

Accelerated aging associated with computerized analysis (Vigor-S) to evaluate the vigor of soybean seeds

Envelhecimento acelerado associado à análise computadorizada de imagem (Vigor-S) para avaliação do vigor de sementes de soja

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ABSTRACT

Tests used to assess the physiological quality of seeds need improvement to optimize the use of seeds, resources, and labor. The development of new tests and/or adjustments to existing ones can allow better screening of vigor between lots and be more predictive of seed performance in the field, especially for new cultivars. Thus, the aim of this study was to verify the effectiveness of a new methodology for assessing the physiological quality of soybean seeds. This methodology is based on the accelerated aging test associated with computerized analysis of the images of seedlings from aged seeds. For this purpose, soybean seeds from three cultivars, and four lots of each cultivar, were used. The physiological quality of the seeds was characterized using germination and vigor tests. Subsequently, the proposed new methodology was applied, where the seeds were aged at 41 °C and 100% relative humidity for periods of 0, 24, 48, and 72 hours, followed by computerized image analysis of the seedlings from the aged seeds. It was observed that accelerated aging, using a temperature of 41 °C, and relative humidity of 100% for 72 hours, followed by computerized image analysis of seedlings, is a potential new methodology to assess the physiological quality of soybean seeds.

KEYWORDS: Vigor test. Physiological quality of seeds. *Glycine max* L. Merrill.

RESUMO

Testes utilizados para acessar a qualidade fisiológica das sementes precisam ser aprimorados, otimizando o uso das sementes, dos recursos e da mão de obra. A elaboração de novos testes e/ou ajustes dos já existentes podem permitir melhor triagem do vigor entre lotes e ser mais preditivos do desempenho das sementes a nível de campo, sobretudo para as novas cultivares. Assim, o presente trabalho teve por objetivo verificar a eficácia de uma nova metodologia para avaliação da qualidade fisiológica das sementes de soja, baseada na execução do teste de envelhecimento acelerado associado à análise computadorizada das imagens de plântulas advindas das sementes envelhecidas. Para isso, foram utilizadas sementes de soja de três cultivares e quatro lotes de cada cultivar. A caracterização da qualidade fisiológica das sementes foi realizada por meio de testes de germinação e vigor. Em seguida, foi realizada a nova metodologia proposta, onde as sementes foram envelhecidas a 41°C e umidade relativa de 100%, pelos períodos de 0, 24, 48 e 72 horas, seguida da análise computadorizada de imagens das plântulas advindas das sementes envelhecidas. Observou-se que o envelhecimento acelerado, utilizando-se a temperatura de 41 °C e umidade relativa de 100% pelo período de 72 horas, seguido da análise computadorizada de imagens das plântulas, apresenta-se como uma nova metodologia potencial para avaliação da qualidade fisiológica de sementes de soja.

PALAVRAS-CHAVE: Teste de vigor. Qualidade fisiológica de sementes. *Glycine max* L. Merrill.

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INTRODUCTION

Soybean (*Glycine max.* (L.) Merrill) is an important crop for global agribusiness (SONI et al. 2023). Due to genetic improvement, the great diversity of use of the oilseed, and the increase in global demand for food, the production and area devoted to soybean cultivation have been increasing annually (ROQUETTE et al. 2023).

The physiological quality of seeds is undoubtedly one of the main factors determining the success of soybean crops worldwide. High-quality seeds perform well in the field, with rapid emergence and plant establishment, even under less favorable environmental conditions (FINCH-SAVAGE & BASSEL 2016). The use of high-quality seeds enables access to genetic advances, with guarantees of quality and adaptation technologies in different regions, ensuring greater productivity (FRANÇA-NETO et al. 2016).

Among the attributes related to seed physiological quality, germination and vigor stand out, both of which are at their maximum at physiological maturity (TRIPATHI & KHARE 2016). From this point onwards, there are several metabolic changes and decreases in viability and vigor parameters, caused by the deterioration process, which can be faster or slower depending on the genetic makeup of the seeds and the conditions to which they are exposed (NAGEL et al. 2014). Therefore, after the physiological maturity point the field and storage conditions influence the loss of quality of this important agricultural input (GASTL-FILHO et al. 2022).

