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Research article

DNA degradation is involved with low physiological potential of soybean seeds

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ABSTRACT: The high quality of soybean seeds is essential for the success of the crop, as it determines the field performance of seedlings. Seed physiological attributes are associated with genotype stability; thus, the maintenance of genome integrity is crucial to ensure seed quality. To evaluate the quality of soybean seed lots at physiological and molecular levels, a completely randomized design was carried out in four replications, with 12 soybean seed lots (cv. BMX Potência RR). Seeds were evaluated through standard germination and vigor tests. Genomic DNA was extracted from the root meristem of seeds from different lots with the Kasvi Spin 50® extraction kit. The analysis was performed through electrophoresis and visualized under ultraviolet (UV) light. The Comet assay was also applied to investigate DNA damage. Soybean seed lots exhibited differences in germination and vigor, as determined by the tests of accelerated aging, field emergence, emergence speed index, shoot length, root length, shoot fresh weight, root fresh mass, and shoot dry mass. In seeds with low germination and vigor, DNA damage and genotoxicity were observed. DNA from samples with high germination and vigor observed by the field emergence and accelerated aging tests was more preserved than DNA extracted from seeds with low physiological quality.
Keywords: DNA integrity, comet assay, genotoxicity, seed vigor

Introduction

Soybean (*Glycine max* L.) is one of the most widely cultivated crops globally and is considered one of the most important crops for the Brazilian agribusiness. Soybean represents the primary source of vegetable protein worldwide (Grassini et al., 2021; Anghinoni et al., 2021).

Given the versatility and economic importance of the soybean crop, the use of high-quality seeds represents one of the most significant technological advances in ensuring uniform productivity and sustainability of soybean crop. Seed quality comprises the physiological attributes of germination and vigor. Seed vigor is a complex trait that involves seed longevity, seedling growth speed, and stress tolerance in the early stages of seedling development (Reed et al., 2022). In this way, the use of vigorous seeds is a strategy for the establishment of plant populations with high agronomic potential, even under adverse conditions (Wijewardana et al., 2019).

Many efforts are directed toward the prevention of seed damage and the enhancement of seed quality. Consequently, the identification and disposal of low-quality lots is of paramount importance to prevent poor field performance (Medeiros et al., 2020). The detection of vigor between seed lots represents a pivotal economic aspect for the soybean seed industry (Powell et al., 2005). However, it is acknowledged that the expression of seed performance is strictly linked to genome stability.

The maintenance of genome integrity is crucial for preventing and/or repairing damage to proteins, cell membranes, and DNA. This is essential for ensuring seed longevity and performance during germination (Waterworth et al., 2015; 2019). Several studies on molecular aspects have involved proteomic analysis and the description of metabolic pathways associated with germination (Rajjou et al., 2012). However, most studies do not use economically important seeds as a study model for investigation, such as soybean.

In soybean seeds, genotoxicity is associated with cellular damage in seeds of different genotypes, demonstrating that intact DNA is crucial for the continuity of cell vital actions that promote embryo growth and normal seedlings emergence (Pereira et al., 2022). RNA fragmentation in dry-stored soybean seeds has been correlated with loss of seed viability (Fleming et al., 2017). It is important to note that DNA electrophoretic profile can help understand the molecular processes underlying differences in physiological quality. In the future, these results may contribute to the development of molecular markers for seed quality.

The objective of this study was to evaluate the physiological potential of soybean seeds (cv. BMX Potência RR) from different lots and to investigate the DNA integrity *in vivo* (Comet assay) and the electrophoretic profile of the genomic DNA of soybean seeds.

Materials and Methods

The experiment was developed in a completely randomized design (CRD) using soybean (*Glycine max* L.) seeds from the cv. BMX Potência RR produced in traditional soybean seed farms, during the 2021/2022 crop season, in Mato Grosso do Sul, Brazil. After

harvesting and during the experimental period, the seeds were kept in a cold and dry chamber (15 °C, 55 % RH = relative humidity).

