

Seedling length and determination of vigor indexes in soybean seeds through image analysis

Hugo Cesar Rodrigues Moreira Catão¹, Laura Martins Vinhais¹, Gabriela Mariano Melazzo¹, Ana Laura Costa Santos¹, Marcella Mara Pereira Almeida¹, Daniel Teixeira Pinheiro², Ricardo Ferreira Domingues¹

¹ Universidade Federal de Uberlândia, Uberlândia, Minas Gerais, Brasil. E-mail: hugo.catao@ufu.br, laura.vinhais@ufu.br, gabriela.melazzo@ufu.br, ana.santos7@ufu.br, marcella.almeida@ufu.br, ricfdomingues@hotmail.com

² Centro Universitário do Triângulo, Uberlândia, Minas Gerais, Brasil. E-mail: pinheiroagroufv@gmail.com

Received: 07/16/2025; Accepted: 07/08/2026.

ABSTRACT

Tests based on seedling performance, such as length measurements, require skilled labor and may become time-consuming depending on the number of seed lots analyzed. Computerized image analysis offers an alternative for quickly and efficiently evaluating the physiological potential of seeds, although details such as seed positioning can influence the results. This study aimed to verify the effectiveness of image analysis and to determine vigor and seedling growth indexes in soybeans based on seed positioning in the seedling length test. Seeds from four cultivars were used to assess quality through water content, germination, electrical conductivity, seedling length and dry mass tests, with BMX DESAFIO cultivar with three vigor levels (high, medium, and low) being used for the image analysis. In the seedling length test, seeds were placed on the substrate with the hilum oriented: (1) to one of the paper's sides, (2) upward, and (3) downward. The experimental design was a completely randomized 3x3 factorial scheme (vigor levels x seed positions). Measurements included hypocotyl length, root length, seedling vigor, growth, uniformity, germination, average length, and dry mass. The position and vigor of soybean seeds influence seedling growth, which was verified through the Vigor-S system. The Vigor-S system is effective for evaluating seedling length in relation to seed vigor and positioning. Positioning the hilum of medium- and high-vigor soybean seeds to the side of the paper is also a viable alternative for seedling length tests.

Keywords: *Glycine max*, Seed vigor, Seedling performance, Computerized image analysis.

Comprimento de plântulas e determinação de índices de vigor em sementes de soja através de análise de imagens

RESUMO

Testes baseados no desempenho de plântulas, como as medições de comprimento, demandam de mão de obra qualificada e podem se tornar morosos a depender do número de lotes analisados. A análise de imagens oferece uma alternativa rápida para avaliar o potencial fisiológico das sementes, embora detalhes como o posicionamento das sementes possam influenciar os resultados. Objetivou-se verificar a eficácia da análise de imagens e avaliar a influência do posicionamento de sementes sobre os índices de vigor e crescimento de plântulas de soja. Foram utilizadas sementes de quatro cultivares para avaliação da qualidade por meio dos testes de teor de água, germinação, condutividade elétrica, comprimento e massa seca de plântulas, sendo escolhida a cultivar BMX DESAFIO com três níveis de vigor (alto, médio e baixo) para a análise de imagens. No teste de comprimento de plântulas, as sementes foram posicionadas no substrato com o hilo orientado: (1) para um dos lados do papel, (2) para cima e (3) para baixo. O delineamento experimental foi em esquema fatorial 3x3 inteiramente casualizado (níveis de vigor x posições das sementes). As medições incluíram comprimento do hipocótilo e da raiz, vigor das plântulas, crescimento, uniformidade, germinação, comprimento médio e massa seca. O posicionamento e o vigor das sementes de soja influenciam o crescimento das plântulas, o que foi verificado pelo sistema Vigor-S. Posicionar o hilo de sementes de soja de vigor médio e alto para o lado do papel também é uma alternativa viável para os testes de comprimento de plântulas.

Palavras-chave: *Glycine max*, Vigor de sementes, Análise de imagens computadorizada, Vigor-S.

1. Introduction

Soybean (*Glycine max* (L.) Merr.) is a legume of great global economic importance due to its yield potential, adaptability to different latitudes, soil types, edaphoclimatic conditions, nutritional characteristics, and versatility of its grains. Recently, Brazil produced 180 million tons of soybean grains (Conab, 2026). To ensure success in production, the use of high quality seeds considering their physical, physiological, genetic, and sanitary attributes is essential for establishing uniform stands and productive fields (Finch-Savage, 2020).

The physiological quality of seeds is evaluated by the germination test, complemented by vigor tests, mainly used to identify performance differences among seed lots during storage or after sowing, aiming to highlight the most efficient lots for stand establishment under variable environmental conditions (Marcos-Filho, 2015b). These tests can be classified as direct (seeds subjected to adverse conditions simulating field environments) and indirect (assessing specific characteristics) (Marcos-Filho, 2020).

Several tests are recommended for evaluating soybean seed vigor, such as accelerated aging, tetrazolium, electrical conductivity, seedling growth, and seedling vigor classification (Vieira et al., 2003; Marcos-Filho et al., 2009; Wendt et al., 2014). Among these, the traditional seedling growth test involves manually measuring seedling length using a ruler or caliper. This process is often slow and prone to errors, especially when evaluating large volumes of seed lots (Gomes-Junior et al., 2014).