Soybean seed storage in Brazil is practically mandatory since the harvest period, which begins in January/February and does not coincide with the planting period, which starts in September/October. As a result, soybean seeds usually remain in storage for a certain period (CUNHA et al. 2019) and are prone to deterioration, often leading to loss of vigor and, ultimately, viability. As a result, after planting, there are stand and emergence failures and uneven plant development, which leads to loss of productivity (EBONE et al. 2020).

Since seed vigor is naturally reduced over time, and the intensity of the deterioration process varies according to the genetic makeup and conditions to which the seeds are exposed, monitoring the physiological quality of seeds is necessary for their production, and marketing (ZUCARELI et al. 2015).

Currently, the physiological quality of soybean seeds has been monitored using numerous germination and vigor tests (KRZYZANOWSKI et al. 2020). However, especially for new cultivars, it has been observed that there are tests that show a greater correlation with the expression of field seed vigor than others (REZVANI et al. 2017, SHEIDAEI et al. 2014) and, sometimes, adjustments to the tests are necessary to make them predictive (RADKE et al. 2018). In addition, the market has increasingly demanded innovation in tests to make them faster, more practical, cheaper, more accurate and predictive. Within this context, computerized seedling image analysis has been a highly sought-after tool and has already proven to be efficient for assessing seed vigor (RIBEIRO et al. 2024, JIN et al. 2022, RODRIGUES et al. 2020).

In addition to computerized image analysis, the accelerated aging test is reported to be one of the tests with the highest correlation with seed performance in the field (MATERA et al. 2019). It causes stress due to exposure to high temperatures and

relative humidity, which leads to rapid deterioration (DELOUCHE & BASKIN 1973), simulating stress in the field. However, the aging method's temperature, humidity, exposure time, and effectiveness depend on various factors, including the species and the cultivar (FANTAZZINI et al. 2018). For soybean seeds, a temperature of 41 °C and 100% relative humidity, with seed exposure for 48 hours, has been indicated as the traditional method for the vigor test (MCDONALD & PHANEENDRANATH, 1978). Although this methodology is widely used and effective for measuring vigor in soybean seeds (MATERA et al., 2019), new vigor tests may emerge from methodological adjustments that enhance its predictive power (SUÑÉ et al., 2021). Additionally, image analysis can be a useful tool for evaluating seedlings after accelerated aging (RIBEIRO et al., 2024). However, further research is needed to validate adjustments to the accelerated aging test when associated with seedling image analysis.

Within this context, the aim of this study was to verify the effectiveness of a new methodology for assessing the vigor of soybean seeds. This methodology was based on the performance of the accelerated aging test combined with the computerized image analysis of seedlings from aged seeds.

MATERIAL AND METHODS

The experiment was conducted at the Seed Laboratory and experimental field of the Universidade Estadual de Maringá – Campus Regional de Umuarama – Paraná – Brazil. The seeds were acquired from seed production fields from the 2022/2023 harvest. Seeds of the cultivars Soytech 591 I2X, Soytech 631 I2X, and Credenz Result I2X were used, with four lots of each cultivar.

The following tests and determinations were performed to characterize the seed lots:

Moisture content - This was determined immediately before the tests were carried out and was determined according to the oven method at 105 ± 3 °C for 24 hours, using four replicates of 25 seeds each (BRASIL 2009).

Germination test (G) - Four replicates of 50 seeds were used. The seeds were sown equidistantly on germination paper moistened with a volume of water equivalent to 2.5 times the weight of the dry substrate and kept in a germinator at 25 °C. Evaluations were carried out to record the percentage of normal seedlings on the 4th and 12th days after sowing (BRASIL 2009).

Accelerated aging (AA) - Four replicates of 50 seeds were used. The seeds were distributed in a single layer on a metal mesh tray attached to a gerbox containing 40 mL of distilled water at the bottom. The boxes were covered to obtain 100% relative humidity (RH) inside and kept in a BOD chamber at 41 °C for 48 hours (KRZYZANOWSKI et al. 2020).