Assessment of the seed physiological potential

Twelve lots of transgenic soybean seeds were used to elucidate the molecular processes occurring between seeds with different physiological characteristics. The seed lots had an average water content of 13 % (wet basis), as determined through an oven drying process (105 ± 3 °C for 24 h) with two replications of 5.0 g of seeds, in accordance with the Rules for Seed Analysis (RAS) (MAPA, 2009). The following seeds characteristics were evaluated, with each characteristic represented by four repetitions. Each repetition was represented by 50 seeds for each soybean seed lot.

Standard germination test

A standard laboratory germination test was conducted with seeds distributed in rolls of germination paper, moistened with water in an amount equivalent to 2.5 times the weight of the dry substrate (MAPA, 2009). Seeds were sown in quadruplicate and were kept in a germination chamber (Biochemical Oxygen Demand, Tecnal) at 25 °C and constant white light. The results were expressed as the mean percentage of normal and abnormal seedlings, according to young plant morphoanatomical characters in terms of survival in optimum field conditions and dead seeds.

Accelerated aging

Seeds were hydrated when exposed to a relatively high temperature (41 °C, 48 h) and humidity (approximately 100 % RH). Seed samples were distributed in single layers on the surface of a wire mesh screen suspended over 40 mL of water inside a plastic box (110 × 110 × 35 mm). Following this aging treatment, the seeds were subjected to a germination test, as previously described. The results were expressed as a percentage of normal seedlings (ISTA, 2024).

Electrical conductivity

For each seed lot, four replications of 50 seeds were individually weighed to an accuracy of 0.01 g and soaked in 75 mL deionized water at 25 °C for 24 h. Electrical conductivity was then measured on a conductivity meter (Tec-4MP, Tecnal) and expressed in $\mu S\ cm^{-1}\ g^{-1}$ (ISTA, 2024).

Field emergence and speed field emergence

Seeds were planted in furrows measuring 2.00 m in length, with a spacing of 0.50 cm and a depth of approximately 0.03 cm in Dystroferric Red Latosol, with a flat soil surface in each replicate to ensure consistent moisture content following rainfall or irrigation. Emergence counts were initiated when the first seedling was visible. Seedling emergence assessments were conducted daily at the same time, with the results expressed as a percentage.

To evaluate the emergence speed of seedlings under field conditions, the formula and criteria described in Maguire (1962) were applied concurrently.

Seedling length and mass

Four replications of 20 seeds were sown over blotting paper moistened with distilled water and were kept in a germination chamber (Biochemical Oxygen Demand, Tecnal) at 25 °C and constant white light. After five days, the shoot and root lengths from the normal seedlings were measured with a millimeter ruler. The seedlings shoot and root lengths were expressed in cm $seedling^{-1}$.

The shoot and roots of the normal seedlings from the previous evaluation were sectioned and the fresh mass was determined in an analytical balance with a precision of 0.001 g. The results were expressed in g $seedling^{-1}$.

The seedlings were divided into individual parts and placed in paper packages. These packages were then dried in an oven with forced air circulation for a period of 24 h at a temperature of 80 °C. The samples were weighed on an analytical balance with a precision of 0.001 g and the results of seedling dry mass were expressed in g $seedling^{-1}$.

DNA extraction and electrophoresis

Following the completion of the physiological quality evaluations, three soybean seed lots were selected for the subsequent electrophoretic DNA profile assessment, in accordance with the germination and vigor criteria described in Table 1. Initially, the seeds of samples A, B, and C were separately placed over blotting paper moistened with distilled water and were kept in a germination chamber at 25 °C for 24 h under constant white light.

Subsequently, the roots were sectioned with a scalpel and macerated in a mortar. They were then placed in tubes with a capacity of 5 mL. Genomic DNA extraction was performed with the Kasvi Spin 50® (Nucleic Acid Extraction Kit) according to the

Table 1 – Germination and vigor criteria of soybean seeds lots for molecular analysis.

