29.5	67.5	44	21

OM: Organic matter; SB: Sum of bases; CEC: Cation exchange capacity; V %: Base saturation; m %: Aluminum saturation.

25°05'34" South latitude and 50°02'53" West latitude, with an altitude of 1027 m. The climate of the region, according to Köppen (1970), is classified as Cfb (mesothermal, humid, subtropical), characterised by uniformly distributed rainfall and no defined dry season. The maximum and minimum temperatures are 22 and 13 °C respectively, with an average annual rainfall of 1550 mm.

The soil was classified as Red Latosol, medium texture (Embrapa, 2013) and presented the following chemical attributes as shown in Table 1.

2.2 EXPERIMENTAL DESIGN

The experimental design was in completely randomized blocks (DBC), in a 2x3 factorial scheme, where the first factor was the application at the Vegetative7 (V7) and Reproductive1 (R1) development stages, interacting with the selenium sources, namely: sodium selenate, sodium selenite and absence (control) of the nutrient, totaling six treatments with four replications totaling 24 plots.

2.3 IMPLEMENTATION AND CONDUCTION OF THE EXPERIMENT

The experimental plots consisted of six planting rows spaced 0.45 m apart and 8.0 m long, totaling an area of 21.6 m². The soil was amended and fertilized according to the nutritional needs of the soybean crop, as per Cantarella et al. (2022). Soybean cultivar Zeus IPRO Brasmax® was sown at a density of 18 seeds per meter and with a spacing of 0.45 m between rows. Base fertilization at sowing was carried out by applying 315 kg ha⁻¹ of the formulation 0-20-20 (N-P₂O₅-K₂O). The soybean seeds had a germination power of 80 % and a purity of 99 %. At sowing, the liquid inoculant Gelfix 5 with *Bradyrhizobium elkanii* (Kuykendall et al. 1992) (SEMIA 587 and SEMIA 5019) was used at a concentration of 5.0 × 10⁹ CFU ml⁻¹, at a dose of 600 ml ha⁻¹ via furrow.

All cultural treatments were performed as needed, with the fungicides copper oxychloride 588 g l⁻¹ active ingredient (a.i.) applied at a dose of 1.0 l ha⁻¹, and Pyraclostrobin (167 g l⁻¹ a.i.) + Fluxapyroxad (333 g l⁻¹ a.i.) at a dose of 350 ml ha⁻¹ of the commercial product. Applica-

tions were made with the aid of a motorized backpack pump, at constant pressure, with a spray volume of 150 l ha⁻¹, with a cone-type nozzle. There was no need to apply insecticides during the soybean crop cycle.

At the V7 R1 development stages of soybeans, selenium sources were applied at a concentration of 10 µM. A motorized backpack pump with constant pressure was used, where a spray volume of 150 l ha⁻¹ was applied, with a cone-type nozzle.

2.4 DEVELOPMENT PARAMETERS

At the Reproductive5.5 development stage, five random plants were selected within the useful area of the experimental plots, totaling a sample of 20 plants per treatment, where the following variables were determined: number of leaves (NL) and number of pods (NP) obtained by direct counting on the plant and the height of the plant (HP) with the aid of a ruler graduated in millimeters.

2.5 LEAF HISTOLOGICAL PARAMETERS

A leaf fragment from the first fully expanded trifoliolate was also collected from the apex of each plant. All plant tissue fragments underwent the appropriate procedures for dehydration, diaphanization, embedding, and histological paraffin. Using a microtome, eight-µm-thick cross sections were cut from each tissue fragment.

The slides were observed under an optical microscope with a camera attached to perform measurements of the histological variables using an imaging program, calibrated with a microscopic ruler at the same magnification, where the palisade parenchyma thickness (PPT); leaf xylem diameter (XD); leaf phloem diameter (PD) (Kraus and Arduim, 1997). For all variables, ten measurements were taken per slide, where each plant was represented by the average of these measurements.

Printing was also performed on the lower or abaxial epidermal surface of the collected leaf fragments using cyanoacrylate ester to determine the stomatal functionality of the lower or abaxial surface (SF) and stomatal density of the lower or abaxial surface (SD) (Castro et

al., 2009). For all variables, 10 measurements were performed per slide. The plants were represented by the mean value obtained from the measurements of each characteristic.

2.6 PRODUCTION PARAMETER

At the Reproductive8 stage, pods from plants within one linear meter were randomly collected along the three central rows of each plot, and the plant population per hectare was estimated. The pods were threshed, and the grains were weighed and converted to 14 % moisture content. Grain yield in kg ha⁻¹ was estimated.

