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Optimizing Basil Seed Vigor Evaluations: An Automatic Approach Using Computer Vision-Based Technique

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Abstract: The short cultivation cycle and high essential oil content of basil plants render them a valuable raw material source for diverse industries. However, large-scale production is hindered by the lack of specific protocols to assess seed vigor; thus, a consistent supply of high-quality seeds that meet consumer demands cannot be ensured. This study investigated the effectiveness of an automated system for seedling analysis as a tool for evaluating basil seed vigor and compared it to traditional tests. For this purpose, seeds from eight commercial lots were evaluated in two separate trials spaced six months apart using the following tests: germination, first germination count, saturated salt accelerated aging, primary root emergence, mean germination time, seedling emergence, seedling emergence speed index, and computerized seedling image analysis. The parameters provided by the system allowed us to clearly and objectively classify the basil seed lots based on vigor, and the results were strongly and significantly correlated with the findings of traditional vigor tests, particularly between the vigor index and seedling length. Digital analysis of four-day-old seedlings proved to be a fast and efficient technique for evaluating basil seed vigor and has the potential for use in automating the data collection and analysis process.

Keywords: *Ocimum basilicum* L.; Lamiaceae; medicinal plant; germination; physiological potential; quality control; seed storage; seedling performance; image analysis; SVIS®

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1. Introduction

The availability of cultivars capable of producing high essential oil content and a wide variety of chemotypes with multiple purposes makes basil (*Ocimum basilicum* L.) a promising raw material source for various industrial sectors [1,2]. Biomolecules synthesized by these plants exhibit antimicrobial and insect-repellent properties, making them valuable for phytosanitary protection during the production and postharvest storage of horticultural products [3]. Furthermore, basil cultivation presents a more sustainable option relative to rosewood (*Aniba rosaeodora* Ducke) overexploitation. This commercially valuable tree species, which is facing endangerment, is prized by perfume, cosmetics, and chemical brands for its essential oil rich in linalool at concentrations exceeding 90% [4]. Unlike the slow-growing rosewood tree, basil plants are herbaceous and annual, allowing for successive harvests to obtain a readily available and renewable source of essential oil, which can contain linalool concentrations of up to 60% [5,6].

To meet the growing demand for basil, commercial cultivation practices must be restructured, starting with the use of high-quality seeds. This is essential for rapid germination, uniform seedling emergence, adverse condition tolerance, and satisfactory plant performance in the field [7]. The germination test, a mandatory requirement for seed commercialization, is carried out under optimal conditions for each species, with standardized

evaluation criteria already established for basil seeds [8]. Therefore, the test results do not always express the real potential of a seed lot and cannot be extrapolated to crops outside the laboratory since the environmental conditions at sowing can be unfavorable for germination and seedling establishment [9,10]. To estimate the potential performance of seeds during storage and field conditions, vigor tests have been developed, based on traits that can vary across species [11]. However, the development of techniques that meet quality standards and market demands for cost-effectiveness, speed, and efficiency remains a major challenge [12]. In fact, only a few commercially important species have protocols that are validated and recommended by independent organizations such as the International Seed Testing Association (ISTA), the Association of Official Seed Analysts (AOSA), and the Brazilian Association of Seed Technology (ABRATES).

Initially, seed vigor assessment methodologies were based on empirical observations that simulated stress conditions encountered in the field, or on theoretical hypotheses related to the biological principles of seed physiology. With the inclusion of accelerated aging and electrical conductivity tests in the International Rules for Seed Testing in 2001, the ISTA Vigor Committee supported the development of new protocols based on the hypothesis linking the deterioration process and repair system, with the potential use of molecular markers and automation [11]. Seedling performance tests have been widely used to assess seed vigor due to their ease of implementation and lack of specialized equipment requirements [13]. However, these tests rely on manual evaluations, making them labor-intensive and time-consuming [14], limiting the number of samples that can be analyzed. Technological advancements have provided computerized tools, such as the Seed Vigor Imaging System (SVIS®), software developed at Ohio State University by Sako et al. [15] and refined by Hoffmaster et al. [16,17], which automates data collection and analysis, leading to increased standardization and expedited results [18]. That software processes multiple digital images of scanned seedlings to provide parameters related to growth rate and uniformity, serving as a benchmark for the development of other systems [19–21].

In an effort to guarantee a steady supply of seeds for farmers, the Brazilian Ministry of Agriculture, Livestock and Food Supply (MAPA) has established a minimum germination standard of 60% for basil seeds sold nationwide. This relatively low percentage requires the purchase of a higher seed quantity per m^2, which not only raises production costs but also does not guarantee successful seedling establishment in the field. The complex reproductive process of basil plants leads to a single lot containing seeds at different stages of maturity and with varying embryo integrity, which affects their germination performance [22]. This variability is due to the asynchronous opening of tiny floral buds at different developmental stages within each inflorescence [23]. In this context, investigating methodologies to enhance the selection of high-quality basil seed lots is justified.

The lack of a standardized and validated protocol for assessing the vigor of basil seed lots poses a problem when estimating their actual physiological performance, particularly for samples that exhibit similar germination rates. As a result, quality control programs resort to general tests, such as the first germination count and seedling emergence, or adopt methodologies developed for other species within the Lamiaceae family [24–27]. To support the selection of high-quality seed lots, this study evaluated the performance of the SVIS® software (version 1.0) in analyzing basil seedling images and the application of this technology in seed quality control programs. To achieve this, the parameters required by the system were adjusted according to the characteristics of basil seedlings, and its performance was evaluated by comparing the results obtained with conventional vigor tests routinely used in seed testing laboratories.

2. Material and Methods

2.1. Seed Samples

Eight commercial seed lots of the basil cultivar "Alfavaca Basilicão", which was free of sanitary treatment, were homogenized, packaged in Kraft paper bags, and stored under controlled environmental conditions (20 °C air temperature and 30% air humidity),

commonly used by seed testing laboratories for maintain the sample quality, ensuring a reliable and readily available supply of material for retesting or future research purposes. The seeds were evaluated for their physiological potential using the tests described below during two experimental trials: before storage and after six months of storage.

2.2. Initial Characterization

2.2.1. Water Content

The water content determined by the oven method. Briefly, two 0.5 g seed samples for each lot were placed in an oven at 105 ± 3 °C for 24 h, and the results were expressed as percentages (wet basis) according to the Rules for Seed Testing [8]. This process was also performed after exposing the seeds to the saturated salt accelerated aging test.

