Ciências Agrárias
SEMINA

DOI: 10.5433/1679-0359.2025v46n6p1889

Soybean germination test: is it possible to dispense premoistening using rolled paper + vermiculite?

Teste de germinação de soja: é possível dispensar o pré-condicionamento utilizando rolo de papel + vermiculita?

Venicius Urbano Vilela Reis[1]; Danilo Cordeiro Maciel[2]; Giulyana Isabele Silva Tavares[2]; Leticia Moreno[3]; Álvaro Alves de Andrade[4]; Vinicius Rodrigues[4]; Everson Reis Carvalho[5]*

Highlights

Premoistening improves the germination of seeds with low moisture content. The RP+V substrate mitigates phytotoxic effects, promoting seedlings growth. Premoistening + RP+V provides reliable results in assessing physiological quality. RP+V does not replace premoistening; it's best to use them together for efficiency.

Abstract

The combination of soybean seeds with low moisture content and treatment with phytosanitary products in seed germination assessment has led to inconsistent results in the rolled paper (RP) germination test. The premoistening technique, which involves slowly hydrating the seeds before the germination test, can be used to mitigate imbibitional injury in seeds with low moisture content. The rolled paper + vermiculite (RP+V) substrate, on the other hand, reduces the phytotoxicity of phytosanitary products. Therefore, this study aimed to assess whether premoistening is necessary when using the RP+V substrate. For this assessment, soybean seeds from a single lot were dried to different moisture contents and were then treated or not treated with phytosanitary products. The experiment was carried out in a 3 × 2 × 2 factorial arrangement, consisting of three seed moisture contents (12%, 10%, and 8%), two

[1] PhD candidate in the graduate Program in Agronomy/Crop Science, Departamento de Agricultura, Universidade Federal de Lavras, UFLA, Lavras, MG, Brazil. Visiting Scholar at the Department of Plant Sciences, University of California, Davis, CA, USA. E-mail: veniciusreis@gmail.com

[2] PhD candidate of the graduate Program in Agronomy/Crop Science, Departamento de Agricultura, Universidade Federal de Lavras, UFLA, Lavras, MG, Brazil. E-mail: macieldaniloagro@gmail.com; giulyanatavares@outlook.com

[3] Research Fellow – Conhecimento Brasil 1, Departamento de Agricultura, Universidade Federal de Lavras, UFLA, Lavras, MG, Brazil. E-mail: leticia.moreno@ufla.br

[4] Undergraduate student in Agronomy, Departamento de Agricultura, Universidade Federal de Lavras, UFLA, Lavras, MG, Brazil. E-mail: alvaroadeandrade2@gmail.com; vinicius.rodrigues1@estudante.ufla.br

[5] Prof. Dr., Departamento de Agricultura, Universidade Federal de Lavras, UFLA, Lavras, MG, Brazil. E-mail: eversoncarvalho@ufla.br

* Author for correspondence

Received: Apr. 14, 2025 Approved: Dez. 02, 2025

types of premoistening activity (with and without), and two substrates (RP and RP+V). Premoistening improved germination rates, especially for seeds with 8% moisture content. Without premoistening, RP+V enhanced seed-quality expression, possibly reducing imbibitional damage. In addition, RP+V resulted in a higher percentage of strong normal seedlings, signaling better initial development. It can be concluded that RP+V does not eliminate the need for premoistening, and both are effective practices in assessing the quality of low moisture content soybean seeds, even when they have been treated with phytosanitary products. This combination of techniques optimizes the germination test, ensuring more reliable results for assessing germination potential.
Key words: *Glycine max* L. Moisture content. Physiological quality. Quality control. Imbibitional injury.

Resumo

A combinação de sementes com baixa umidade e tratadas com produtos fitossanitários tem gerado inconsistências nos resultados do teste de germinação em rolo de papel (RP). A técnica de pré-condicionamento, que consiste na hidratação lenta da semente antes do teste de germinação, pode ser utilizada para amenizar danos por embebição nessas sementes, enquanto o substrato rolo de papel + vermiculita (RP+V) reduz a fitotoxicidade dos produtos fitossanitários. Sendo assim, o objetivo deste estudo foi avaliar se a utilização do pré-condicionamento é necessária quando se faz uso do substrato RP+V, visto que há a hipótese de lenta absorção de água quando se utiliza este substrato, em sementes com e sem tratamento. Para isso, utilizou-se sementes de soja de um mesmo lote, que por secagem foram obtidas diferentes umidades e então tratadas ou não com produtos fitossanitários. O experimento foi realizado em fatorial 3 × 2 × 2, sendo três umidades das sementes (12, 10 e 8%), dois tipos de pré-condicionamento (condicionadas ou não), e dois substratos (RP e RP+V). O pré-condicionamento proporcionou melhor condição de germinação, especialmente em sementes com 8% de umidade. Sem pré-condicionamento, o RP+V favoreceu a expressão da qualidade, reduzindo possivelmente, danos por embebição. Além disso, o RP+V promoveu maior percentagem de plântulas fortes, indicando proporcionar melhor desenvolvimento inicial. Conclui-se que o RP+V não dispensa o pré-condicionamento, sendo ambos eficazes para avaliar a qualidade de sementes de soja com baixa umidade, mesmo quando tratadas com produtos fitossanitários. Essa combinação de técnicas otimiza o teste de germinação, assegurando resultados mais confiáveis para a avaliação do potencial germinativo.
Palavras-chave: *Glycine max* L. Umidade. Controle de qualidade. Qualidade fisiológica. Dano por embebição.

Introduction

Soybeans are one of the world's most important agricultural commodities, and Brazil is the largest producer worldwide (Companhia Nacional de Abastecimento [CONAB], 2025). High yield and total production are directly related to the use of technologies, most of which are applied to seeds. In addition, the use of high-quality seeds is essential, since high yield and seed quality are directly related (Reis et al., 2022).

Reliable assessment of seed physiological quality is a current concern in the soybean seed production chain, since the type of substrate or even germination time can often affect results, especially when seeds have been treated with phytosanitary products (Rocha et al., 2025). The results of the assessment of the germination rate of treated seeds using conventional substrates such as rolled paper (RP) have been inconsistent with what is observed in the field, as the assessed germination rate has been lower than emergence in the field. This may be caused by several factors, including types of substrates, the phytosanitary products applied, the moisture content of the seed lot, seed quality, and imbibitional damage resulting from the low moisture content of the seed lot (Carvalho et al., 2024).