Seedling growth test (SG) - Four replicates of 20 seeds were used. These were sown equidistantly on germination paper and moistened with a volume of water corresponding to 2.5 times the weight of the dry paper. Germination rolls were prepared (KRZYZANOWSKI et al. 2020) and kept at 25 °C for three days. The seedlings were scanned using an HP Ink Tank 416 printer, resulting in 300 dpi images. These were processed using the Vigor-S software, and the parameters of seedling

between the variables analyzed. The results of the computerized image analysis of seedlings from seeds aged for different periods were subjected to analysis of variance, followed by Pearson's correlation analysis at 5% probability using the F test, to check their correlation with the field emergence test. The statistical analyses were carried out using the R Core Team software.

RESULTS AND DISCUSSIONS

The seeds of all lots and cultivars had a moisture content of around 9%. In addition, all the seed lots were preconditioned at 25 °C for 16 hours before the tests were carried out (BRASIL 2009). The results of the analysis of the characterization of the physiological quality of the seeds are presented in Table 1.

Table 1. Results of the germination (G), accelerated aging (AA), seedling emergence (EM), vigor index (VIG), uniformity index (UNIF) and seedling length (SL) from lots and cultivar of soybean seeds.

Cultivars	Lots	G	AA	EM	VIG	UNIF	SL
Soytech 591 I2X	1	100 A	95 A	100 A	460 A	722 A	4.83 A
Soytech 591 I2X	2	100 A	91 A	96 A	505 A	672 A	5.88 A
Soytech 591 I2X	3	100 A	96 A	100 A	553 A	678 A	6.30 A
Soytech 591 I2X	4	100 A	91 A	96 A	316 B	573 A	2.95 B
Soytech 631 I2X	1	98 A	72 B	79 C	559 A	742 A	6.2 A
Soytech 631 I2X	2	99 A	74 B	74 C	408 A	624 A	4.53 A
Soytech 631 I2X	3	98 A	74 B	79 C	235 B	446 B	1.95 B
Soytech 631 I2X	4	99 A	76 B	74 C	181 B	421 B	1.40 B
Credenz Result I2X	1	96 B	92 A	98 A	465 A	634 A	5.13 A
Credenz Result I2X	2	99 A	91 A	90 B	488 A	720 A	5.10 A
Credenz Result I2X	3	96 B	95 A	98 A	575 A	752 A	6.23 A
Credenz Result I2X	4	99 A	86 A	90 B	379 A	612 A	3.78 B
CV (%)		1.66	5.75	4.33	30.52	19.94	6.73

*** Means followed by the same capital letter do not differ by the Scott - Knott test at 5% probability. CV = coefficient of variation

All seed lots used in this study exhibited high physiological quality, with germination rates above 96%. However, there was a difference in vigor between the seed lots (Table 1). The germination of lots 1 and 3 of the Credenz Result I2X cultivar was lower than that of the other lots and cultivars. It was also evident that the four lots of the Soytech 631 I2X cultivar had lower vigor than the other cultivars, as they exhibited the lowest seedling emergence and the lowest percentage of normal seedlings after accelerated aging.

The germination test expresses the maximum potential for production of normal seedlings under optimal conditions for the resumption of embryo growth (BRASIL 2009). This test allowed lots to be grouped into two quality levels: high (A) and low (B). To distinguish vigor levels between lots with similar germination, vigor tests are commonly adopted, being accelerated aging, seedling length, vigor index and seedling emergence tests indicated for investigating the vigor of soybean seeds (RODRIGUES et al. 2020, KRZYZANOWSKI et al. 2020). In this experiment, the accelerated aging test, seedling length and uniformity and vigor indices were able to distinguish the lots into two vigor classes: high (A) and low (B), while the seedling emergence test distinguished them into three classes levels: high (A), intermediate (B) and low (C)

(Table 1). These results are in line with what is reported in the literature, which points to the emergence test as one of the most indicative for evaluating seed vigor (KRZYZANOWSKI et al. 2020, MARCOS-FILHO 2015).

