

IMPROVING ACCELERATED AGING AND ELECTRICAL CONDUCTIVITY TESTS TO ASSESS POPCORN SEED VIGOR

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Abstract

Laboratories constantly evaluate seed lot quality under controlled conditions; however, these tests may overestimate actual field values, making vigor tests indispensable. This study fine-tuned the methods for testing electrical conductivity and accelerated aging to determine popcorn seed vigor. Four lots of hybrid AP8203 popcorn seeds underwent testing for the parameters of germination, first germination count, seedling length, emergence, emergence speed index, water content, electrical conductivity (immersion of 50 or 100 seeds in 50, 75, and 100 ml of distilled water for two, four, five, eight, and 24 hours), and accelerated aging (conventional with water, unsaturated NaCl solution, and saturated NaCl solution). The study had a completely randomized design. The accelerated aging test efficiently aged popcorn seeds using a conventional solution for 48 hours and a saturated saline solution for 72 hours. The electrical conductivity test effectively discriminated seed lots into vigor classes when using 50 popcorn seeds in 50 ml of water at 25°C after six hours of imbibition.

Keywords: Salt solution. Soaking period. Vigor. *Zea mays* L. var. everta.



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1. Introduction

Commercial popcorn (*Zea mays* L. var. *everta*) varieties are highly profitable and economically valuable. The Brazilian availability of hybrids is insufficient to supply the internal demand for seeds, making importation essential (Catão and Caixeta 2017; Catão et al. 2020).

Laboratories constantly evaluate seed lot quality, following the pre-established standards of the rules for seed analysis (RAS) (Brasil 2009). The germination test occurs under controlled conditions, which allow the required physiological and biochemical reactions for seed germination. However, this test may overestimate the actual field values. Hence, vigor tests are indispensable (Rocha et al. 2018; Leite et al. 2019).

Vigor tests combined with germination tests aim to refine the evaluations of seed physiological potential, thus supporting decision-making, increasing efficiency and agility, and improving seed lot quality control (Rodrigues et al. 2022). Among these tests, accelerated aging exposes seeds to high relative humidity and temperatures, influencing the speed and intensity of biological membrane deterioration (Bewley and Black 2014; Marcos-Filho 2015a). Therefore, combinations of temperature, exposure periods, and solution types are essential to improve the response speed and the likelihood of comparability to field results (Marcos-Filho 2015).

Rocha et al. (2018) verified that the accelerated aging test performed at 42°C for 48 hours provided consistent information to discriminate popcorn seed lots. However, this finding raises questions about a potential increment in the coefficient of variation, possible shorter exposure periods for seed lot stratification, and different solutions for the test. Examples of these solutions are saturated and unsaturated NaCl saline solutions, which delay the deterioration rate and equalize water absorption by seeds (Catão et al. 2021).

Electrical conductivity is another vigor test that verifies physiological seed deterioration. It is directly associated with enzymatic and respiratory activities and cell membrane integrity (Souza et al. 2014; Rech Cassol et al. 2016). Seed lots with high electrical conductivity present less vigorous seeds. Therefore, increased results may infer membrane integrity loss, poorly structured membranes, lower repair ability, and other cellular damage (Prado et al. 2019).

The test uses a conductivity meter to measure the exudates leached from the seed to the imbibing solution after submerging the seeds in water for a determined period and temperature (Khan et al. 2020). The method by Alvarenga et al. (2003) is widely used to evaluate popcorn seed vigor.

Depending on seed size, the test may require fine-tuning seed quantity in the imbibing solution (Hampton and Tekrony 1995; Vieira and Marcos-Filho 2020). Another relevant factor is water volume, which must be compatible with seed sizes in the sample to submerge all seeds. The solution should not be too concentrated or diluted (Vieira and Marcos-Filho 2020).

Considering the need to improve vigor tests and aiming to increase the agility and accuracy of seed quality assessments, the present study fine-tuned the methods of accelerated aging and electrical conductivity tests to determine popcorn seed vigor.