2.2.2. Germination Test

Four replicates of 50 seeds each were distributed on two sheets of blotting paper (previously moistened with distilled water in an amount equivalent to 2.5 times the substrate mass) and placed in transparent plastic boxes (11 × 11 × 3.5 cm). The containers were kept in a Bio-Oxygen Demand (BOD) chamber, remaining under an alternating temperature regime of 20/30 °C and a photoperiod of 8 h. Normal seedlings were counted on the fourth day, considering the first germination count, and on the 14th day after sowing, according to the criteria established by the Rules for Seed Testing [8]. The results were expressed as a percentage of normal seedlings.

2.3. Complementary Physiological Tests

2.3.1. Saturated Salt Accelerated Aging Test

Approximately 2 g of seeds were distributed in a single uniform layer on stainless-steel screens suspended inside transparent plastic boxes (11 × 11 × 3.5 cm), containing 40 mL of saturated sodium chloride solution at the bottom of the box to create an environment with 76% air humidity [28]. The containers were placed in a water-jacketed accelerated aging chamber and held at 42 °C for 48 h [24]. Following aging, the seeds were subjected to the germination test and evaluated after four days. The results were expressed as a percentage of normal seedlings.

2.3.2. Primary Root Emergence Test

Four replicates of 50 seeds each were distributed on two sheets of blotting paper, which were previously moistened with distilled water in an amount equivalent to 2.5 times the substrate mass, and placed in transparent plastic boxes (11 × 11 × 3.5 cm). The containers were kept in a BOD chamber, remaining under an alternating temperature regime of 20/30 °C in the dark. The number of seeds that emitted a primary root, specifically a minimum of 2 mm, were counted at 12 h intervals spanning the time limit from 12 to 84 h. At the end of the counts, the mean germination time was calculated as described by Marin et al. [26]. The results were expressed as a percentage of seeds with radicle protrusion.

2.3.3. Seedling Emergence Test

Four replicates of 50 seeds each were sown 0.5 cm deep in individual cells of 200-cell polystyrene trays containing a commercial substrate (Basaplant®) for vegetable seedling production. The trays were kept under benches inside the laboratory and irrigated daily until excess water was observed draining from the cell drainage hole. The number of seedlings that emerged with fully expanded cotyledon leaves was recorded daily to calculate the seedling emergence speed index, using the method proposed by Maguire [29]. The final evaluation was carried out on the 21st day after sowing, and the result was expressed as a percentage of seedlings emerged.

2.4. Acquisition and Analysis of Seedling Images

Four replicates of 25 seeds each were distributed in two parallel rows in the upper third of two sheets of blotting paper, previously moistened with distilled water in an amount equivalent to 2.5 times the substrate mass, and placed in transparent plastic boxes (11 × 11 × 3.5 cm). The containers were arranged in a BOD chamber forming an angle of 70° with the horizontal, remaining under alternating temperature regime of 20/30 °C in the dark (Figure 1A).

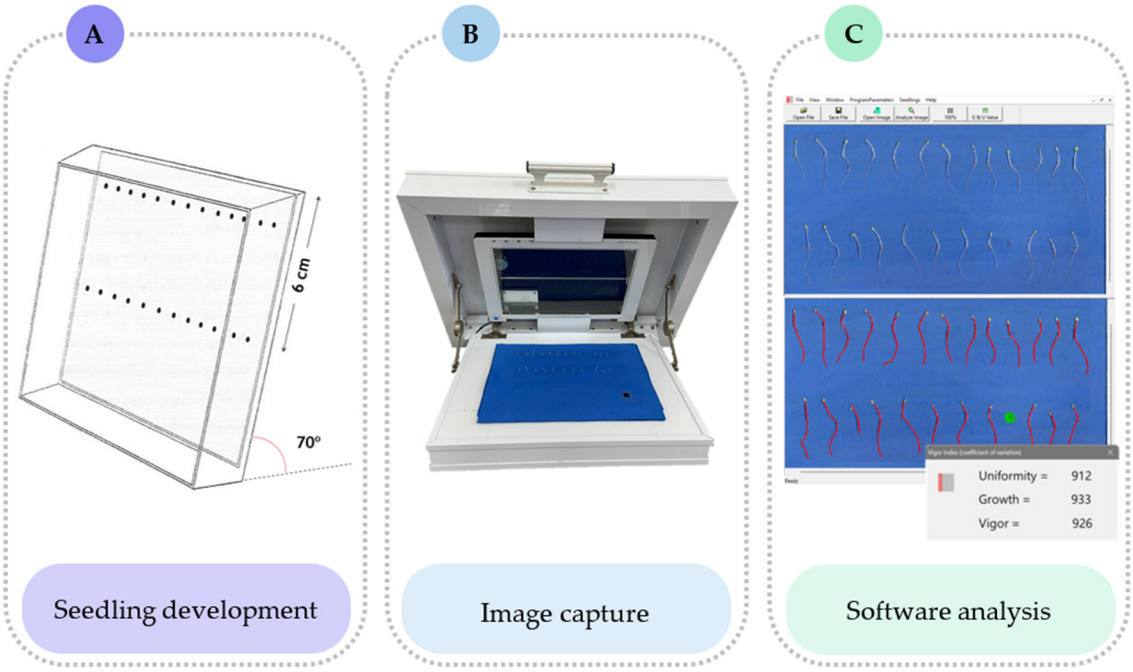

Figure 1. Step-by-step procedure for automated image analysis of basil seedlings using SVIS® software. Seeds were sown on blotting paper at 2 cm (top row) and 6 cm (bottom row) from the top of the paper; after sowing, the containers were placed in a germination chamber at a 70° angle with the horizontal (**A**). Seedlings and non-germinated seeds were transferred to a blue ethylene vinyl acetate sheet and scanned to capture images (**B**). Captured images were processed to generate development uniformity, growth, and vigor indices. Analyzed seedlings are highlighted in red, while non-germinated seeds are marked in green (**C**).