Seed Lot	Seed Germination	FE	AA
	---------- % ----------		
A	100	100	> 90
B	≥ 85 < 95	≥ 80 < 90	> 80
C	≤ 80	≤ 80	≤ 80

FE = field emergence; AA = accelerated aging.

manufacturer's methodology. Subsequently, a 5 µL aliquot of DNA from A, B, and C samples was diluted in 5 µL of MiliQ water. In order to assess the DNA integrity, the samples were subjected to agarose gel electrophoresis (1 %) in Tris-HCl/EDTA buffer (TRIS hydrochloride/ethylenediaminetetraacetic acid, 20 %) containing ethidium bromide (0.5 %). The samples were then compared with a standard 100-bp DNA ladder (Promega®) using 2 µL of DNA marker of bromophenol blue Promega®.

The electrophoretic run was conducted over a period of 90 min at an applied voltage of 110 V. The resulting agarose gel was observed under UV light and the image was captured using a L-Pix Loccus Molecular Imaging® photo documenter.

Comet assay

The Comet assay was carried out in accordance with the methodology proposed by Pereira et al. (2022). In brief, 25 radicles/roots were collected from each treatment and immersed in 500 µL of phosphate buffered saline (PBS) solution. Subsequently, the material was centrifuged at 10,950 x g for 10 s, after which 200 µL of the supernatant was discarded. Prior to the Comet assay, super frosted microscope slides were first coated with a 1.5% agarose solution in PBS (i.e., 10 mM, pH 7.4) and placed to dry overnight at room temperature.

Following the preparation of the slides in duplicate for each treatment, each slide contained 60 µL of cell suspension and 240 µL of 0.5 % low-melting point agarose at 37 °C. From this point onward, all subsequent steps were conducted in the dark with inactinic light to prevent additional DNA damage. The slides were incubated in lysing solution for 4 h (i.e., 2.5 M NaCl, 100 mM Na_2EDTA, 10 mM Tris, 1 % Triton X-100, and 10 % dimethyl sulfoxide (DMSO) added immediately before use, pH 10) at a temperature of 4 °C in the dark. Following cell lysis, the slides were gently placed in a horizontal electrophoresis chamber filled with a freshly prepared alkaline solution (i.e., 300 mM NaOH, 1 mM Na2EDTA, pH > 13) for 20 min to denature the DNA.

Subsequently, the samples were electrophoresed at 37 V, 300 mA for 25 min. This time proved to be sufficient to discriminate different levels of DNA damage and avoid overlapping comets with our cell density. Lysis, DNA unwinding, and electrophoresis were performed at 4 °C. Following electrophoresis, the slides were washed in a neutralization buffer (0.4 mol L^{-1} Tris) and fixed with 95 % ethanol. They were then stored at 4 °C until the counts were performed. The slides were stained with 50 µL of ethidium bromide (0.08 mg mL^{-1}) and the nucleoids were observed under a fluorescence microscope (LABMED, Lx 400) on the 40× objective.

Slide scoring was conducted via visual assessment in accordance with the method outlined by Collins et al. (1997). A total of 100 nucleoids per slide were counted and classified into five categories of Comets based on the degree of DNA damage, ranging from 0 (i.e., no tail) to 4 (i.e., almost all DNA in the tail, indicating highly damaged DNA). The DNA damage levels were expressed in arbitrary units (AU). The arbitrary unit, which ranged from 0 to 400, was calculated using the following formula: $AU = [(0 \times NO) + (1 \times N1) + (2 \times N2) + (3 \times N3) + (4 \times N4)] / \Sigma \text{ Comet} \times 100$

Statistical test

The experimental design was based on a completely randomized design. For each soybean seed lot, physiological quality evaluations were carried out with four replications of 50 seeds each. The normality of the data was verified by the Shapiro-Wilk test. The ANOVA parametric test (represented by critical difference at the 0.05 probability level) was applied for both tests of the physiological quality of the seeds (germination and vigor tests) and for the Comet assay evaluations. The ANOVA analyses were also conducted to ascertain whether there were significant variations between seed lots and cell damage.