2.7 STATISTICAL ANALYSIS

For statistical evaluation, the variables were subjected to normality tests using the Shapiro-Wilk test. After complying with the test precepts, analysis of variance was performed using the F-test ($p < 0.05$) and their means compared using the Scott-Knott test at 5 % probability (Banzatto and Kronka, 2013). To identify the dependent variables, a Pearson correlation analysis ($p \leq 0.05$) was performed and presented as a colored heat map. In addition, a principal component analysis (PCA) was performed (Lisboa *et al.*, 2024) using the R statistical program (R Core Team, 2019).

3 RESULTS

The significant effect was observed in isolation only for the selenium source factor, where selenate presented a higher average for the leaf number (NL), which was approximately 17.02 % higher than the selenite source, which presented a lower average, as shown in Table 2.

For plant height (PH), the effect of the factors was observed in isolation, where the application at the V7 stage presented the highest average, and the use of selenite presented the highest average, which represented approximately 13.06 % higher in relation to the absence of selenium (Control), as shown in Table 2.

Once again, the effect of the selenium source on the number of pods (NP) was observed in isolation, where selenate presented a higher average, 33.62 % higher than the treatment without selenium (control) (Table 2).

In grain production kg ha⁻¹, a significant effect was observed only in the selenium source, where selenate provided greater grain production, 24.53 % higher than the treatment without the use of selenium (control) as observed in Table 1.

Significant Pearson correlations were observed between plant height (PH) and grain production in kg ha⁻¹, also between palisade parenchyma thickness (PPT) and xylem diameter (DX) and stomatal density (SD), and between stomatal density (SD) and stomatal functionality (SF), as shown in Figure 1.

An interaction between the factors in the palisade

Table 2: Average values of leaf number (NL); plant height (PH); number of pods (NP) and production in kg ha⁻¹ of soybean cultivated after foliar application of selenium sources at different development stages.

Stage(S)	NL	PH (cm)	NP	kg ha ⁻¹
V7	14.01a	56.81a	15.75a	3605.59a
R1	13.10a	54.80b	14.75a	3485.59a
p-value	0.0876ns	0.0300*	0.0610ns	0.2521ns
Selenium (Se)				
Absence	12.95b	51.30b	11.55b	3165.55b
Selenate	15.15a	57.11a	17.40a	4194.61a
Selenite	12.57b	59.01a	16.80a	3276.62b
p-value	0.0002**	0.0001**	0.0001**	0.0001**
p-value SxSe	0.9931ns	0.9993ns	0.0710ns	0.9990ns
p-value Block	0.0002**	0.0001**	0.0001**	0.0008**
CV%	21.45	8.94	18.94	16.08
OM	13.55	55.80	15.25	3545.59

** - significant at the 1 % probability level ($p < 0.01$); ns - not significant ($p > = 0.05$). Lowercase letters in the same column do not differ statistically from each other. The Scott-Knott method was applied at the 5 % probability level. CV: coefficient of variation; OM: overall mean.

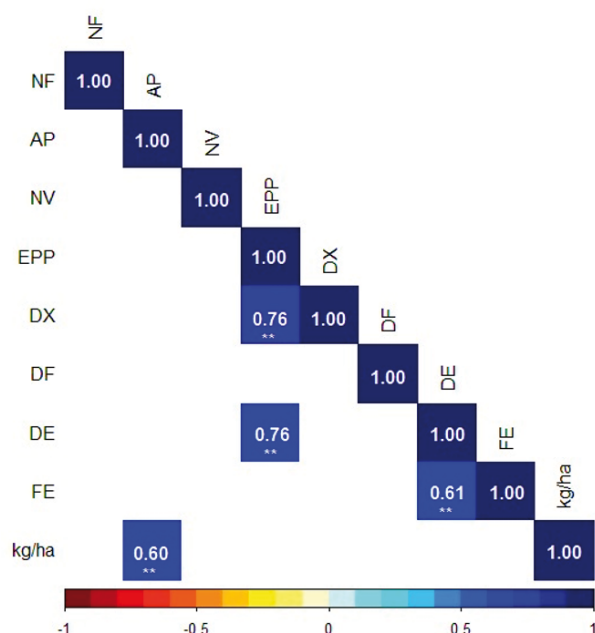


Figure 1: Significant Pearson correlations between the variables evaluated in soybeans cultivated after foliar application of selenium sources at different development stages.

parenchyma thickness (EPP) parameter was observed, as shown in Table 3.

