

Quality of soybean cultivar seeds in response to pod position in the plant canopy

Qualidade de sementes de cultivares de soja em função da posição da vagem no dossel da planta

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<https://doi.org/10.19084/rca.46925>

Received/recebido: 2026.05.01
Accepted/aceite: 2026.05.08

ABSTRACT

The quality of soybean seeds is a determining factor for crop establishment and productivity, being influenced by physiological and sanitary aspects, as well as the position of the pods in the plant canopy. The objective of the study was to evaluate the physiological and sanitary quality of seeds from two soybean cultivars based on the position of the pods (upper, middle, and lower third) in the plant canopy. The experiment was conducted in the field using a randomized block design with split plots, evaluating the cultivars Brasmax Olimpo IPRO and CZ 58B23 I2X, with four replications. Analyses were performed for germination, vigor (accelerated aging, emergence, and emergence speed index), electrical conductivity, dry mass, and seed health, followed by statistical analysis using ANOVA and multivariate methods (canonical analysis and GGE biplot). The results indicated that the position of the seeds in the canopy significantly influences physiological quality, with the middle third being the most favorable, showing higher germination, vigor, stability, and better overall performance of the Brasmax Olimpo IPRO cultivar. The sanitary quality regarding the presence of *Fusarium* sp. and *Cercospora* sp. was also influenced by the position of the seeds on the plant, with the best performance in the middle third in both cultivars.

Keywords: *Glycine max*, pathogens, seed vigor.

RESUMO

A qualidade de sementes de soja é um fator determinante para o estabelecimento da cultura e produtividade, sendo influenciada por aspectos fisiológicos, sanitários e pela posição dos legumes no dossel da planta. O objetivo do estudo foi avaliar a qualidade fisiológica e sanitária de sementes de duas cultivares de soja em função da posição dos legumes (terço superior, médio e inferior) no dossel. O experimento foi conduzido em campo, em delineamento de blocos ao acaso com parcelas subdivididas, avaliando as cultivares Brasmax Olimpo IPRO e CZ 58B23 I2X, com quatro repetições. Foram realizadas análises de germinação, vigor (envelhecimento acelerado, emergência e índice de velocidade de emergência), condutividade elétrica, massa seca e sanidade de sementes, com posterior análise estatística por ANOVA e métodos multivariados (análise canônica e GGE biplot). Os resultados indicaram que a posição das sementes no dossel influencia significativamente a qualidade fisiológica, sendo o terzo médio o mais favorável, apresentando maior germinação, vigor e estabilidade, além de melhor desempenho global da cultivar Brasmax Olimpo IPRO. A qualidade sanitária em relação à presença de *Fusarium* sp. e *Cercospora* sp. também foi influenciada pela posição das sementes na planta, com o melhor desempenho no terzo médio em ambas as cultivares.

Palavras-chave: *Glycine max*, doenças, vigor de sementes.

INTRODUCTION

Soybean [*Glycine max* (L.) Merrill] is one of the main agricultural crops in the world. The legume stands out as an important source of plant protein (Guo *et al.*, 2022). In Brazil, soybean cultivation holds a prominent position among the commodities produced. It is estimated that in the 2025/2026 harvest, national production will reach 177 million tons, representing a 3.6% increase compared to the 2024/2025 harvest (CONAB, 2025). The expansion of soybean cultivation in Brazil is associated with the advancement of agriculture in the Cerrado biome, which has established itself as one of the main grain-producing regions of the country in recent decades (Mello *et al.*, 2025). In this context, states belonging to the agricultural frontier of MATOPIBA (an acronym for Maranhão, Tocantins, Piauí, and Bahia), such as the state of Maranhão, have shown significant growth in the area cultivated with soybeans, highlighting the importance of the crop for regional agricultural development (Cunha and Espíndola, 2016; Loaysa *et al.*, 2023).

