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Treated Soybean Cultivars and Subjected to Storage under Controlled and Uncontrolled Conditions during Different Periods

Cultivares de Soja Tratadas e Submetidas ao Armazenamento sob Condições Controladas e não Controladas Durante Diferentes Períodos

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Abstract

One of the challenges in establishing soybean crops has been the short timeframe for carrying out pre-planting processes, with seed treatment often being rushed and occurring simultaneously with rainfall. Therefore, this study aimed to evaluate the soybean seeds' performance stored under controlled and uncontrolled conditions for three different periods after seed treatment. Two soybean cultivars, two storage conditions, and three seed treatment time intervals were used to evaluate germination, vigor, potential seedling health damage, and field performance. An interaction between cultivar and storage time treatments was observed, with the TMG 2776 IPRO cultivar achieving the best result, with 94.50% normal seedlings after 30 days of storage, while HO Aporé IPRO reached 94.31%. Germination, vigor, and normal seedling performance of HO Aporé IPRO presented the following values: 95.5%, 96.22%, and 97.15%, respectively. Meanwhile, the TMG 2776 IPRO cultivar achieved 96.25% germination, 96.29% vigor, and 95.98% performance. A reduction in the number of normal seedlings was observed as the storage period increased. Seeds stored under controlled conditions exhibited a higher percentage of normal seedlings compared to those stored under uncontrolled conditions. The TMG 2776 IPRO cultivar performed better in laboratory tests; and, in field tests, HO Aporé IPRO showed superior performance.

Keywords: *Glycine max*. Moisture Damage. Defective Seedlings. Normal Seedlings.

Resumo
Um dos problemas na instalação da cultura da soja tem sido o curto prazo para a realização dos processos antes do plantio, sendo que o tratamento de semente, ocorre de modo apressado e simultaneamente às chuvas, com isso, o trabalho teve como objetivo avaliar o desempenho das sementes de soja submetidas ao armazenamento sob condições controladas e não controladas durante três períodos após o tratamento de semente. Duas cultivares de soja, duas condições de armazenamento e três intervalos de tempo de tratamento de sementes foram utilizados para avaliar a germinação; o vigor e possíveis danos para a sanidade das plântulas e o comportamento em condições de campo. Foi observado interação entre os tratamentos cultivar e tempo, em que a cultivar TMG 2776 IPRO obteve melhor resultado com 94,50% de plântulas normais aos 30 dias de armazenamento, e a HO Aporé IPRO com 94,31%. Germinação, vigor e comportamento das plântulas normais da HO Aporé IPRO apresentaram os seguintes valores 95,5; 96,22 e 97,15%, respectivamente. Entretanto, a cultivar TMG 2776 IPRO obteve 96,25% de germinação; 96,29% de vigor e 95,98% comportamento. Ocorre redução no número de plântulas normais conforme período crescente de armazenamento. Sementes armazenadas em ambiente controlado apresentam maior porcentagem de plântulas normais em relação àquelas armazenadas em ambiente não controlado. A cultivar TMG 2776 IPRO é melhor nos testes em laboratório e, no teste a campo, a HO Aporé IPRO apresenta um melhor desempenho.

Palavras-chave: *Glycine max*. Danos por Umidade. Plântulas Anormais. Plântulas Normais.

1 Introduction

The USDA (United States Department of Agriculture) estimates that global soybean production in the 2024/25 crop will reach approximately 422.3 million tons, a new record and a 6.7% increase (+26.3 million) compared to the 2023/2024 crop, which ended with 395.9 million tons (USDA, 2024). In Brazil, the 2023/2024 crop totaled 147.3 million tons on 45.9 million hectares planted (CONAB, 2024a; 2024b), accounting for 37.9% of global production.

Brazil is the largest producer and exporter of soybeans worldwide, as well as the second largest holder of ending stocks (Coelho, 2024). For production to continue growing as it has in previous years, producers must pay attention to the quality of seeds used, as they directly affect the plant stand in cultivated areas. Operations in the early stages of soybean establishment must also be closely monitored as they are directly linked to final productivity. Therefore, it is essential to make precise and effective decisions to prevent losses and address any issues in time (Dan *et al*., 2010).