After four days, the seedlings (normal and abnormal) and non-germinated seeds were transferred to a blue ethylene vinyl acetate sheet to provide the necessary contrast for analysis by the system. The images were captured with an HP Scanjet 200 scanner (Hewlett-Packard, Palo Alto, CA, USA), installed in an inverted position inside an aluminum box (60 × 50 × 12 cm), adjusted to a resolution of 100 dpi, and connected to a computer (Figure 1B). Next, the images were processed by SVIS® software to generate development uniformity (Equation (1)), growth (Equation (2)), and vigor (Equation (3)) indices, comprising values from 0 to 1000 (Figure 1C) [17]. The mean length of the seedlings (cm) was also determined by using the conversion of 0.0254 cm pixel^{-1}.

$$\text{Uniformity} = 1000 - \left[\frac{\sum_{i=1}^{N}\left(X_i - \overline{X}\right)^2}{N}\right]^{0.5} \times 500 - 20 \times n_{dead}, \quad (1)$$

$$\text{Growth} = 1000 \times \left(\frac{\sum_{i=1}^{N} X_i}{N \times \text{MaxSL}}\right), \quad (2)$$

$$\text{Vigor} = w_u \times \text{uniformity} + w_g \times \text{growth}, \quad (3)$$

where X_i is the length of each individual seedling, $\overline{X}$ is the mean seedling length, N is the total number of seedlings, n_{dead} is the number of non-germinated seeds comprising the image analyzed, MaxSL is the value for the maximum growth possible for basil seedlings, and w_u and w_g represent weights associated with the parameters being multiplied.

To calculate the vigor index, the maximum expected development for four-day-old basil seedlings was determined based on the maximum seedling length (hypocotyl and primary root), with 30% of the vigor index being attributed to uniformity and 70% to growth. These procedures were repeated on new sets of three-day-old seedlings to investigate the possibility of earlier evaluations.

2.5. Statistical Analyses

The experiment was conducted using a completely randomized experimental design with four replicates. The original data obtained were tested for normality using the Shapiro–Wilk test. The analysis of variance (ANOVA) was performed separately for each experimental period, and the means were grouped by the Scott–Knott test ($p \leq 0.05$); this approach offers a distinct advantage over traditional multiple comparisons tests (e.g., Tukey, Duncan, SNK, and Dunnett) by partitioning the means into non-overlapping groups [30], providing a clearer classification of seed lots performance. The degree of association between the results of the different tests was determined using Pearson's correlation coefficients (r), where the significance of the r values was determined by the t-test ($p \leq 0.05$). The analyses were conducted using the R software, version 4.4.1.

3. Results and Discussion

All samples achieved the minimum germination percentage of 60% required for the commercialization of basil seeds. However, Lots 1, 2, and 8 showed lower viability according to the germination test, differing significantly from the others in both experimental periods (Table 1). Based on this result, these lots could have been excluded from the subsequent analysis, as vigor tests are generally recommended to identify differences between lots with similar germination percentages, such as Lots 3, 4, 5, 6, and 7. In the present study, all lots were included to consider the importance of having samples with different physiological potentials when establishing or improving vigor analysis methods [31]. This approach provides a comprehensive evaluation of the effectiveness of the adopted procedure while also adding reliability to the results produced.

Table 1. Characterization of the physiological potential of basil seed lots.

Lots	WC	WC_{AA}	GE	FGC	SSAA	PRE	SE	ESI	MGT
					%			Index	h
First experimental trial †									
1	6.7	9.0	68 ± 1.30 ^{b}	66 ± 1.50 ^{d}	54 ± 3.83 ^{d}	52 ± 3.67 ^{d}	70 ± 1.92 ^{c}	5.38 ± 0.08 ^{c}	58.7 ± 1.09 ^{a}
2	6.8	9.2	72 ± 1.87 ^{b}	61 ± 2.55 ^{d}	56 ± 1.92 ^{d}	68 ± 2.17 ^{c}	70 ± 1.78 ^{c}	5.43 ± 0.27 ^{c}	53.6 ± 0.57 ^{b}
3	6.8	9.3	86 ± 1.92 ^{a}	86 ±1.87 ^{b}	68 ± 1.09 ^{c}	82 ± 4.82 ^{b}	74 ± 4.85 ^{c}	5.64 ± 0.27 ^{c}	53.5 ± 0.42 ^{b}
4	6.8	9.2	90 ± 3.27 ^{a}	88 ± 2.55 ^{b}	89 ± 2.06 ^{b}	77 ± 1.80 ^{b}	70 ± 2.45 ^{c}	5.54 ± 0.31 ^{c}	54.8 ± 1.15 ^{b}
5	6.8	9.7	92 ± 1.87 ^{a}	91 ± 1.66 ^{b}	84 ± 1.30 ^{b}	83 ± 1.66 ^{b}	82 ± 1.87 ^{b}	7.00 ± 0.30 ^{b}	50.9 ± 0.50 ^{c}
6	6.1	8.9	90 ± 2.12 ^{a}	86 ± 1.64 ^{b}	86 ± 1.79 ^{b}	88 ± 1.41 ^{b}	88 ± 2.05 ^{a}	7.20 ± 0.22 ^{b}	51.5 ± 0.62 ^{c}
7	6.5	9.8	97 ± 1.12 ^{a}	97 ± 1.12 ^{a}	96 ± 1.22 ^{a}	98 ± 1.09 ^{a}	94 ± 1.92 ^{a}	8.41 ± 0.32 ^{a}	47.6 ± 0.32 ^{d}
8	7.0	9.0	75 ± 2.60 ^{b}	74 ± 2.77 ^{c}	62 ± 0.83 ^{d}	56 ± 1.92 ^{d}	76 ± 0.71 ^{c}	5.13 ± 0.05 ^{c}	60.0 ± 0.50 ^{a}
p-value	-	-	**1.98×10^{-8}**	**2.99×10^{-10}**	**6.72×10^{-13}**	**4.81×10^{-10}**	**5.21×10^{-6}**	**6.87×10^{-8}**	**1.63×10^{-9}**
CV (%)	-	-	5.82	5.78	6.08	7.98	7.25	9.18	3.03