Among the factors contributing to the success of soybean production, the use of seeds with high physiological and sanitary quality stands out. The physiological quality of soybean seeds is reflected in higher germination and vigor rates, directly influencing the initial establishment of the crop in the field (Conceição *et al.*, 2023). Seed quality can be negatively affected by sanitary factors related to the presence of diseases in the seeds (Luz and Waureck, 2025). The morphology of soybeans and the distribution of pods throughout the plant canopy can result in variations in the physiological and sanitary quality of the seeds. The vertical distribution of pods on the soybean plant is an important genetic and agronomic factor that affects the number of seeds formed and yield (Ning *et al.*, 2018). The position of pods along the canopy influences the physiological quality of the seeds, generating variability in vigor (Oliveira *et al.*, 2025).

The heterogeneity in seed quality can be explained by the source-sink relationship, in which the leaves adjacent to the pods act as the main sources of photoassimilates. During the grain filling period, the photosynthetic rate of the leaves and the subsequent translocation of assimilates via phloem to

the pods are determinants for seed growth and mass accumulation (Chiluwal *et al.*, 2022; Du *et al.*, 2024). Additionally, variations in the position of the pods within the canopy can alter the seed development microenvironment, such as light availability, temperature, and assimilates, resulting in differences in the composition and quality of seeds formed in different strata of the plant (Huber *et al.*, 2016).

In soybean cultivars with an indeterminate growth habit, flowering initially occurs at the lower nodes of the plant, which provides seeds formed in the basal portion of the canopy a potentially longer period of development (Huber *et al.*, 2016). Additionally, these seeds develop under distinct micro-environmental conditions compared to those located in the upper portions of the plant. Factors such as temperature, intensity and quality of light radiation, as well as relative humidity within the canopy, can vary along the crown and influence physiological processes during grain filling. These environmental variations are recognized to impact soybean seed development and composition (Carrera *et al.*, 2009). Furthermore, reduced fungicide penetration in the lower portions of the canopy may favor a higher incidence of foliar diseases in these regions of the plant, resulting in senescence and premature leaf loss. This process can reduce photosynthetic activity and compromise the remobilization of photoassimilates and nutrients from leaves to developing pods, potentially affecting the quality of seeds formed in these plant strata.

Given the above, the position of the pods along the plant canopy can influence the physiological and sanitary quality of soybean seeds, and this response may vary among cultivars due to genetic differences related to plant architecture, the pattern of pod distribution, and the efficiency in the source-sink relationship. Differences in the availability of photoassimilates, exposure to the micro-environment, and the grain filling process among different plant strata may result in variations in seed vigor, germination, and pathogen incidence. Therefore, the objective of the present study is to evaluate the physiological and sanitary quality of seeds from different soybean cultivars based on the position of the pods in the plant canopy.

MATERIAL AND METHODS

Characteristics of the study area

The experiment was conducted under field conditions at Fazenda Pequizeiro, at the Experimental Station of Accert Agronomic Research and Consulting Company, located in the municipality of Balsas, Maranhão, Brazil (07°31'57" S, 46°02'08" W; approximate altitude of 283 m), during the 2024/2025 agricultural season. The region's climate, according to the Koppen-Geiger classification, is tropical hot and humid (Aw), characterized by a rainy summer and dry winter (Peel *et al.*, 2007). The climatic data recorded during the experiment, along with historical climatic data from 1991 to 2020, are presented in Figure 1.

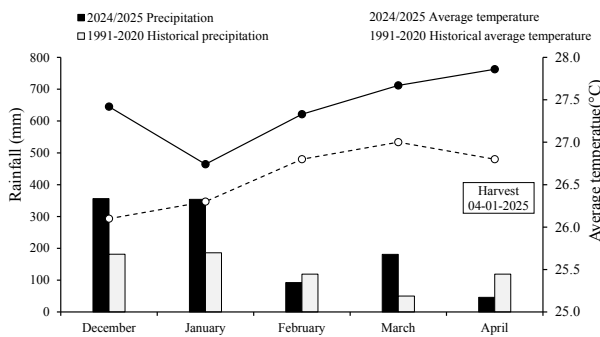


Figure 1 - Monthly averages of rainfall and temperature during the 2024/2025 agricultural season, compared with historical averages (1991-2020) in Balsas, Maranhão, Brazil. Source: INMET (2025).