2. Material and Methods

The Seed Laboratory of the Universidade Federal de Uberlândia, Umuarama campus, hosted the experiments, which used four seed lots of the AP8203 hybrid from AG Alumni Seeds produced in the 2020 season in Indiana, USA. Initially, the following tests characterized the seed lots:

Water content: Each lot used two replicates of 5.0 g of seeds. The seeds were set in an oven at 105°C for 24 hours following the RAS method (Brasil 2009). The findings were expressed as percentages (%).

Germination: The test comprised four replicates of 50 seeds from each lot, distributed between two germination paper sheets moistened with distilled water equivalent to 2.5 times the substrate's dry weight and later organized in rolls. The rolls were set in a germination chamber at 25°C for a 12-hour photoperiod. Evaluations were performed on the fourth day for the first germination count and on the seventh day for the final germination count, as proposed by the RAS (Brasil 2009). The findings were expressed as regular seedling percentages.

Seedling length: After the germination tests, 20 normal seedlings from each replicate were randomly separated, and shoot and root lengths were measured with a ruler. The findings were expressed as lengths in centimeters.

Emergence: The test performed four replicates of 50 seeds from each lot in plastic trays with sand. The seeds were sowed at 3.0 cm deep, with spacings of 0.5 cm, in 40 cm-long grooves. The substrate was saturated to 60% of its water-holding capacity, and trays were set under greenhouse conditions. The evaluations ended on the tenth day after sowing when the stand was stabilized, and they considered only the emerged seedlings. The findings were expressed in percentages.

Emergence speed index (ESI): Performed with the emergence test, with daily evaluations for ten consecutive days. After data collection, the ESI was calculated as in Maguire (1962):

$$ESI = \frac{G1}{N1} + \frac{G2}{N2} + \dots + \frac{Gn}{Nn}$$

G = The number of normal seedlings computed on each count.

N = The number of days from sowing to counting.

The accelerated aging test relied on a completely randomized design in a 4x3x3 factorial arrangement (seed lots x aging methods x aging periods). Three accelerated aging methods used three aging solutions. The solutions were I - conventional solution with distilled water; II - unsaturated NaCl solution with 11 g of NaCl diluted in 100 ml of distilled water; and III - saturated NaCl solution with 40 g of NaCl diluted in 100 ml of distilled water. Each plastic box (Gerbox) received 40 ml of the solutions, and a metal mesh prevented the direct contact of the solution and seeds set over the metal mesh in a uniform single layer (Krzyzanowski et al., 2020). The boxes were set at 41°C in a BOD incubator for 24, 48, and 72 hours.

Seed water content was verified after the aging periods, as described. Then, germination tests ended as reported, and the fourth-day regular seedlings were counted. The findings for each lot were expressed as percentages (%).

The electrical conductivity test followed the mass method (Vieira and Marcos-Filho 2020). It used a combination of seed quantities (50 or 100), deionized water volumes (50, 75, or 100 ml), and immersion periods (two, four, five, eight, and 24 hours). The seeds undergoing four replicates of each treatment were previously weighed in an analytical balance (0.001g precision), added to plastic cups (200ml capacity) with deionized water, and set on a BOD incubator at 25°C for the designated immersion period. After completing the immersion periods, cups containing the solution and seeds were stirred to homogenize the leached content. Electrical conductivity was measured immediately, aided by a conductivity meter (MCA 150) with constant electrode 1, and the findings were expressed as $\mu\text{S} \cdot \text{cm}^{-1} \cdot \text{g}^{-1}$ of seeds.

The study applied a completely randomized statistical design with a T-test at a 5% probability. The Scott-Knott test compared the qualitative means when detecting significant effects. It applied a 5% probability in SISVAR 5.0 software (Ferreira, 2011). Regression analysis evaluated the electrical conductivity test according to statistical significance (F test) and the coefficient of determination (R^2).