The data were subjected to the analysis of variance by the F test ($p < 0.01$). For the seed physiological evaluations, the means were grouped by the Scott Knott test ($p \leq 0.05$) through Sisvar® software. For the Comet assay, the means were compared by the Tukey test ($p \leq 0.05$). The analyses were performed using the R platform (R Development Core Team, 2020).

Results

Physiological attributes of seed quality

Soybean seed lots showed significant differences ($p \leq 0.05$) in their responses to the standard germination (SG), accelerated aging (AA), field emergence (FE), and emergence speed index (ESI) tests, while the electrical conductivity (EC) did not reveal significant differences in vigor (Table 2). As accelerated aging and field emergence accurately detected seed vigor differences between soybean seed lots, this parameter, even as germination, was selected for further investigations.

The standard germination method proved to be the most effective in assessing the physiological potential of the seed lots (Table 2). The seed lots with the highest germination rates were 1, 2, 5, 7, 8, 9, and 10, with an average of germination rate of 98 %. The next highest germination rates were observed in the seed lots 3 and 6, with an average germination rate of 93 % (Table 2).

Seed lot 4 exhibited a germination rate of 85 %. In contrast, seed lots 11 and 12 displayed the lowest germination rates compared to the other soybean seed lots. In accordance with the AA test, the germination of soybean seed lots 7, 8, 9, and 10 exhibited the highest vigor levels, followed by seed lots 1, 3, 4, 5, and 9 (Table 2). Seed lots 2 and 6 showed similar vigor levels, which were notably lower than those of the other lots. Based

on the germination test, seed lots 1, 2, and 5 did not differ from each other and were contemplated to have high germination rates (Table 2). However, according to the AA tests, these seed lots were believed to have low vigor levels compared to seed lots 7 to 10 (Table 2). In contrast, seed lots 11 and 12 were pondered to have the lowest vigor levels in relation to the other seed lots, corroborating the results obtained from the standard germination test.

The statistical analysis showed no significant difference in field emergence between seed lots 1 to 10, with an average of field emergence of 100 %. High field emergence is an important quality prerequisite for the desired number of plants in the field, which is necessary to achieve high crop production. In contrast, seed lots 11 and 12 exhibited poor field performance, in accordance with the AA test results (Table 2).

For the assessment of the ESI, seed lots 1, 3, 5, 7, and 8 did not exhibit significant differences and were considered more vigorous (on average, 11) in comparison to the others. However, seed lots 2, 4, 6, 9, and 10 demonstrated intermediate vigor (on average, 10), while seed lot 12 exhibited the lowest ESI (on average, 7). These results are consistent with the previously conducted vigor tests (Table 2).

The biometric data (seedling length, and seedling fresh and dry weight) are shown in Table 3. The data indicate that there were significant differences ($p \leq 0.05$) among the soybean seed lots in terms of shoot length (SL), shoot fresh mass (SFM), root fresh mass (RFM), and root dry mass (RDM). However, the root length (RL) and shoot dry mass (SDM) analyses did not yield significant results in detecting vigor differences between the seed lots (Table 3).

The seed lots 1, 3, 4, 5, 6, 7, 8, 9, and 10 showed the highest results for SL, with an average of 4.5 cm, without significant differences (Table 3). The seed lots 2 and 12 presented intermediate results, with an average of 3 cm, and seed lot 11 exhibited the lowest SL values (Table 3).

Similar results were observed for RL without significant differences between the seed lots 1, 3, 4, 5, 6, 7, 8, 9, and 10, with an average of 10.47 cm. These seed lots exhibited the highest RL results. In contrast, seed lots 2, 11, and 12 showed lower results (on average, 6.87 cm) compared to the other seed lots (Table 3).

The SFM indicates that the seed lots 6, 7, 9. and 10 exhibited the highest results, without significant differences, with an average of 0.24 g. Seed lots 1, 3, 4, 5, and 8 presented intermediate results, with an average of 0.21 g, while the seed lots 2, 11, and 12 showed the lowest performance in relation to SL, as these parameters are interrelated (Table 3). The low performance of the seed lots 2, 11, and 12 was also observed in RFM (on average, 0.03 g). However, seed lot 4 exhibited the highest RFM, differing from the other seed lots that did not present significant differences in RFM (on average, 0.17 g) (Table 3).

