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VALIDATION OF DIFFERENT SUBSTRATES IN THE GERMINATION TEST OF SOYBEAN SEEDS TREATED WITH THIAMETHOXAM, METALAXYL-M, AND FLUDIOXONIL

VALIDAÇÃO DE DIFERENTES SUBSTRATOS NO TESTE DE GERMINAÇÃO DE SEMENTES DE SOJA TRATADAS COM THIAMETHOXAM, METALAXYL-M E FLUDIOXONIL

EVALUACIÓN DE DIFERENTES SUSTRATOS EN LA GERMINACIÓN DE SEMILLAS DE SOJA TRATADAS CON THIAMETHOXAM, METALAXYL-M Y FLUDIOXONIL

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Resumo

O tratamento de sementes é amplamente adotado na agricultura devido à sua eficiência na proteção contra patógenos e insetos-praga durante as fases iniciais de desenvolvimento das culturas. No entanto, permanecem questionamentos quanto aos efeitos do intervalo entre o tratamento de sementes e a semeadura, bem como sobre a adequação dos substratos utilizados nos testes de germinação. Este estudo avaliou como diferentes substratos e curtos períodos de armazenamento após o tratamento químico afetam a qualidade fisiológica de sementes de soja tratadas com thiamethoxam, metalaxyl-M e fludioxonil. O experimento foi conduzido em esquema trifatorial, considerando a presença ou ausência de tratamento de sementes, quatro períodos entre o tratamento e a instalação do teste (0, 2, 4 e 6 dias) e diferentes substratos para o teste de germinação (papel germitest, areia e vermiculita, utilizados isoladamente e em combinação). As variáveis analisadas incluíram porcentagem de germinação, primeira contagem de germinação, comprimento da parte aérea, comprimento radicular, comprimento total de plântulas, número de raízes e massa seca total. Os resultados indicaram que curtos períodos de armazenamento após o tratamento de sementes não afetaram significativamente a germinação e o vigor, e que o tipo de substrato exerceu influência mínima sobre a qualidade fisiológica. Embora a vermiculita tenha promovido desenvolvimento ligeiramente superior das plântulas, esse efeito não justificou sua utilização rotineira em substituição aos substratos tradicionais. Conclui-se que curtos períodos de armazenamento após o tratamento químico não comprometem a qualidade fisiológica das sementes de soja, e que o papel germitest permanece como substrato adequado para testes de germinação com sementes tratadas.

Palavras-chave: substrato; controle químico; desempenho de plântulas; germinação, Glycine max; vigor.

Abstract

Seed treatment is widely adopted in agriculture due to its effectiveness in protecting seeds against pathogens and insect pests during early crop development. However, questions remain regarding the effects of the interval between seed treatment and sowing, and the suitability of substrates used in germination tests. This study evaluated how different substrates and short storage periods after chemical treatment affect the physiological quality of soybean seeds treated with thiamethoxam, metalaxyl-M, and fludioxonil. The experiment used a trifactorial scheme, considering the presence or absence of seed treatment, four periods between treatment and test installation (0, 2, 4, and 6 days), and different substrates for the germination test (germitest paper, sand, and vermiculite, individually and in combination). The analyzed variables included germination percentage, first germination count, shoot length, root length, total seedling length, number of roots, and total dry mass. Results indicated that short storage periods after seed treatment did not significantly affect germination and vigor, and substrate type had minimal influence on physiological quality. Although vermiculite promoted slightly greater seedling development, this did not warrant its routine use over traditional substrates. In conclusion, short storage periods after chemical treatment do not compromise the physiological quality of soybean seeds, and germitest paper remains an appropriate substrate for germination tests with treated seeds.

Keywords: Substrate; chemical control; seedling performance; germination; Glycine max; vigor.

Resumen

El tratamiento de semillas es una práctica ampliamente adoptada en la agricultura debido a su