The application of selenate at the V7 stage of soybean showed higher averages in the thickness of the palisade parenchyma (PPT), representing a difference of

approximately 34 % higher in relation to the non-application of selenium at the R1 stage, as shown in Table 4.

In the xylem diameter (XD) parameter, a statistical difference was observed only in the selenium source factor, where again the use of selenate presented a higher average, which implied approximately 37.65 % higher in relation to the non-use of selenium (control), as observed in Table 3.

An isolated statistical difference was observed between the factors application at the developmental stage (V7 and R1) and selenium sources, in phloem diameter (PD) parameter, where the application of selenium at the V7 stage was 12.26 % higher than the R1 application, and the use of selenite was superior to the absence of selenium (control), which implied a difference of approximately 12.26 % between the sources (Table 3). These differences in the tissues are notable when the selenium sources were applied at different development stages, as observed in Figures 2 B; C; D and E, in relation to Figure 2 A (absence), which shows the internal tissues of the leaves of soybean cultivated after foliar application of selenium sources at different development stages.

Regarding the principal component analysis (PCA), when considering the application stages and their effect on the contribution to the responses of the evaluated parameters, it was observed in Figure 3A that the application of selenium in the development stages in Vegetative7 (Red) and Reproductive1 (Blue) presented similar contributions, thus confirming the results presented in Tables 2 and 3.

Table 3: Average values of palisade parenchyma thickness (PPT); xylem diameter (XD); phloem diameter (PD); stomatal density (DE) and stomatal functionality (SF) of soybean leaves cultivated after foliar application of selenium sources at different development stages.

Stage (S)	PPT (μm)	XD (μm)	PD (μm)	SD ($\text{n}^\circ/\text{mm}^2$)	SF
V7	64.23a	16.68a	5.38a	208.63a	2.18a
R1	61.73b	17.66a	4.72b	212.31a	2.23a
p-value	0.0051**	0.0710ns	0.0001**	0.3221ns	0.3069ns
Selenium (Se)					
Absence	50.44c	14.29b	4.73b	197.91b	2.24b
Selenate	75.76a	22.92a	5.17a	227.35a	2.41a
Selenite	62.74b	14.31b	5.24a	206.16b	1.96c
p-value	0.0001**	0.0001**	0.0102*	0.0001**	0.0001**
p-value SxSe	0.0075**	0.4795ns	0.3689ns	0.4843ns	0.7307ns
p-value Block	0.0001**	0.0002**	0.0008**	0.0001**	0.0011**
CV%	7.57	17.13	15.64	9.61	14.01
OM	62.98	17.17	5.05	210.47	2.20

** - significant at the 1 % probability level ($p < 0.01$); ns - not significant ($p > 0.05$). Lowercase letters in the same column do not differ statistically from each other. The Scott-Knott method was applied at the 5 % probability level. CV: coefficient of variation; OM: overall mean.

Table 4: Average values of the interaction of the thickness of the palisade parenchyma (PPT) of soybean leaves cultivated after foliar application of selenium sources at different development stages.

Stage (S)	Selenium (Se)			p-value (S)
	Absence	Selenate	Selenite	
V7	50.74aC	75.99aA	65.97aB	0.0001**
R1	50.15aC	75.54aA	59.51bB	0.0001**
p-value (Se)	0.6992ns	0.7638ns	0.0001**	

** - significant at the 1% probability level ($p < 0.01$); ns - not significant ($p > 0.05$). Lowercase letters in the same column do not differ statistically from each other. Uppercase letters in the same row do not differ statistically from each other. The Scott-Knott method was applied at the 5 % probability level.

When selenium sources are considered, it is clear that selenate, represented by the color blue, presented the greatest contribution to the evaluated parameters, followed by the selenite source (Green) and finally the absence of selenium use (Red) as demonstrated in Figure 3B. These results contribute to and reinforce the answers already presented in Tables 2 and 3.