For SDM, soybean seed lots were significantly differentiated into only two groups (Table 3). The seed lots 1, 3, 6, 9, and 11 showed higher results with an average of 0.13 g, in comparison to the other seed lots (on average, 0.09 g; Table 3). In contrast, for RDM, there was no significant difference among the seed lots, which averaged 0.03 g (Table 3).

DNA electrophoretic profile and comet assay

The physiological attributes were examined by standard germination tests and a wide range of vigor tests. The objective was to elucidate the effects and the

Table 2 – Standard germination (SG), accelerated aging (AA), field emergence (FE), emergence speed index (ESI), and electrical conductivity (EC) of soybean seed lots cv. BMX Potência RR, 2021/2022 crop season.

Seed lots	SG	AA	FE	ESI	EC
	%				$\mu S\ cm^{-1}\ s^{-1}$
1	97[a]	89[b]	100[a]	11[a]	35[a]
2	97[a]	69[c]	100[a]	10[b]	36[a]
3	93[b]	88[b]	100[a]	11[a]	34[a]
4	85[c]	83[b]	100[a]	10[b]	31[a]
5	96[a]	83[b]	100[a]	11[a]	35[a]
6	92[b]	69[c]	100[a]	10[b]	46[a]
7	100[a]	98[a]	100[a]	11[a]	37[a]
8	98[a]	92[a]	100[a]	11[a]	21[a]
9	98[a]	99[a]	100[a]	10[b]	33[a]
10	100[a]	97[a]	100[a]	10[b]	43[a]
11	53[e]	0[e]	24[c]	2[d]	26[a]
12	70[d]	37[d]	79[b]	7[c]	37[a]

Different letters in the columns are significantly different ($p < 0.05$) using a Tukey test.

Table 3 – Seedling performance and biometric from twelve soybean seed lots cv. BMX Potência RR, crop 2021/22.

Seed lots	SL	RL	SFM	RFM	SDM	RDM
	cm		g			
1	4.47[a]	11.31[a]	0.22[b]	0.07[b]	0.13[a]	0.06[a]
2	3.22[b]	7.20[b]	0.18[c]	0.04[c]	0.09[b]	0.02[a]
3	4.93[a]	11.76[a]	0.23[b]	0.06[b]	0.12[a]	0.03[a]
4	4.20[a]	9.00[a]	0.21[b]	0.17[a]	0.10[b]	0.01[a]
5	4.90[a]	10.30[a]	0.22[b]	0.05[b]	0.08[b]	0.02[a]
6	4.50[a]	11.30[a]	0.24[a]	0.06[b]	0.14[a]	0.03[a]
7	4.56[a]	10.46[a]	0.24[a]	0.08[b]	0.09[b]	0.03[a]
8	4.96[a]	10.80[a]	0.21[b]	0.07[b]	0.11[b]	0.02[a]
9	4.04[a]	9.21[a]	0.25[a]	0.07[b]	0.13[a]	0.12[a]
10	4.19[a]	10.17[a]	0.26[a]	0.08[b]	0.11[b]	0.05[a]
11	2.36[c]	7.19[b]	0.12[d]	0.01[c]	0.14[a]	0.01[a]
12	3.46[b]	6.21[b]	0.14[d]	0.02[c]	0.08[b]	0.01[a]

Different letters in the columns are significantly different ($p < 0.05$) using a Tukey test. SL = shoot length; RL = root length; SFM = shoot fresh mass; RFM = root fresh mass; SDM = shoot dry mass; RDM = root dry mass.

underlying mechanism of the DNA status examined by gel electrophoresis. In addition, the genotoxic aspects of germination and vigor loss were assessed by performing an alkaline comet assay. The results are shown in Figure 1.