In some Brazilian regions, such as the Midwest, despite topographical features called "chapadões" that favor agricultural operations, climatic conditions can create setbacks, providing an ideal environment for the emergence and proliferation of pests and diseases that can affect or even harm production chains (Silva; Cunha; Souza, 2021).

Especially in the early stages of seed development, it is crucial to assess recurring factors to avoid significant losses, such as rainfall forecasts, planter calibration, machinery passage within the crop, and, most importantly, pests and soil diseases that feed on seeds and seedlings, causing planting failures (Dan *et al*., 2010; Santos *et al*., 2024).

Producers have been using agricultural pesticides for seed treatment, a practice that is increasingly indispensable and economically recommended. This strategy prevents pest and disease attacks, ensuring plant protection from seed emergence. In addition to minimizing damage caused by defoliating pests, it helps regulate and prevent damage from stress (Ioris Junior, 2019).

In the seed treatment process, chemical products protect the plant from pest attacks and provide micronutrients such as cobalt, nickel, and molybdenum, which can promote more vigorous plant growth, making cell walls more resistant and roots deeper. Biological products, which have been gaining ground in the agricultural input market, also play a role (Avelar *et al*., 2011).

The positive effects of seed treatment have been observed through the seedlings' behavior in the field, with no issues in germination and emergence (Bem Junior *et al*., 2020; Dalgalo; Borsoi; Slovinski, 2019; Kaefer *et al*., 2019; Nakao *et al*., 2018; Coradi *et al*., 2015). However, Ioris Junior (2019) stated in his research that some fungicide products can interfere with seed development, potentially having a toxic effect due to the individual product's action or even incompatibility with other products added during treatment.

Therefore, seed storage has been studied and improved over time to maintain the plant's maximum vigor. To store seeds efficiently, appropriate conditions must be maintained to reduce biochemical reactions in the seeds, minimize the effects of quality loss, and discourage the development of fungi and insects (Kaefer *et al*., 2019; Martins Filho *et al*., 2001).Thus, the aim of this work was to evaluate the performance of soybean cultivars subjected to storage under controlled and uncontrolled conditions during different storage periods after seed treatment.

2 Material and Methods .

The study was conducted using a completely randomized experimental design with a 2x2x3 factorial scheme, consisting of: two soybean varieties (TMG 2776 IPRO and Aporé IPRO, with respective cycles of 108 and 105 days), two environments (controlled and uncontrolled), and three storage periods after seed treatment (0, 15, and 30 days), with 4 repetitions for each treatment.

For seed treatment, the following were used for 1kg of seeds: 1.8 mL of Certeza N (AI - Methyl Thiophanate + Fluazinam); 0.54 mL of Cruiser (AI - Thiamethoxam); 0.8 g of the micronutrient NiCoMo DRY; 2 mL of Bio 10; 0.6 mL of Bio 31; and graphite, an inorganic polymer that, in addition to forming a protective layer, aids in seed sliding during sowing operations.

The chemical and biological products were added to the mixer with the seeds, which were homogenized, graphitized, and placed in big bags ready for storage. This process was carried out on the same day for all seeds used in the tests from both varieties to avoid differences between the seeds analyzed.

Three storage periods were evaluated after treatment (0, 15, and 30 days), with tests starting on the treatment day. Two types of storage were used: controlled environment storage (cold chamber with a constant temperature from 13 to 16 °C and relative humidity around 35%) and uncontrolled environment storage with a maximum temperature varying from 48 °C (max.) to 18 °C (min.) and relative humidity ranging from 20 to 60%.

The treated seeds were separated and stored according to each treatment. For the 0-day treatment, no storage was applied, so as soon as the seeds were treated, the first battery of tests began, replicating what happens in the field. The 15- and 30-day treatments were the storage periods during which the treated seeds were stored before the tests were conducted.

All tests were carried out based on the Seed Analysis Rules (Brazil, 2009). For each treatment, 200 seeds were used (4 subsamples of 50 seeds per treatment with 4 repetitions).

The seedlings were analyzed and classified according to physical characteristics and observed damage, as normal, weak, defective, and dead, following the methodology of Zorato (2003).