To overcome these limitations, seed technology research has prioritized computerized procedures for seed vigor evaluation, offering speed, error elimination, and the ability to save images for later analysis (Wendt et al., 2014; Gomes-Junior, 2020; Leite et al., 2024). Among the available software, Vigor-S allows for rapid and accurate seedling evaluation, significantly reducing analysis time (Rodrigues et al., 2020). Besides soybean seeds (Rodrigues et al., 2020), Vigor-S has been effective for analyzing seed vigor in other species such as maize (Castan et al., 2018), common bean (Medeiros et al., 2019), melon (Leite et al., 2020), and cowpea (Rego et al., 2021).

In both manual and Vigor-S seedling growth tests, it is recommended to position seeds on the substrate with the radicle tip facing downward, i.e., the micropyle should point toward the bottom of the germination paper (Krzyzanowski et al., 2020; Rodrigues et al., 2020). A major drawback in this procedure is the time and effort required to position soybean seeds accurately, as they may shift from the ideal orientation.

Given this, the objective was to verify the efficacy of computerized image analysis and to determine vigor and growth indexes of soybean seedlings according to seed positioning in the seedling length test.

2. Material and Methods

The experiment was conducted at the Seed Laboratory (LASEM) of the Federal University of Uberlândia (UFU), in Uberlândia, MG, Brazil. Initially, seeds from the cultivars M 5917 IPRO, M 6410 IPRO, BMX Desafio, and NS 7505 IPRO were used to select the material with the highest physiological quality through the following tests:

Water content: Determined by the oven method at 105 °C for 24 hours, using two replicates of 4-5 grams of seeds (Brasil, 2025).

Germination: Four replicates of 50 seeds were placed between two sheets of germination paper, moistened with distilled water at a volume of 2.5 times the dry paper weight, and placed in a Mangelsdorf-type germinator at 25 °C under a 12-hour photoperiod. Evaluations were done on the eighth day post-sowing, with results expressed as the percentage of normal seedlings (Brasil, 2025).

Electrical conductivity: Four replicates of 50 seeds were weighed and placed in disposable plastic cups (200 mL capacity) containing 75 mL of deionized water for 24 hours at 25 °C. After soaking, conductivity was measured using an MCA 150 conductivity meter (constant 1), with results expressed in $\mu\text{S cm}^{-1} \text{ g}^{-1}$ (Vieira and Marcos Filho, 2020).

Seedling length: Twenty seeds were placed on germination paper in two longitudinal rows of ten seeds, evenly spaced to allow free development. Each treatment had four replicates. Rolls were incubated in a B.O.D. chamber under germination test conditions. On the third day, the lengths of hypocotyls, roots, and entire normal seedlings were measured using a millimeter ruler (Krzyzanowski et al., 2020).

Dry mass: Seedlings from the germination test were placed in paper bags and dried to constant weight in a forced-air oven at 65 °C for 72 hours. After drying, they were cooled in a desiccator and weighed on an analytical scale with 0.001 g precision (Krzyzanowski et al., 2020). Dry mass of hypocotyls, roots, cotyledons, and whole seedlings was recorded.

After obtaining the results of the physiological tests, the cultivar BMX Desafio was selected for computerized image analysis. For this purpose, three lots with different vigor levels (high, medium, and low) were previously classified using the seedling emergence test (92%, 78%, and 56%, respectively).

Seeds were placed on the substrate in the following positions: 1 - Hilum positioned to one side of the paper

(HLP); 2 - Hilum positioned facing up on the paper (HCP); 3 - Hilum positioned facing downward on the paper (HIP) (Krzyzanowski et al., 2020) (Figure 1). The

experimental design was completely randomized in a 3 (vigor levels) $\times$ 3 (positions) factorial scheme, with four replications.

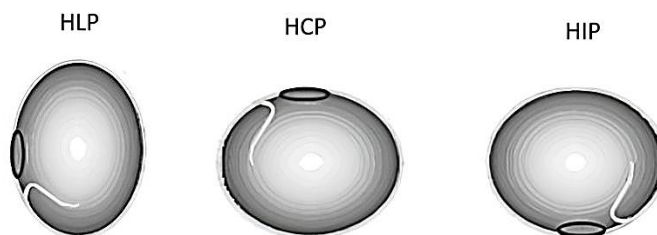


Figure 1. Sowing positions of soybean seeds on the substrate for conducting the seedling length test.

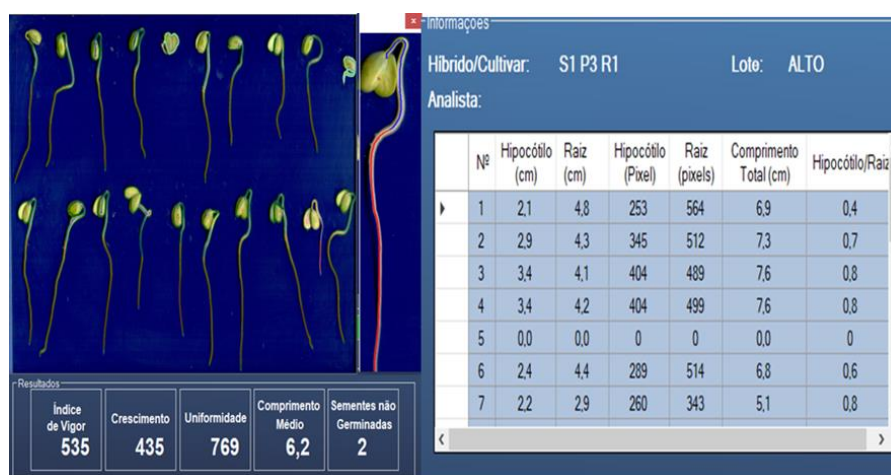


Figure 2. Analyzed images of 3-day-old soybean seedlings from a high-vigor seed lot, cultivar BMX DESAFIO, illustrating the data obtained by Vigor-S: vigor index, seedling uniformity, and seedling length values.