Table 1. *Cont.*

Lots	WC	WC_{AA}	GE	FGC	SSAA	PRE	SE	ESI	MGT
					%			Index	h
Second experimental trial [††]									
1	6.0	9.9	76 ± 2.92 [b]	60 ± 4.36 [c]	46 ± 0.71 [e]	50 ± 1.87 [e]	69 ± 1.80 [c]	5.23 ± 0.13 [c]	58.8 ± 1.10 [a]
2	6.0	9.3	75 ± 3.64 [b]	64 ± 2.55 [c]	57 ± 2.69 [d]	66 ± 2.05 [d]	71 ± 2.06 [c]	5.22 ± 0.06 [c]	53.2 ± 0.25 [b]
3	6.1	9.0	90 ± 2.86 [a]	86 ± 2.17 [b]	69 ± 2.06 [c]	85 ± 1.66 [c]	76 ± 2.17 [c]	5.57 ± 0.19 [c]	52.5 ± 0.61 [b]
4	6.4	9.4	90 ± 2.12 [a]	86 ± 2.17 [b]	84 ± 2.45 [b]	83 ± 2.69 [c]	74 ± 1.30 [c]	5.43 ± 0.06 [c]	54.0 ± 0.40 [b]
5	6.4	9.0	88 ± 2.12 [a]	85 ± 2.29 [b]	84 ± 2.55 [b]	92 ± 0.71 [b]	81 ± 0.50 [b]	6.94 ± 0.13 [b]	50.1 ± 0.38 [c]
6	5.7	8.8	93 ± 1.12 [a]	88 ± 1.30 [b]	82 ± 3.70 [b]	90 ± 1.79 [b]	88 ± 2.92 [a]	7.17 ± 0.06 [b]	50.2 ± 0.50 [c]
7	6.2	8.8	98 ± 0.71 [a]	98 ± 1.09 [a]	96 ± 0.83 [a]	98 ± 0.43 [a]	92 ± 1.92 [a]	8.33 ± 0.20 [a]	46.1 ± 0.63 [d]
8	6.9	9.0	83 ± 1.80 [b]	66 ± 1.79 [c]	54 ± 0.43 [d]	68 ± 3.27 [d]	74 ± 1.79 [c]	5.27 ± 0.10 [c]	58.0 ± 0.53 [a]
p-value	-	-	**2.03×10^{-5}**	**2.29×10^{-9}**	**1.72×10^{-12}**	**9.59×10^{-13}**	**6.54×10^{-7}**	**5.35×10^{-14}**	**2.73×10^{-11}**
CV (%)	-	-	6.24	7.02	7.14	5.86	5.69	4.79	2.62

Bold values indicate significant *p*-values. For each evaluation at each experimental period, numbers followed by different letters within lots indicate significant difference at $p \leq 0.05$ by the Scott–Knott test. Analysis before ([†]) and after six months ([††]) of storage, CV: coefficient of variation, WC: initial water content, WC_{AA}: water content after the aging procedure, GE: germination, FGC: first germination count, SSAA: saturated salt accelerated aging, PRE: primary root emergence after 60 h, SE: seedling emergence, ESI: seedling emergence speed index, MGT: mean germination time. Values represent the mean ± standard error ($n = 4$).

During the germination test, 90% of viable seeds germinated on the fourth day in all lots and experimental periods and the increments stabilized until the tenth day after the test was set up, with sufficient seedlings development for an adequate evaluation of their structures. Considering that the germination test should have sufficient duration to allow the germination process to occur without prolonging it to the point of breaking dormancy or overestimating the sample performance [32], this result provides significant insights into the timing specified by the Rules for Seed Testing [8] for *O. basilicum* L., indicating the potential to reduce the total test duration from fourteen to ten days, which could lead to substantial reductions in operational costs related to equipment usage and labor in the laboratory.

The first germination count (FGC) classified the lots into different levels, highlighting Lot 7 as the most vigorous in both evaluation periods (Table 1). The ability of the FGC to distinguish between lots compared to the germination test, conducted under optimal conditions, stems from the fact that seeds with higher levels of deterioration require additional time to restore the metabolic functions necessary for germination [33]. Thus, the FGC provides information on physiological potential that complements the germination test in a quick and practical way [34].

The primary root emergence test (PRE) performed similarly to the FGC and saturated salt accelerated aging test (SSAA), but with a shorter execution time (Table 1). The time required to perform the PRE was inversely proportional to the range between the percentages of seeds with radicle protrusion, with significant differences in the behavior of the lots observed 60 h after sowing, including those with the lowest viability (Figure 2). The principle of the PRE is similar to that of the FGC in terms of the speed at which the evaluated structures are formed in each test, emphasizing the importance of determining the optimal time for data collection to ensure reliable results. Seeds in an advanced state of deterioration exhibit slow and uneven germination [35], leading to multiple counts at different intervals until a stabilization in the number of germinated seeds. However, the demand for rapid results and the high volume of samples to be evaluated can limit the application of this test in routine seed quality control programs [36].

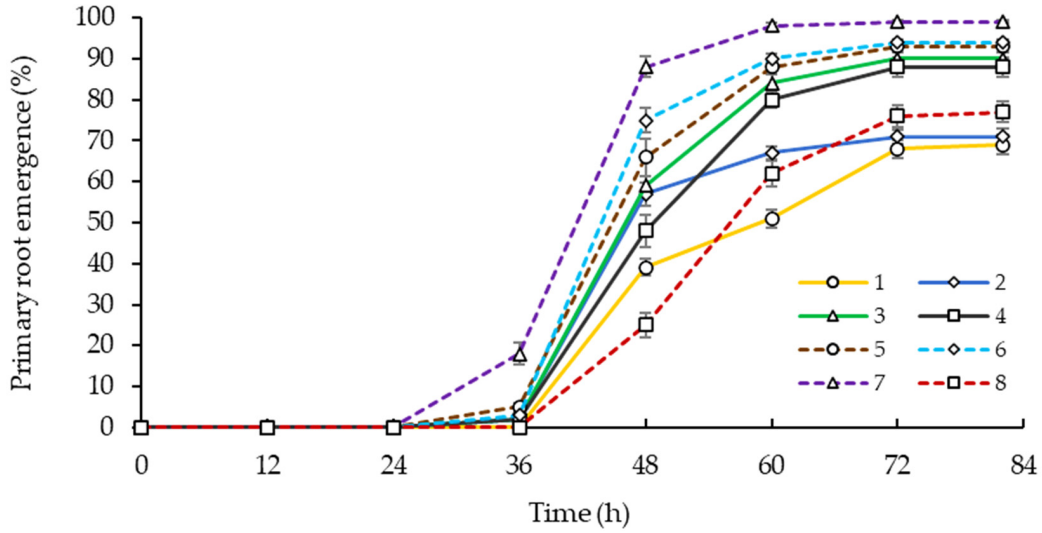

Figure 2. Primary root emergence progress curves for basil seed lots. Values represent the mean ± standard error (n = 8).