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eficacia para proteger las semillas contra patógenos e insectos plaga durante las etapas iniciales del desarrollo de los cultivos. Sin embargo, aún existen interrogantes sobre los efectos del intervalo entre el tratamiento de semillas y la siembra, así como sobre la idoneidad de los sustratos utilizados en las pruebas de germinación. Este estudio evaluó cómo diferentes sustratos y cortos períodos de almacenamiento después del tratamiento químico afectan la calidad fisiológica de semillas de soja tratadas con thiamethoxam, metalaxyl-M y fludioxonil. El experimento se llevó a cabo bajo un esquema trifactorial, considerando la presencia o ausencia de tratamiento de semillas, cuatro períodos entre el tratamiento y la instalación de la prueba (0, 2, 4 y 6 días) y diferentes sustratos para la prueba de germinación (papel germitest, arena y vermiculita, utilizados individualmente y en combinación). Las variables analizadas incluyeron porcentaje de germinación, primer conteo de germinación, longitud de la parte aérea, longitud de la raíz, longitud total de plántulas, número de raíces y masa seca total. Los resultados indicaron que los cortos períodos de almacenamiento después del tratamiento de semillas no afectaron significativamente la germinación ni el vigor, y que el tipo de sustrato tuvo una influencia mínima sobre la calidad fisiológica. Aunque la vermiculita promovió un desarrollo ligeramente superior de las plántulas, este efecto no justificó su uso rutinario en sustitución de los sustratos tradicionales. En conclusión, los cortos períodos de almacenamiento posteriores al tratamiento químico no comprometen la calidad fisiológica de las semillas de soja, y el papel germitest continúa siendo un sustrato adecuado para las pruebas de germinación de semillas tratadas.

Palabras clave: Sustrato; control químico; desempeño de plántulas; germinación; Glycine max; vigor.

1. Introdução

Soybeans stand out among the major agricultural crops worldwide, alongside corn, wheat, rice, and cotton, and consolidate their position as one of the most economically, nutritionally, and industrially important commodities on a global scale. For the 2025/26 growing season, global soybean production is expected to exceed 427 million tons, setting a record, with Brazil, the United States, and Argentina ranking as the leading producers. Its importance is directly associated with its high protein content and balanced amino acid profile, characteristics that expand its use in human food, animal feed, biofuel production, and several industrial sectors (Karges et al., 2022).

However, achieving high crop productivity requires high-quality seeds, since adequate seedling establishment represents one of the most critical stages for crop success. Uniform emergence and vigorous initial development directly influence crop performance and final yield potential (Finch-Savage & Bassel, 2016). In this context, the evaluation of seed physiological quality plays a fundamental role and

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can be performed using specific tests to identify differences among seed lots and estimate their field performance potential (Ebone et al., 2019).

Among the technologies widely adopted in soybean seed production, seed treatment has become an essential practice for protecting seeds against pathogens and insect pests during the early stages of crop development. However, the increasing use of industrial seed treatment is a challenge. Depending on the chemical composition used and the storage period after treatment, phytotoxic effects may occur, compromising seed physiological quality (Bem-Júnior et al., 2020; Carvalho et al., 2020).

Some active ingredients present the potential to directly interfere with seed cellular metabolism. At high concentrations, benomyl, for example, may inhibit mitotic activity in root cells, impairing early seedling development (Dane & Dalgic, 2005). Likewise, chemical combinations frequently used in industrial seed treatment, such as imidacloprid + thiodicarb and pyraclostrobin + thiophanate-methyl + fipronil, may reduce seed germination and vigor (Ferreira et al., 2016). Nevertheless, information on the physiological effects at short storage periods after seed treatment for less than one week remains limited.

In addition to the phytotoxic effects associated with seed treatments, the suitability of germitest paper as the standard substrate for evaluating the germination of treated seeds has also been questioned. In field conditions, seeds interact directly with soil components, such as clay and organic matter, which can adsorb part of the chemical compounds applied to the seeds. This adsorption reduces the availability and potential toxicity of these compounds during germination (Sartori et al., 2020). Consequently, laboratory results obtained exclusively using germitest paper may not adequately reflect actual field conditions during seedling establishment.

Another relevant factor associated with seed imbibition dynamics. The use of alternative substrates, such as sand and vermiculite combined with germitest paper, may alter water availability and modify the water absorption rate by seeds, directly influencing the interaction between the seed coat and the chemical products applied. Studies have demonstrated that these modifications may provide results closer to those observed under field conditions, thereby increasing the reliability of physiological tests (Rocha et al., 2020).

Based on the issues discussed above, the objective of this study was to evaluate the interference of different substrates on the results of physiological quality analyses of treated soybean seeds, as well as to investigate the effects of short storage periods on seed germination, vigor, and early seedling characteristics.

2. Revisão da Literatura

Seed physiological quality directly determines crop establishment by affecting seedling emergence, stand uniformity, and yield. In soybean (*Glycine max* L.), a crucial global source of vegetable oil and protein, the use of high-quality seed is

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essential for optimal agronomic performance and sustainable production (EMBRAPA, 2023). Evaluating seed physiological and sanitary quality ensures planting viability and minimizes establishment losses. High-vigor seeds, as noted by Costa et al. (2011), lead to more uniform and productive stands, even under stress.