The seeds were distributed between two sheets of germination paper, moistened with distilled water at a volume equivalent to 2.5 times the dry weight of the paper. Four paper rolls were then prepared, each containing 20 seeds, totaling 80 seeds per treatment. The rolls containing the seeds were placed in plastic bags and incubated in a B.O.D. (Biological Oxygen Demand) chamber at 25 °C. Seedling length was evaluated on the third day, and the images were automatically processed using the Vigor-S software (Figure 2).

The seedlings from each replicate were transferred from the germination paper roll to a blue EVA (ethylene-vinyl acetate) sheet measuring 30 cm x 22 cm (corresponding to the scanner's useful area). Image scanning was then performed using an HP Scanjet 200 scanner installed in an inverted position inside an aluminum box (60 x 50 x 12 cm). The scanner was set to 300 dpi and connected to a Core i7 computer (2.80 GHz and 16 GB RAM).

The images were processed individually by the software, recording hypocotyl length, root length, vigor,

growth, uniformity, germination, and average length (Rodrigues et al., 2020). For the growth calculation, the hypocotyl and primary root lengths were weighted at 10% and 90%, respectively. The contributions of the growth and uniformity parameters to the vigor index calculation were 70% and 30%, respectively.

After measurements, hypocotyls, roots, and cotyledons were separated and dried in a forced-air circulation oven at 65 °C for 72 hours (Krzyzanowski et al., 2020). After this period, the dry mass of the hypocotyl, root, cotyledons, and entire seedlings was measured using an analytical balance with a precision of 0.001 g.

For statistical analysis, the F-test and analysis of variance (ANOVA) at a 5% probability level were used. When significant effects were observed, means were compared using the Scott-Knott test at a 5% significance level. Hypocotyl length, root length, and total length data were transformed using $\sqrt{(X+1)}$. In addition, principal component analysis (PCA) was performed for the overall dataset. All analyses were conducted using R software (R Core Team, 2022).

3. Results and Discussion

Seed moisture content ranged from 7.8% to 8.3% (Figure 3A). Uniform seed moisture is a critical factor that affects germination speed and seedling development, as it directly influences the initial rates of enzyme activation and the mobilization of seed reserves (Krzyzanowski et al., 2020). In this context, the observed moisture content values can be considered

uniform, allowing for consistent seed quality assessment (Marcos-Filho, 2015a).

The NS7505 IPRO cultivar showed 68% germination in the initial characterization (Figure 3B), below the minimum germination standard (80%) required for commercial soybean seed lots (Brasil, 2013) and was therefore not included in the seed positioning tests.

