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POTENTIAL USE OF VERMICULITE IN COFFEE SEED GERMINATION TEST

Venicius Urbano Vilela Reis[1,3], Giulyana Isabele Silva Tavares[1], Rafaela Rezende Mizael[1], Gabriela Ribeiro Gontijo[1,4], Everson Reis Carvalho[1], Sttela Dellyzete Veiga Franco da Rosa[2], Heloisa Oliveira dos Santos[1]

[1]Department of Agriculture, Universidade Federal de Lavras, Lavras, Minas Gerais, Brazil.
[2]Empresa Brasileira de Pesquisa Agropecuária, Embrapa Café, Brasília, Distrito Federal, Brazil.
[3]Seed Biotechnology Center, Department of Plant Science, University of California, Davis, California, USA.
[4]BioDiscovery Institute & Department of Biological Sciences, University of North Texas, Denton, Texas, USA

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Abstract
The quality of the seeds and the subsequent germination process are critical for the successful cultivation of coffee seedlings that meet the desired standards. However, coffee seed germination is notoriously slow, which can hinder the efficiency of coffee seedling producers and seed testing laboratories. In this context, the substrate utilized in the germination test exerts a direct influence on the germination process. The objective of this study was to evaluate the use of rolled paper with vermiculite (RP+V) as an alternative substrate for coffee seed germination testing. Five germination substrates were evaluated: rolled paper (RP) and rolled paper with vermiculite (RP+V) at four water-to-vermiculite ratios (1×, 2×, 3×, and 4× the vermiculite weight). The results indicated that the RP+V substrate significantly enhanced germination and seedling length compared to RP, particularly the RP+V 1× treatment, attributable to the enhanced water retention and aeration properties of vermiculite. Conversely, increasing water levels, particularly the RP+V 4× treatment, was observed to reduce radicle protrusion. This phenomenon can be attributed to excess water limiting oxygen availability. In general, the application of RP+V has been shown to have a positive influence on the development of coffee seedlings, particularly the root system. This suggests that the RP+V 1× substrate may be a promising alternative for assessing the germination potential of seed lots.

Keywords: *Coffea arabica L*. Quality control. Seed quality. Seedling development. Substrate.

Corresponding author:
Heloisa Oliveira dos Santos
heloisa.osantos@ufla.br

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1. Introduction

Coffee (*Coffea arabica* L.) is one of the most valuable agricultural commodities worldwide, generating employment, income, and foreign exchange for producing countries. Brazil is the world's largest producer and exporter of coffee, and the 2026 harvest is projected to reach 66.2 million 60-kg bags, a 17.1% increase over the previous season, driven by a positive biennial cycle, expansion of cultivated area, and favorable climatic conditions (CONAB 2026). Global coffee consumption reached 175.1 million 60-kg bags in coffee year 2024, with *C. arabica* accounting for 57.5% of world production (ICO 2026). Despite this relevance, establishing productive crops still depends on the production of vigorous seedlings, which require high-quality seeds and efficient germination processes.

Seed germination is a multifaceted process involving physiological and biochemical events that culminate in seedling establishment (Bewley and Black 1994). For coffee seeds, this process is particularly delicate and influenced by numerous factors, including temperature, moisture content, oxygen, light, and the substrate used (Brasil 2025). Germination in coffee is particularly slow, often requiring several weeks for radicle protrusion and up to 30 days for seedling evaluation under standard conditions (Brasil 2025). This delay poses challenges for seed testing laboratories and nurseries, as rapid and reliable germination results are critical for lot approval and seedling production.

The substrate used in germination tests plays a decisive role in this context. An ideal substrate must provide physical support, maintain adequate aeration and water retention, and allow uniform contact with seeds (Martins et al. 2008). In Brazil, the rolled paper (RP) method is the official substrate for coffee germination tests (Brasil 2025). Although it provides standardized conditions, it is often associated with prolonged evaluation time, high labor demand, and susceptibility to deterioration caused by secondary metabolites released during germination, such as phenolic acids (Area and Cheradame 2011).