For the germination test, Germitest paper was used, and for the emergence test, a sand substrate was used, with a test also conducted in the seedbed, all according to the Seed Analysis Rules (RAS) (Brazil, 2009). The vigor count was performed on the seventh and fourteenth days, as well as the classification and germination percentage, determining the seedlings according to their development in the test as: normal, weak, defective, and dead (Brazil, 2009).

After analyzing and counting the seedlings, the averages for each classification within each treatment were obtained. The data were subjected to an arcen$\sqrt{x}$ transformation to meet the assumptions of variance analysis, and statistical analysis was performed using the Agroestat program (Maldonado Jr, 2022). When significant, the data were subjected to mean tests using Tukey's test at a 5% probability.

3 Results and Discussion

The cultivar and storage time treatments showed interaction with each other. A significant difference ($P<0.01$) was observed between the cultivars regarding storage time. The TMG 2776 IPRO cultivar had a higher germination percentage (98.5%) of normal seedlings compared to the HO Aporé IPRO cultivar (96.87%).

At 15 days of storage, a significant difference ($P<0.01$) was observed between the evaluated cultivars. In this case, the HO Aporé IPRO cultivar had a higher percentage of normal seedling emergence (97.56%) compared to the TMG 2776 IPRO cultivar (95.87%). However, after 30 days of storage, no significant difference ($P>0.05$) was observed between the evaluated cultivars (Table 1).

Table 1 – Germination percentage (%) in a sand test of treated soybean seed cultivars as a function of storage time

Time	**Cultivar**			
	HO Aporé IPRO	**TMG 2776 IPRO**	**HO Aporé IPRO**	**TMG 2776 IPRO**
	Normal		**Weak**	
0 days	96.87Ba	98.50Aa	2.00Ba	0.63Aa
15 days	97.56Aa	95.87Bb	1.25Aa	2.31Bb
30 days	94.31Ab	94.50Ac	2.25Aa	2.50Ab

Means followed by different uppercase letters in the row and lowercase letters in the column differ from each other according to Tukey's test at a 5% significance level.
Source: Research data.

For weak seedlings, among the evaluated cultivars with zero days of storage, the TMG 2776 IPRO cultivar had a lower percentage of weak seedling emergence (0.63%) compared to the HO Aporé IPRO cultivar (2.00%). However, after 15 days, the HO Aporé IPRO cultivar showed a lower percentage of weak seedling emergence (1.25%) compared to the TMG 2776 IPRO cultivar (2.31%). After 30 days of storage, no significant difference (P>0.05) was observed between the evaluated cultivars (Table 1).

Analyzing the emergence of normal seedlings in relation to storage time, for treated seeds of the HO Aporé IPRO cultivar, no difference was observed between 0 and 15 days of storage (96.87% and 97.56%). However, after 30 days, a reduction in the percentage of normal seedling emergence was observed (94.31%). For the TMG 2776 IPRO cultivar, the 0-day period showed a higher percentage of normal seedling emergence (98.50%) compared to the 15- and 30-day periods (95.87% and 94.50%) (Table 1).

For the HO Aporé IPRO cultivar, no difference was observed between storage times in relation to the percentage of weak seedling emergence. However, for the TMG 2776 IPRO cultivar, differences were observed between storage times, with an increase in the percentage of weak seedling emergence at 15 and 30 days (Table 1).

Ludwig *et al.* (2011) observed an interaction between seed lots and storage time. Regarding the germination percentage, they found that after 180 days of storage in a cold chamber, the results were more satisfactory compared to storage in a controlled environment. According to Dalgalo, Borsoi, and Slovinski (2019), seed treatment under cold chamber storage provided protection against storage fungi. These authors stated that the polymer used forms a protective layer around the seed cell.

Camargo (2018) and Bem Junior *et al.* (2020) observed negative results between seed treatment and storage time, particularly in treatments stored in warehouses without any environmental control. They reported lower germination rates in seeds stored for longer periods, attributing this effect to the interference of thiamethoxam in germination. Schons *et al.* (2018) and Dan *et al.* (2010) explained that treatments involving thiamethoxam could accelerate seed deterioration, even in controlled environments, as it is a bioactivator that enhances nitrogen metabolism.