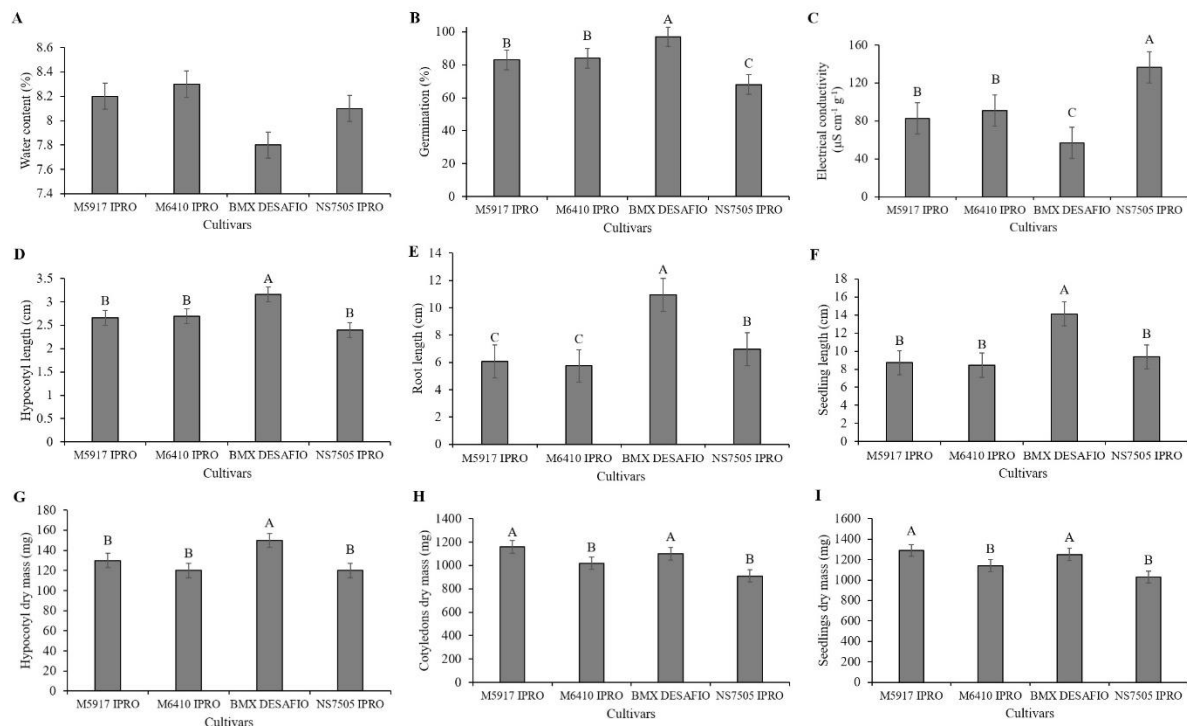


Figure 3. Initial characterization of soybean seed cultivars through the determination of moisture content (A), germination (B), electrical conductivity (C), hypocotyl length (D), root length (E), total seedling length (F), dry mass of the hypocotyl (G), cotyledons (H), and seedlings (I).

Germination tests are often insufficient to reveal differences in physiological potential, highlighting the need to jointly assess results from vigor tests. Upon analyzing the vigor tests, it was observed that the seeds of the BMX Desafio cultivar exhibited lower electrical conductivity (Figure 3C) and higher seedling length and dry mass (Figure 3D, I), indicating higher vigor compared to the others.

Vigorous seeds give rise to seedlings with faster growth rates due to their greater capacity for reserve allocation and more efficient transformation and incorporation into the embryonic axis (Krzyzanowski et al., 2020). In other words, more vigorous seeds are more efficient at mobilizing and transferring dry matter from the reserve tissues to the embryonic axis (Catão et al., 2024). Thus, the reduction in cotyledonary dry mass generally reflects an increase in shoot and root biomass production (Henning et al., 2010). Even when translocation rates are low, the efficiency of conversion may still be high (Catão et al., 2024).

In general, seeds with low vigor show impaired metabolism, RNA synthesis, and protein production,

resulting in reduced growth rates (Bewley et al., 2013). In contrast, high-vigor seeds have greater metabolism capacity and reserve supply, producing seedlings with superior growth rates (Nerling et al., 2022). In this context, Padilha et al. (2024) evaluated reserve mobilization in common bean seeds with different vigor levels under salt stress and observed that high-vigor seed lots showed greater hydrolytic activity for carbohydrates and proteins, resulting in more vigorous seedlings. Pinheiro et al. (2023) found that soybean seeds with higher levels of deterioration had greater protein degradation, directly affecting physiological potential.

Another important aspect involves the genetic factors associated with seed quality, which are essential for understanding seed performance and are directly related to improvements in agricultural productivity (Reed et al., 2022). Based on the traits evaluated, the BMX Desafio cultivar was selected for computerized image analysis using three seed lots with different vigor levels (92%, 78%, and 56% emergence). A significant interaction was observed between seed vigor and seed

position in the seedling length test for all analyzed

variables, except for uniformity (Figure 4).