It was also observed that seedling length, vigor, and uniformity indices from computerized image analysis, which are described as good indicators of seed vigor (RODRIGUES et al. 2020, JIN et al. 2022, MARCOS-FILHO 2015), were not significantly correlated with emergence in the field (Figure 2).

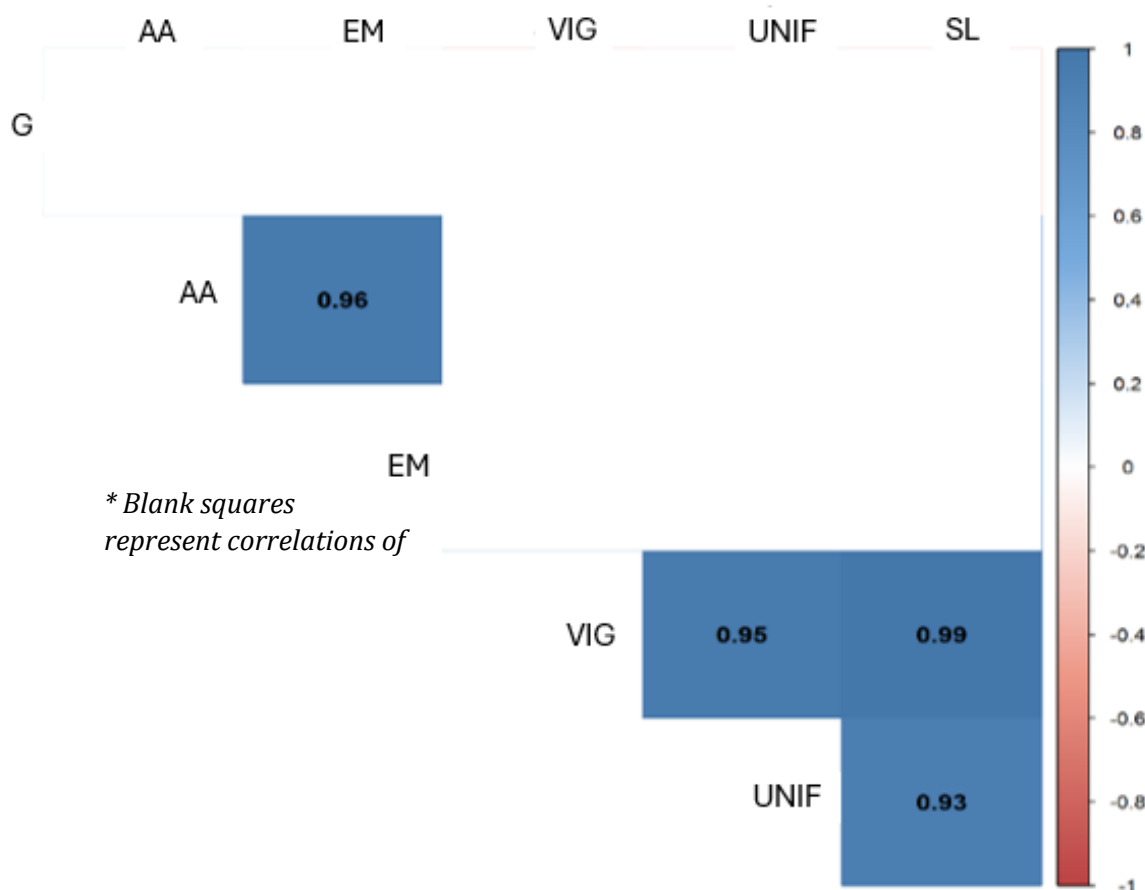


Figure 2. Correlation of germination and vigor test variables, which infer the physiological quality of seed lots. G - germination, AA - accelerated aging, EM – seedling emergence in the field, VIG - vigor index, UNIF- uniformity index, SL - seedling length. Blank squares mean that there was no significant correlation between the variables at 5% probability by the F test; blue squares mean that there was a positive correlation; red squares represent a significant negative correlation between the variables.