An interaction was observed between cultivars and storage times. However, no significant difference (P>0.05) was found between the cultivars across storage times. When analyzing each cultivar individually, differences were observed within storage times regarding the percentage of normal seedling emergence. Specifically, after 30 days of storage, there was a reduction in seedling emergence percentage (Table 2).

Table 2 – Percentage (%) of emergence in a seedbed test of treated soybean seed cultivars as a function of storage time

Time	**Cultivar**	
	HO Aporé IPRO	**TMG 2776 IPRO**
	Normal	
0 days	97.938Aa	97.938Aa
15 days	97.938Aa	97.938Aa
30 days	95.563Ab	95.563Ab

Means followed by distinct uppercase letters in the row and lowercase letters in the columns differ from each other according to Tukey's test at a 5% significance level.
Source: Research data**.**

Andrade (2018), evaluating 15 soybean cultivars, found that the Aporé cultivar exhibits a resistant reaction, aiding in the recovery of plants subjected to injury or stress. At 30 days of seed storage, even when treated and coated with graphite, they remain susceptible to environmental exchanges. According to Schons *et al.* (2018), the seeds' hygroscopic capacity is directly related to their deterioration, which directly affects quality, vigor, and germination, occurring during storage - whether refrigerated or not - ultimately affecting the entire seed batch.

According to the results presented in the studies by Avelar *et al*. (2011) and Martins Filho *et al.* (2001), it was possible to observe that between zero and 30 days of storage of treated seeds, there was a reduction not only in seed emergence but also in the plants' development with low physiological quality (Forti; Cícero; Pinto, 2010).

The percentage of normal seedlings is directly linked to seed germination and vigor. As observed by Rodrigues (2020), the longer the storage period, the greater the likelihood of a reduction in the percentage of normal seedlings. Over time, seeds stored in a non-refrigerated environment increase their metabolic activity and reduce their germination capacity. This occurs due to temperature fluctuations in an uncontrolled environment; cellular respiration intensifies, initiating the seed oxidation process. When not stored in an appropriate location for germination, deterioration occurs (Bem Junior *et al*., 2020).

On paper substrate, the TMG 2776 IPRO cultivar showed a higher percentage of normal seedling germination (96.25%) compared to the HO Aporé IPRO cultivar (95.50%). It also had a lower percentage of weak seedling germination (2.04%) than HO Aporé IPRO (2.60%). Regarding defective seedlings, no significant difference (P>0.05) was observed between the evaluated cultivars. For dead seedlings, the TMG 2776 IPRO cultivar had a lower percentage of dead seedling germination (0.44%) compared to the HO Aporé IPRO cultivar (0.90%) (Table 3).

Table 3 - Percentage (%) of Germination on Paper Substrate and Emergence on Sand Substrate and Seedbed of Two Soybean Cultivars Subjected to Seed Treatment

Variable	**Cultivar**	
	HO Aporé IPRO	**TMG 2776 IPRO**
Paper substrate		
Normal	95.50b	96.25a
Weak	2.60b	2.04a
Defective	1.00a	1.27a
Dead	0.90b	0.44a
Sand substrate		
Normal	96.22a	96.29a
Weak	1.81a	1.81a
Defective	1.25a	1.10a
Dead	0.73a	0.65a
Seedbed		
Normal	97.15a	95.98b
Weak	1.48a	2.30b
Defective	0.77a	1.15a
Dead	0.58a	0.96a

Means followed by distinct uppercase letters in the row and lowercase letters in the columns differ from each other according to Tukey's test at a 5% significance level.
Source: research data**.**

Regarding normal, weak, defective, and dead seedlings evaluated through the emergence test on a sand substrate, no significant difference was observed between cultivars (P>0.05) (Table 3).

Concerning the emergence test in the seedbed for the classification of normal seedlings, the cultivar TMG 2776 IPRO showed a lower percentage of normal seedling emergence (95.98%) compared to the cultivar HO Aporé IPRO (97.15%); it had a higher number of weak seedlings (2.30%) compared to HO Aporé IPRO (1.48%); and for defective and dead classifications, no significant difference was observed (P>0.05). This occurs due to the genetic diversity of each variety; therefore, HO Aporé IPRO stands out in field tests. Being less productive, it may contain more protective and resistant genes against external factors (Coradi *et al*., 2015).