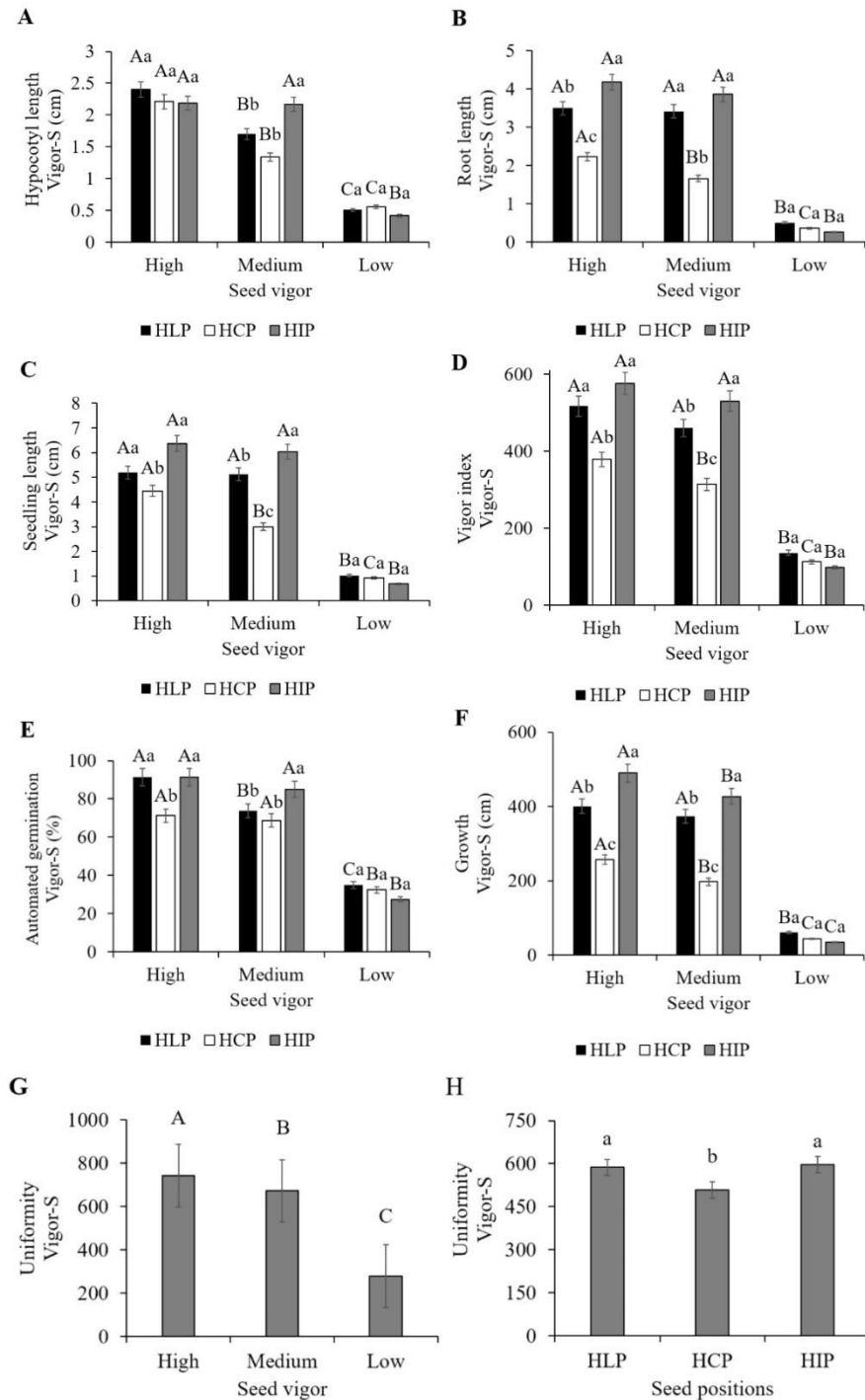


Figure 4. Hypocotyl length (A), root length (B), seedling length (C), vigor index (D), automated germination (E), growth (F), and uniformity (G) as a function of seed vigor and seed position in the seedling length test analyzed using the Vigor-S software. Means followed by the same uppercase letter differ among vigor levels and means followed by the same lowercase letter differ among seed positions in the seedling length test.

Seed positioning during testing can influence seedling length outcomes. Depending on the orientation of the micropyle relative to the substrate, seedling development may demand more energy, affecting results (Masilamani et al., 2023). For high-vigor seeds, there was no significant difference in hypocotyl length based on position (Figure 4A). However, the HIP position (hilum down) promoted greater hypocotyl development in medium-vigor seeds. For low-vigor

seeds, no differences were observed among positions. The seedling length test is highly influenced by seed vigor, with high-vigor lots producing longer and more vigorous seedlings (Guedes et al., 2015), which promotes rapid and uniform emergence. This behavior is associated with reserve translocation to the embryonic axis and the efficiency of reserve conversion (Catão et al., 2024).

For high-vigor seeds, root development was greater in the HIP position (Figure 4B), while the HCP position (hilum up) showed the lowest development, likely due to increased energy expenditure. Seeds placed with the hilum down showed greater vigor, possibly because the radicle, which emerges near the hilum, requires less movement to reach the substrate (Cardoso et al., 2008). In medium-vigor seeds, the HLP and HIP positions did not differ according to Vigor-S data. Recently, this software was also validated for identifying soybean seedlings with phytotoxic symptoms from seed treatments (Leite et al., 2024), demonstrating its versatility in detecting subtle differences in seedling length and physiological potential.

Seedling length also varied according to seed position in high-vigor seeds, with the highest values observed in HLP and HIP positions (Figure 4C). For medium-vigor seeds, the HIP position (hilum down) resulted in better seedling performance. Regardless of position, there were no significant differences in seedling length for low-vigor seeds, indicating that seed orientation does not strongly influence the outcome when using low-quality seeds. Lower-vigor seed lots typically show greater chemical composition variability and reduced seedling length (Henning et al., 2010).

Vigor index and automated germination data from Vigor-S showed no significant differences between HLP and HIP positions for high-vigor seeds (Figures 4D and 4E), suggesting that these positions do not reduce performance. However, the HCP position should be avoided even for high-vigor seeds due to observed reductions in these indices. For medium-vigor seeds, the

HIP position yielded the best performance in both vigor index and automated germination.

Although manual seedling length testing is considered efficient (Marcos-Filho, 2015b), the Vigor-S software also provides seedling vigor indices that effectively classified seed lots (Rego et al., 2021). The HIP position yielded the highest automated growth indices from Vigor-S for both high- and medium-vigor seeds (Figure 4F). It is important to note that HLP did not differ from HIP for these vigor levels, suggesting that orienting seeds with the hilum to the side (HLP) can also be a viable alternative in seedling growth tests.

Uniformity of seedlings did not differ when seeds were positioned in HLP and HIP orientations (Figure 4H). However, as expected, uniformity differed among the three seed vigor classes (Figure 4G). It is important to emphasize that high uniformity in seedling development does not necessarily guarantee well-developed seedlings, since poorly developed seedlings may still exhibit high uniformity. Therefore, the uniformity index should not be considered in isolation; instead, further studies should examine its relationship with other variables (Rego et al., 2021).