Vermiculite, a hydrated aluminum–magnesium silicate, has emerged as a promising alternative substrate due to its structural and physicochemical properties. It is lightweight and porous, with high water retention capacity, which ensures continuous water availability while maintaining adequate oxygen diffusion (Nunes et al. 2023; Reis et al. 2025). Studies in soybean, maize, rice, and common bean have demonstrated that rolled paper combined with vermiculite (RP+V) enhances germination speed, improves root system development, and reduces the time required for final evaluation (Rocha et al. 2020; Xavier et al. 2021; Rossetti et al. 2022; Rocha et al. 2023; Carvalho et al. 2024; Reis et al. 2025). To date, RP+V has only been officially approved for use with chemically treated corn and soybean seeds (Brasil 2025). In addition to its physical properties, vermiculite may also play a role in mitigating the phytotoxic effects associated with the release of phenolic acids during coffee seed germination, a characteristic that reinforces its evaluation as a substrate for this species (Pereira et al. 2002).

In the case of coffee, the potential use of vermiculite in germination testing remains unexplored. Given the slow and irregular germination of coffee seeds, identifying substrates that accelerate the process and better express seedling vigor would be of considerable practical importance. A faster and more reliable test could optimize seed lot approval and seedling production, thereby benefiting producers and the coffee industry.

We hypothesized that adding vermiculite to the rolled paper substrate would improve the expression of germination potential and promote seedling development in *C. arabica* seeds. Therefore, the objective of this study was to evaluate the use of rolled paper with vermiculite (RP+V) as an alternative substrate for coffee seed germination testing, with emphasis on seed germination potential and seedling development.

2. Material and Methods

A completely randomized design was used, with five germination substrates (RP; RP+V moistened with 1× the weight of vermiculite; RP+V moistened with 2× the weight of vermiculite; RP+V moistened with 3× the weight of vermiculite; RP+V moistened with 4× the weight of vermiculite), all with eight replicates of 25 *C. arabica* L. seeds.

Coffee fruits of *C. arabica* L. cv. MGS Paraíso 2 were selectively harvested in Brazil at the coordinates 21°13'40"S, 44°57'38"W during the 2023/2024 harvest season. After harvesting, the fruits were washed, followed by manual sorting to remove fruits that were underdeveloped, misshapen, or damaged by the coffee berry borer (*Hypothenemus hampei*), as well as other impurities.

The ripe fruits were mechanically pulped, and the seeds were subjected to a fermentation process in water at 25 ± 2 °C for 24 hours to remove the mucilage. The seeds were then thoroughly washed to ensure complete removal of the mucilage, after which they underwent rigorous manual selection to eliminate defective seeds, those without parchment, those with shells, and any other remaining impurities. The seeds were shade-dried under open-air conditions at mean daily ambient temperatures ranging from 17.8 to 18.5 °C and mean daily relative humidity ranging from 69 to 73%, without humidity control, and with natural ventilation. Seeds were not covered and remained exposed to the air throughout the process. To ensure uniform drying, they were arranged in a single layer on nylon sieves without overlapping and were periodically turned over during the three-day drying period.

The seeds were then dried using silica gel until they reached a water content of 25%. During silica gel drying, seeds were arranged in a single layer on metal screens inside acrylic Gerbox-type boxes (11 × 11 × 3.5 cm), without overlap, to ensure uniform dehydration. A total of 60 g of activated silica gel was placed beneath the metal screen in each box. Boxes were maintained in a BOD-type germination chamber, model EL102 G (Eletrolab, São Paulo, Brazil), at 25 °C in the absence of light. Silica gel was replaced periodically as indicated by its characteristic color change from blue to pink (Coelho et al. 2015). After drying, all seeds were pooled and stored in a sealed plastic bag in a cold chamber (Climazon, Brazil; 10 ± 2 °C; 50% relative humidity) for 15 days prior to the start of the experiments.