The correlation analysis showed that the only test with a significant correlation with emergence in the field was the accelerated aging test (AA) (0.96) (Figure 2), demonstrating the effectiveness of the AA test in assessing the vigor of soybean seeds. This confirms the studies of MATERA et al. (2019), who describe accelerated aging as a test that has a high positive correlation with seed vigor in the field. In addition, a positive and significant correlation was observed between the parameter's vigor index, uniformity index, and seedling length (Figure 2). This high and significant correlation was expected since the vigor index (SAKO et al. 2001) and uniformity index

(CHRISTIANSEN 1942), automatically obtained by Vigor-S, are both based on seedling length parameters (CASTAN et al. 2018).

The results obtained in this study reinforce the need to develop new tests that are predictive of seed vigor in the field. In this research, the germination, seedling length, and vigor and uniformity indices tests, which are conducted under ideal conditions for seed germination, presented no correlation with the seedling emergence in the field.

This may be due to the genetic characteristics of the cultivars since all the varieties used in this research are genetically modified and were recently launched on the market. Thus, these cultivars may not respond to the tests in the same way as older cultivars. Furthermore, the length of soybean seedlings was obtained automatically by Software Vigor-S after 72 hours of sowing on paper (RODRIGUES et al. 2020). According to MENEGUZZO et al. (2021), the length of soybean seedlings conducted under ideal conditions for germination must be performed after at least 96 hours of sowing. It may be that, under ideal temperature and humidity conditions, soybeans require more time to grow, to allow for the differentiation of vigor between lots. In this way, aging the seeds by accelerated aging before performing the seedling length test in an automated way by Vigor-S, may be a viable alternative, as a new methodology, as proposed in this work.

The results of this new proposed methodology can be seen in Table 2.

Table 2. Seedling length after 0 hours (SL_0h), 24 hours (SL_24h), 48 hours (SL_48h) and 72 hours (SL_72h) and vigor after 0 hours (VIG_0h), 24 hours (VIG_24h), 48 hours (VIG_48h) and 72 hours (VIG_72h) of accelerated aging in lots and cultivars soybean seeds.

Cultivars	Lots	SL_0h	VIG_0h	SL_24h	VIG_24h	SL_48h	VIG_48h	SL_72h	VIG_72h
Soytech 591 I2X	1	4.83 A	460 A	9.18 A	751 A	5.53 A	509 A	4.75 A	469 A
Soytech 591 I2X	2	5.88 A	505 A	5.63 B	508 B	4.48 A	415 A	2.53 C	272 B
Soytech 591 I2X	3	6.30 A	553 A	2.1 C	220 C	4.43 A	437 A	1.55 C	201 B
Soytech 591 I2X	4	2.95 B	316 B	6.08 B	574 B	2.6 B	256 B	0.85 D	113 C
Soytech 631 I2X	1	6.2 A	559 A	2.95 C	313 C	3.55 A	338 A	2.53 C	273 B
Soytech 631 I2X	2	4.53 A	408 A	2.18 C	258 C	2.25 B	255 B	1.05 D	146 C
Soytech 631 I2X	3	1.95 B	235 B	1.9 C	240 C	0.95 C	139 C	0.35 C	36 D
Soytech 631 I2X	4	1.40 B	181 B	2.7 C	294 C	0.43 C	56 C	0.35 C	49 D
Credenz Result I2X	1	5.13 A	465 A	4.35 C	432 B	1.7 B	202 B	5.118 A	503 A
Credenz Result I2X	2	5.10 A	488 A	6.18 B	572 B	2.5 B	275 B	2.18 C	258 B
Credenz Result I2X	3	6.23 A	575 A	3.7 C	364 B	4.48 A	434 A	3.33 B	335 B
Credenz Result I2X	4	3.78 B	379 A	1.23 C	124 C	1.00 C	128 C	0.40 D	62 D
CV (%)		6.73	30.52	8.88	34.73	1.78	33.55	2.77	10.76