The seedling emergence test (SE) categorized the seed lots into three vigor classes but showed limited precision in ranking the lots within each class, as in the case of Lot 6 being grouped in the same category as Lot 7 (Table 1); this result supports the argument that the SE has limitations in estimating the seed vigor. The interaction of multiple environmental and biological factors can influence seedling development and mask the results, as uncontrolled environmental conditions make it challenging to accurately assess the real physiological potential of the sample [37].

The basil seeds are small and have a mucilage composed of a strong network of fibrils which are gelatinous and have a high capacity for water absorption and emulsification [38]. These characteristics can affect the results of the accelerated aging test, as they can promote the proliferation of microorganisms during the test. To regulate water uptake and mitigate the negative effects of microbial growth on germination, the accelerated aging test was conducted with a salt solution. This approach ensures that the test remains sensitive required to evaluate seed vigor [28,39]. This methodology was initially proposed by Jianhua and McDonald [28] for small seeds and later adapted by Lima et al. [24] to evaluate the vigor of *Ocimum gratissimum* L. seeds, which have characteristics similar to basil seeds. Therefore, we expected to prevent abrupt and excessive increases in seed water content during the accelerated aging test.

The variation in water content among the basil seed lots, both initially and after accelerated aging, was below the recommended limit of 2% (Table 1), complying with the established principles regarding the influence of seed moisture on the results of vigor analysis methods [40]. Variations exceeding the indicated percentage can compromise the analyses and hinder the correct interpretation of the results; this is because the water content is directly associated with the level of metabolic activity of the seeds and the intensity of the deterioration process [41]. Based on the foregoing, it can be inferred that the impact of water content on the results of the tests conducted in this study was minimal or negligible.

The number of normal seedlings from the SSAA was not significantly reduced compared to the values obtained in the germination test (Table 1). This result suggests that the saline solution mitigated excessive stress during the aging period, thereby minimizing its impact on subsequent germination. The percentage variation in the values for the lots with lower viability showed a greater reduction (up to 20%), while the values for Lot 7 decreased by only 1.5%, evidencing its high physiological performance. According to Marcos-Filho [37], the larger the difference between the standard germination results and

those of the accelerated aging test, the lower the vigor of the seed sample. Conversely, smaller variations indicate greater tolerance to stress in the field and during storage.

To perform computerized image analysis using the SVIS® software, the analyst must provide the maximum seedling length observed among the samples in the shortest possible time. Given its critical importance for the success of the analysis, this procedure must be carried out meticulously. In the case of basil seeds, it was necessary to establish the optimal time for seedling measurement, taking as a reference the recommendation in the Rules for Seed Testing [8] for FGC and verifying the possibility of anticipating the evaluation by 24 h. Although most seeds germinated three days after sowing, the hypocotyl and primary root structures were not yet sufficiently developed to enable the software to accurately stratify the seed lots (Figure 3). According to Hoffmaster et al. [16], limited growth in underdeveloped seedlings can lead to errors in the software algorithm measurements. To determine the maximum size of seedlings to be used in system calibration, it is essential to use samples identified by other routine tests such as high vigor, capable of expressing the maximum growth potential of seedlings of a species [42]. Thus, the evaluations were carried out with data obtained on the fourth day after sowing, when the seedlings reached a maximum length of 5.08 cm (Figure 4), with good development of the hypocotyl and primary root, allowing verification of the differences in seedling growth.

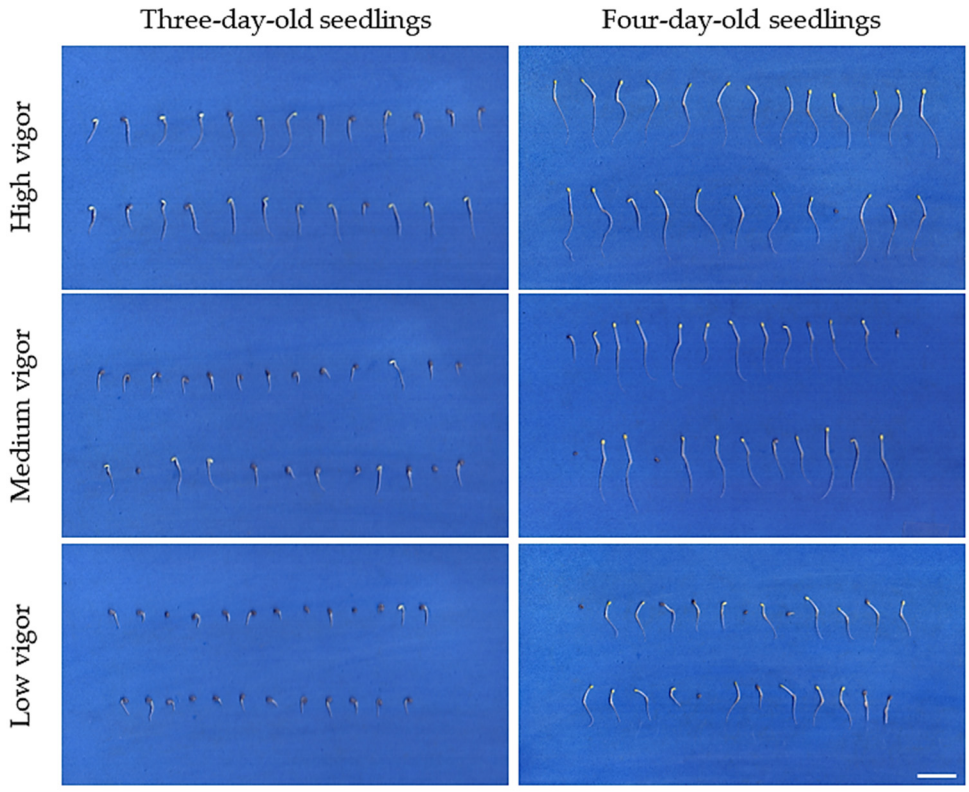

Figure 3. Visual appearance of three- and four-day-old basil seedlings. The white line indicates the image scale (2.0 cm).

The parameters provided by SVIS® allowed for the identification of vigor differences from the initial evaluation, positioning Lot 7 as the highest vigor (Table 2), in alignment with the results of traditional vigor tests (Table 1). The vigor index (VI) indicated the largest number of vigor levels in the initial evaluation and is among the fastest and most sensitive indicators for evaluating physiological potential [43]. However, depending on the number of samples to be evaluated, the number of strata provided by the statistical analysis results may increase the difficulty of classifying the lots into the categories of high, medium, and low vigor. Thus, a scale was proposed by researchers at Ohio State University that classifies lots as exceptional vigor if they have VI values of 800–1000; high

vigor = 600–799; medium vigor = 400–599; and low vigor = 200–399 [44]. When applying this scale to interpret the data available in Table 2, most lots can be classified as high vigor, including those that showed lower physiological potential in the results of traditional tests (Table 1). This finding reveals that this scale should not be used in a generalized way as there is a risk of producing incorrect results.