A comparison of genomic DNA extracted from seeds exhibiting high physiological performance and DNA extracted from seeds exhibiting low vigor revealed notable differences. The DNA isolated from the seeds of sample A demonstrated high stability, as evidenced by the absence of degradation in the electrophoretic profile (Figure 1A).

In general, an aliquot of preserved DNA was observed in samples B and C. However, it was also observed that the isolated DNA presented some degradation. It should be noted that the genomic DNA was extracted from a sample of seeds. Furthermore, none of the evaluations (germination and vigor) demonstrated the complete loss of physiological attributes of the studied seed lots (Tables 2 and 3).

The total DNA strand breaks were quantified in radicles isolated from seeds lots consisting of a pool of seeds lots with high or low germination and vigor. The results are shown in Figure 1B. The Comet assay demonstrated significant differences ($p \leq 0.05$) in DNA damage, as evidenced by the DNA electrophoretic profile (Figure 1A). The seed lots with low physiological potential exhibited a higher number of arbitrary units (AU) in damaged cells than the seed lots with high physiological potential (Figure 1B).

Due to the associations between the prevalence of abnormal seedlings and the presence of dead seeds and the loss of DNA integrity, the results of incomplete germination were also included in Figure 1. Seed lots with poor performance under both controlled and uncontrolled conditions exhibited a higher number of abnormal seedlings and dead seeds than seed lots demonstrating high germination and vigor (Figure 1C).

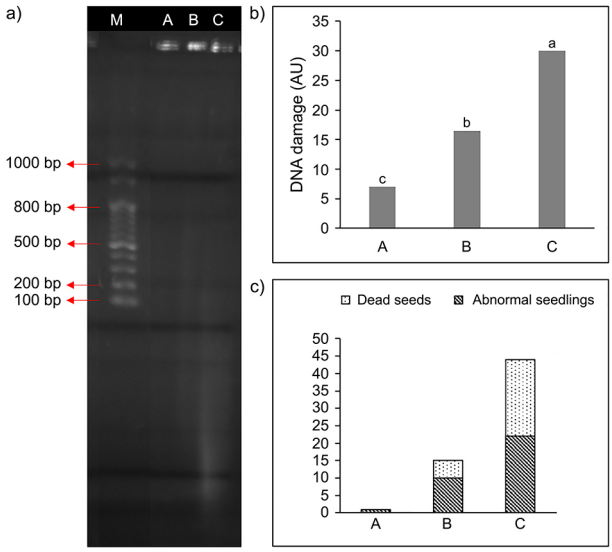

Figure 1 – a) Gel electrophoresis of DNA fragments, column 'M' represents a 100 bp molecular mass marker; b) DNA damage in arbitrary units; c) Abnormal seedlings and dead seeds in the standard germination test. Inside the figure, A, B, and C represents: A = soybean seeds with 100 % germination and field emergence (FE) and accelerated aging (AA) > 90 %; B = soybean seeds with ≥ 85 % < 95 % germination, ≥ 85 % < 95 % FE and > 80 % AA; C = soybean seeds with ≤ 80 % germination, ≤ 80 % FE and ≤ 80 % AA. Common letter did not differ ($p \geq 0.05$), as analyzed by the Tukey test.

Discussion

The quality of soybean seeds is of great relevance to the global soybean crops, as high-quality seeds can withstand extreme conditions after planting, such as high or low temperatures, and of display rapid and uniform emergence (Finch-Savage and Bassel, 2016). Notably, the predominant categorization method to predict the performance of seed lots under abiotic factors is conditioned by high temperature and high relative humidity.