*** Means followed by the same capital letter do not differ by the Scott - Knott test at 5% probability. CV = coefficient of variation

The Scott-Knott test used to compare the length and vigor means of seedlings from unaged seeds (SL_0h and VIG_0h) grouped the lots into two vigor classes. When accelerated aging was applied for 24 or 48 hours, three vigor classes were distinguished. On the other hand, when accelerated aging was applied for 72 hours, it was possible to distinguish four vigor classes (Table 2). Vigor tests are considered

better, the better they show differences between lots (KRZYZANOWSKI et al. 2020). In this way, it is possible to verify that there was greater evidence of difference between the batches, the greater the aging carried out before the length test (Table 2). This highlights the effectiveness of the new proposed test.

Also, there was a significant positive correlation between the variables EM and SL_48h, and Vigor_48h; and between EM and SL_72h and VIG_72h (Figure 3). However, when the seeds were not aged (SL_0h, VIG_0h) or were aged for 24 hours (SL_24h, Vigor_24h) there was no significant correlation with seedling emergence. These results indicate that conducting the seedling length test without prior aging, using Vigor-S, as described by MARCOS-FILHO et al. (2009), or after stress for 24 hours at a temperature of 41 °C/RH 100%, does not always correlate with emergence in the field.

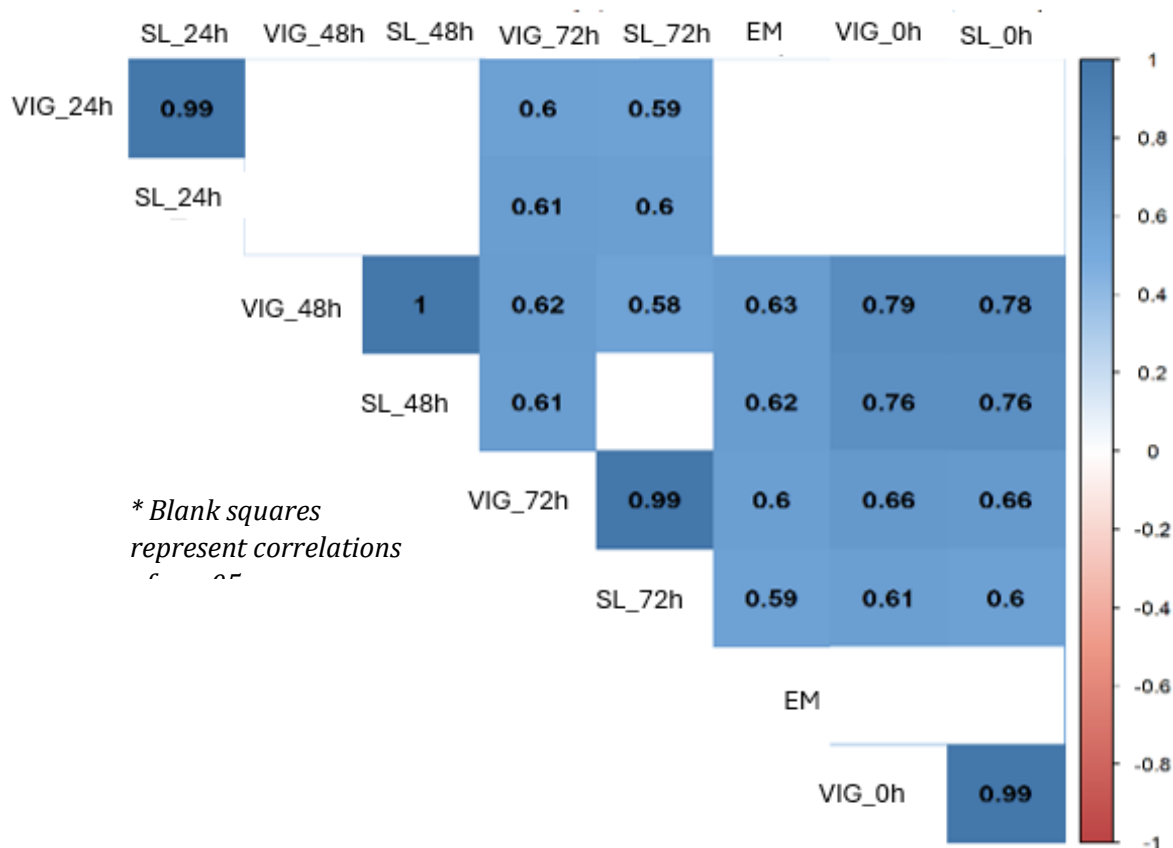


Figure 3. Correlation between the variables of the new proposed methodologies and field seedling emergence (EM). SL_0h - seedling length after 0 hours of aging; SL_24h - seedling length after 24 hours of aging; SL_48h - seedling length after 48 hours of aging; SL_72h - seedling length after 72 hours of aging; VIG_0h - vigor index after 0 hours of aging; VIG_24h - vigor index after 24 hours of aging; VIG_48h - vigor index after 48 hours of aging; VIG_72h - vigor index after 72 hours of aging. Blank squares indicate that there was no significant correlation between the variables at 5% probability by the F test; blue squares indicate that there was a positive correlation; red squares represent a significant negative correlation between the variables.

The computerized image analysis of seedlings using the Vigor-S software has been described as an efficient tool for assessing the physiological potential of bean seeds (MEDEIROS et al. 2019), cowpea seeds (REGO et al. 2021) and soybean seeds (RODRIGUES et al. 2020). However, it should be noted that in these studies, aged lots

were used, either through artificial aging (MEDEIROS et al. 2019) or through natural aging (REGO et al. 2021, RODRIGUES et al. 2020). We believe that computerized analysis of seeds is more effective in showing the differences in vigor between lots when the seeds are subjected to aging than when they are analyzed freshly harvested, without aging, as shown in our research (Figure 3).