The soil at the experimental site was classified as Latossolo Vermelho Amarelo with a sandy texture, according to the Brazilian soil classification system

(Santos *et al.*, 2018). Soil samples were collected from two layers: 0–20 cm and 20–40 cm, and their chemical and physical properties are summarized in Table 1.

Experimental design and description of treatments

The experimental design used was a randomized block design arranged in a split-plot scheme with four replications. In the plots, two soybean cultivars were evaluated: Brasmax Olimpo IPRO (relative maturity group 8.0, indeterminate growth habit) and CZ 58B23 I2X (relative maturity group 8.2, indeterminate growth habit). In the split-plots, three seed collection positions along the plant were evaluated (Figure 2), obtained by dividing the main stem into three equal portions based on the total number of nodes (1/3 of the nodes in each segment). Thus, the seeds were collected in the upper third (apical portion, corresponding to the last third of the nodes), middle third (intermediate portion, corresponding to the central third of the nodes), and lower third (basal portion, corresponding to the first third of the nodes). Each plot consisted of four rows spaced 0.50 m apart and 4.0 m in length, totaling an area of 8 m². The useful area considered was the two central rows, with 0.5 m discarded at each end, resulting in an area of 4 m².

Soybean sowing was carried out on December 7, 2024, using a seed-fertilizer drill equipped with a rod-type furrow opener, with seeds placed at an approximate depth of 3 cm. The crop was established with row spacing of 0.50 m and a seeding density of 15 seeds per meter. Throughout the crop cycle, weeds, pests, and diseases were monitored

Table 1 - Main chemical properties of the soils used in the experiment

Depth	pH	OM	TOC	H+Al	Al ³⁺	Ca ²⁺	Mg ²⁺	K ⁺	CEC	BS
cm	H ₂ O	--- dag/kg ---				cmol _c dm ⁻³				%
0-20	5.92	1.57	0.91	1.58	0.01	3.39	1.04	0.15	6.16	74.34
20-40	5.03	1.07	0.62	1.76	0.37	1.81	0.49	0.29	4.35	59.58
	P ^{Mehlich⁻¹}	B	Cu	Fe	Mn	Zn	S	Clay	Silt	Sand
		---mg dm ⁻³ ---							---%---	
0-20	45.71	0.16	0.25	6.92	38.16	0.01	10.29	24.24	9.26	66.49
20-40	30.24	0.18	0.24	20.52	11.51	0.37	19.32			

Abbreviations used: pH - Soil pH (Potential of Hydrogen), OM - Organic Matter, TOC - Total Organic Carbon, H+Al - Potential Acidity (Hydrogen + Aluminum), Al³⁺ - Exchangeable Aluminum, Ca²⁺ - Exchangeable Calcium, Mg²⁺ - Exchangeable Magnesium, K⁺ - Exchangeable Potassium, CEC - Cation Exchange Capacity, BS - Base Saturation, P (Mehlich⁻¹) - Phosphorus, B - Boron, Cu - Copper, Fe - Iron, Mn - Manganese, Zn - Zinc, S - Sulfur.

and controlled through the application of chemical pesticides whenever necessary to ensure proper crop development.

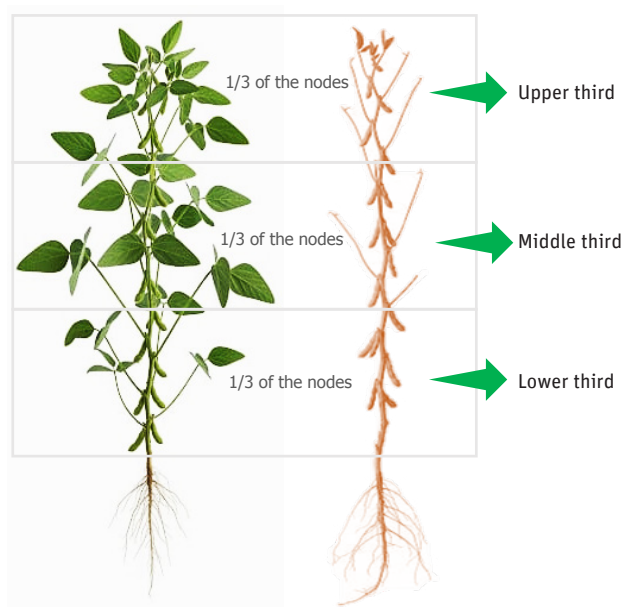


Figure 2 - Illustrative diagram of the seed collection positions along the soybean plant, obtained by dividing the main stem into three equal parts (1/3 of the nodes): upper third (apical portion), middle third (intermediate portion), and lower third (basal portion).