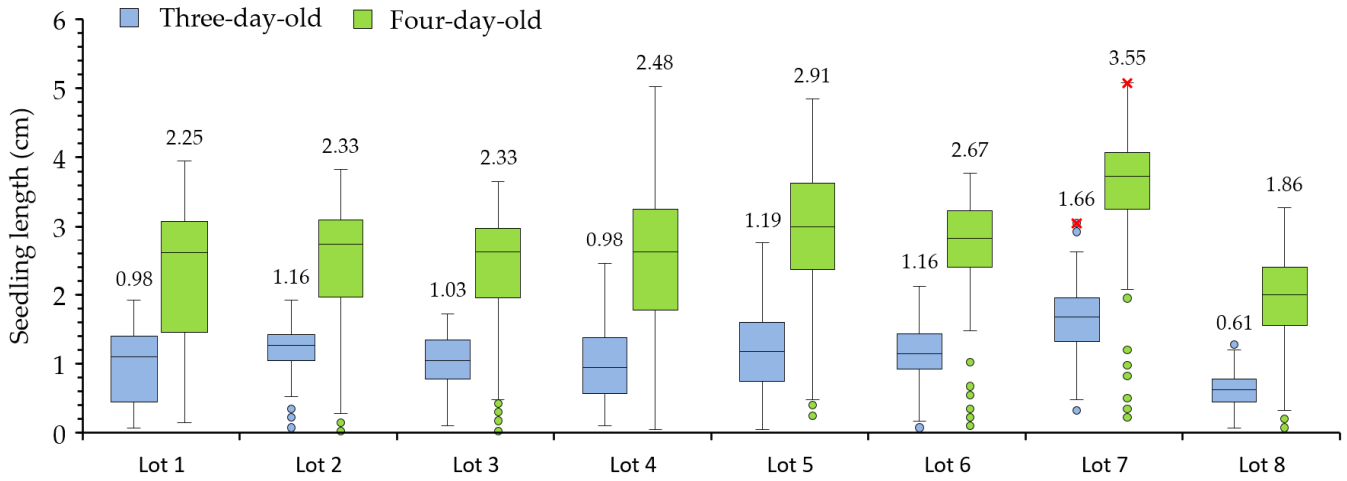

Figure 4. Length of three- and four-day-old basil seedlings from seed lots subjected to computerized image analysis using the SVIS® software. The red crosses indicate the values used as a reference for calculating the growth index (3.05 and 5.08 cm). Values represent the mean (n = 200).

Table 2. Computerized analysis of three- and four-day-old basil seedling images from seed lots using the SVIS® software.

Lots	Three-Day-Old Seedlings				Four-Day-Old Seedlings			
	UI	GI	VI	SL	UI	GI	VI	SL
		Index		cm		Index		cm
First experimental trial †								
1	704 ± 19.23 ^{c}	410 ± 35.78 ^{b}	498 ± 29.67 ^{b}	0.70 ± 0.10 ^{c}	724 ± 25.46 ^{c}	550 ± 7.33 ^{d}	602 ± 12.35 ^{e}	1.68 ± 0.12 ^{d}
2	720 ± 6.42 ^{c}	486 ± 24.95 ^{b}	556 ± 18.20 ^{b}	0.81 ± 0.03 ^{b}	754 ± 20.73 ^{c}	600 ± 40.19 ^{d}	646 ± 34.18 ^{d}	1.93 ± 0.15 ^{c}
3	831 ± 10.81 ^{b}	420 ± 19.82 ^{b}	543 ± 14.48 ^{b}	0.94 ± 0.04 ^{b}	830 ± 4.89 ^{b}	604 ± 18.19 ^{d}	671 ± 12.56 ^{d}	2.22 ± 0.04 ^{c}
4	799 ± 22.91 ^{b}	431 ± 23.19 ^{b}	541 ± 14.60 ^{b}	0.87 ± 0.05 ^{b}	813 ± 20.42 ^{b}	579 ± 21.71 ^{d}	649 ± 17.31 ^{d}	1.99 ± 0.09 ^{c}
5	811 ± 20.90 ^{b}	436 ± 10.85 ^{b}	548 ± 4.93 ^{b}	0.93 ± 0.03 ^{b}	872 ± 9.83 ^{a}	750 ± 2.14 ^{b}	786 ± 2.86 ^{b}	2.85 ± 0.02 ^{b}
6	865 ± 18.77 ^{a}	459 ± 19.27 ^{b}	580 ± 18.75 ^{b}	1.04 ± 0.06 ^{b}	884 ± 6.67 ^{a}	667 ± 14.41 ^{c}	732 ± 10.09 ^{c}	2.64 ± 0.05 ^{b}
7	907 ± 6.86 ^{a}	659 ± 29.55 ^{a}	733 ± 22.28 ^{a}	1.57 ± 0.08 ^{a}	913 ± 8.10 ^{a}	846 ± 5.08 ^{a}	866 ± 3.02 ^{a}	3.39 ± 0.02 ^{a}
8	804 ± 18.28 ^{b}	246 ± 9.44 ^{c}	413 ± 11.33 ^{c}	0.50 ± 0.04 ^{c}	805 ± 15.96 ^{b}	433 ± 9.88 ^{e}	544 ± 7.50 ^{f}	1.54 ± 0.06 ^{d}
p-value	**1.30 × 10^{-6}**	**4.42 × 10^{-8}**	**3.56 × 10^{-8}**	**1.04 × 10^{-8}**	**1.15 × 10^{-6}**	**5.43 × 10^{-11}**	**8.48 × 10^{-11}**	**2.21 × 10^{-12}**
CV(%)	4.78	12.05	7.60	14.90	4.41	6.88	5.25	8.19
Second experimental trial ††								
1	652 ± 26.09 ^{c}	392 ± 27.12 ^{c}	470 ± 21.68 ^{d}	0.58 ± 0.07 ^{d}	784 ± 8.25 ^{c}	574 ± 24.35 ^{c}	637 ± 14.54 ^{c}	1.92 ± 0.02 ^{c}
2	728 ± 28.35 ^{b}	483 ± 18.64 ^{b}	556 ± 20.71 ^{c}	0.79 ± 0.07 ^{c}	699 ± 9.21 ^{d}	562 ± 11.06 ^{c}	603 ± 8.96 ^{d}	1.64 ± 0.05 ^{d}
3	849 ± 17.81 ^{a}	421 ± 17.27 ^{c}	549 ± 16.87 ^{c}	0.92 ± 0.07 ^{c}	843 ± 4.62 ^{b}	560 ± 31.40 ^{c}	645 ± 23.33 ^{c}	2.15 ± 0.10 ^{c}
4	818 ± 11.36 ^{a}	386 ± 22.81 ^{c}	515 ± 18.84 ^{c}	0.84 ± 0.06 ^{c}	848 ± 18.44 ^{b}	654 ± 17.87 ^{b}	712 ± 16.49 ^{b}	2.49 ± 0.12 ^{b}
5	828 ± 18.90 ^{a}	558 ± 24.42 ^{b}	639 ± 12.25 ^{b}	1.20 ± 0.04 ^{b}	862 ± 19.17 ^{b}	705 ± 15.57 ^{b}	752 ± 13.74 ^{b}	2.68 ± 0.07 ^{b}
6	866 ± 7.11 ^{a}	506 ± 19.19 ^{b}	614 ± 14.89 ^{b}	1.16 ± 0.04 ^{b}	862 ± 8.23 ^{b}	664 ± 6.47 ^{b}	724 ± 6.27 ^{b}	2.56 ± 0.07 ^{b}
7	893 ± 18.22 ^{a}	723 ± 33.08 ^{a}	773 ± 28.08 ^{a}	1.71 ± 0.10 ^{a}	910 ± 6.90 ^{a}	929 ± 3.11 ^{a}	923 ± 1.56 ^{a}	3.68 ± 0.03 ^{a}
8	812 ± 18.35 ^{a}	258 ± 9.42 ^{d}	424 ± 7.27 ^{d}	0.53 ± 0.03 ^{d}	831 ± 9.29 ^{b}	494 ± 12.88 ^{d}	594 ± 10.88 ^{d}	1.80 ± 0.06 ^{d}
p-value	**1.25 × 10^{-6}**	**4.14 × 10^{-10}**	**1.04 × 10^{-9}**	**5.32 × 10^{-10}**	**4.58 × 10^{-9}**	**3.79 × 10^{-12}**	**1.54 × 10^{-12}**	**1.01 × 10^{-13}**
CV(%)	5.56	11.15	7.55	15.03	3.24	6.33	4.46	7.06