The evaluated substrates were rolled paper (RP) and rolled paper combined with vermiculite (RP+V), prepared as described below.

Germination on Rolled Paper (RP): two sheets of germination paper (Jprolab, Brazil; 28 × 38 cm; basis weight 65 g m^{-2}; pH neutral) were moistened with distilled water at 2.5 times the weight of the paper. Seeds were evenly distributed over the two base sheets and covered with a third moistened sheet of the same paper. The sheets were then rolled into cylinders, secured with rubber bands, and placed vertically in a Mangelsdorf-type germinator, model 4001 (Biomatic, Porto Alegre, Brazil), at 30 ± 2 °C. Eight replicates of 25 seeds were used per treatment. The percentage of normal seedlings was recorded at each evaluation date, following the criteria established by the Rules for Seed Analysis (Brasil 2025).

Germination on Rolled Paper between Vermiculite (RP+V): two sheets of germination paper (Jprolab, Brazil; 28 × 38 cm; basis weight 65 g m^{-2}; pH neutral) were moistened with distilled water at 3.0 times the weight of the paper. A thin, uniform layer of 100 mL of fine vermiculite (particle size: 65–95% > 1.2 mm) was spread over the moistened paper (Carvalho et al. 2024). The vermiculite was moistened with distilled water according to each treatment: 1×, 2×, 3×, or 4× its weight in water. Seeds were then distributed over the vermiculite layer using a seed counter, with eight replicates of 25 seeds per treatment, and covered with a third moistened sheet of germination paper. Rolls were assembled and maintained under the same conditions as the RP treatment, at 30 ± 2 °C in a Mangelsdorf-type germinator, model 4001 (Biomatic, Porto Alegre, Brazil). The percentage of normal seedlings was recorded at each evaluation date (Brasil 2025).

After the germination tests were set up according to the methodology described with the different substrates, the following characteristics were evaluated:

At 15 days after sowing (DAS), the percentage of radicle protrusion (Brasil 2025), the percentage of seedlings at stage S1 (Rosa et al. 2010), total seedling length and primary root length (Reis et al. 2022) were evaluated.

At 30 DAS, the percentage of normal seedlings was evaluated (Brasil 2025), and total seedling length and primary root length (Reis et al. 2022) were evaluated.

At 45 DAS, the percentage of seedlings with expanded cotyledonary leaves (Rosa et al. 2010), total seedling length and primary root length (Reis et al. 2022), and root, shoot, and total dry matter of the seedling (Rosa et al. 2011) were evaluated from the same seedlings.

Measurements were taken using GroundEye®, version S120 (Tbit, Brazil), for total seedling length and primary root length. The seedlings were placed on a tray in the capture module to obtain high-resolution images. Then, the CIELab color model was used to calibrate the background color, with a luminosity index ranging from 0 to 100, a dimension "a" ranging from -13.9 to 46.1, and a dimension "b" ranging from -57.1

to 40.6. After calibrating the background color, the images were automatically analyzed and the average values of the parameters were extracted (Reis et al. 2022).

These seedlings were obtained 45 days after sowing. First, the aerial part of the seedlings was separated from the roots using a scalpel. The plant material was placed in kraft paper bags and dried in a forced-circulation oven at 60 ± 2 °C until it reached a constant mass (approximately 120 hours). Dry weight was determined using a precision scale (0.0001 g), and the results were expressed in milligrams per seedling (Rosa et al. 2011).

The data were analyzed using the F-test at a significance level of 5%. When a significant effect of the treatments was observed, the means were compared using the Tukey test. All analyses were performed using RStudio version 2024.04.2+764 with the ExpDes.pt package (Ferreira et al. 2021; R Core Team 2026).

3. Results

A comprehensive statistical analysis revealed significant differences among the treatment means for all variables assessed at 15 and 30 DAS. However, at 45 DAS, significant variations were observed only for the percentage of seedlings with expanded cotyledonary leaves, primary root length, and total seedling length.