Seed moisture content is an important physical property that affects seed viability (Mbofung et al., 2013). At low moisture content levels (< 11%), soybean seeds are susceptible to imbibitional injury caused by rapid uptake of water (Toledo et al., 2010). Rapid uptake of water can cause irreversible cellular damage, leading to cell death in soybeans. Genotypic differences also affect susceptibility to cell damage (Bahry et al., 2017; Pereira et al., 2022; Sato et al., 2019). Germination testing can reveal the symptoms of imbibitional injury through changes in seedling morphology, such as abnormalities in the root system (short or missing radicles) or changes in the color of seedling tissues (França & Krzyzanowski, 2018; Satya Srii et al., 2022).

The seed moisture content factor combined with the substrate used in the germination test may directly affect the level of imbibitional injury to the seeds. In general, more marked imbibitional injury in the soybean germination test using the RP substrate occurs at seed moisture content levels below 11%, whereas moisture content higher than 15% does not result in this type of injury (Toledo et al., 2010; Nardelli et al., 2025).

In general, soybean seeds often reach low moisture content levels (< 10%) in regions with high- temperatures and low relative humidity, due to hygroscopic equilibrium. Seed analyses in the laboratory through physiological tests, such as the germination test, are then conducted on seed lots with low moisture content. These conditions can prevent expression of full seed germination potential, primarily as a result of imbibition damage caused by rapid water uptake.

Premoistening of seeds prior to sowing them in the substrate is recommended to mitigate imbibitional injury during the germination test. The Rules for Seed Testing (*Regras para Análise de Sementes* - RAS) (Ministério da Agricultura, Pecuária e Abastecimento [MAPA], 2025) recommend premoistening when seeds exhibit sensitivity to damage from rapid imbibition. Moreover, Toledo et al. (2010) recommend this procedure for seeds with a moisture content below 11%, whether or not sensitivity has been confirmed.

Premoistening can help alleviate problems and abnormalities resulting from rapid water uptake, as premoistening can result in reduced electrolyte leaching (Pereira et al., 2022; Rodrigues et al., 2006). However, a major disadvantage of premoistening is related to seed testing laboratory routines; premoistening adds an extra day to test procedures and increases operational costs. Therefore, alternatives that enhance the reliability of analyses in a faster, more cost-effective, and safer manner should be evaluated.

The RP+V substrate methodology (Carvalho et al., 2024) uses vermiculite in the germination test and may represent an alternative that mitigates injury caused by water uptake. This hypothesis is based on the ability of vermiculite may have in reduce the seed imbibition rate (Reis et al., 2024).

The objective of this study was to determine whether premoistening is necessary when using the RP+V substrate methodology under different seed moisture contents and substrates in the germination test.

Materials and Methods

Location

The experiment was performed at the central seed research laboratory, and seed treatment was performed at the seed processing plant, both at the Department of Agriculture (DAG), School of Agricultural Sciences of Lavras (ESAL) of the Universidade Federal de Lavras (UFLA), Lavras, Minas Gerais, Brazil.

Experimental design

The experiment was performed in a completely randomized design (CRD) in a 3 × 2 × 2 factorial arrangement, with four replicates of 50 seeds each. The factors included three seed moisture content levels at the time of testing (12%, 10%, and 8%), two types of premoistening activity (with and without), and two germination test substrates (RP and RP+V). Treated and untreated seeds were evaluated separately.

Initial seed lot characterization and seed drying

Seeds were taken from a single lot of the cultivar NS 8080 IPRO. They met the physical and physiological parameters for moisture content, first germination count on RP substrate (FG), germination rate on RP substrate (G), seedling emergence in trays (SE), and accelerated aging (AA), as shown in Table 1, and were dried to the desired moisture content levels. Drying was performed in a stationary, longitudinal experimental dryer set to 35 °C (± 2 °C) with an airflow of 23 m^3 min^{-1}, and seed moisture content was monitored until the target level was reached.

Table 1
Initial seed lot quality, before drying and seed treatment

Physical and physiological parameters (%)				
Initial moisture	FC	G	SE	AA
12.57	83	83	85	60

FC = first germination count (5 days), G = germination (8 days), SE = seedling emergence in trays (8 days), and AA = accelerated aging.

After drying, the seeds were kept in Kraft-paper packaging and covered with a plastic bag for a period of 12 hours to achieve uniform moisture content within the seed mass. This process provided three seed lots with moisture contents of 8%, 10%, and 12%.

Seed treatments and moisture content determination

After the seeds were dried and the moisture content was stabilized, the seeds were weighed and divided into 500-g portions for a subsequent phytosanitary treatment process. The treatment was performed using the Momesso Arktos Laboratory L2K BM machine set to 15 hertz on the equipment inverter, followed by application and mixing for 20 seconds to replicate the industrial batch process. All seeds received the Fortenza Duo® formulation that includes the fungicides and insecticides shown in Table 2. Additionally, the Biocroma Red Biogrow® polymer was added at the rate of 100 mL per 100 kg of seeds.

Table 2
Active ingredients (AI) and commercial products used in the chemical treatment of soybean seeds

AI	AI concentration	Commercial product	Type[1]	Dose of commercial product[2]
Thiamethoxam	350 g L^{-1}	Cruiser 350 FS®	I	200 mL
Cyantraniliprole	600 g L^{-1}	Fortenza 600 FS®	I	60 mL
Metalaxyl-M	20 g L^{-1}	Maxim Advanced®	F	100 mL
Thiabendazole	150 g L^{-1}			
Fludioxonil	25 g L^{-1}			

[1]Type – I: insecticide, F: fungicide; [2]Dose: mL per 100 kg of seeds

After treatment, the seeds were packed in multi-ply Kraft paper bags and subsequently tested for germination on different substrates. The different moisture content levels after drying and chemical treatment of the seeds were confirmed using the laboratory oven method at 105 °C, as described in MAPA (2025). The results are shown in Table 3.

Table 3
Moisture content of the seed lots after drying and seed treatment

Phytosanitary treatment	Desired moisture content (%)		
	12%	10%	8%
Untreated	12.57	9.75	7.91
Treated	12.01	9.84	7.72

Methodologies and evaluations

For the treatments involving premoistening prior to sowing, plastic boxes were used with suspended aluminum screens and the addition of 40 mL of water at the bottom. The seeds were placed on the screens and remained for a period of 24 hours in biochemical oxygen demand (B.O.D) chambers at 25 ± 2 °C (MAPA, 2025). These premoistened seeds were then tested for germination on the different substrates described below, as were the seeds that did not undergo premoistening.