The methodology of imposing high temperature and relative humidity stress on the seeds for 48 or 72 hours, then assessing the seedling length, and the vigor index, showed a significant positive correlation with field seedling emergence. However, it was also possible to observe that the 72 hour aging methodology, followed by computerized analysis of seedlings, made it possible to distinguish four vigor classes among the seed lots (Table 2). It has made a better screening even than the emergence test, demonstrating the potential of the new methodology we proposed.

In addition to the computerized analysis of seedlings from aged seeds having demonstrated better vigor screening between seed lots (Table 2), compared to accelerated aging or the seedling length test performed individually (Table 1), it is important to highlight that this methodology saves on the use of germination paper (since smaller papers are used – cut in half of the original paper), on the number of seeds and faster evaluation, as it is carried out automatically.

Since the accelerated aging test is one of the most widely used tests for evaluating the vigor of soybean seeds and has a high correlation with vigor in the field (Figure 2) and the analysis of seedling vigor using Vigor-S is a tool that allows for quick, agile and accurate analysis (RIBEIRO et al. 2024, MARCOS-FILHO et al. 2009), the combination of these tests has the potential to be used as a new methodology for evaluating the vigor of soybean seeds (Table 2 and Figure 3).

CONCLUSION

Promoting accelerated aging at 41 °C and 100% relative humidity for 72 hours, followed by computerized image analysis of seedlings from aged seeds, represents a promising new methodology for assessing the physiological quality of soybean seeds..

NOTES

AUTHOR CONTRIBUTIONS

Conceptualization, methodology, and formal analysis, **Cleverton, Martha and José Henrique**; software and validation, **Cleverton, and Martha**; investigation, **Cleverton, and Martha**; resources and data curation, **Cleverton, Martha and José Henrique**; writing-original draft preparation, **Cleverton, and Martha**; writing-review and editing, **Cleverton, and Martha**; visualization, **Cleverton, Martha and José Henrique**; supervision, **Martha**. All authors have read and agreed to the published version of the manuscript.

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Not applicable, as this study did not involve humans or animals.

INFORMED CONSENT STATEMENT

Not applicable because this study did not involve humans.

DATA AVAILABILITY STATEMENT

The data can be made available upon request.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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