At 15 DAS, the percentage of radicle protrusion was negatively affected in RP+V 4×, which showed a reduction of up to 7 percentage points (pp) compared to RP (Figure 1A). In contrast, RP+V 1× exhibited a marked advantage, promoting an increase of 48 pp in the proportion of seedlings at stage S1 compared with RP. This indicates that a higher proportion of seeds had advanced to an early developmental stage in RP+V 1×, while in RP the majority remained with only radicle protrusion. Seedling length parameters followed a similar pattern. RP+V 1× produced seedlings with an average increase of 1.22 cm in primary root length and 1.20 cm in total seedling length relative to RP (Figure 1B). These results indicate that differences among substrates were already detectable at initial stages of germination.

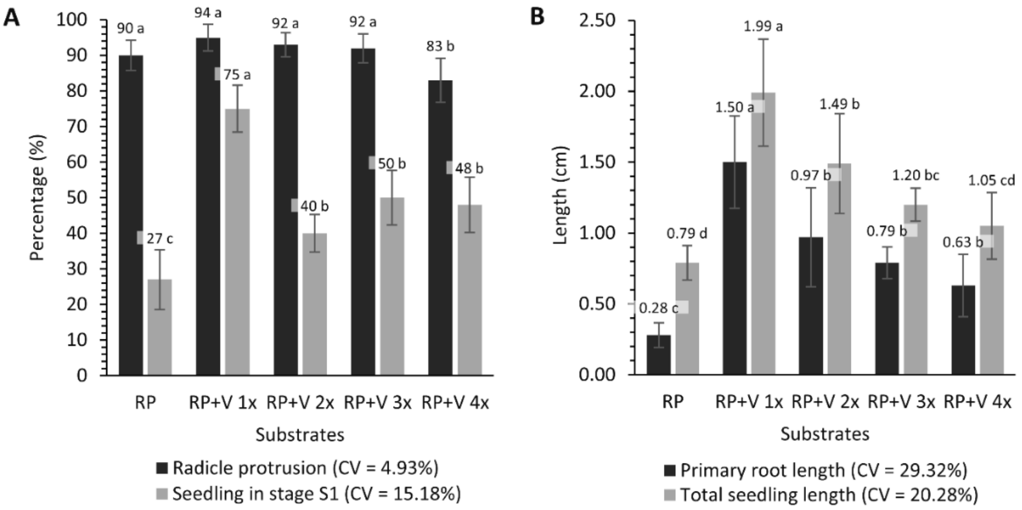

Figure 1. Evaluation at 15 days after sowing of *Coffea arabica* L. seeds in different germination substrates. A: Percentage of radicle protrusion and percentage of seedlings at S1 stage. B: Primary root length and total seedling length (cm). *When averages within a variable are compared using the Tukey test, averages that share the same letter are not significantly different at a 5% significance level.

At 30 DAS, corresponding to the final count for coffee germination testing, significant differences among substrates became more evident (Figures 2A and 3). The percentage of normal seedlings in RP (69%) fell below the 70% threshold required for commercial seed lot approval. In contrast, all RP+V treatments yielded germination percentages above this threshold, ranging from 83% (RP+V 4×) to 90% (RP+V 1×), and formed a common statistical group that differed significantly from RP.

Morphological traits were also significantly influenced by substrate composition. For both primary root length and total seedling length, RP+V 1× and RP+V 3× formed a common statistical group, presenting the highest mean values, 4.01 and 3.61 cm for primary root length, and 10.98 and 10.60 cm for total seedling length, respectively. RP+V 4× showed intermediate performance, being statistically similar to both RP+V 1× and RP+V 3× for primary root length, while RP+V 2× presented intermediate values for both variables. All RP+V treatments differed from RP, which recorded the lowest means across all morphological variables (Figure 2B), indicating that the incorporation of vermiculite into the substrate consistently promotes superior seedling development.