The substrates used in the evaluations were RP and RP+V, as follows:

Germination on rolled paper (RP): The seeds were sown between two sheets of germination paper moistened with distilled water in an amount of 2.5 times the weight of the paper. The rolls were closed and incubated in a Mangelsdorf-type germinator set at 25 ± 2 °C. Germination was assessed five and eight days after sowing, calculating the percentage of normal seedlings (MAPA, 2025).

Germination on rolled paper with vermiculite (RP+V): For each replicate, a pre-moistened sheet of germination paper (with distilled water in the amount of three times the weight of the paper) received 100 mL of vermiculite (65% to 95% of the particles with fine granulation) in a thin, uniform layer, moistened at a ratio of 1 g of vermiculite to 1 mL of water. The 50 seeds for each replicate were then distributed using a seed counter and covered with another moistened sheet of paper. Then the rolls were closed, as in the RP substrate tests. The rolls were incubated in a Mangelsdorf-type germinator at 25 ± 2 °C. Normal seedlings were counted five and seven days after sowing (Carvalho et al., 2024).

For both substrates, the strong normal seedlings were also counted five days after sowing. Strong normal seedlings are defined as those without defects and longer than 5 cm (Krzyzanowski et al., 2020)

Statistical analysis

Analysis of variance (ANOVA) was performed on the data at a 5% significance level using the F-test. When differences were significant, means were compared using Tukey's test, also at a 5% significance level. All analyses were performed with RStudio software, using the ExpDes.pt package (Ferreira et al., 2021; R Core Team [R], 2024).

Results

Significant differences were observed in all the variables studied, both for untreated and treated seeds.

Untreated seeds

For the first germination count in the untreated seed lots, no statistical differences were observed between the substrates for the seed lots with 12% moisture content. However, for the seed lots with 10% and 8% moisture contents, the RP+V substrate resulted in a higher percentage of normal seedlings, by 6 and 11 percentage points (pp), respectively. Within the same substrate, only the seed lots with moisture contents above 10% showed no significant differences from each other (Figure 1A).

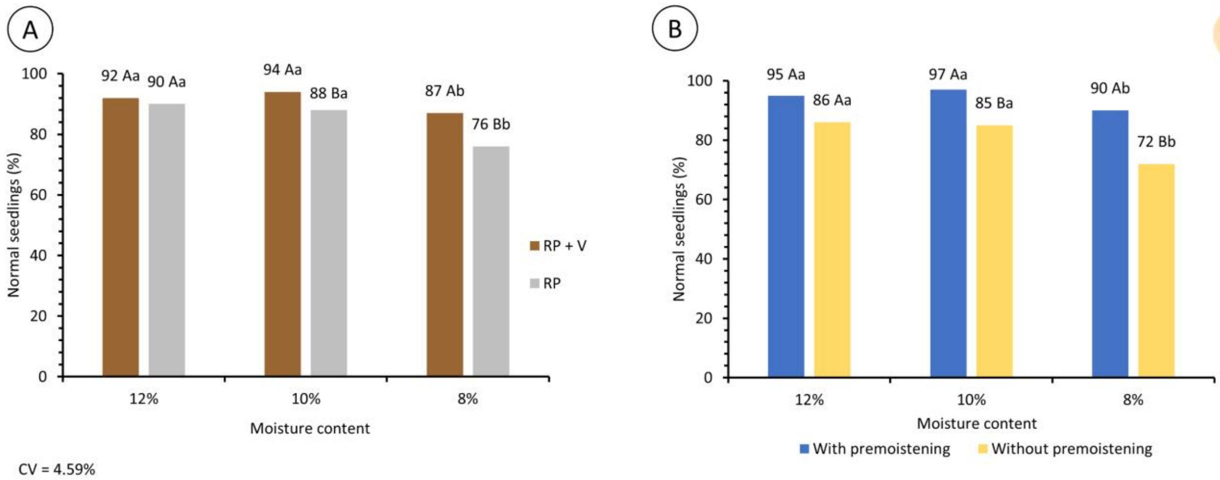

Figure 1. Percentage of normal seedlings in the first germination count of untreated soybean seeds, based on moisture content and substrate (A), and based on moisture content and the use of premoistening status (B). Means with the same letter are not significantly different according to Tukey's test at 5% significance (uppercase for substrate comparison within the same moisture content and lowercase for moisture content comparison within the same substrate in A; uppercase for premoistening status comparison within the same moisture content and lowercase for moisture content comparison within the same premoistening status in B).

Premoistening improved the seed-quality expression in untreated seed lots at 10% and 8% moisture content, with an increase in normal seedlings of 12 pp and 18 pp, respectively (Figure 1B). Regardless of premoistening, soybean seed lots with 8% moisture content had a lower percentage of normal seedlings in the first germination count compared to lots with higher moisture contents (Figure 1B).

In the germination test, the RP+V substrate was superior to RP, regardless of premoistening, though the difference was more pronounced without premoistening, where the germination rate on the RP substrate was below 80%. Furthermore, the use of premoistening increased the germination rate in both substrates (Figure 2A).