Among the technologies used to preserve seed quality and protect seedlings during early development, seed treatment with fungicides and insecticides is particularly important. This practice provides protection against seed- and soil-borne pathogens and reduces pest damage during early crop growth (Shaban et al., 2017). Consequently, seed treatment is widely adopted in modern agriculture, with more than 95% of soybean seed crops receiving some form of phytosanitary treatment before sowing.

Despite its benefits, studies show that some active ingredients in seed treatments can affect germination, vigor, and early seedling development. Rizvi et al. (2019) noted that inappropriate or excessive use of fungicides and insecticides may induce metabolic alterations that impair seed quality. Similarly, Dias et al. (2017) emphasized that germination and vigor tests must account for phytosanitary products, as these compounds can alter both the rate and uniformity of germination.

Beyond direct effects of chemical compounds, interactions between seed treatment and laboratory test substrates can affect results. Thus, choosing the right substrate is critical for accurate assessment of seed physiological quality. Germitest paper is the traditional choice for germination tests due to its practicality and standardization. However, recent research explores alternative substrates that better mimic field conditions.

The use of materials such as vermiculite and sand between Germitest paper sheets has been studied to alter seed water-uptake dynamics. This makes the process more like what is seen in soil (Rocha et al., 2020). França Neto et al. (2001) found that these substrates help root development and allow more consistent seed vigor evaluation. Vermiculite's high water-holding capacity, good aeration, and exchange capacity help maintain stable conditions, improving germination test reliability and consistency. This is important because testing substrates, such as Germitest paper in sunflower testing, can promote more fungal growth and reduce accuracy. When

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vermiculite was added, fungal problems dropped, and seed quality assessments became more accurate. These results show that vermiculite can improve the reliability of lab seed analysis.

In soybeans, Hartwig et al. (2016) observed that using vermiculite between Germitest paper sheets produced better results compared with the conventional substrate for seeds treated with fludioxonil, metalaxyl-M, thiabendazole, bifenthrin, and imidacloprid. This was attributed to vermiculite's ability to mitigate initial phytotoxic effects and create conditions favorable for seedling development. These findings reinforce the need for more studies on substrate effects in evaluating seed treatments.

Before vermiculite can be officially included in the Rules for Seed Testing (RAS), technical criteria need to be established for standardized use. One main limitation involves the different commercial particle sizes (micron, superfinOne main limitation is the different commercial particle sizes (micron, superfine, fine, and medium), which exhibit distinct physical characteristics that affect water retention, aeration, and interactions with treated seeds (Martins et al., 2023).uracy, and reliability in germination tests.

Another relevant aspect is the time between seed treatment and sowing. Early treatment brings logistical and operational benefits. However, studies report that storing treated seeds may speed up physiological deterioration. Khan et al. (2019) showed that specific chemical treatments can affect germination and seedling growth, depending on storage duration. Similarly, Smith et al. (2020) found that some active ingredients may affect seed metabolism, leading to oxidative stress and reduced vigor during storage.

More pronounced effects were reported by Sales (2021), who observed substantial reductions in the germination of treated maize seeds stored for 90 days. Seeds treated with imidacloprid + thiodicarb showed a 43% reduction in germination. Treatment with imidacloprid + fipronil resulted in a 44% reduction. Both values were considerably greater than those for untreated seeds. This demonstrates that specific active ingredient combinations during storage significantly decrease germination compared to untreated seeds. It highlights the importance of understanding these

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interactions for seed performance.

Given this context, understanding how various substrates and storage durations affect the physiological quality of treated seeds is crucial. Such information helps improve seed testing protocols, validate alternative substrates, and guide recommendations to maximize seed treatment benefits without compromising quality. These studies aid in modernizing methodologies and developing more efficient, reliable, and sustainable agricultural systems.

3. Metodologia

The experiment was conducted at the Seed Analysis Laboratory (LAS) of the Faculty of Agronomy and Veterinary Medicine (FAMV) at Universidade de Passo Fundo. Soybean seeds of the BMX Zeus® cultivar from the 2018/2019 growing season were used, presenting initial vigor above 90%. The seeds were subjected to chemical treatment using the commercial products Cruiser®, Maxim XL®, and polymer, at the recommended doses for soybean cultivation. The treatment consisted of applying the active ingredients thiamethoxam, metalaxyl-M, and fludioxonil at doses of 200, 100, and 100 mL of commercial product per 100 kg of seeds, respectively. The spray solution was applied using an industrial seed treatment machine (Momesso Arktos África model), to simulate the operational conditions commonly adopted in commercial seed processing units. The evaluations were performed at four periods after seed treatment: 0, 2, 4, and 6 days.