Bold values indicate significant p-values. For each evaluation at each experimental period, numbers followed by different letters within lots are significantly different at $p \leq 0.05$ by the Scott–Knott test. Analysis before (†) and after six months (††) of storage, CV: coefficient of variation, UI: development uniformity index, GI: growth index, VI: vigor index, SL: seedling length. Values represent the mean ± standard error (n = 4).

The development uniformity index (UI) can lead to erroneous conclusions about the vigor of basil seeds because it groups lots with different growth rates into the same category (Table 2). Since the UI is calculated based on the standard deviation of the length of the seedlings present in the repetition, regardless of the development stage they present, similar values may be observed between lots with different physiological potentials. This occurs because the index reflects the uniformity of both well-formed seedlings and underdeveloped seedlings [42,45,46]. Thus, during the interpretation of this parameter, the value provided by the index must be analyzed together with the images (Figure 3) in order to avoid the erroneous classification of lots with inferior performance as high vigor or vice versa.

The inclusion of new procedures for analyzing seed vigor depends on the equivalence of their results with those of traditional tests [13]. However, before labeling a test as "good" or "bad", it is important to consider that the criteria adopted in each methodology vary according to the characteristics evaluated. For example, in the present study, computerized image analysis quantified seedling growth and development uniformity, while traditional tests (SSAA, PRE, and SE) focused on the percentage of germination or emerged seedlings. Tests that evaluate similar parameters tend to show a strong and significant correlation with each other ($r \geq 0.70$, $p \leq 0.05$), as observed between the methods that evaluated the percentages of germinated seeds: GE, FGC, SSAA, and PRE (Table 3). This pattern was repeated among procedures that used the speed of the germination process (FGC, PRE, ESI, and MGT), as well as among those that considered seedling development (SL and UI; GI and VI). In this context, Pearson's correlation coefficient is a useful tool for selecting tests that can be combined to assess the physiological potential of seed lots [47–49], as long as the relative nature of vigor is considered.

Table 3. Pearson's correlation coefficients between traditional vigor tests and computerized seedling analyses for basil seed lots.

Parameter	Traditional Seed Vigor Tests						Computerized Analysis			
	FGC	**SSAA**	**PRE**	**SE**	**ESI**	**MGT**	**UI**	**GI**	**VI**	**SL**
GE	**0.91** *	**0.78** *	**0.81** *	0.63 *	0.66 *	−0.64 *	**0.77** *	0.59 *	0.66 *	**0.73** *
FGC	-	**0.86** *	**0.82** *	0.64 *	**0.70** *	−0.67 *	**0.78** *	0.63 *	**0.70** *	**0.76** *
SSAA		-	**0.82** *	0.62 *	**0.72** *	**−0.73** *	**0.71** *	**0.70** *	**0.74** *	**0.78** *
PRE			-	0.67 *	**0.72** *	**−0.86** *	0.69 *	0.68 *	**0.73** *	**0.77** *
SE				-	**0.91** *	−0.63 *	0.63 *	0.68 *	**0.71** *	**0.73** *
ESI					-	**−0.75** *	0.66 *	**0.83** *	**0.85** *	**0.86** *
MGT						-	−0.55 *	**−0.81** *	**−0.81** *	**−0.81** *
UI							-	0.63 *	**0.74** *	**0.82** *
GI								-	**0.99** *	**0.95** *
VI									-	**0.98** *

* Significant difference at $p \leq 0.05$ by the *t*-test. Bold values indicate a strong correlation coefficient ($r \geq 0.70$). GE: germination, FGC: first germination count, SSAA: saturated salt accelerated aging, PRE: primary root emergence after 60 h, SE: seedling emergence, ESI: seedling emergence speed index, MGT: mean germination time, UI: development uniformity index, GI: growth index, VI: vigor index, SL: seedling length.