3. Results

The initial characterization showed seed lots with uniform water content, a 1.4% difference between the highest and lowest percentages (Table 1), and uniform germination. Lots 1 and 2 showed higher percentages in the first germination count and higher seedling shoot and root lengths (Table 1). The popcorn seed lots had germination percentages above 94%. Lots 1 and 2 showed higher emergence percentages, and lot 2 presented the highest ESI, followed by lots 3, 1, and 4 (Table 1). Therefore, the seed lots of the present study exhibited high physiological quality.

The accelerated aging test demonstrated significant interactions between seed lots and aging methods and between aging methods and aging periods. Figure 1A evidences the interaction between seed lots and aging methods, in which lots 1 and 2 have higher vigor values regardless of the aging solution.

The accelerated aging method analyses showed that conventional and saturated techniques evidenced the segregation of lots in three vigor classes. Lot 3 yielded lower values with conventional and

saturated methods. The unsaturated solution method only evidenced two vigor classes among seed lots (Figure 1A). However, the unsaturated solution presented the lowest vigor for lots 3 and 4.

Figure 1B shows the interaction of aging methods and aging periods. The 48-hour period presented the highest stratification compared to accelerated aging methods. The conventional method caused seeds to age more in 48 and 72 hours, resulting in lower percentages of regular seedlings.

The 72-hour accelerated aging period using a saturated saline solution statistically differed from all other aging periods, showing the possibility of stratifying popcorn seed vigor. Comparing water content before and after accelerated aging (Figure 1C) evidenced a water content increase but at a lower percentage in seed lots under the saturated NaCl solution.

Table 1. Water content (WC), first germination count (FC), germination (G), shoot length (SL), root length (RL), emergence (E) and emergence speed index (IVE) for the initial characterization of physiological quality in lots of popcorn seeds.

Lot	WC (%)	FC (%)	G (%)	SL (cm)	RL (cm)	E (%)	IVE
1	7.8	89 ^b	98 ^a	5.1 ^a	13.0 ^a	98 ^a	9.4 ^a
2	7.7	94 ^a	97 ^a	5.2 ^a	12.2 ^a	96 ^a	10.3 ^a
3	9.1	82 ^b	94 ^a	4.1 ^a	7.9 ^b	92 ^a	9.8 ^a
4	9.1	86 ^b	97 ^a	3.9 ^a	7.4 ^b	91 ^a	9.3 ^a
CV (%)		4.4	2.9	19.6	30.5	5.8	5.99

*Means followed by the same small letter between seed lots are not statistically different by the Scott-Knott test at 5% probability.