In the stomatal density (SD) parameter, a statistical difference was observed only in the selenium source factor, where the selenate source presented a higher average, representing 12.94 % higher in relation to the absence of selenium (control). A statistical difference was also observed in stomatal functionality (SF) only in the selenium source factor, where again the use of selenate presented a higher average, with approximately 18.67 %

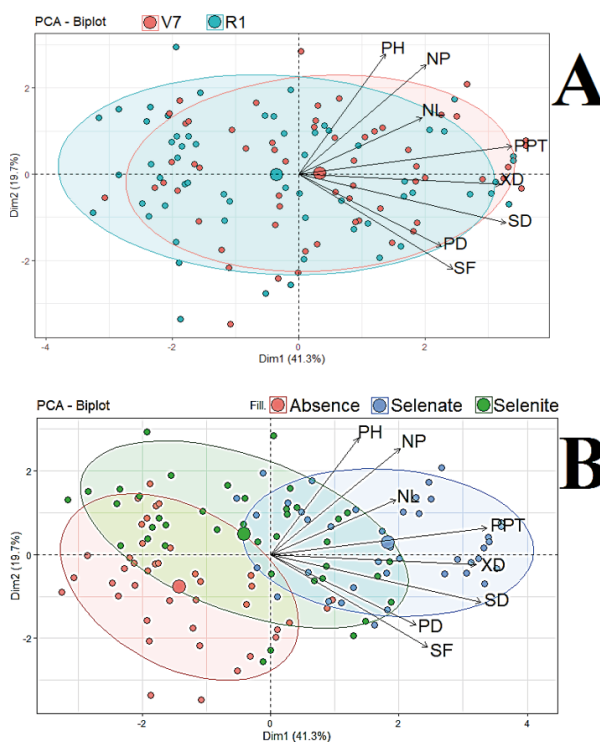


Figure 3: Principal component analysis between variables in soybean cultivated after foliar application of selenium sources at different development stages. Development stages V7 and R1; Red = Absence of Se; Blue = Organic Selenate and Green = Selenite.

higher in relation to the selenite source, which presented a lower average, as shown in Table 3.

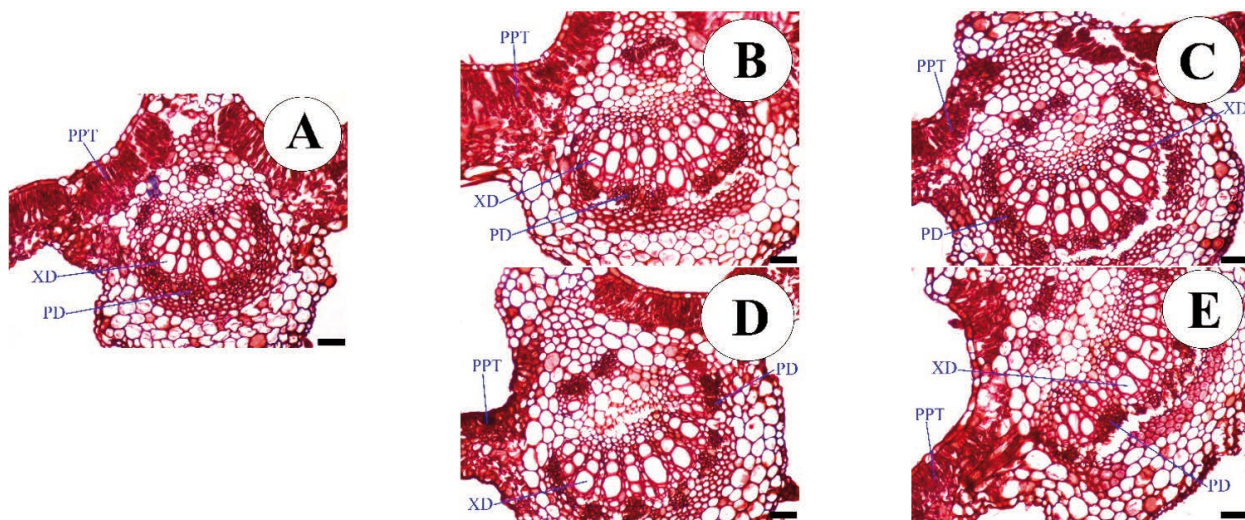


Figure 2: Internal tissues of soybean leaves after foliar application of selenium sources at different development stages. A – Absence of selenium; B – Selenate applied at V7, C – Selenite applied at V7, D – Selenate applied at R1, and E – Selenite applied at R1. PPT – Palisade parenchyma thickness, XD – Xylem diameter, and PD – Phloem diameter. Bar = 50 μ m.

4 DISCUSSION

4.1 DEVELOPMENT AND PRODUCTION PARAMETERS

Start the paragraph with your results and then compare the with the literature data! Selenate has greater absorption compared to selenite; therefore, the correct choice of selenium source will provide greater soybean development. In general, selenite in the form SeO_2^{-3} is known to be more toxic to organisms than selenate and other organic Se compounds, as this element becomes associated with some amino acids, such as selenomethionine (SeMet), selenocystine (SeCys₂) and methyl selenocysteine (MeSeCys) by Huang et al. (2022). Therefore, selenite may have caused a negative effect on the number of leaves in the plant, equaling the absence of selenium (control) (Table 2).