At full maturity (R_8 growth stage), soybean plants were manually harvested and threshed. Seed water content was determined immediately after harvest, following the methodology described by Brasil (2025). Subsequently, seed samples were standardized to a moisture content of 13% by slow drying under shaded conditions. After drying, the seed lots were uniformly mixed, thousand-seed mass was determined (Brasil, 2025), and the standardized seed samples were used for physiological and sanitary quality analyses. Several seed quality parameters were evaluated:

Germination: Seeds were arranged on three sheets of germination paper moistened with distilled water in an amount corresponding to 2.5 times the dry weight of the paper. The sheets were then rolled and placed in a BOD-type germination chamber maintained at 25 °C. Evaluations of germination

were conducted on the 5th and 8th days after sowing, following the standards described by Brasil (2025).

Shoot and Root Dry Matter: Ten seedlings were randomly selected per treatment. These were separated into shoot and root portions and subsequently dried in a forced-air oven at 60 °C for 72-hours. The dry matter was then quantified and expressed in mg per seedling.

Accelerated Aging: Seeds were distributed on stainless steel screens positioned inside plastic boxes containing 40 mL of water, as described by Marcos Filho (2020). The boxes were kept in a BOD chamber at 41 °C for 96-hours. After this period, the seeds were subjected to a germination test, with the number of normal seedlings recorded on the 5th day after sowing, in accordance with Brasil (2025).

Seedling Emergence: Seeds were sown in plastic trays filled with Carolina Soil® substrate, using four replicates of 50 seeds per treatment. The trays were maintained under greenhouse conditions, with temperatures ranging between 25 and 30 °C. Seedling emergence (E) was assessed at 12 days after sowing. Additionally, the emergence speed index (ESI) was calculated following the methodology proposed by Maguire (1962), which is based on the daily count of emerged seedlings in relation to the time required for emergence.

Electrical Conductivity: For each replicate, fifty seeds were weighed and placed in plastic containers containing 75 mL of deionized water, remaining at 25 °C for 24 hours (Vieira and Marcos-Filho, 2020). After soaking, the electrical conductivity of the solution was measured using a conductivity meter (MS Tecnoport® – mCA150), and the results were expressed in $\mu\text{S cm}^{-1} \text{ g}^{-1}$.

Seed Health: The sanitary quality of the seeds was evaluated using the Blotter test. Five replicates of 40 seeds were incubated in a controlled environment at 20 °C, under a 12-hour photoperiod, for seven days (Brasil, 2025). Following incubation, the seeds were examined under a light microscope for fungal identification, according to the methodology described by Barnett and Hunter (1998).

Statistical Analyses

The experimental data were subjected to tests to verify the assumptions of normality and homogeneity. When these assumptions were met, they were submitted to analysis of variance (ANOVA). All analyses were performed using the R statistical software (R Core Team, 2024; v. 4.4.1) with the following packages: *dplyr* (Wickham *et al.*, 2023) for data manipulation and aggregation; *MASS* (Venables & Ripley, 2002) for Box–Cox transformations in linear models; *lme4* (Bates *et al.*, 2015) for mixed model; *agricolae* (Mendiburu, 2023); and functions from the *utils* and *base* packages. For GGE Biplot analysis, graphical outputs, and other analyses, additional functions from *GGEbiplot*, *statistics*, and *graphics* packages were used.