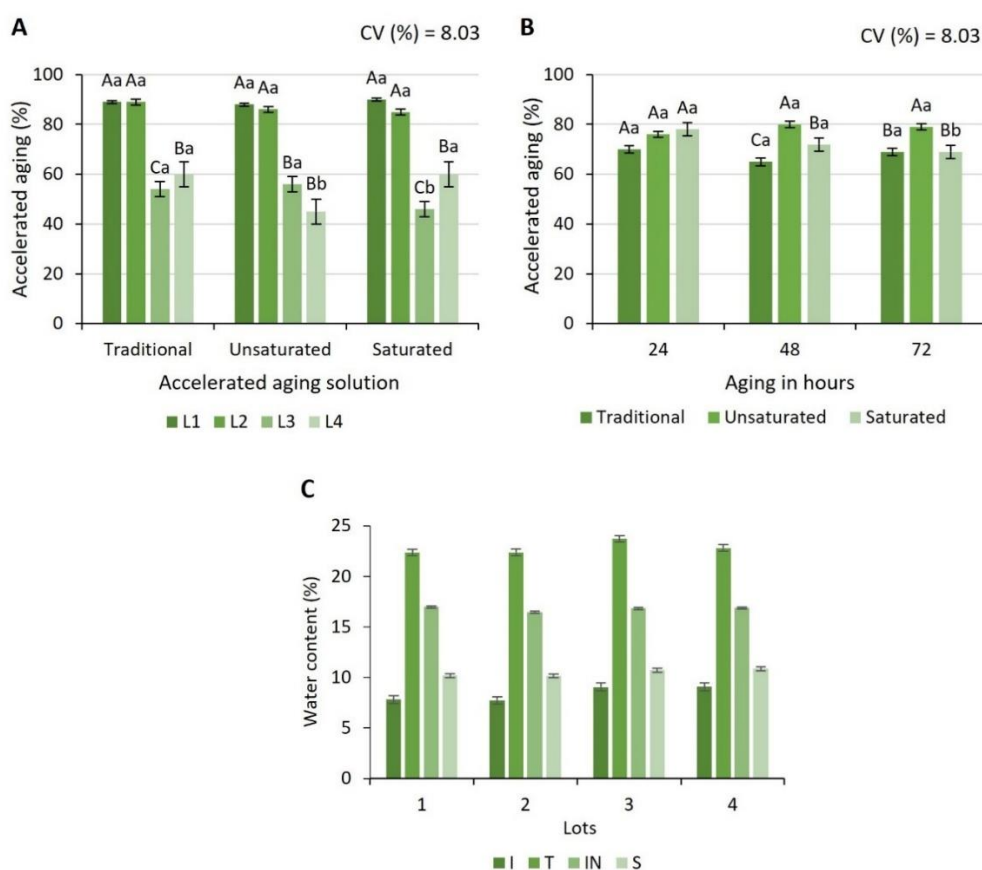


Figure 1. Accelerated aging (%) in popcorn seeds treated with: **A** - different solutions; and **B** - different periods and solutions; **C** - Water content (%) from different popcorn seed lots, prior and after accelerated aging using Traditional solution (T), Unsaturated solution (IN), Saturated solution (S) and Initial water content (I). ***A** - Means followed by the same capital letter between popcorn seed lots and small letter between accelerated aging methods; **B** - same capital letter between accelerated aging methods and small letter between hours of accelerated aging; do not differ by the Scott-Knott test at 5% probability.

Table 2 shows the electrical conductivity data for popcorn seed lots according to the number of seeds, water volume, and immersion periods. Lots 1 and 2 had the lowest electrical conductivity values.

Table 2 and Figure 2A show a higher discrimination between lots when combining 50 seeds in 50 ml of water. These parameters indicated that periods of six, eight, and 24 hours classified lots in three vigor levels. Lots 1 and 2 were the most vigorous, showing higher physiological quality regardless of the immersion period. Such findings are similar to those of the initial characterization (Table 1) in the first germination count, seedling length, and emergence in sand.

Table 2 - Electric conductivity ($\mu\text{S}\cdot\text{cm}^{-1}\cdot\text{g}^{-1}$) from lots of hybrid popcorn seeds under different combinations of 50 and 100 seeds, water volumes and immersion periods.