For the physiological tests, three substrate conditions were evaluated: germitest paper alone, germitest paper combined with sand, and germitest paper combined with vermiculite. The germination test was conducted with four replications of 50 seeds per treatment, arranged on three sheets of germitest paper moistened with distilled water to 2.5 times the dry paper mass. In the treatments containing sand and vermiculite, a layer of 70 g of sand or 17.5 g of vermiculite was added between the paper sheets, and the substrate was moistened with distilled water equivalent to three times the dry paper mass. The seeds were maintained in a germination chamber at a constant temperature of 25 °C. Germination evaluation was conducted 8 days after test installation, according to the Rules for Seed Testing (Brasil, 2009). The first germination count was performed on the fifth day after sowing by recording the percentage of normal seedlings. Shoot length, root length, and total seedling length were determined using four replications of 20 seeds distributed longitudinally on the substrate, with the micropyle facing the lower end of the paper.

After the germination period, ten seedlings per replication were randomly selected for evaluation. Measurements were performed using a graduated ruler, and the results were expressed in cm seedling^{-1}. The number of secondary roots was determined using the same seedlings evaluated for length measurements by

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manual counting. For total dry mass determination, the seedlings were placed in a forced-air circulation oven at 60 °C until constant weight was achieved (Ebone et al., 2020). Subsequently, the samples were weighed using an analytical balance with 0.001 g precision, and the results were expressed in mg seedling^{-1}. The experimental design was completely randomized in a 2 × 4 × 3 factorial arrangement, corresponding to the presence or absence of seed treatment, four storage periods after treatment, and three germination substrates, with four replications.

The data were subjected to normality and homogeneity tests, and transformations were performed when necessary. Subsequently, the data was submitted to analysis of variance (ANOVA), followed by mean comparisons among treatments. Pearson correlation coefficients were also estimated among the evaluated variables, and their significance was checked by Student's t-test at 1% and 5% probability levels. Correlation networks were constructed using only coefficients with absolute values of 0.50, with positive correlations represented in green and negative correlations in red. Additionally, principal component analysis (PCA) was performed after data standardization to mean zero and unit variance to reduce dimensionality and summarize the variability of the evaluated traits (Hair et al., 2009). Components with eigenvalues lower than 1.0 were disregarded according to Kaiser's criterion (1958). All statistical analyses were performed using RStudio software (R Core Team, 2021) with the "factoextra" package.

4. Resultados e Discussão

Analysis of variance revealed a significant effect by the F-test ($p \leq 0.05$) for the presence or absence of chemical seed treatment on the variable's germination and number of roots (Table 1).

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Table 1. Summary of the analysis of variance for germination (G), first germination count (PCG), number of roots (NR), shoot length (CPA), root length (CR), total length (CT), and dry mass (MS) as a function of seed treatment, test substrate, and storage period.

SOURCE OF VARIATION	GL	Mean Squares						
		G	PCG	NR	CPA	CR	CT	MS
Treatment	1	297,51*	7,04	177,39*	1,9	0,83	5,27	261,9*
Substrate (S)	2	156,26*	271,32*	0,45	35,32*	11,08*	60,64*	455,6*
Time (T)	3	24,17*	30,58*	247,73*	3,29*	15,09*	31,50*	96,7*
TRAT x S	2	173,32*	186,57*	34,62*	1,18	0,46	3,11	16,4
TRAT x T	3	6,12	40,01*	4,83	1,14	0,68	3,17	3,3
S x T	6	13,84	20,82	19,41	0,6	2,88	4,54*	20,0
TRAT x S x	6	7,35	15,12	14,12	1,11	1,59	2,86	13,2
Resíduo	72	7,74	11,1	8,76	0,79	1,42	1,42	15,0
Total	95							
CV (%)		2,94	3,74	20,64	17,84	11,45	7,74	12,88

*** Significant at the 5% probability level by the F-test.
DF – Degrees of Freedom. **

Source: The authors, 2026.

Regarding the interaction between seed treatment and substrates, significant effects were observed for germination, first germination count, and number of roots. For the interaction between seed treatment and storage periods, significance was detected only for the first germination count. In the interaction between substrates and storage periods, significance was observed only for total seedling length. No significant interaction was observed among the three evaluated factors (seed treatment × storage periods × substrates) for any of the analyzed variables.