RESULTS AND DISCUSSION

The cultivar × position interaction was significant only for the variables water content (WC), electrical conductivity (EC), and incidence of *Cercospora* sp. (CER) (Table 2), suggesting that the effect of plant position is largely consistent among the evaluated cultivars. In general, a significant effect of cultivars was observed for the variables germination (G), accelerated aging (AA), emergence speed index (ESI), and total dry matter (TDM). In turn, the seed

collection position along the plant had a significant effect not only for these same variables but also for the first germination count (FGC) and electrical conductivity (EC). Previous studies demonstrate that the seed position within the plant canopy plays an important role in determining physiological quality, particularly for traits such as germination, vigor, and electrical conductivity, with the influence of genotype and its interaction with canopy position tending to be significant (Ferreira *et al.*, 2023; Oliveira *et al.*, 2025).

After identifying significant effects through analysis of variance (ANOVA), the decision was made to use multivariate analyses, specifically canonical variate analysis and the GGE biplot, with the aim of integrating and simultaneously interpreting the set of physiological, physical, and health variables evaluated, since seed quality constitutes a multidimensional trait. These approaches allow reducing the dimensionality of the data, identifying patterns of association between treatments and variables, and jointly assessing the average performance and stability of genotypes and collection positions, providing a more comprehensive and biologically consistent interpretation of the results.

The canonical variate analysis revealed distinct patterns of association between treatments and the physiological, physical, and health variables of the

Table 2 - Estimates of mean squares for water content (WC), first germination count (FGC), germination (G), electrical conductivity (EC), accelerated aging (AA), seedling emergence (E), emergence speed index (ESI), total dry matter (TDM), and incidence of *Fusarium* sp. (FUS) and *Cercospora* sp. (CER) in two soybean cultivars (Brasmax Olimpo IPRO and CZ 58B23 I2X) and three seed collection positions along the plant (upper third, middle third, and lower third)

	WC	FGC	G	EC	AA
Cultivar (C)	0.10 ^{ns}	180.50 ^{ns}	288.00 ^{**}	16.05 ^{ns}	264.50 [*]
Position (P)	0.02 ^{ns}	903.50 ^{**}	516.50 ^{**}	275.98 ^{**}	703.50 ^{**}
C × P	0.35 ^{**}	18.50 ^{ns}	4.50 ^{ns}	65.13 ^{**}	81.50 ^{ns}
CV Plot (%)	3.42	4.70	1.02	12.67	4.89
CV Split-plot (%)	2.62	3.22	3.77	9.32	11.70
	E	ESI	TDM	FUS	CER
Cultivar (C)	10.66 ^{ns}	9.62 ^{**}	240.79 [*]	213.55 ^{ns}	1283.55 ^{ns}
Position (P)	58.50 ^{ns}	26.08 [*]	54.97 [*]	272.66 ^{ns}	683.55 ^{ns}
C × P	10.50 ^{ns}	0.36 ^{ns}	20.51 ^{ns}	137.55 ^{ns}	1718.22 [*]
CV Plot (%)	2.95	0.61	9.70	23.45	33.14
CV Split-plot (%)	5.88	5.40	9.06	31.66	19.77

^{ns} = not significant; ^{*} and ^{**} indicate statistical significance at the 5% ($p \leq 0.05$) and 1% ($p \leq 0.01$) levels, respectively, according to the F-test. CV= coefficient of variation.

seeds, indicating that the collection position along the plant and the cultivar significantly influenced the quality of soybean seeds (Figure 3). In general, it was observed that treatments associated with the middle third of both cultivars showed greater proximity to the variables germination (G), first germination count (FGC), seedling emergence (E), emergence speed index (ESI), accelerated aging (AA), and total dry mass (TDM), suggesting a higher physiological potential of these seeds. Oliveira and Carvalho (2021) found that the seed position on the plant influences physiological quality, where seeds from the middle stratum tend to exhibit superior performance in vigor, germination, and seed mass compared to seeds located in lower positions. Souza *et al.* (2020) observed in their study that seeds located in upper and intermediate

positions show better physiological performance. According to Ferreira *et al.* (2023), the physiological potential of seeds increases as they progress from the lower third to the upper third of the plant.