50 seeds/50 ml water					
Lots	Immersion periods				
	2 h	4 h	6 h	8 h	24 h
1	16.8 ^a	18.8 ^a	20.2 ^a	21.8 ^a	28.0 ^a
2	18.4 ^a	20.4 ^a	21.6 ^a	23.8 ^a	31.4 ^a
3	24.3 ^b	28.0 ^b	30.3 ^b	33.2 ^b	43.3 ^b
4	30.0 ^b	33.7 ^b	36.7 ^c	39.2 ^c	50.0 ^c
CV (%)	26.91	27.47	28.50	27.65	26.86
50 seeds/75 ml water					
Lots	Immersion periods				
	2 h	4 h	6 h	8 h	24 h
1	11.6 ^a	12.9 ^a	13.7 ^a	14.4 ^a	19.5 ^a
2	11.8 ^a	13.3 ^a	14.2 ^a	14.9 ^a	20.2 ^a
3	14.7 ^b	17.2 ^b	18.7 ^b	19.8 ^b	28.8 ^b
4	14.8 ^b	17.3 ^b	18.9 ^b	20.1 ^b	29.6 ^b
CV (%)	13.33	15.83	17.15	17.74	22.08
50 seeds/100 ml water					
lots	Immersion periods				
	2 h	4 h	6 h	8 h	24 h
1	8.8 ^a	10.4 ^a	11.4 ^a	12.0 ^a	16.7 ^a
2	9.6 ^a	10.8 ^a	11.6 ^a	12.2 ^a	16.8 ^a
3	12.3 ^b	14.7 ^b	16.0 ^b	17.1 ^b	23.8 ^b
4	12.9 ^b	14.9 ^b	16.2 ^b	17.4 ^b	23.9 ^b
CV (%)	18.39	19.15	19.26	20.29	20.20
100 seeds/50 ml water					
Lots	Immersion periods				
	2 h	4 h	6 h	8 h	24 h
1	16.5 ^a	18.3 ^a	19.3 ^a	21.0 ^a	26.2 ^a
2	16.7 ^a	18.6 ^a	19.4 ^a	21.0 ^a	26.3 ^a
3	21.9 ^b	24.7 ^b	26.5 ^b	28.6 ^b	38.5 ^b
4	22.1 ^b	25.1 ^b	26.7 ^b	29.0 ^b	39.5 ^b
CV (%)	16.16	17.21	18.22	18.10	22.60
100 seeds/75 ml water					
Lots	Immersion periods				
	2 h	4 h	6 h	8 h	24 h
1	10.7 ^a	12.5 ^a	13.5 ^a	14.1 ^a	20.0 ^a
2	11.1 ^a	12.9 ^a	13.9 ^a	14.6 ^a	20.8 ^a
3	14.0 ^b	16.3 ^b	17.9 ^b	19.0 ^b	27.4 ^b
4	14.0 ^b	16.5 ^b	18.0 ^b	19.1 ^b	27.6 ^b
CV (%)	14.44	14.74	15.54	16.30	17.17
100 seeds/100 ml water					
Lots	Immersion periods				
	2 h	4 h	6 h	8 h	24 h
1	7.5 ^a	9.3 ^a	10.5 ^a	11.2 ^a	14.7 ^a
2	7.9 ^a	9.7 ^a	10.9 ^a	11.7 ^a	15.2 ^a
3	9.5 ^b	13.6 ^b	14.0 ^b	15.0 ^b	20.5 ^b
4	9.7 ^b	12.6 ^b	14.5 ^b	15.3 ^b	21.3 ^b
CV (%)	12.86	18.80	16.56	16.16	19.28

*Means followed by the same small letter between seed lots are not statistically different by the Scott-Knott test at 5% probability.

The polynomial regression (Figure 2) evidenced a direct influence of the imbibition period on electrical conductivity, showing an exponential increment in the results according to the imbibition period extension. Lots 3 and 4 showed higher values, regardless of the relationship between the imbibition period, the number of seeds, and the solution, thus signaling lower vigor. Figure 2 (B, C, D, E, and F) presents an overlap of tendency lines in lots 1, 2, 3, and 4, allowing stratification in only two vigor levels.