Germination and SSAA showed a strong correlation but diverged in the ranking of the lots (Tables 1 and 3). This result can be justified by the fact that lots classified as high vigor by the accelerated aging test generally had high germination; however, the reverse is not always true, since not all lots with high viability are classified as vigorous in the vigor tests. The sum of the individual behavior of each seed, which is influenced by the intensity of its deterioration process, determines the categorization of the germination performance of the samples into three vigor categories: high, medium, and low.

The stratification of seed lots into high or low vigor categories is relatively easily determined by traditional seed analysis methods (Figure 5). At one extreme are the high vigor lots, where majority of seeds retain their germination potential almost virtually unaltered, even under adverse conditions, as they have a high level of cell membrane

structure, an efficient enzymatic system, and rapid repair of the metabolic apparatus. At the opposite extreme are the low vigor lots, in which most of their seeds are intensely affected by deterioration and therefore often do not perform satisfactorily, even under ideal conditions. The challenge in detecting medium vigor lots arises from the diverse and complex factors that cause deterioration, as well as the speed and intensity with which they act, affecting individual seeds within a lot and even different parts of the same seed. As a result, medium vigor lots have a range that intersperses between the two extremes, as they are composed of individuals at different stages of deterioration, where even if the membrane system is damaged, it can repair the enzymatic mechanism with relative efficiency. This means that under stressful conditions, performance may be reduced, but if the conditions are ideal, germination occurs satisfactorily.

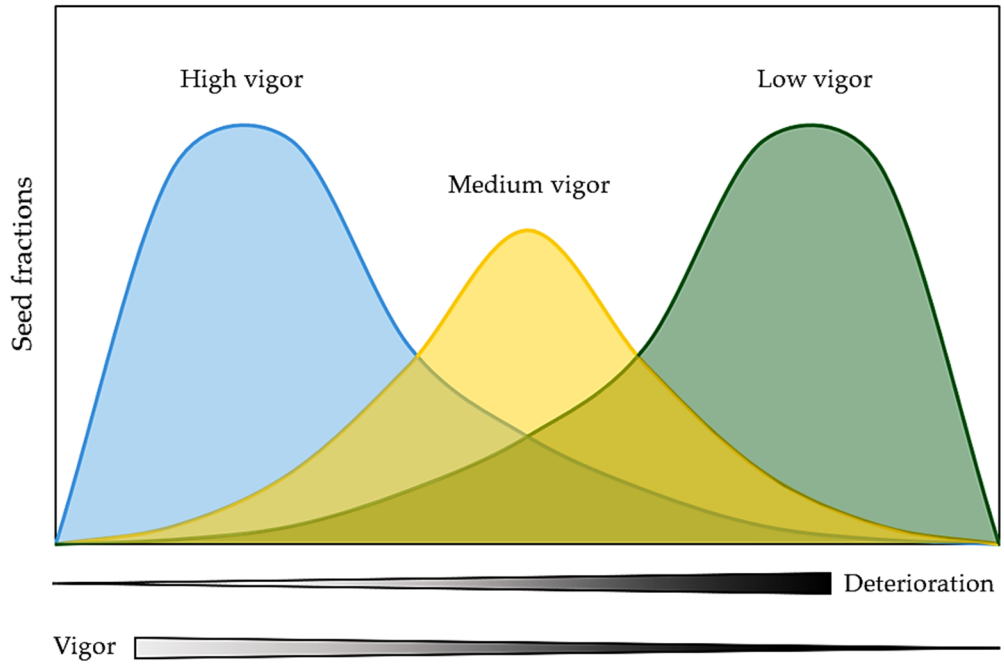

Figure 5. Hypothetical scheme of the distribution of seed lots in the categories of high, medium, and low vigor as a function of the different phases of the deterioration process.

This reasoning is supported by findings reported by Abud et al. [50], as differences between methodologies and the parameters evaluated can explain the possible variations in the classification of lots, especially for those with intermediate performance. According to Marcos-Filho [37], identifying the physiological potential of medium vigor lots can be challenging due to their inconsistent behavior, sometimes equaling that of higher or lower vigor lots, depending on the test used. It is important to remember that vigor is a constituent attribute of seed quality that should not be oversimplified as a single quantifiable property. Instead, it should be understood as a characteristic that encompasses the potential performance of seeds in an agricultural context, influenced by the interactions between genes and the environment [51]. It is unlikely that a single test can provide a satisfactory and accurate diagnosis of all aspects related to seed quality [52]; therefore, the joint analysis of the results of different tests is essential for obtaining consistent and reliable information. Thus, based on the degree of correlation, the strong and significant correlations verified in this study could be used to identify promising or alternative tests for the classification of basil seed lots regarding vigor, with emphasis on the SSAA, PRE, SL, and VI because they combined strong correlation with a similar statistical ranking.

Finally, we add an observation regarding the storage conditions for basil seeds. At the beginning of the research, the authors were concerned with the preservation of physiological potential of seed samples in the absence of specific information for this species.

We opted for a dry chamber (20 °C and 30% relative humidity), similar to those shown to maintain the physiological potential of *O. gratissimum* L. seeds for up to 12 months [53]. This protocol successfully ensured the maintenance of the basil seed samples during the analysis period, a fact evidenced by the data in Tables 1 and 2.

4. Conclusions

The optimal time for measuring the growth of basil seedlings was determined to be four days after sowing. This timeframe allowed the SVIS® software to generate reliable vigor assessment parameters, yielding results comparable to those obtained through conventional vigor tests routinely used in seed testing laboratories.

This protocol enabled a reduction in both the costs and time associated with additional evaluations, as the analysis can be conducted concurrently with the germination test. Furthermore, the software can compile data and generate results within minutes, allowing for a larger volume of samples to be evaluated through a simple, rapid, and standardized approach. This greatly enhances the accuracy and efficiency of estimating the physiological potential of basil seeds.

Given that seed vigor is a complex, multifaceted trait influenced by the interaction between genetic characteristics and environmental factors that cannot be reduced to a single quantitative measure, the SVIS® software offers many possibilities. For instance, the creation of a database for comparisons among analysts and laboratories could establish a readily accessible, interconnected support channel. This network would be populated with data on evaluation results and the seed's origin, allowing the identification of ideal growing conditions and improving basil seed quality. In the future, this data-driven approach could serve as a valuable tool for strategic decision-making in seed production.

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