The seeds of the Brasmax Olimpo IPRO cultivar, when harvested from the middle third, showed a stronger association with the variables FGC, G, and AA (Figure 3), indicating greater physiological potential and initial seedling performance. In turn, the seeds of the CZ 58B23 I2X cultivar, also from the middle third, were more associated with the E and ESI variables, demonstrating higher speed and uniformity in the emergence process. Differences related to initial vigor are linked to intrinsic genetic characteristics of the cultivars. Variations in protein expressions in energy metabolism are a key biological determinant for high-vigor seeds to exhibit physiological superiority (Sampaio *et al.*, 2025).

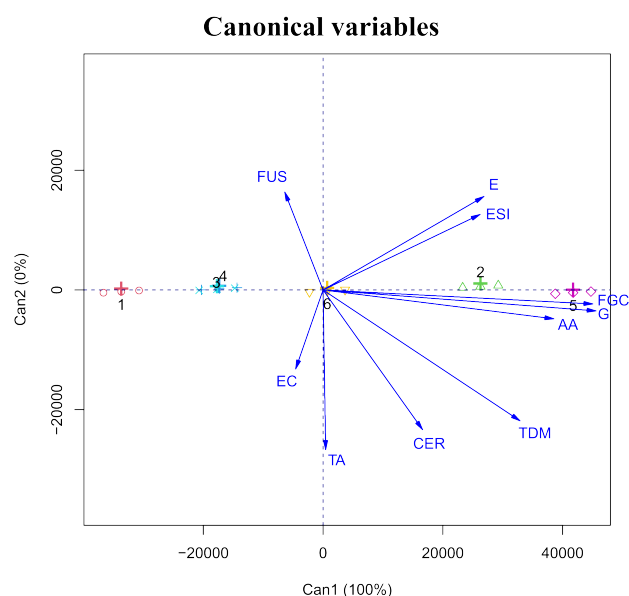


Figure 3 - Canonical analysis of variables: WC (water content), FGC (first germination count), G (germination), E (seedling emergence), ESI (emergence speed index), TDM (total dry mass), EC (electrical conductivity), AA (accelerated aging), FUS (*Fusarium* sp.), and CER (*Cercospora* sp.). The treatments correspond to the combination of two soybean cultivars and three seed collection positions along the plant, being: 1) CZ 58B23 I2X (lower third), 2) CZ 58B23 I2X (middle third), 3) CZ 58B23 I2X (upper third), 4) Brasmax Olimpo IPRO (lower third), 5) Brasmax Olimpo IPRO (middle third), and 6) Brasmax Olimpo IPRO (upper third).

The GGE biplot allowed for the simultaneous evaluation of the average performance and stability of the cultivars and seed collection positions in relation to the set of physiological, physical, and sanitary variables analyzed (Figure 4). In Figure 4A, it can be observed that the Brasmax Olimpo IPRO cultivar showed a greater projection along the principal axis (PC1), positioning itself closer to the ideal performance vector, indicating better overall performance and greater stability when compared to the CZ 58B23 I2X cultivar. This behavior suggests that Brasmax Olimpo IPRO has a greater capacity to express superior seed quality consistently, regardless of the inherent variations in the evaluated variables. According to Felisberto *et al.* (2015), this behavior was already expected, considering that soybean cultivars have different genetic origins, growth habits, maturity groups, and other agronomic characteristics, factors that naturally contribute to the occurrence of variations among the evaluated materials.

In Figure 4B, regarding the ranking of seed collection positions, it is observed that the middle third showed the closest proximity to the ideal point, indicating a better combination between average performance and stability. This result demonstrates that seeds formed in the intermediate portion of the plant generally exhibit a greater balance between vigor, mass, and health variables,