These findings were further corroborated by visual observations at 30 DAS (Figure 3). Seedlings germinated on RP (panel A) exhibited shorter hypocotyls, reduced primary root length, and a sparse lateral root system. In contrast, seedlings from RP+V treatments (panels B–E) displayed longer and more intensely pigmented hypocotyls, a well-developed primary root, and a denser lateral root system with abundant secondary roots. Furthermore, seedlings from RP+V treatments (panels B–E) already showed early cotyledon opening at 30 DAS, whereas seedlings grown on RP alone (panel A) had not yet reached this developmental stage, remaining with cotyledons still enclosed.

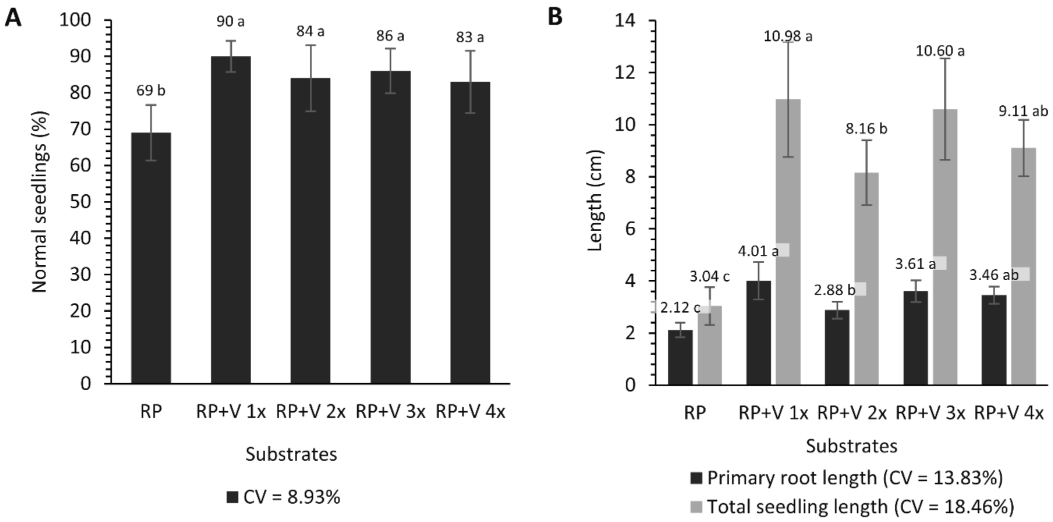

Figure 2. Evaluation at 30 days after sowing *Coffea arabica* L. seedlings in different germination substrates. A: Percentage of normal seedlings. B: Primary root length and total seedling length (cm). *When averages within a variable are compared using the Tukey test, averages that share the same letter are not significantly different at a 5% significance level.