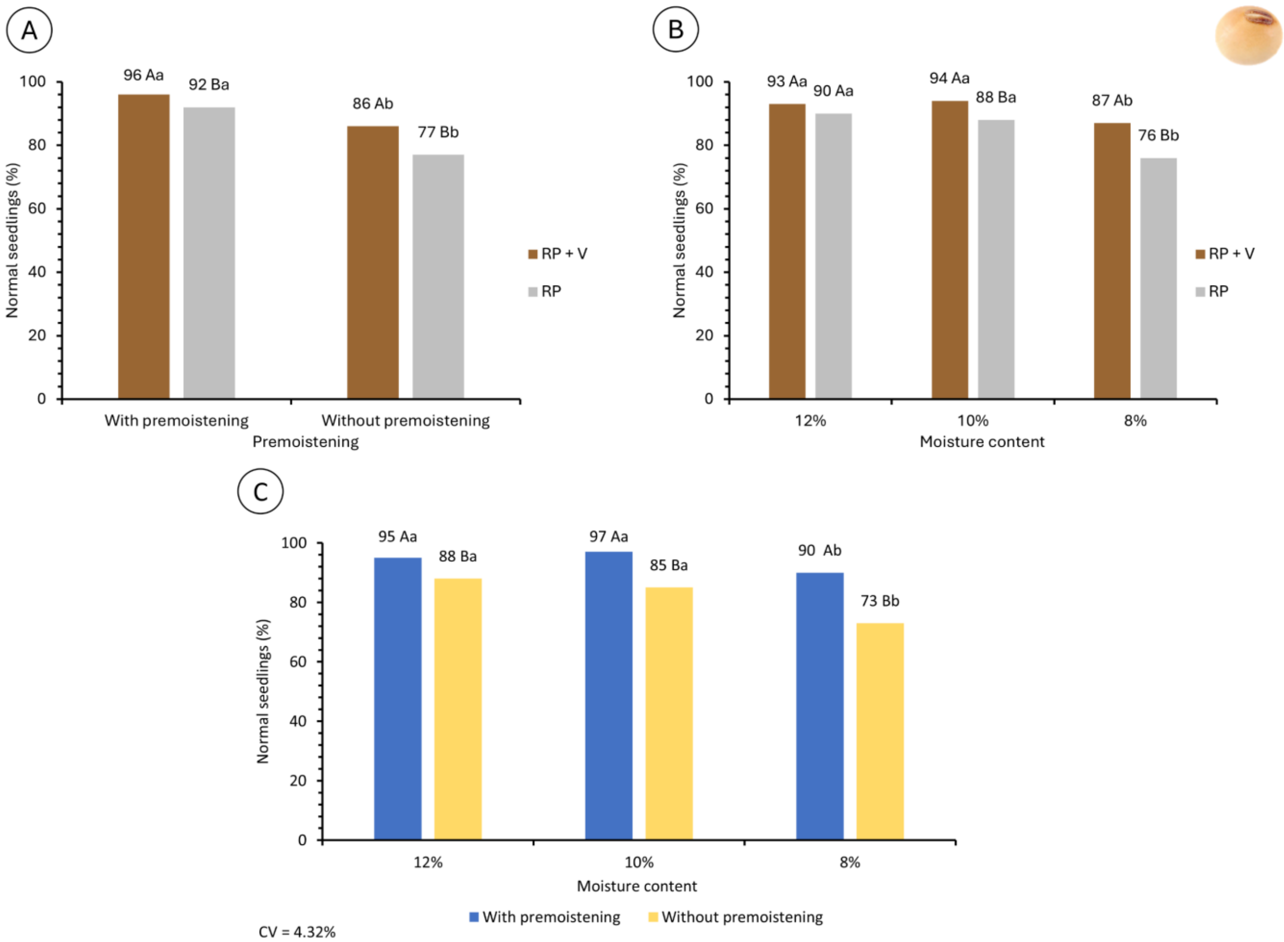

Figure 2. Percentage of normal seedlings in evaluation of germination of untreated soybean seeds, based on substrate and premoistening status (A), moisture content and substrate (B), and moisture content and premoistening status (C). Means with the same letter do not differ significantly according to Tukey's test at 5% significance (uppercase for substrate comparison within the same premoistening status and lowercase for premoistening status comparison within the same substrate in A; uppercase for substrates within the same moisture content and lowercase for moisture content levels within the same substrate in B; uppercase for premoistening status within the same moisture and lowercase for moisture content levels within the same premoistening status in C).

As in the first germination count (Figure 1A), untreated seed lots with 12% moisture content did not show differences between substrates in germination test results (Figure 2B). For 10% and 8% moisture content, the RP+V substrate maintained its superiority, resulting in higher expression of germinative potential. The germination rates were below 80% for the RP substrate and 8% moisture content. Additionally, for both substrates, the difference between seed lots was more evident at lower moisture content levels, which showed strikingly lower germination rates compared to moisture content levels above 10% (Figure 2B).

Furthermore, the use of premoistening allowed seed lots to express their germinative potential at all moisture content levels evaluated, especially at the lower moisture content of 8%, which showed an increase of 17 pp, regardless of the substrate (Figure 2C). This highlights the importance of premoistening regardless of the germination methodology used.

The use of premoistening promoted greater seedling development, resulting in a higher percentage of strong normal seedlings (Figure 3A). This phenomenon was more evident in the RP substrate, with an increase of 23 pp in strong normal seedlings.

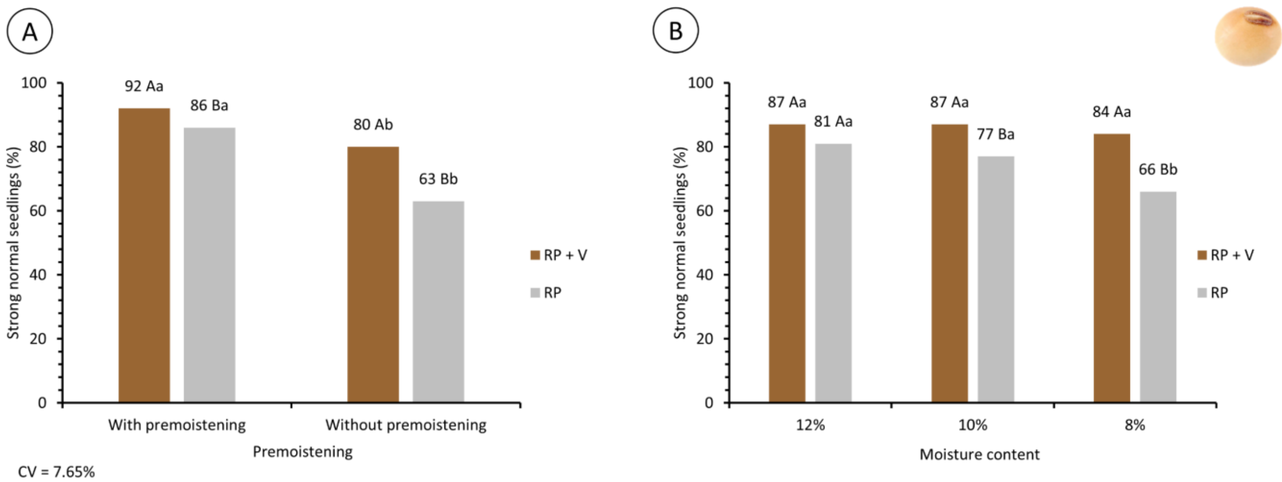

Figure 3. Percentage of strong normal seedlings at five days after sowing untreated soybean seeds, based on substrate and premoistening status (A), and on moisture content and substrate (B). Means with the same letter do not differ significantly according to Tukey's test at 5% significance (uppercase for substrate comparison within the same premoistening status and lowercase for premoistening status comparison within the same substrate in A; uppercase for substrates within the same moisture content and lowercase for moisture contents within the same substrate in B).