When the interaction between the presence or absence of seed treatment and the substrate was analyzed, it was observed that, for seeds treated with phytosanitary products, there was a difference between sand and the other two substrates, with increases of 9% for vermiculite and 6% for germitest paper (Table 2). When comparing the presence and absence of seed treatment, a significant difference was observed only for the sand substrate, with untreated seeds showing a 9% lower germination percentage compared to treated seeds. The highest germination values were observed in the vermiculite substrate; however, they did not differ statistically from those obtained with germitest paper.

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Table 2. Interaction between seeds treated × substrate for germination (G), first germination count (PCG), and number of roots (NR). CT – with seed treatment; ST – without treatment; A – sand; V – vermiculite; P – paper.

Seed treatment	***G (%)*** ***Substrate***			***PCG (%)*** ***Substrate***			***NR*** ***Substrate***		
CT	A	V	P	A	V	P	A	V	P
	97 aA	97 aA	96 aA	89 aAB	92 aA	87bB	14 aA	13bA	12bA
ST	88 bB	97 aA	94 aA	83 bB	93 aA	91 aA	15 aA	15 aA	17 aA

[1]Means followed by the same lowercase letter in the column and uppercase letter in the row do not differ from each other by Tukey's test ($p < 0.05$).

Source: The authors, 2026.

For the first germination count, treated seeds showed significant differences among substrates, with vermiculite giving better results than germitest paper but not differing statistically from sand. The differences between substrates were 3% for vermiculite and sand and 5% for vermiculite and germitest paper (Table 3). For untreated seeds, vermiculite still had the highest values; however, sand and germitest paper switched positions, with germitest paper 8% higher than sand. Comparing treatments across substrates, vermiculite showed no significant difference between treated and untreated seeds. In sand, treated seeds performed better physiologically than untreated seeds. In germitest paper, untreated seeds performed better. Substrates showed no significant differences in root numbers. In both vermiculite and germitest paper, untreated seeds had more roots than treated seeds, averaging 2 and 5 roots, respectively (Table 2). In sand, untreated seeds also had more roots, but the difference was not statistically significant.

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Table 3. Interaction between storage period × seed treatment for first germination count (PCG) and interaction between storage period × substrates used for total seedling length (CT). CT – with seed treatment; ST – without seed treatment; A – sand; V – vermiculite; P – paper.

Time	PCG (%)		CTOTAL (cm.pl^{-1})		
	CT	ST	A	V	P
0	91 A	90 A	15,86 A	17,16 A	14,04 B
2	89 A	89 A	16,97 A	16,94 A	15,92 A
4	88 A	91 A	14,94 B	17,90 A	13,65 B
6	89 A	86 B	14,01 B	15,09 A	12,47 B

[1]Means followed by the same in the column and uppercase letter in the row do not differ from each other by Tukey's test ($p < 0.05$).

Source: The authors, 2026.

When the interaction between storage period after seed treatment and the first germination count test was analyzed, a significant difference was observed only on the sixth day between treated and untreated seeds (Table 3). Treated seeds showed a 3% higher germination rate than untreated seeds when the test was performed 6 days after treatment.

For the total seedling length variable, no significant differences were observed among the storage periods between seed treatment and test execution, with the effect being influenced only by the substrates used (Table 4). In most cases, the highest values were obtained with vermiculite, followed by sand and then germitest paper. On day 0, only the germitest paper differed from the other substrates. On day 2, no differences were observed among treatments. On day 4, vermiculite was superior to both sand and germitest paper, whereas at day 6, only germitest paper showed inferior results compared to the other substrates.

Table 5. Mean comparison for shoot length (SL), root length (RL), and dry mass (DM) as a function of the substrate used for the tests.

Substrate	SL (cm per seedling^{-1})	RL (cm per seedling^{-1})	DM (cm per seedling^{-1})
Vermiculite	**6,20 a**	**10,57 a**	**29,23 a**
Sand	**4,54 b**	**10,90 b**	**34,15 a**
Paper	**4,26 b**	**9,76 b**	**26,72 b**

[1]Means followed by the same lowercase letter in the column and uppercase letter in the row do not differ from each other by Tukey's test ($p < 0.05$).

Source: the authors, 2026.