Andrade (2018), when evaluating soybean varieties, observed that among the studied varieties, HO Aporé IPRO has genetic traits resistant to certain nematodes, which explains the lower percentage of weak seedlings in the seedbed.

To assess germination and vigor, different tests are performed, as each test expresses similar averages but highlights different damage points. The HO Aporé IPRO variety, due to its more resistant genes, performs better in sand tests, as this test demands more from the seeds due to high light intensity, just like the germination test on paper.

Seed treatment may affect each evaluated cultivar differently. According to Rocha *et al*. (2017), one of the evaluated cultivars was affected by the storage period after seed treatment. According to Dan *et al*. (2010), the reduction in seed quality is explained by the combination with insecticides in seed treatment before storage. Therefore, it would be advisable to apply insecticides during sowing.

The significant difference between cultivars is relevant to their intrinsic characteristics, demonstrating better development of TMG 2776 in tests with constant exposure, while in scarcity tests, such as the seedbed test, HO Aporé performed better. The TMG 2776 cultivar showed lower development in the seedbed test compared to HO Aporé, which exhibits good stress response. This cultivar can grow and maintain its production potential due to its resistance traits (Andrade, 2018).

Regarding the classification of normal and dead seedlings, no difference was observed between storage times after seed treatment on a paper substrate. In relation to weak seedlings, a lower percentage of weak seedling emergence was observed at 30 days of storage (1.78%) on a paper substrate. In this type of substrate, for the classification of defective seedlings, the 30-day period showed a higher number of defective seedlings (1.97%) compared to 0 and 15 days (0.69% and 0.78%) (Table 4).

Table 4 - Percentage (%) of germination on paper substrate and emergence on sand substrate and seedbed of treated soybean seeds subjected to different storage periods

Variable	**Time**		
	0 days	**15 days**	**30 days**
Paper substrate			
Normal	96.13a	96.00a	95.50a
Weak	2.63b	2.63b	1.78a
Defective	0.69a	0.78a	1.97b
Dead	0.56a	0.59a	0.84a
Sand substrate			
Normal	97.69a	96.72b	94.41c
Weak	1.31a	1.78ab	2.38b
Defective	0.50a	1.03b	2.02c
Dead	0.50a	0.47a	1.16a
Seedbed			
Normal	97.50a	96.56b	94.62c
Weak	1.53a	1.66ab	2.48b
Defective	0.66a	0.78b	1.44c
Dead	0.34a	1.00ab	0.97b

Means followed by distinct uppercase letters in the row and lowercase letters in the columns differ from each other according to Tukey's test at a 5% significance level.
Source: Research data**.**

Ludwig *et al*. (2011) explain that the use of fungicides and powdered polymers combined in seed treatment prevents the proliferation of fungi and favors seed germination after storage, as it reduces the seeds' biochemical activities, ensuring that they only spend energy when necessary, specifically during sowing.

For the sand substrate emergence test, at zero days of storage, a higher percentage of normal seedlings (97.69%) was observed, whereas at 15 and 30 days of storage, there was a significant reduction in this classification (96.72% and 94.41%, respectively) ($P<0.01$). Regarding the classification of weak seedlings, no significant difference ($P>0.05$) was observed between zero and 15 days of storage of treated seeds. However, at 30 days of storage, a higher number of weak seedlings was observed, although no significant difference ($P>0.05$) was found between 15 and 30 days of storage (Table 4).

Still in the sand substrate, regarding the germination of defective seedlings, an increase in the number of defective seedlings was observed at 30 days of storage. However, for dead seedlings, no significant difference ($P>0.05$) was found between the storage periods (Table 4).

Rodrigues (2020) explains that in non-refrigerated environments, seeds exhibit increased electrical conductivity compared to refrigerated storage. This results in increased physiological activity, consequently reducing seed vigor.