The RP+V substrate produced a higher percentage of strong normal seedlings, both with and without premoistening. However, this effect was more evident when premoistening was not applied (Figure 3A).

The strong normal seedling results in the use of the RP+V substrate were not affected by the moisture contents of the seed lots, meaning there was no statistical difference between the different moisture content levels. However, the use of the RP substrate showed significant differences; seed lots with 8% moisture content resulted in 11 pp lower strong normal seedling development compared to seed lots with 10% moisture content. Comparison of the substrates shows that for moisture content of 12% or less, the RP+V substrate was superior, particularly at 8%, where the difference between the substrates was 18 pp (Figure 3B).

Treated seeds

Premoistening improved seed-quality expression in soybean seed lots with phytosanitary treatment at all moisture content levels and on both substrates, except for 12% moisture content on the RP+V substrate, with increases of up to 15 pp for normal seedlings on the RP substrate and 14 pp on the RP+V substrate. Moreover, as observed in untreated seeds, seed lots with 8% moisture content expressed lower quality (Figure 4).

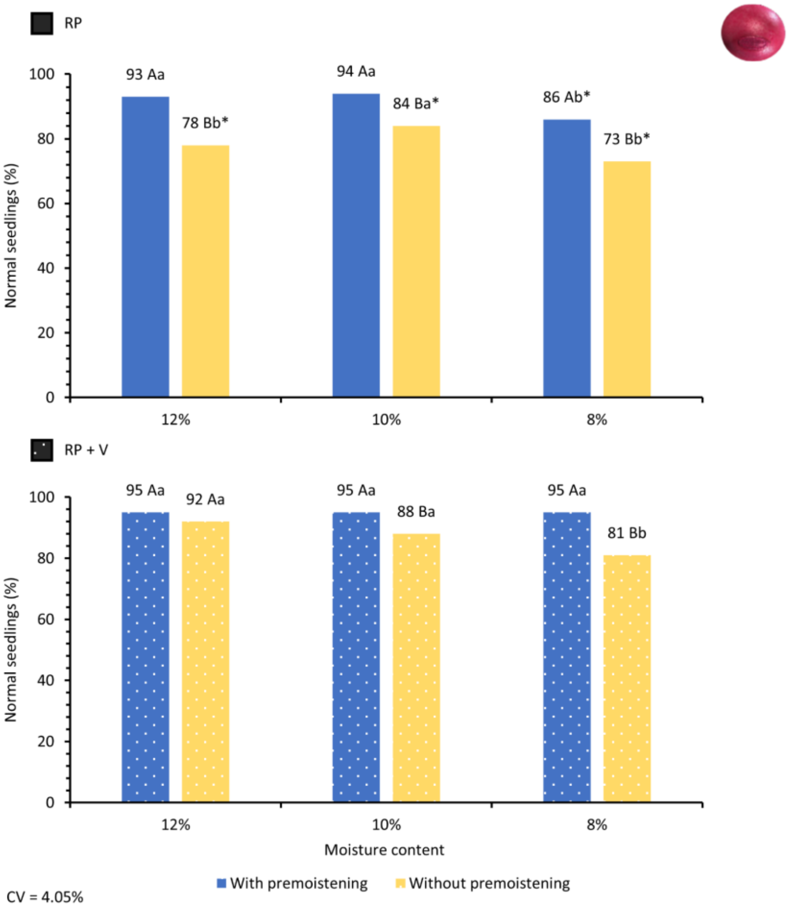

Figure 4. Percentage of normal seedlings in the first germination count at five days after sowing soybean seeds with a phytosanitary treatment, based on seed lot moisture content, premoistening status, and substrate. Means with the same letter do not differ significantly according to Tukey's test at 5% significance (uppercase for premoistening status comparison within the same moisture content and substrate, and lowercase for moisture content comparison within the same premoistening status and substrate). The symbol '*' indicates a statistical difference between substrates within the same premoistening status and moisture content level.

With the use of premoistening, the substrates showed differences only at 8% moisture content. However, without premoistening, RP+V produced a higher percentage of normal seedlings at all moisture content levels. The greatest difference was 14 pp at 12% moisture content (Figure 4).

For germination, the factors exhibited independent effects. Premoistening increased the germination rate by 9 pp, and use of the RP+V substrate increased by 5 pp Furthermore, the germination rate was statistically the same for the 12% and 10% moisture content, both were 4 pp higher than the 8% moisture content (Figure 5).

Premoistening favored seedling development at all moisture content levels; the 8% moisture content level showed the greatest effect, with a 26-pp increase in strong normal seedlings. When premoistening was used, no differences were observed among moisture content levels; however, without premoistening, the seed lots with moisture content levels of 8% showed an 11-pp decrease in strong normal seedlings (Figure 6A). Comparison of the substrates shows that, apart from other factors, the RP+V substrate increased strong normal seedling development by 5 pp.

Discussion

Premoistening soybean seeds is a routine practice in analysis laboratories; yet it does not always follow the technical recommendations established in the literature. As highlighted by Toledo et al. (2010), the use of this technique should be restricted to seeds with moisture content levels below 11%, aiming to mitigate injury from rapid water uptake. However, commercial laboratories often use this technique on all the seed lots received, regardless of their initial moisture content.

This methodological discrepancy calls for a critical reflection on the importance of standardizing premoistening procedures in seed analysis. Premoistening allows seeds to take up a portion of water in controlled exposure to a humid atmosphere. This approach limits rapid water uptake immediately after sowing, as described by Chen and Arora (2013).

During premoistening, the seed reaches hygroscopic equilibrium with the environment, increasing seed moisture content and delaying complete metabolic activation. This allows time for cells to repair damage accumulated during storage (Ataíde et al., 2016; Varier et al., 2010).