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When the effects of the substrates on shoot length were analyzed, vermiculite showed superior performance, with an average length of 6.2 cm seedling^{-1}. This value was 36.5% higher than the 4.54 cm seedling^{-1} observed in the sand substrate and 45.5% higher than the 4.26 cm seedling^{-1} obtained using germitest paper alone. In contrast, root length presented different results, with the highest values observed in the sand substrate, reaching 10.9 cm seedling^{-1}. This value was 3% higher than that obtained with vermiculite, which presented a mean root length of 10.57 cm seedling^{-1}, and 10.4% higher than that observed with germitest paper, where root length averaged 9.76 cm seedling^{-1}. Dry mass results followed a pattern like that observed for root length, with the highest values in the sand substrate, followed by vermiculite and germitest paper. Dry mass in vermiculite was 14.4% lower than in sand, while germitest paper showed values 21.7% lower than in sand.

The first germination count showed a positive correlation with germination (0.79) (Figure 1), shoot length (0.53), and total seedling length (0.40) (Figure 1). Germination, in addition to its positive correlation with the first germination count, showed only a negative correlation with total dry mass (-0.47) (Figure 1). For shoot length, besides the positive correlation with the first germination count, positive correlations were also observed with root length (0.43), total seedling length (0.84), and number of roots (0.42) (Figure 1). Root length, in addition to its correlation with shoot length, also showed positive correlations with total seedling length (0.84) and number of roots (0.62) (Figure 1). Total seedling length, besides its positive correlations with the first germination count, shoot length, and root length, also showed a positive correlation with the number of roots (0.62) (Figure 1).

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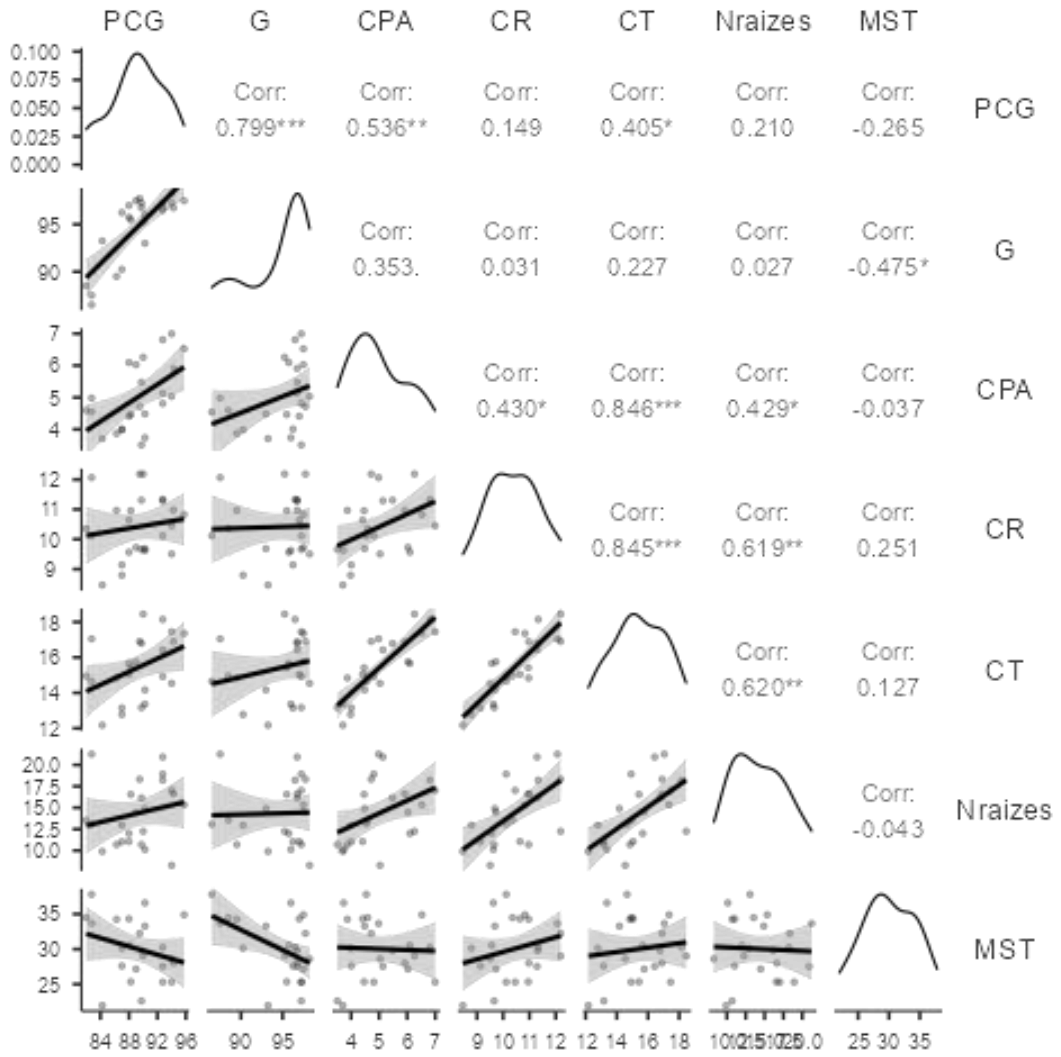


Figure 1. Pearson correlation network and dispersion of the observed values among germination (G), first germination count (PCG), total dry mass (MST), number of roots (NRaizes), total seedling length (TL), root length (RL), and shoot length (SL). * Significant at 5%, ** significant at 1%, and *** significant at 0.01% probability levels.