When a plant has a greater number of leaves (NL), its photosynthetic complex is more efficient, providing a higher rate of carbon assimilation in its dry mass, as the carbohydrates produced in photosynthesis are allocated to plant growth and grain production. These responses are demonstrated by the correlation between plant height (PH) and grain production (Figure 1), where larger plants can yield higher productivity in soybean crops. Foliar application of selenium salts may be the best option to reduce the risk of selenium immobilization in the soil. However, the selenium source used in foliar fertilizers must be considered to avoid negative effects on crop yield (Mrltina et al., 2024).

When selenium was applied at the V7 development stage, it allowed the plant a longer period to assimilate the nutrient from its leaves and act on cellular metabolism (Table 2). Similar results were observed by Yang et al. (2003), who reported that foliar application of selenate provided greater efficiency and resulted in an increase in selenium content in soybean than soil application. Foliar application of selenium is a suitable method for crop supplementation and can improve grain yield and quality, particularly the quality of organic Se and selenium-containing amino acids (SU et al., 2024).

4.2 LEAF HISTOLOGICAL PARAMETERS

The influence of Se on the secondary structure of raw soybean protein has not yet been clarified. These changes in protein components and structure may affect their functional properties and antioxidant capacity (Huang et al., 2022) and may impact the development of parenchymal tissue cells. When the plant responds efficiently to reactive oxygen species, it exhibits greater

development of the internal tissues. When ROS neutralization is inefficient, lipid peroxidation of cell membranes and organelles begins, causing changes in the palisade parenchyma (Table 4). Changes in palisade parenchyma thickness compromise the photosynthetic rate and the rate of carbon assimilation, thus affecting plant growth and grain yield (Cunha et al., 2023; Cunha et al., 2022). The selenate source contributed the most to the results of the evaluated parameters, presenting a larger positive surface (Figure 3B). Studies conducted by Burmistrov et al. (2025) when evaluating selenium sources, stated that selenate shows better absorption by plants and does not present toxicity at high doses when compared to the selenite source.

When the plant is grown under excess selenium with doses above 10 μM , the cells present deformities in the cell wall and also in the organelles, as reported by Hu et al. (2024). Desouky et al. (2024) reported that the increase in cells in the epidermis region is a direct consequence of the secondary growth of soybean, which involves the thickening and increase in the diameter of the vascular tissues, particularly xylem and phloem, and this increase in vascular tissues can be influenced by the concentration of selenium present in these tissues (Zian et al., 2024). Murtaza et al. (2025) observed that the use of selenium improved the xylem structure in *Nicotiana tabacum* L. plants when exposed to high concentrations of boron.

A study carried out by Habibi (2013) with barley under water stress conditions highlighted that leaf stomata play an important role in the survival of plants grown under drought conditions, and when the plant is under water restriction, stomatal density (SD) can be reduced. However, Zeeshan et al. (2024) reports that spraying Se on soybean plants under water stress facilitated stomatal opening, organized the shape of stomatal cells and increased their number in a manner dependent on the selenium dose, showing the importance of this element in the morphological changes in soybean leaves.

With a greater number of stomata, gas exchange is increased, ensuring greater development of the palisade parenchyma, where this tissue is responsible for the highest rate of photosynthesis in leaves due to the large number of chloroplasts present in its cells, showing a positive correlation between stomatal density (SD) and palisade parenchyma thickness (PPT) (Figure 1). With selenium present in the plant's metabolism, its physiology may have improved through enzyme activation, and this may have been reflected in the development of stomatal structures. With a well-developed stomatal apparatus, its functionality (FE) is enhanced, ensuring greater efficiency in gas burrows due to the greater opening of the ostiole, which

increases proportionally to the size of the guard cells that make up the stomata (Castro *et al.*, 2009).

5 CONCLUSION

The selenium influences soybean plant development. The use of 10 μ M selenate improves soybean crop development, grain yield, palisade parenchyma thickness, xylem diameter, phloem diameter, stomatal density and stomatal functionality. Selenium application at the Vegetative7 development stage can result in increased plant height, palisade parenchyma thickness, and phloem diameter.

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Data availability statement

The authors state that there was no public funding for the implementation and conduct of the experiment, the costs were paid with private money, and they have no conflict of interest regarding the subject of the work.

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