The results of this study show that seed lots with 8% moisture content exhibited lower physiological quality, except when germinated on the RP+V substrate (Figure 4). This result confirms the hypothesis that initial moisture content directly affects the expression of germinative potential. Seeds below 10% moisture content showed significant reductions in germination rate and seedling vigor, in agreement with the findings of Toledo et al. (2010). These authors associate rapid water uptake on substrates that have high water availability with irreversible damage to cellular membranes and genetic material.

Pereira et al. (2022) showed that slow hydration through premoistening minimizes toxic effects from abrupt water uptake, preserving the integrity of the cell cycle and normal seedling growth. Additionally, premoistening reduces excessive production of reactive oxygen species, possibly by limiting exposure to free oxygen during controlled imbibition. Moreover, studies on seed coat permeability of soybeans, water uptake, and germination rate indicate that artificial aging leads to deterioration of the seed coat. This structural damage and increased permeability increase water uptake during the initial phase of imbibition. Rapid water uptake in aged seeds is associated with a decline in germination rate and a higher incidence of abnormal seedlings (Satya Srii et al., 2022).

The application of premoistening to seeds with moisture content between 8% and 12% was essential for maximizing physiological quality, whether the seeds were treated with phytosanitary products or not. This result is in agreement with Silva and Villela (2011), who credit the technique with the ability to mitigate metabolic damage caused by accelerated rehydration, allowing for repair of plasma membranes and DNA.

Premoistening can prevent soybean lots from being discarded due to low germination, as observed in Figure 2A. This is an important aspect, given the legal requirement of an 80% germination rate for seed commercialization in Brazil (MAPA, 2013). The benefits were more pronounced in seeds with 8% moisture content, especially on the RP substrate, reflecting the reality of lots stored at hygroscopic equilibrium with environments of low relative humidity and high temperature, common in the Center West region of Brazil in the winter, when most soybean seed germination tests are conducted.

Pereira et al. (2022) emphasize that modern cultivars require greater water availability to germinate, which explains the superiority of the RP+V substrate. The addition of vermiculite increases water-holding capacity in the substrate, simulating field conditions in soil, and it may reduce imbibitional injury (Carvalho et al., 2024; Reis et al., 2025). This vermiculite substrate also minimizes the phytotoxicity of phytosanitary products applied to the seeds, making it a viable option for treated seeds (Rocha et al., 2020; Tunes et al., 2021). This reduction in toxicity may have occurred regarding the treated seed lots and can explain the difference between the two substrates (Figures 4, 5, and 6). Studies indicate that the laminar structure of vermiculite absorbs toxic molecules, reducing their availability to the seeds and making laboratory tests more representative of field conditions (Carvalho et al., 2024; Cuong et al., 2019).

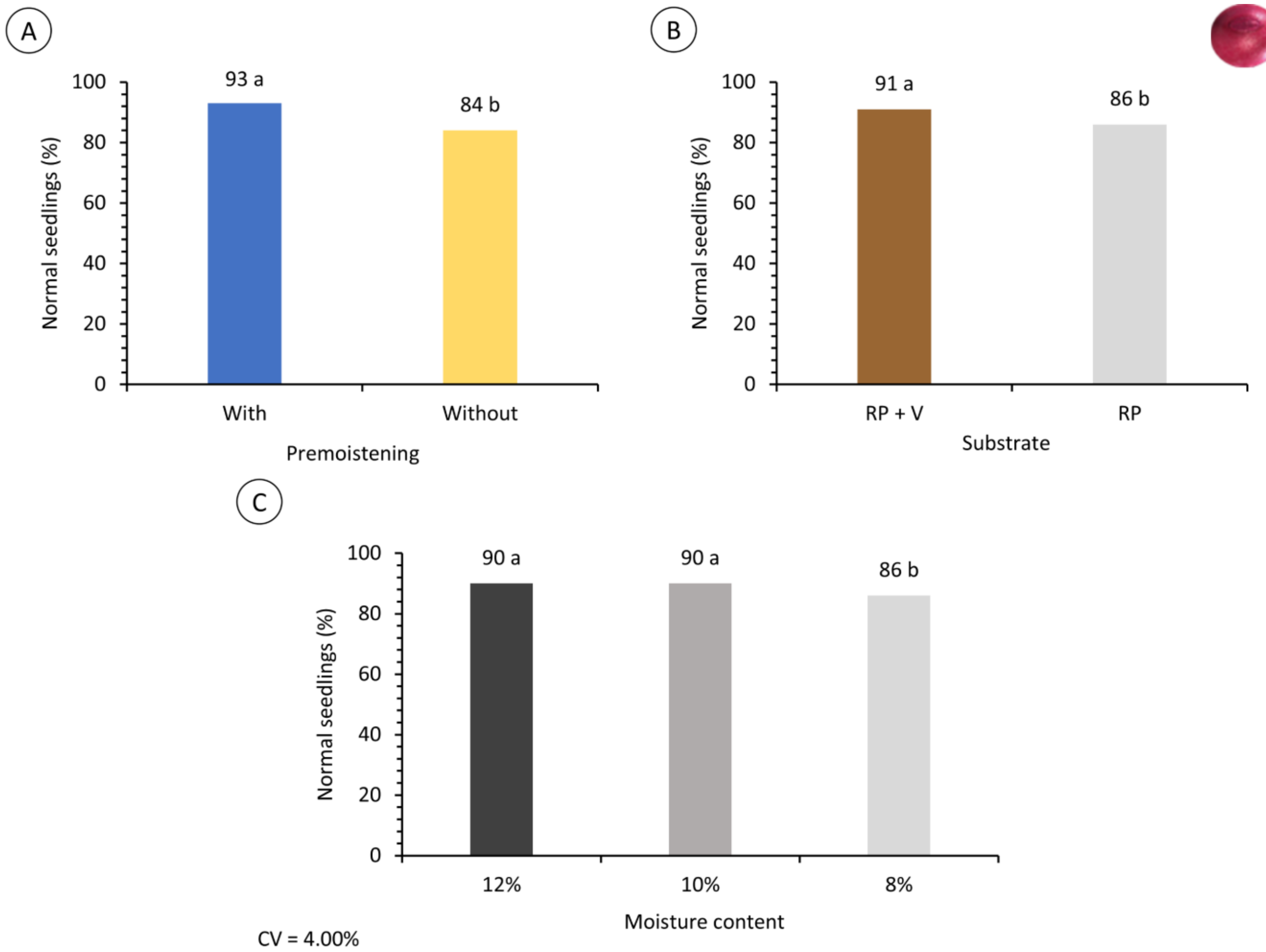

Figure 5. Percentage of normal seedlings in the germination of soybean seeds without phytosanitary treatment, based on premoistening status (A), substrate (B), and seed lot moisture content (C). Means with the same letter do not differ significantly according to Tukey's test at 5% significance.