No differences were observed in the dry mass of hypocotyl, root, cotyledons, or total seedling dry mass when high-vigor seeds were used, regardless of seed position in the seedling length test (Figure 5A, B, C, and D). Differences in dry mass allocation to the hypocotyl, root, and total seedling were observed among medium-vigor seeds depending on seed positioning. The HIP position allowed for greater reserve allocation, followed by HLP and HCP, respectively (Figure 5).

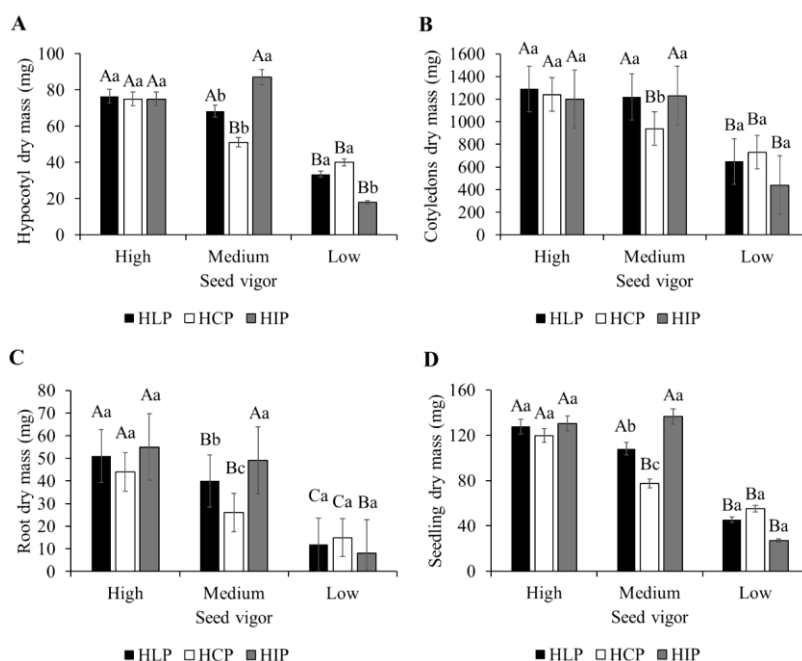


Figure 5. Dry mass of hypocotyl (A), cotyledons (B), roots (C), and total seedlings (D) as a function of seed vigor and seed position in the seedling length test. *Means followed by the same uppercase letter differ between vigor levels and means followed by the same lowercase letter differ between seed positions in the seedling length test.

There was no difference in the dry mass of cotyledons between the HIP and HLP positions, indicating that the translocation of seed reserves to the seedlings occurred equally in these positions. In low-vigor seeds, lower reserve translocation to the hypocotyl was observed in the HIP position (Figure 5A). For the other traits, no significant differences were found among seed positions in the low-vigor group. High-vigor seeds produce more vigorous seedlings and exhibit greater efficiency in repair mechanisms, mobilizing reserves from storage tissues and incorporating them into the growth of the embryonic axis, thereby promoting the synthesis of new tissues (Marcos-Filho, 2015b).

Through principal component analysis, it was observed that components 1 (PC1) and 2 (PC2) explained 96.45% and 2.23% of the total data variability, respectively, totaling 98.68% (Figure 6).

It is possible to observe that all the vectors of the analyzed variables - CDM, HDM, HL, SDM, RDM, AG, SL, V, RL, and G - are positioned in the positive scores of PC1, close to the treatments of high and medium vigor in HLP and HIP. This indicates that the soybean seed hilum positioned towards one of the sides of the paper (HLP) efficiently allows for the translocation and mobilization of reserves and seedling growth. This position may also be used during the test conduction for seeds of medium and high vigor. On the other hand, the treatments with low-vigor seeds were positioned in the negative scores of PC1, on the opposite side of the vectors of physiological quality. This reinforces that these seeds, regardless of the hilum position, are unable to express their physiological potential through growth (Figure 6).

The variability of the data is primarily explained by two components, as the variables are highly correlated, indicated by the similar direction of the vectors.

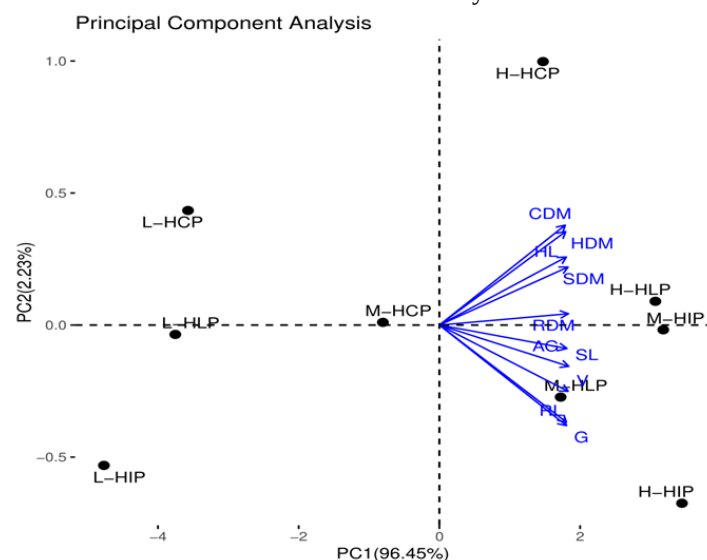


Figure 6. A: Principal component analysis (PCA) obtained by the linear combination of variables in soybean seeds based on seed vigor and position in the seedling length test. Principal Component 1 (PC1); Principal Component 2 (PC2); CDM: cotyledon dry mass; HDM: hypocotyl dry mass; SDM: seedling dry mass; RDM: root dry mass; HL: hypocotyl length; AG: automated germination - Vigor-S; SL: seedling length; V: vigor index - Vigor-S; RL: root length; G: growth - Vigor-S.