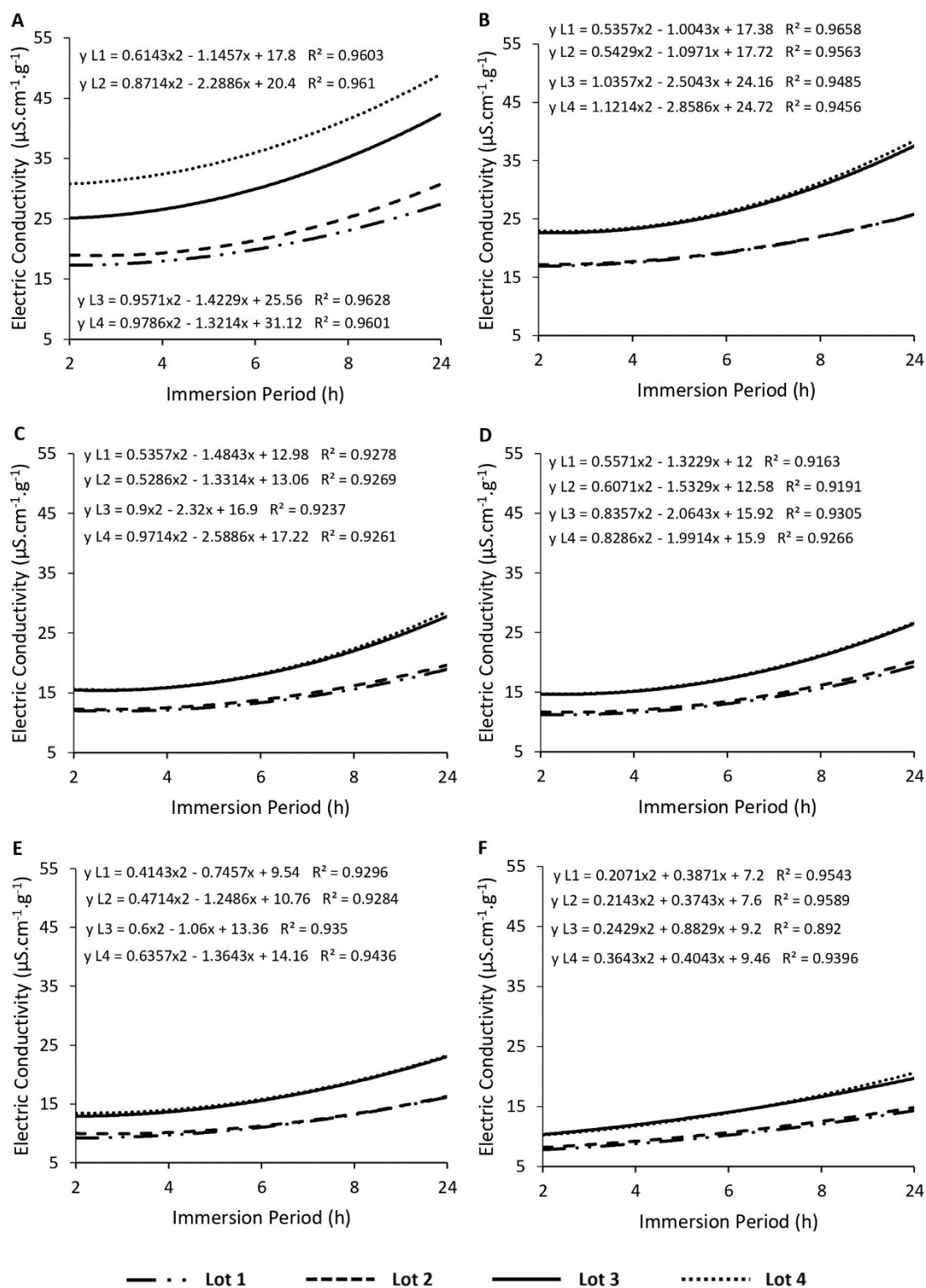


Figure 2. Electric conductivity in different popcorn seed lots after imbibition periods of 2, 4, 6, 8 and 24 h. A – with 50 seeds in 50 mL; B - with 100 seeds in 50 mL; C - with 50 seeds in 75 mL; D – with 100 seeds in 75 mL; E - with 50 seeds in 100 mL; F - with 100 seeds in 100 mL.

Seed vigor classification was poorly efficient when using 50 seeds and 75 and 100 ml volumes. It was impossible to separate seed lots according to different physiological quality levels, probably due to a higher leached content dilution, regardless of the immersion periods, as in the method in Figure 3. Only two vigor levels were distinguished using these water volumes (Table 2 and Figures 2C and 2E). Therefore, these volumes were unfit to evaluate popcorn seed vigor.