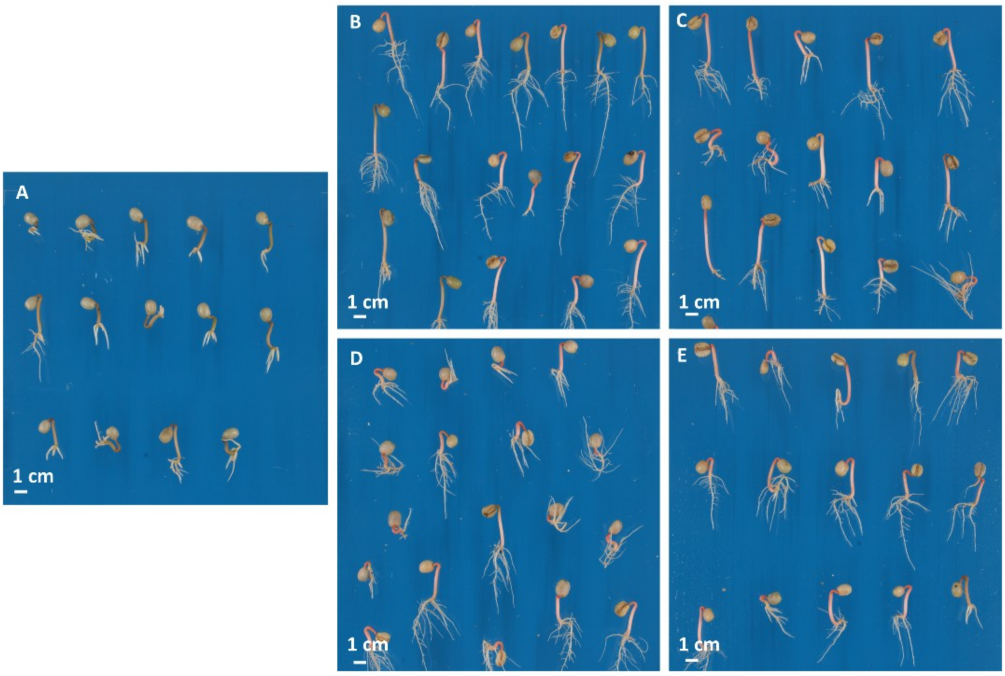

Figure 3. *Coffea arabica* L. seedlings at 30 days after sowing in different germination substrates. A: RP; B: RP+V 1×; C: RP+V 2×; D: RP+V 3×; E: RP+V 4×.

At 45 DAS, the superiority of the RP+V treatments persisted, particularly in RP+V 1× and RP+V 3×, which did not differ statistically from each other, and both reached 91% germination, representing a 9 pp increase over RP (82%) (Figure 4A). These treatments also produced seedlings with the greatest total seedling length and primary root length, with values up to 1.55 cm and 1.97 cm greater than those observed in RP for total seedling length and primary root length, respectively (Figure 4B). Regarding biomass accumulation, differences among substrates were less pronounced; root dry matter was significantly higher only in RP+V 1× (0.0031 g) compared to RP+V 4× (0.0025 g), while no significant differences were detected among treatments for shoot dry matter or total seedling dry matter (Figure 5).

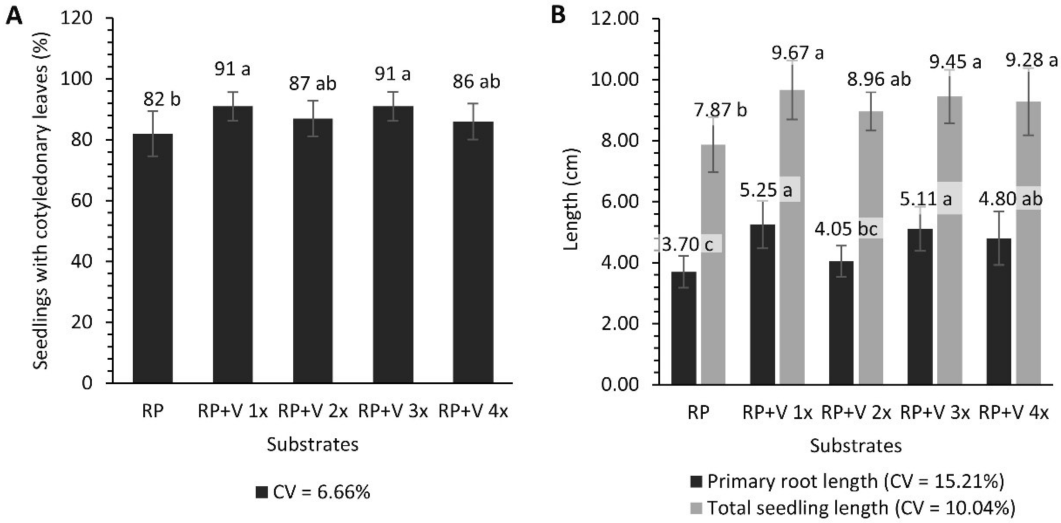

Figure 4. Evaluation at 45 days after sowing of *Coffea arabica* L. seeds in different germination substrates. A: Percentage of seedlings with expanded cotyledonary leaves. B: Primary root length and total seedling length (cm). *When averages within a variable are compared using the Tukey test, averages that share the same letter are not significantly different at a 5% significance level.