The PCA results (Figure 2) showed that the first two principal components (PCs) could be used to study the relationship between the physiological quality variables and the treatments evaluated in this study, since their eigenvalues (λi) were greater than 1 (PC1: 2).

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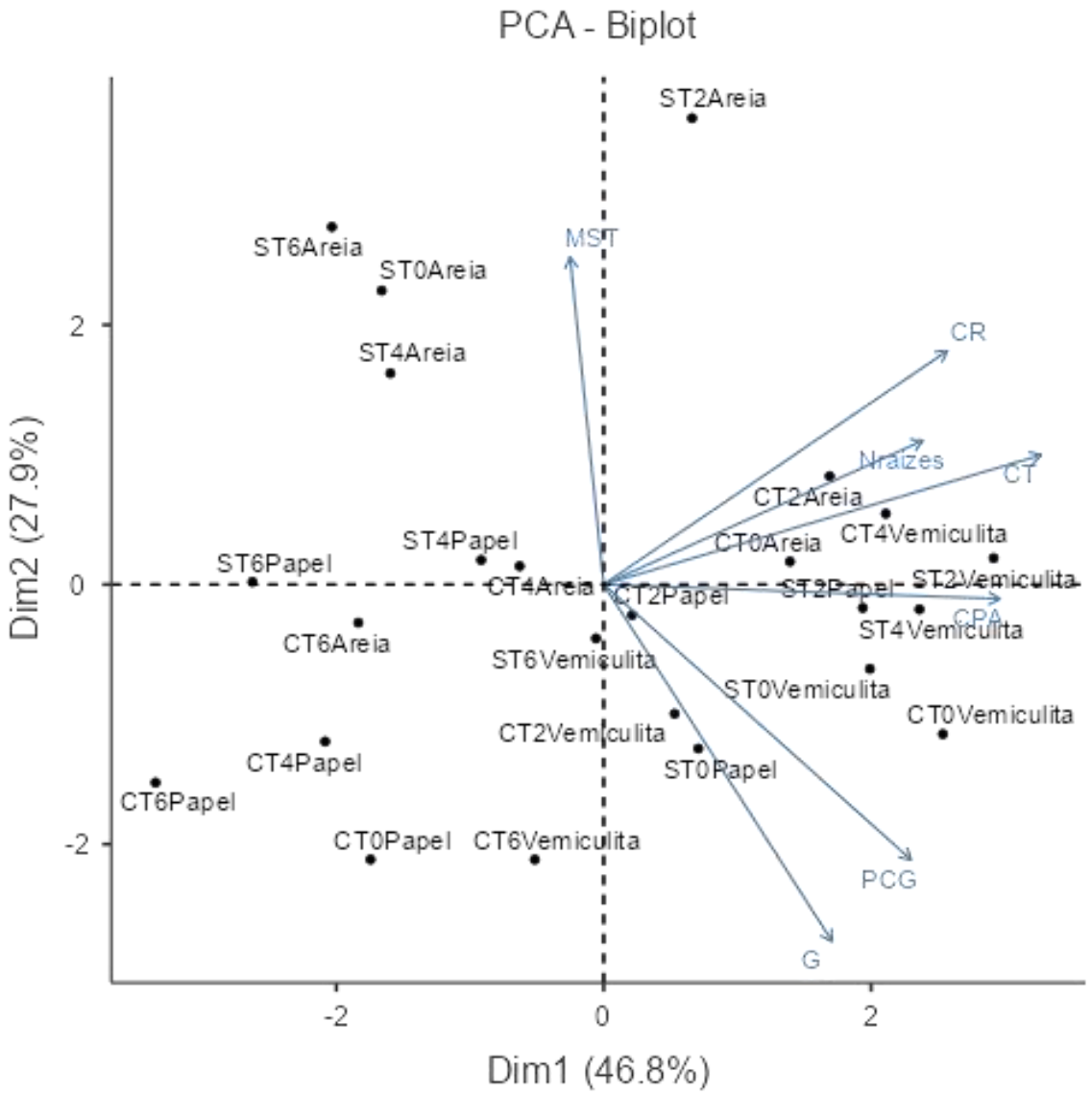


Figure 2. Principal component analysis (PCA) based on the first two principal components considering treatments and seed variables. CT: with treatment; ST: without treatment; 0, 2, 4, and 6 days of storage period. Substrates: sand, germitest paper, and vermiculite.