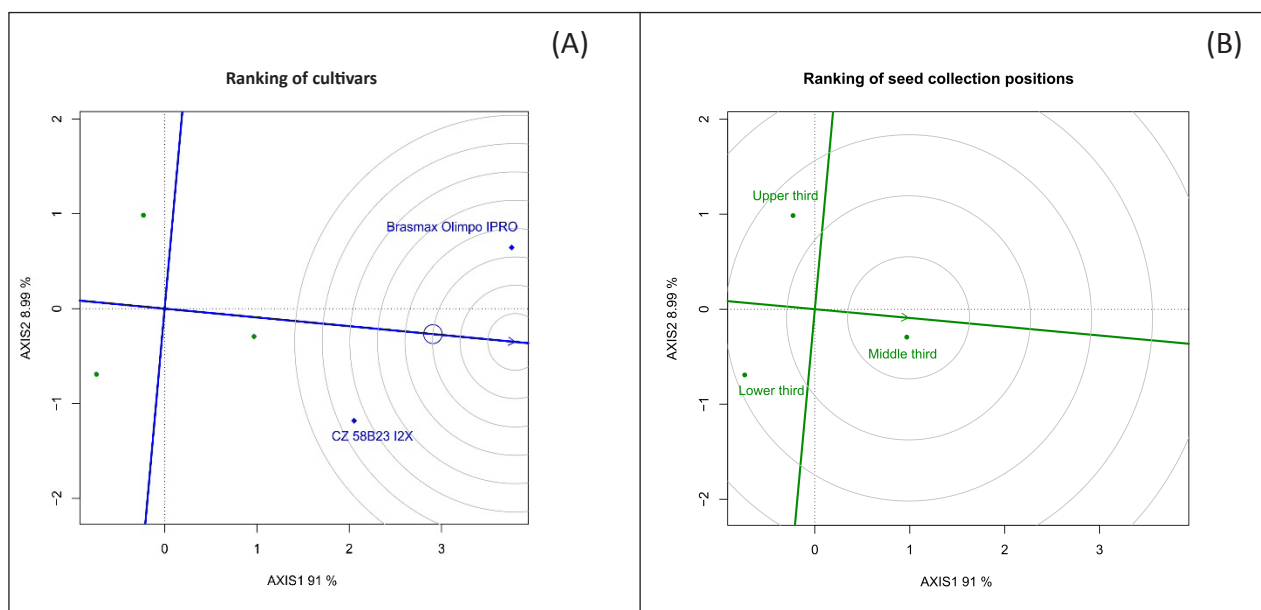


Figure 4 - GGE biplot showing the classification of the best cultivar compared to other treatments and studied variables (A) and the ranking of the best seed positions (B).

establishing themselves as the most favorable position for obtaining high-quality seeds. The superiority of seeds from the middle third can be attributed to the formation of microenvironments along the soybean plant canopy, where positions with greater light availability favor photosynthetic activity, seed filling, and reserve deposition (Oliveira *et al.*, 2025). Oliveira and Carvalho (2021) found in their study that seeds from the middle third are located in a favorable position for greater photosynthetic efficiency and translocation of photoassimilates.

Thus, the GGE results corroborate the previous multivariate analyses, reinforcing that both the genotype and the position of seed formation in the canopy exert a determining influence on the quality of soybean seeds. The superiority of the Brasmax Olimpo IPRO cultivar and the middle third highlights that management strategies prioritizing these conditions can result in seed lots with greater physiological potential and higher uniformity, with direct implications for seed production and certification programs, since multivariate approaches allow the simultaneous selection of superior genotypes based on multiple attributes, increasing the robustness of agronomic inferences (Zuffo *et al.*, 2020).

The Pearson correlation matrix revealed consistent relationships between the physiological, physical, and sanitary variables of the seeds, allowing for an integrated understanding of the determining factors of physiological quality (Figure 5). A strong positive correlation was observed between germination (G) and first germination count (FGC) ($r = 0.96$; $p < 0.001$), indicating that seeds with higher initial germinative capacity tend to maintain high performance throughout the test, reinforcing FGC as an efficient vigor indicator. According to Ebone *et al.* (2020), high-quality seed lots exhibit greater vigor and germination. Araújo *et al.* (2024), when analyzing the quality of seeds subjected to delayed harvest, observed a progressive decrease in FGC as seed quality declined, directly reflecting a reduction in the final germination percentage.