4. Conclusions

The position and vigor of soybean seeds influence seedling growth. The Vigor-S system is efficient in evaluating seedling length based on seed vigor and position. The soybean seed hilum positioned towards one side of the paper is also a viable alternative for the seedling length test when using seeds of high and medium vigor.

Authors' Contribution

Laura Martins Vinhais and Hugo Cesar Rodrigues Moreira Catão: Conduct of study and development of material and wrote original draft of the article. Laura Martins Vinhais, Marcella Mara Pereira Almeida and

Gabriela Mariano Melazzo: Conduct of study. Ana Laura Costa Santos and Ricardo Ferreira Domingues: Conduct of Statistical analysis. Hugo Cesar Rodrigues Moreira Catão and Daniel Teixeira Pinheiro: Gave research idea and hypothesis. Daniel Teixeira Pinheiro and Hugo Cesar Rodrigues Moreira Catão: Helped in editing the manuscript. Daniel Teixeira Pinheiro and Hugo Cesar Rodrigues Moreira Catão: Provided resources and gave research idea and hypothesis.

Bibliographic References

Bewley, J.D., Bradford, K.J., Hilhorst, H.W.M., Nonogaki, H., 2013. Seeds: physiology of development, germination and

dormancy. 3rd edition. Seed Sci. Res., 23(4), 392 pp. <https://doi.org/10.1007/978-1-4614-4693-4>

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. Regras para Análise de Sementes. Ministério da Agricultura, Pecuária e Abastecimento. Secretaria de Defesa Agropecuária. Coordenação-Geral de Laboratórios Agropecuários – CGAL. Brasília: MAPA, 2025. https://wikisda.agricultura.gov.br/pt-br/Laborat%C3%B3rios/Metodologia/Sementes/RAS_2024

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento, 2013. Instrução Normativa no 45, de 17 de setembro de 2013. Padrões para a produção e a comercialização de sementes. Diário Oficial da União.

Cardoso, E.A., Alves, E.U., Bruno, R.L.A., Alves, A.U., Silva, K.B., 2008. Emergência de plântulas de *Erythrina velutina* em diferentes posições e profundidades de semeadura. *Ciência Rural*, 38(9), 26182621. <https://doi.org/10.1590/S0103-84782008000900034>

Castan, D.O.C., Gomes-Junior, F.G., Marcos-Filho, J., 2018. Vigor-S, a new system for evaluating the physiological potential of maize seeds. *Scientia Agricola*, 75(2), 167172. <http://dx.doi.org/10.1590/1678-992x-2016-0401>

Catão, H.C.R.M., Pontes, B.S., Pinheiro, D.T., Oliveira Filho, M.A., Santos, A.L.C., Zolla, M.C., 2024. Chemical treatment and mobilization of reserves of soybean seeds under water deficit. *Journal of Seed Science*, 46, e202446005. <https://doi.org/10.1590/2317-1545v46278828>

CONAB. Companhia Nacional de Abastecimento, 2026. Acompanhamento de safra brasileira: grãos, décimo segundo levantamento, junho 2026. Brasília: CONAB, 2026. Disponível em: <https://www.conab.gov.br/info-agro/safras/graos/boletim-da-safra-de-graos>.

Finch-Savage, W.E., 2020. Influence of seed quality on crop establishment, growth, and yield. In: Seed quality. CRC Press, p. 361-384. <https://doi.org/10.4324/9781003075226-11>

Gomes-Junior, F.G. Análise computadorizada de imagens de plântulas. In: Krzyzanowski, F.C.; Vieira, R.D.; França-Neto, J.B.; Marcos-Filho, J. (Eds.). Vigor de sementes: conceitos e testes Londrina: Abrates, 2020. p.141-183

Gomes-Junior, F.G., Chamma, H.M.C.P., Cicero, S.M., 2014. Automated image analysis of seedlings for vigor evaluation of common bean seeds. *Acta Scientiarum. Agronomy*, 36(2), 195200. <https://doi.org/10.4025/actasciagron.v36i2.21957>

Guedes, R.S., Alves, E.U., Santos-Moura, S.S., Galindo, E.A., 2015. Teste de comprimento de plântula na avaliação da qualidade fisiológica de sementes de *Amburana cearensis*. *Semina: Ciências Agrárias*, 36(4), 23732382. <https://doi.org/10.5433/1679-0359.2015v36n4p2373>

Henning, F.A., Mertz, L.M., Jacob Junior, E.A., Machado, R.D., Fiss, G., Zimmer, P.D., 2010. Composição química e mobilização de reservas em sementes de soja de alto e baixo vigor. *Bragantia*, 69(3), 727734. <https://doi.org/10.1590/S0006-87052010000300026>

Krzyzanowski, F.C., Franca-Neto, J.B., Gomes-Junior, F.G., Nakagawa, J., 2020. Testes de vigor baseados em desempenho de plântulas. In: Krzyzanowski, F.C., Vieira, R.D., França-

Neto, J.B., Marcos-Filho, J. (Eds.) Vigor de sementes: conceitos e testes. Londrina: Abrates, p.79-140.