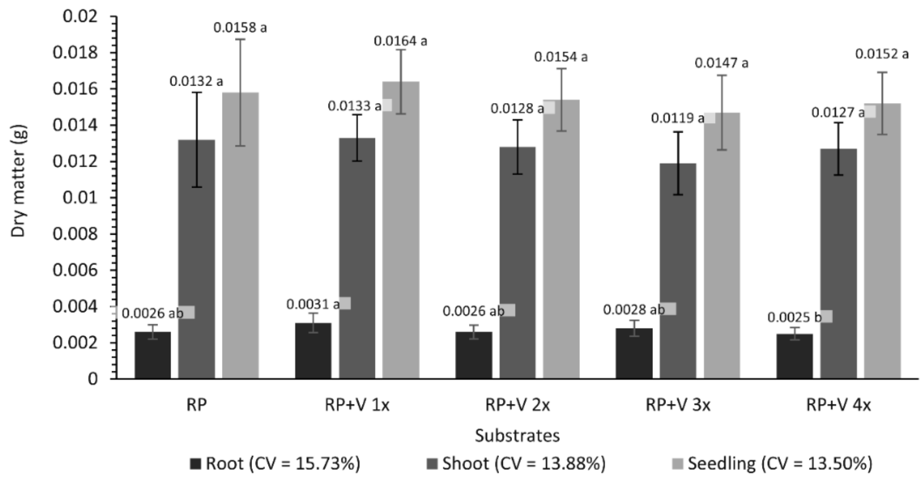

Figure 5. Evaluation of dry matter at 45 days after sowing *Coffea arabica* L. seedlings in different germination substrates. *When averages within a variable are compared using the Tukey test, averages that share the same letter are not significantly different at a 5% significance level.

4. Discussion

The results demonstrate that the addition of vermiculite to rolled paper alters coffee seed germination dynamics and seedling development. At 15 DAS, the reduction in radicle protrusion observed in RP+V 4× can be explained by the excessive water content of this treatment. During germination, excess moisture reduces oxygen diffusion and consequently restricts respiration, negatively affecting seed performance (Park and Hasenstein 2016). This condition is especially critical in coffee, a species known to be less tolerant to waterlogging (León-Burgos et al. 2022).

In contrast, RP+V 1× markedly increased the proportion of seedlings at stage S1, indicating accelerated germination. Evaluations at S1 are equivalent to the 30-day count in the standard RP method, highlighting the potential of RP+V to shorten the evaluation period for coffee seeds (Guimarães et al. 2013).

Vermiculite has been recognized as a favorable substrate component due to its unique structural and physicochemical characteristics. When used in adequate quantities, it provides greater aeration and water retention, maintaining optimal conditions for germination and seedling growth (Martins et al. 2008).

In addition to serving as physical support, its porosity and absorbent surface ensure gradual water availability while preserving oxygen diffusion. This explains the higher soaking rate of seeds in RP+V, which favors faster germination and subsequent seedling growth, as reported in soybean by Reis et al. (2025). Another advantage is that RP+V reduces the need for frequent moistening, which is particularly beneficial for species such as coffee that require long germination periods (Rossetti et al. 2022).

At 30 DAS, the superiority of RP+V substrates over RP became more evident. RP produced less than 70% normal seedlings, a level below the minimum established by Brazilian legislation (Brasil 2012), which would lead to lot rejection. In contrast, RP+V treatments allowed approval, showing that this substrate more accurately expresses the physiological quality of seed lots. In addition, RP+V 1× significantly increased primary root length and total seedling length compared to RP, indicating better expression of vigor potential. These improvements are consistent with the properties of vermiculite, which ensure adequate water supply and oxygen diffusion during germination testing (Rossetti et al. 2022; Reis et al. 2025).