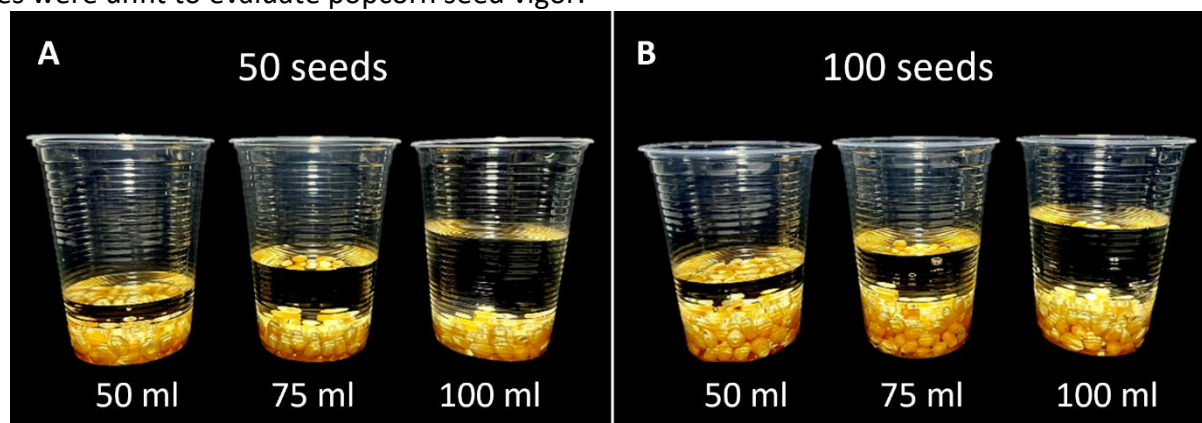


Figure 3. Illustration of all solution volumes tested in popcorn seed lots after 24 hours imbibition. Plastic cups with 200 ml capacity were used for each volume. A - Cups with 50 popcorn seeds. B - Cups with 100 popcorn seeds.

All three tested water volumes caused low electrical conductivity variations when using 100 seeds, hindering seed lot discrimination within the tested periods (Table 2 and Figures 2B, 2D, and 2F). Even the lengthiest period (24 hours) failed to improve seed lot stratification across vigor classes due to the higher number of seeds. Lots 1 and 2 showed higher vigor through the electrical conductivity evaluation using either 50 or 100 seeds.

4. Discussion

Water content uniformity is evidenced when analyzing the initial characterization of seed lots (Table 1). Marcos-Filho (2015) found that water content differences between 1% and 2% are acceptable. The homogeneity observed in this test is relevant when comparing the physiological quality between seed lots to prevent a variation source from influencing the results (Marcos-Filho 2015).

The germination test also showed a homogeneous outcome between lots, as all lots met the minimal germination standard (70%) established by the popcorn seed trading legislation (Brasil 2013). Araújo et al. (2022) and Marques Rodrigues et al. (2019) verified that lots with similar germination are crucial for studies determining seed vigor evaluation methods, as more vigorous seeds present uniform germination with higher rates of seedling structure growth and development (Marcos-Filho 2015).

Regarding the vigor tests and due to the analogous simulation of field conditions with seeds subjected to adverse situations, the emergence and ESI tests were the best to infer seed vigor, revealing differences in seeds' physiological quality undetected in the germination test (Guedes et al. 2011; Grzybowski et al. 2015). Marcos-Filho (2015) found that the ESI is a significant predictor of differences in the physiological potential of seed lots or genotypes, considering the ESI directly relates to seed vigor.

Laboratories certified by the Brazilian Ministry of Agriculture, Livestock, and Food Supply constantly apply accelerated aging and electrical conductivity tests to determine seed vigor. Accelerated aging of popcorn seeds (Figure 1) promoted degenerative modifications in the membrane system, reducing its integrity and selectivity, causing an uncontrolled water and solute exchange between cells and the external medium, and reducing seed viability (Moraes et al. 2016). Consequently, the primary objective of this test was fulfilled by verifying the method's efficiency in causing deterioration.

Munareto et al. (2021) found that a conventional solution with 12 hours of aging at 45°C promoted better stratification of quinoa seed lots. It is worth noting that accelerated aging outcomes may vary according to seed lot or genotype. The aging test using the unsaturated solution provided only two vigor classes between seed lots (Figure 1A).