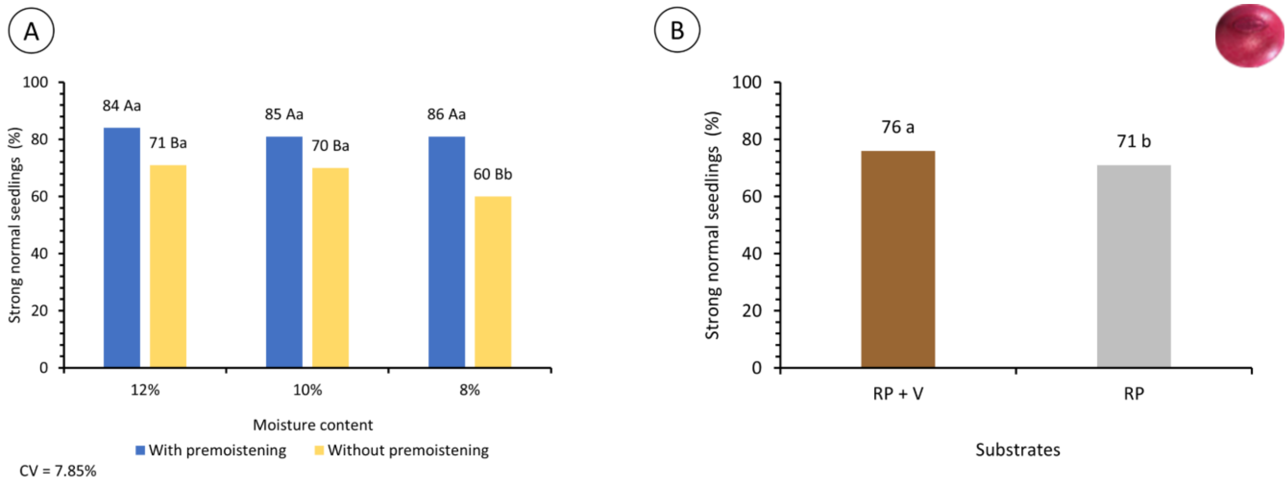

Figure 6. Percentage of strong normal seedlings (> 5 cm length) at five days after sowing soybean seeds with a phytosanitary treatment, based on moisture content and premoistening (A) and on substrate (B). Means with the same letter do not differ according to Tukey's test at 5% significance (uppercase for premoistening status within the same moisture content and lowercase for moisture content within the same premoistening status in A and B).

Additionally, the RP+V substrate promoted the development of vigorous seedlings (> 5 cm length), facilitating test interpretation and increasing the precision of the results (Figures 1, 2, and 3). This characteristic is particularly relevant for treated seeds, where vermiculite lessens phytotoxic effects, and also for seeds with low moisture content, allowing for a more representative assessment of the seed lot germinative potential.

Synergistically combining a premoistening treatment, initial seed moisture content, and the choice of a suitable substrate emerges as a strategy to optimize seed quality evaluation, especially in seed lots with low moisture content. Vermiculite, in particular, shows potential for standardizing testing methodologies, minimizing confounding variables, and producing laboratory results that better reflect agronomic performance.

Conclusions

The use of the RP+V substrate does not eliminate the need for premoistening soybean seed lots with moisture content between 12% and 8% in germination testing.

Premoistening improves the expression of seed quality in both treated and untreated soybean seed lots with moisture content between 12% and 8%, with the most noticeable effect in lots with moisture content lower than 10%.

In the absence of premoistening, the RP+V substrate results in better expression of the physiological quality of the seed lot compared to the RP substrate alone.

Statements and Declarations

Acknowledgments

The authors extend their sincere gratitude to the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), the Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for their generous financial support, including scholarships and a research productivity grant.

Funding

This study was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq (Process: 314408/2021-5), the Fundação de Amparo à Pesquisa do Estado de Minas Gerais – FAPEMIG (Process: APQ-01869-18), and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES (Finance code: 001).

Consent for publication

All the authors consent to the publication of the article and associated data.

Availability of data and materials

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

References

Ataíde, G. D. M., Borges, E. E. D. L. E., Gonçalves, J. F. D. C., Guimarães, V. M., & Flores, A. V. (2016). Alterações fisiológicas durante a hidratação de sementes de Dalbergia nigra ((vell.) fr. All. Ex benth.). *Ciência Florestal, 26*(2), 615-625. doi: 10.5902/1980509822761

Bahry, C. A., Perboni, A. T., Nardino, M., & Zimmer, P. D. (2017). Physiological quality and imbibitions of soybean seeds with contrasting coats. *Revista Ciência Agronômica, 48*(1), 125–133. doi: 10.5935/1806-6690.20170014

Carvalho, E. R., Reis, V. U. V., Carvalho, M. L. M. D., Rocha, D. K., Caetano, C. C., & Fernandes, N. (2024). Validation of the methodology of the germination test using a rolled paper plus vermiculite for treated soybean seeds. *Journal of Seed Science, 46*(1), e202446020. doi: 10.1590/2317-1545v46282001

Chen, K., & Arora, R. (2013). Priming memory invokes seed stress-tolerance. *Environmental and Experimental Botany, 94*(1), 33-45. doi: 10.1016/j.envexpbot.2012.03.005

Companhia Nacional de Abastecimento (2025). Acompanhamento da safra brasileira de grãos: safra 2024/25 - quarto levantamento. CONAB. https://

www.conab.gov.br/info-agro/safras/graos/boletim-da-safra-de-graos

Cuong, N. D., Hue, V. T., & Kim, Y. S. (2019). Thermally expanded vermiculite as a risk-free and general-purpose sorbent for hazardous chemical spillages. *Clay Minerals, 54*(3), 235-243. doi: 10.1180/clm.2019.34

Ferreira, E. B., Cavalcanti, P. P., & Nogueira, D. A. (2021). ExpDes: an R Package for ANOVA and experimental designs. *Applied Mathematics, 5*(19), 2952-2958. doi: 10.4236/am.2014.519280

França, J. de B., Neto, & Krzyzanowski, F. C. (2018). *Metodologia do teste de tetrazólio em sementes de soja.* EMBRAPA Soja. https://ainfo.cnptia.embrapa.br/digital/bitstream/item/193315/1/Doc-406-OL.pdf

Krzyzanowski, F. C., França, J. D. B., Neto, Gomes, F. G., Jr., & Nakagawa, J. (2020). Testes de vigor baseados em desempenho de plântulas. In F. C. Krzyzanowski, R. D. Vieira, J. de B. França-Neto, & J. Marcos-Filho, *Vigor de sementes: conceitos e testes* (2a ed., pp. 79-140). Londrina: Associação Brasileira de Tecnologia de Sementes - ABRATES.