At 45 DAS, the benefits of RP+V persisted, as evidenced by the higher percentage of seedlings with expanded cotyledonary leaves and greater seedling length, particularly in RP+V 1× and RP+V 3×. These results demonstrate that the positive effects of vermiculite extend beyond germination, sustaining seedling development in later stages.

Root dry matter accumulation was also higher in RP+V 1× compared to RP+V 4×, while excessive hydration in the latter reduced root biomass. This observation aligns with findings that vermiculite enhances root biomass in coffee seedlings when used at optimal levels (Silva et al. 2023). Conversely, the reduction in biomass under RP+V 4× illustrates the detrimental effects of substrate saturation, which limits aeration and inhibits root respiration, a phenomenon widely documented in the literature (Hussain et al. 2023).

The beneficial role of vermiculite in promoting root development and distribution has also been reported in other species, reinforcing its importance for ensuring reliable and reproducible germination test results (Xavier et al. 2021; Rossetti et al. 2022; Rocha et al. 2023; Reis et al. 2025).

The mineral used in this study is structurally similar to mica and is composed of hydrated aluminum–magnesium silicate. Its high surface area, porosity, negative surface charge, and water-holding capacity make it an efficient absorbent material suitable for seed germination testing (Nunes et al. 2023). Its low density and structural arrangement also facilitate root penetration and elongation, fundamental processes for seedling establishment (Carvalho et al. 2024). Additionally, by preventing direct contact between seeds and the paper substrate, vermiculite minimizes degradation of paper caused by phenolic acids released during coffee germination (Area and Cheradame 2011).

The integration of vermiculite into rolled paper substrates therefore provides several advantages: improved aeration, increased water availability, and greater uniformity in seed-substrate contact. These factors contribute to enhanced repeatability and reproducibility of germination tests, as already validated in maize and soybean (Rocha et al. 2023; Carvalho et al. 2024). Physiologically, vermiculite enables faster soaking, accelerates germination, and improves seedling development due to its absorbent and porous properties, which function as regulatory mechanisms for water and oxygen availability (Reis et al. 2025).

Overall, the results of this study confirm that RP+V, particularly at the 1× proportion, provides optimal conditions for coffee seed germination and early seedling development. This substrate accelerates evaluation, improves the accuracy of seed quality testing, and maintains favorable conditions for seedling growth. Beyond the practical benefits for seed testing laboratories, the method has the potential to reduce the time required for lot approval, increasing efficiency in the coffee seed supply chain. Future studies should explore its performance across different coffee cultivars, seed storage conditions, and initial moisture content of seeds.

Although RP+V 1× and RP+V 3× did not differ statistically for most variables evaluated, RP+V 1× can be considered the most efficient substrate for coffee germination testing based on two complementary criteria. First, RP+V 1× required the lowest amount of water for substrate preparation among the RP+V treatments, representing a practical advantage in terms of resource efficiency and sustainability in seed testing laboratories. Second, RP+V 1× yielded the highest proportion of seedlings at the S1 development stage, indicating superior expression of seedling vigor. Taken together, these considerations support the adoption of RP+V moistened at 1× the weight of vermiculite as the recommended substrate for coffee germination testing, balancing analytical precision, seedling vigor expression, and operational efficiency.

5. Conclusions

The use of rolled paper with vermiculite (RP+V) improved germination performance and seedling development of *Coffea arabica* L. cv. MGS Paraíso 2, particularly enhancing root system expression. Among the evaluated treatments, RP+V with water equivalent to 1× the weight of vermiculite was the most effective. Thus, RP+V 1× represents a practical and cost-effective alternative substrate for coffee seed germination testing, contributing to more accurate assessments of physiological seed quality.

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Ethics Approval: Not applicable.

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