This study found that conventional and saturated methods effectively segregated popcorn seed lots. Rocha et al. (2018) also verified that accelerated aging with a 48-hour exposure and a conventional solution more efficiently segregated popcorn seed lots. Also, 72-hour periods were efficient. It is worth noting that other authors emphasize the relevance of speed to executing and obtaining consistent results (Wendt et al. 2017).

The adaptation and validation of solutions for accelerated aging tests must be evaluated. A conventional solution (water) may cause high germination discrepancy (Freitas et al. 2018). A saturated NaCl solution is recommended for some species, as it promotes humid atmosphere suitability, the rate of water absorption by the seed, and deterioration intensity, thus providing a lower outcome variation (Lima et al. 2015; Radke et al. 2016).

The saturated saline solution (40 g NaCl/100 ml water) reduces the relative humidity within the Gerbox from 100% to 76%, promoting slow seed imbibition and reducing seed stress. Therefore, a longer time is required to discriminate the lots, as in Figure 1B. The other methods also had the same result regardless of the seed imbibition period. Santos et al. (2021) found that using a saturated NaCl solution in the accelerated aging test of soybean seeds provides more accurate and representative results of actual storage conditions than the conventional method.

The speed of completion is key when selecting between two methods of vigor tests to be performed in a laboratory, as decisions are based on the results. Consequently, the conventional 48-hour method is recommended. Nonetheless, if lot discrimination is not attained, the indication is the saturated saline solution for 72 hours.

Seed water content may initially characterize seed lots, as may the comparative method after accomplishing accelerated aging. Accelerated aging using saturated solutions promotes lower deterioration rates because the seed water content is lower and more consistent. That delays seed imbibition during test execution and provides less drastic outcomes (Oliveira et al., 2020). Therefore, the saturated saline solution is more consistent regarding seed deterioration, which is essential when evaluating seed vigor (Catão et al. 2021).

Higher electrical conductivity values in seeds are associated with lower seed vigor due to slower membrane organization speed during imbibition and, consequently, higher solute leaching, promoting lower storage potential for these seeds (Vieira and Marcos-Filho 2020). The literature considers shorter imbibition periods efficient for evaluating seed vigor. Marques and Dutra (2018) determined that the optimal conditions for the electrical conductivity test on sorghum seeds involve a 16-hour imbibition period at 30°C in 25 mL of water.

It is worth noting that immersion periods of eight and 12 hours are not as functional as 24 hours, which is the best to read electrical conductivity (Araújo et al. 2022). This period is often used to evaluate seed vigor in *Fabaceae*, such as common beans (Silva et al. 2013; Suzana et al. 2017), *Vigna unguiculata* (Moura et al. 2017), and soybeans (Vieira and Marcos-Filho 2020). Torres et al. (2015) affirmed that fast results favor seed producers' interests; therefore, the six-hour immersion period may be considered to evaluate popcorn seed vigor.

Inserting the conductivity measuring electrode in the solution to obtain (read) the data was easy even when our experiment used the lowest water volume (50 ml), regardless of seed quantities (50 or 100 seeds) (Figure 3). The Association of Official Seed Analysts (AOSA) recommends no direct contact between the conductivity meter cell and seeds during the conductivity test (Vieira and Marcos-Filho, 2020; Krzyzanowski et al. 2023).

Additionally, our study highlights that electrical conductivity tests may show inaccurate results for methods using excessive water. Araújo et al. (2022) evidenced this fact while evaluating chickpea seeds, verifying leached electrolyte dilution that hampered seed lot stratification.

5. Conclusions

The accelerated aging test effectively aged popcorn seeds using a conventional solution for 48 hours and a saturated saline solution for 72 hours. The electrical conductivity test efficiently discriminated seed lots in vigor classes, using 50 popcorn seeds in 50 ml of water at 25°C after six hours of imbibition.

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