Mbofung, G. C. Y., Goggi, A. S., Leandro, L. F. S., & Mullen, R. E. (2013). Effects of storage temperature and relative humidity on viability and vigor of treated soybean seeds. *Crop Science, 53*(3), 1086-1095. doi: 10.2135/cropsci2012.09.0530

Ministério da Agricultura, Pecuária e Abastecimento [MAPA] (2013). Instrução Normativa nº 45, de 17 de setembro de 2013. Diário Oficial da União, Seção 1. https://www.gov.br/agricultura/pt-br/assuntos/insumos-agropecuarios/insumos-agricolas/sementes-e-mudas/publicacoes-sementes-e-mudas/copy_of_INN45de17desetembrode2013.pdf

Ministério da Agricultura, Pecuária e Abastecimento [MAPA] (2025). *Teste de germinação. In: Regras para Análise de Sementes* (RAS). Ministério da Agricultura e Pecuária, MAPA. https://wikisda.agricultura.gov.br/pt-br/Laborat%C3%B3rios/Metodologia/Sementes/RAS_2025/cap_4_Germinacao_rev_1

Nardelli, A. C. P., Carvalho, E. R., Reis, V. U. V., Rocha, D. K., Reis, L. V., & Cunha, A. R. D., Neto. (2025). Moisture content and storage temperature on the physical and physiological quality of soybean seeds treated with phytosanitary products. *Journal of Seed Science, 47*(1), e202547020. doi: 10.1590/2317-1545v47291209

Pereira, L. S., Masetto, T. E., Crispim, B. D. A., Nascimento, H. D. S., & Barufatti, A. (2022). Toxicogenetic effects are involved in the occurrence of imbibitional damage in soybean seeds. *Acta Scientiarum. Agronomy, 44*(1), e53555. doi: 10.4025/actasciagron.v44i1.53555

R Core Team (2024). *R: A language and environment for statistical computing.* R Foundation for Statistical Computing. https://www.R-project.org/

Reis, V. U. V. (2024). *Uso da vermiculita no teste de germinação de sementes tratadas de soja: implicações na avaliação e a validação metodológica.* Dissertação de mestrado em Agronomia/Fitotecnia, Universidade Federal de Lavras, Lavras, MG, Brasil. http://repositorio.ufla.br/jspui/handle/1/59138

Reis, V. U. V., Carvalho, E. R., Maciel, D. C., Tavares, G. I. S., Medeiros, J. C., Brito, R. B. de, & Pereira, J. E. J. (2025). Benefits of using vermiculite in the soybean seed germination testing. *Discover Plants, 2*(1), 165-175. doi: 10.1007/s44372-025-00248-7

Reis, V. U. V., Penido, A. C., Carvalho, E. R., Rocha, D. K., Reis, L. V., & Semolini, P. H. Z. (2022). Vigor of maize seeds and its effects on plant stand establishment, crop development and grain yield. *Journal of Seed Science, 44*(1), e202244020. doi: 10.1590/2317-1545v44257527

Rocha, D. K., Carvalho, E. R., Pires, R. M. D. O., Santos, H. O. D., Penido, A. C., & Andrade, D. B. D. (2020). Does the substrate affect the germination of soybean seeds treated with phytosanitary products? *Ciência e Agrotecnologia, 44*(1), e020119. doi: 10.1590/1413-7054202044020119

Rocha, D. K., Reis, V. U. V., Carvalho, E. R., Nardelli, A. C. P., Morais, G. M., & Reis, L. V. (2025). How do the components used in chemical seed treatment affect physiological quality over the storage period? *Bragantia, 84*(1), e20240131. doi: 10.1590/1678-4499.20240131

Rodrigues, M. B. C., Villela, F. A., Tillmann, M. Â. A., & Carvalho, R.. (2006). Pré-hidratação em sementes de soja e eficiência do teste de condutividade elétrica. *Revista Brasileira de Sementes, 28*(2), 168–181. https://doi.org/10.1590/S0101-31222006000200023

Sato, K., Jitsuyama, Y., Yamada, T., Liu, B., & Abe, J. (2019). Structural features of the aleurone layer of the seed coat associated with imbibition injury in soybean. *Breeding Science, 69*(2), 364-370. doi: 10.1270/jsbbs.18181

Satya Srii, V., Nagarajappa, N., & Vasudevan, S. N. (2022). Is seed coat structure at fault for altered permeability and imbibition injury in artificially aged soybean seeds? *Crop Science, 62*(4), 1573-1583. doi: 10.1002/csc2.20750

Silva, K. D. R. G. D., & Villela, F. A. (2011). Pré-hidratação e avaliação do potencial fisiológico de sementes de soja. *Revista Brasileira de Sementes, 33*(2), 331-345. doi: 10.1590/S0101-31222011000200016

Toledo, M. Z., Cavariani, C., França, J. D. B., Neto, & Nakagawa, J. (2010). Imbibition damage in soybean seeds as affected by initial moisture content, cultivar and production location. *Seed Science and Technology, 38*(2), 399-408. doi: 10.15258/sst.2010.38.2.13

Tunes, C. D., Meneghello, G. E., Gonçalves, V. P., Meneguzzo, M. R. R., Silva, J. B. da, Teixeira, S. B., Zimmer, G., & Maass, D. W. (2021). Alternative substrates for the germination test with treated soybean seeds. *Brazilian Journal of Development, 7*(9), 93210-93224. doi: 10.34117/bjdv7n9-470

Varier, A., Vari, A. K., & Dadlani, M. (2010). The subcellular basis of seed priming. *Current Science, 99*(4), 450-456. http://www.jstor.org/stable/24109568