The vigor variables showed positive and significant associations with seedling growth attributes. A positive correlation was observed between germination (G) and total dry mass (TDM) ($r = 0.79$; $p < 0.001$), as well as between first germination count (FGC) and TDM ($r = 0.68$; $p < 0.01$), indicating that batches with better germination performance tend to produce seedlings with greater biomass accumulation. According to Meneguzzo *et al.* (2021), high-vigor seeds exhibit greater physiological

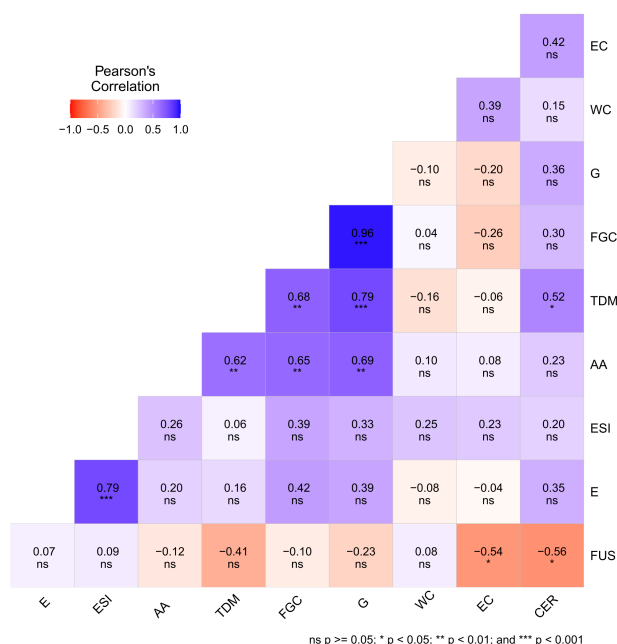


Figure 5 - Pearson correlations between the evaluated variables. Abbreviations used: 1000-S: thousand-seed mass; WC: water content; E: seedling emergence; ESI: Emergence speed index; EC: electrical conductivity; FGC: first germination count; G: germination; TDM: total dry mass; AA: accelerated aging; CER: *Cercospora* sp.; FUS: *Fusarium* sp. (ns $p \geq 0.05$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

potential, resulting in seedlings with greater length and consequently greater biomass accumulation.

In addition, accelerated aging (AA) was positively correlated with TDM ($r = 0.52$; $p < 0.05$) and also with G ($r = 0.69$; $p < 0.01$) and FGC ($r = 0.65$; $p < 0.01$), reinforcing that maintaining vigor after stress is associated with better physiological performance and initial seedling growth. According to Jo *et al.* (2023), high-vigor seeds subjected to the accelerated aging test exhibit better germination and field emergence. Seed vigor is crucial for mitigating stresses during field germination (Woga and Degaga, 2026).

Furthermore, seedling emergence (E) showed a strong positive correlation with the emergence speed index (ESI) ($r = 0.79$; $p < 0.001$), indicating that batches with higher emergence speed tend to have a higher percentage of emerged seedlings. Soybean seeds with high vigor levels exhibit greater uniformity and emergence speed, leading to better vegetative development (Ebene *et al.*, 2020).

The incidence of *Fusarium* sp. (FUS) showed a significant negative correlation with *Cercospora* sp. (CER) ($r = -0.56$; $p < 0.05$) and with electrical conductivity (EC) ($r = -0.54$; $p < 0.05$), indicating that the presence of these pathogens is associated with reduced physiological integrity of the seeds. The genus *Fusarium* performs aggressive colonization of seed tissues and has a high capacity to dominate the ecological niche of the seed, causing severe deterioration (Zhao *et al.*, 2022). The fungus *Cercospora* is responsible for severe impairment of soybean seeds, negatively affecting physiological quality, morphological characteristics of the seeds, and the initial development of seedlings (Amaral Júnior *et al.*, 2025).

CONCLUSIONS

Seeds originating from the middle third of the canopy demonstrate qualitative superiority compared to the upper and lower thirds, establishing themselves as the preferred position for obtaining high-vigor seeds. Additionally, the cultivar Brasmax Olimpo IPRO shows the best performance in relation to the cultivar CZ 58B23 I2X.

ACKNOWLEDGMENTS

The authors are grateful to "Accert Pesquisa e Consultoria Agronômica" for the financial support with agricultural inputs and for providing the experimental area.

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