In the field emergence test for normal seedlings, higher emergence (97.50%) was observed at zero days of storage compared to 15 and 30 days (96.56% and 94.62%, respectively). For weak seedlings, no significant difference ($P>0.05$) was found between 0 and 15 days of storage, nor between 15 and 30 days. However, at zero days of storage, a lower number of weak seedlings was observed compared to 30 days. Regarding defective seedlings, a lower percentage (0.66%) was found at zero days compared to 30 days of storage (1.44%). For dead seedlings, no difference was observed between zero and 15 days of storage, but a higher number of dead seedlings was recorded at 30 days compared to zero days (Table 4).

According to Forti, Cicero and Pinto (2010), weak and defective seedlings are more likely to result from moisture damage, which generally occurs in the embryonic axis and hypocotyl. These damages are easily observed in laboratory tests, as they evolve and cause the plant to exhibit disordered cell growth, leading to deformities in the roots, plumule, or cotyledons—characteristics that drastically reduce the plant's vigor.

Rainfall close to harvest contributes to the deterioration of future seedlings, as it causes fissures in seed's structures, leading to defective seed's germination, reduced germination rates, and decreased vigor. Even if the seed manages to emerge, the seedling may fail to develop due to these damages (Krzyzanowski; França Neto; Henning, 2018).

The controlled environment resulted in a higher percentage of normal seedling emergence

(96.17%) in paper substrate compared to the non-controlled environment (95.59%). However, no significant difference (P>0.05) was observed between storage environments for the analyzed variables: weak, defective, and dead seedlings. In the sand substrate germination test, no differences were observed between storage environments for normal, weak, defective, or dead seedlings. In the field emergence test, the controlled environment resulted in a higher percentage of normal seedlings (96.81%) compared to the non-controlled environment (96.31%). However, no significant difference was found between storage environments for the analyzed variables: weak, defective, and dead seedlings (Table 5).

Table 5 - Percentage (%) of Germination in Paper Substrate and Emergence in Sand Substrate and Field Bed of Treated Soybean Seeds Subjected to Different Storage Conditions

Variable	**Ambiente**	
	Non control	**Control**
Paper substrate		
Normal	95.59b	96.17a
Weak	2.60a	2.08a
Defective	1.04a	1.25a
Dead	0.73a	0.60a
Sand substrate		
Normal	96.21a	96.33a
Weak	1.75a	1.89a
Defective	1.31a	1.08a
Dead	0.75a	0.67a
Seedbed		
Normal	96.31b	96.81a
Weak	1.90a	1.88a
Defective	1.08a	0.83a
Dead	0.96a	0.58a

Means followed by distinct uppercase letters in the row and lowercase letters in the columns differ from each other according to Tukey's test at a 5% significance level.
Source: research data**.**

For this reason, storage in a controlled environment becomes an alternative to minimize the loss of germination capacity and seed vigor. The fewer temperature and air humidity fluctuations experienced by the seeds, the lower the chances of caloric expenditure (Krzyzanowski; França Neto; Henning, 2018). In the analyses performed, it was observed that seeds stored in a controlled environment produced more vigorous seedlings compared to those stored in an uncontrolled environment.

Between the two types of storage, it was possible to observe that seeds are more susceptible and active in an uncontrolled environment. Thus, the longer the seeds remained in this environment, it is believed that there was greater metabolic activity, leading to the germination of a higher number of weak, defective, and dead seedlings. Primon, Primon, and Pontes (2019) demonstrated that a climatized environment improves the condition and stability of soybean seed quality, as it prevents the seeds from experiencing stress due to edaphoclimatic factors.

Coradi *et al*. (2015) observed that after more than 100 days of storage, vigor and germination at temperatures above 20°C begin to decline significantly. This is due to temperature fluctuations, which cause loss of membrane integrity, changes in seed respiratory activity, modifications in enzymatic activity, and an inability to maintain the electrochemical gradient.

4 Conclusion

There is a reduction in the number of normal seedlings as the storage period increases.

Seeds stored in a controlled environment have a higher percentage of normal seedlings compared to those stored in an uncontrolled environment.

The TMG 2776 IPRO cultivar performs better in laboratory tests; however, in field tests, the HO Aporé IPRO cultivar shows better performance.

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