Source: The authors, 2026.

The results demonstrated only slight distinctions between Germitest paper and the evaluated substrates, with vermiculite showing superior performance compared to sand in maintaining seed germinative capacity. Similar findings were reported by Rocha et al. (2020), who observed higher germination percentages in vermiculite than in sand, although both substrates statistically neutralized the effects of the phytosanitary products tested in their study. These results reinforce the importance of substrate selection in seed physiological analyses, especially when evaluating seeds treated with chemical compounds.

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The superior performance of vermiculite was expected due to its intrinsic physicochemical properties, particularly its high-water retention capacity and cation exchange capacity, which are associated with its expansive 2:1 clay mineral structure and provide a buffering effect (Indrasumunar and Gresshoff, 2013). In this context, the slower release of water and vermiculite's ability to adsorb and temporarily retain chemical compounds (Rocha et al., 2023) likely minimized the immediate phytotoxic effects of the seed treatment. This buffering capacity may have reduced the initial formation of reactive oxygen species (ROS), which are considered one of the main factors associated with the deleterious effects of seed treatment on seed physiology (Gódói et al., 2023).

The phytotoxicity caused by ROS is mainly due to their interaction with membrane lipids, leading to lipid peroxidation and the formation of malondialdehyde, a compound that can alter membrane permeability and impair membrane integrity (Agmon et al., 2018). Furthermore, malondialdehyde is recognized as a mutagenic compound that can form DNA Adducts, intensifying oxidative damage in seed tissues (Ebone et al., 2019). Beyond reducing germination, oxidative stress also compromises seedling growth and vigor (Gódói et al., 2023). This phenomenon was evidenced in the present study by the greater total seedling length, root length, and shoot length observed in seedlings developed in vermiculite. The buffering effect promoted by vermiculite likely slowed the release and availability of phytotoxic compounds, allowing the enzymatic and non-enzymatic antioxidant systems of the seeds to efficiently detoxify free radicals and mitigate their deleterious effects.

Initially, it was expected that lipid peroxidation damage would be more pronounced in the embryonic root region than in the shoot, since the portion of the embryonic axis responsible for radicle protrusion is located near the end of the funiculus and presents greater exposure to external environmental conditions, making it more susceptible to oxidative damage (Hartmann Filho et al., 2016). However, the most expressive differences in seedling development were observed in the shoot rather than in the root system. This behavior suggests that the deterioration process caused by the seed treatment during the evaluated storage period was relatively mild and insufficient to induce severe structural damage to the embryonic root tissues.

Among the evaluated variables, the most pronounced impacts on physiological quality were observed in the first germination count, whereas germination percentage itself showed only minor differences among treatments. These findings indicate that vigor tests are more sensitive than standard germination tests for detecting subtle physiological alterations caused by seed treatment and short-term storage. Similar results have been described in the literature, including reports of no reduction in the germination potential of maize seeds treated with thiamethoxam during 90 days of storage (Silva et al., 2020), as well as studies demonstrating only a 6% reduction in soybean seed germination

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after 30 days of storage following thiamethoxam treatment (Bem-Junior et al., 2020).

Therefore, the absence of significant germination losses during the 6-day short storage period observed in the present study is consistent with previous reports and suggests that soybean seed treatment with thiamethoxam, metalaxyl-M, and fludioxonil can be applied shortly before sowing without substantial impairment of seed physiological quality. From a practical perspective, these findings provide important information for farmers and seed companies, supporting the operational feasibility of pre-sowing seed treatment while maintaining satisfactory germination and seedling establishment potential.

5. Conclusion

The results demonstrated that short storage periods do not significantly compromise the physiological quality of soybean seeds treated with thiamethoxam, metalaxyl-M, and fludioxonil. Although vermiculite promoted greater initial seedling development, especially in shoot growth, the observed gains do not justify its inclusion in routine laboratory analyses. Furthermore, the first germination count proved more sensitive in detecting early physiological changes than the final germination test, highlighting the potential of vigor assessments for analyzing treated seeds.

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