Leite, C.A.M., França-Neto, J.B., Krzyzanowski, F.C., Gomes-Junior, F.G., Lopes, I.O.N., Henning, F.A., 2024. Validation of the Vigor-S image analysis system for the characterization of phytotoxicities in soybean seedlings. *Journal of Seed Science*, 46, e202446014. <https://doi.org/10.1590/2317-1545v46279386>

Leite, M.S., Torres, S.B., Gomes Junior, F.G., Rego, C.H.Q., Paiva, E.P., Leite, T.S., 2020. Viability of seedling image analysis (Vigor-S) to determine the physiological potential of melon seeds. *Journal of Seed Science*, 42, e202042043. <https://doi.org/10.1590/2317-1545v42237826>

Marcos-Filho, J., 2015a. Fisiologia de sementes de plantas cultivadas. FEALQ, Piracicaba, 495p.

Marcos-Filho, J., 2015b. Seed vigor testing: an overview of the past, present and future perspective. *Scientia Agricola*, 72(4), 363374. <https://doi.org/10.1590/0103-9016-2015-0007>

Marcos-Filho, J., 2020. Testes de vigor: importância e utilização. In: Krzyzanowski, F.C., Vieira, R.D., França-Neto, J.B., Marcos-Filho, J. (Eds.) Vigor de sementes: conceitos e testes. Londrina: Abrates, p. 17-72.

Marcos-Filho, J., Kikuti, A.L.P., Lima, L.B., 2009. Métodos para avaliação do vigor de sementes de soja, incluindo a análise computadorizada de imagens. *Revista Brasileira De Sementes*, 31(1), 102112. <https://doi.org/10.1590/S0101-31222009000100012>

Masilamani, P., Venkatesan, S., Navamaniraj, K.N., Rajarathinam, P., Alagesan, A., Thiagu, K., 2023. Impact of the orientation of seed placement and depth of its sowing on germination: a review. *Journal of Applied and Natural Science*, 15(1), 314324. <https://doi.org/10.31018/jans.v15i1.4265>

Medeiros, A.D.D., Silva, L.J.D., Capobianco, N.P., Fialho, C.A., Dias, D.C.F.D.S., 2019. Assessing the physiological quality of common bean seeds using the Vigor-S system. *Journal of Seed Science*, 41(2), 187195. <https://doi.org/10.1590/2317-1545v41n2211401>

Nerling, D., Coelho, C.M.M., Brummer, A., 2022. The hydrolysis dynamic of storage reserves in maize seed germination. *Journal of Seed Science*, 44, e202244019. <https://doi.org/10.1590/2317-1545v44258701>

Padilha, M.S., Coelho, C.M.M., Siega, Y.P., Ehrhardt-Brocardo, N.C.M., 2024. Vigor and reserve mobilization of common bean seed during germination under salt stress. *Bragantia*, 84, e20240079. <https://doi.org/10.1590/1678-4499.20240079>

Pinheiro, D.T., Dias, D.C.F.S., Silva, L.J.D., Martins, M.S., Finger, F.L., 2023. Oxidative stress, protein, and physiological potential of soybean seeds under weathering deterioration. *Acta Scientiarum. Agronomy*, 45, e56910. <https://doi.org/10.4025/actasciagron.v45i1.56910>

R Core Team, 2022. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>

Reed, R.C., Bradford, K.J., Khanday, I., 2022. Seed germination and vigor: ensuring crop sustainability in a changing climate. *Heredity*, 128(6), 450-459. <https://doi.org/10.1038/s41437-022-00497-2>

Rego, C.H.Q., Cicero, S.M., França-Silva, F., Gomes-Junior, F.G., 2021. Assessing the vigor of cowpea seeds using the Vigor-S software. *Journal of Seed Science*, 43, e202143018. <https://doi.org/10.1590/2317-1545v43244858>

Rodrigues, M., Gomes-Junior, F.G., Marcos-Filho, J., 2020. Vigor-S: System for Automated Analysis of Soybean Seed Vigor. *Journal of Seed Science*, 42, e202042039. <https://doi.org/10.1590/2317-1545v42237490>

Vieira, R.D., Bittencourt, S.R.M., Panobianco, M., 2003. Seed vigour - an important component of seed quality in Brazil. *ISTA - Seed Testing International*, 126, 2122.

Vieira, R.D., Marcos-Filho, J. 2020. Teste de condutividade elétrica. In: Krzyzanowski, F.C., Vieira, R.D., França-Neto, J.B., Marcos-Filho, J. (Eds.) *Vigor de sementes: conceitos e testes*. Londrina: Abrates, p.93-97.

Wendt, L., Gomes-Junior, F.G., Zorato, M.F., Moreira, G.C., 2014. Avaliação do potencial fisiológico de sementes de soja por meio de imagens. *Pesquisa Agropecuária Tropical*, 44(3), 280-286. <https://doi.org/10.1590/S1983-40632014000300011>