The current results corroborate previous observations. The AA test proved to be more sensitive in detecting initial signs of deterioration between seed lots with similar germination (Table 2). These results agree with the assumption that seed vigor decreases before the loss of germination capacity during seed deterioration (Ellis, 2019). Moreover, soybean seed lots with high germination in the laboratory exhibited low vigor, as evidenced by low seedling emergence in uncontrolled conditions, such as the field. The ability to emerge early in the field is one of the primary attributes of vigor and is a more sensitive indicator of physiological performance than standard germination (Marcos Filho, 2015). The results demonstrate that seed lots with low germination also exhibit a low capacity to convert embryonic reserves to seedling formation, as observed by seedling biometry data. For soybean seeds, Hartmann Filho et al. (2016) highlight that the mechanisms directly connected with the processes of translocation and transformation of cotyledonary reserves into substances that the embryonic axis can assimilate might be affected during seed deterioration, restricting the accumulation of dry matter coming from the cotyledons. Subsequently, Forti et al. (2018) conducted a flow cytometry analysis of soybean seed lots, revealing that the quantity of DNA present in the cotyledons is higher in seeds with high vigor and lower in seeds with low vigor. These findings indicate that the cotyledons of vigorous seeds are more effective at synthesizing compounds essential for germination and early seedling growth than those of less vigorous seeds.

Seed deterioration may occur through different mechanisms, with an accumulation of reactive oxygen species and lipid peroxidation generally considered to be major contributors (Ballesteros and Walters,

2019). Furthermore, it is associated with controlled biochemical activities, such as programmed cell death, as evidenced by DNA fragmentation in aged sunflower seeds (El-Maarouf-Bouteau et al., 2011), loss of RNA integrity in aged pea seeds (Kranner et al., 2011) and in dry-stored soybean seeds (Fleming et al., 2017). These studies have emphasized the crucial role of maintaining genome integrity in seed performance.

Nevertheless, predicting seed vigor remains a significant challenge for many seed industries. Evidence suggests that even seed lots with high germination and vigor (as determined by the AA and FE tests) presented low-speed emergence and/or did not differ from low-quality seed lots regarding cell membrane integrity (Table 2). While better performance would have been expected if the intra-specific variation between the seed lots was not undeniably significant, the observed results indicate that this is not the case. For instance, Colville and Pritchard (2019) reported that the observed intra-specific variation in seed life span may be attributed to genetic variation between populations, which in turn leads to variability in seed lot performance.

It is well known that mature dry seeds gradually accumulate cellular damage during aging. Once seeds are imbibed, the cytoplasm of seed cells undergo transformation from a glassy to a fluid state, accompanied by the activation of metabolism (Sano et al., 2016). In this condition, seeds can repair the damage if the protective cellular components are present and active. This allows the seed to re-establish the cell machinery to withstand the resumption of seedling growth (Pagano et al., 2017; Ballesteros and Walters, 2019). This is a global aspect of the high quality of seeds.

The current study provided information on the DNA status extracted from seeds with high or low germination and vigor, determined through the AA and FE tests. The results demonstrated DNA damage in soybean seeds with satisfactory germination but with the beginning of vigor loss, and more pronounced in seed lots with low germination and vigor (Figure 1A and B). In addition, DNA disassembly was related to the loss of physiological potential of seed lots evaluated through several vigor tests.

The DNA degradation pattern observed in this study was similar to those previously extracted from soybean seeds submitted to fast imbibition. This pattern was related to the chromosomal aberrations, a reduction in the mitotic index, and was associated with the high results of abnormal seedlings (Pereira and Masetto, 2021; Pereira et al., 2022). The employment of chemical modifications to DNA introduces an additional layer of complexity to cellular processes, as these modifications enable the DNA to function within a regulatory network that modulates chromatin structure and genome function (Plitta-Michalak et al., 2022).

In seeds that were imbibed early, damage to the embryo genome must be repaired prior to the initiation of cell division to minimize the inhibition of growth and the mutation of genetic information (Kurek et al., 2019; Pereira et al., 2022). Information concerning DNA repair in plants remains limited, although gene profiling and mutant analysis results suggest possible differences in repair mechanisms between plants and other eukaryotes (Balestrazzi et al., 2011).

The alterations produced on the embryo, particularly on the meristematic cells from which the plant will develop, may have been masked in the analysis of a pool of seeds. DNA extracts from pooled seeds are a mix of physiological stages (dead and alive seeds) and a pool of tissues, which may have different susceptibilities to aging (Mira et al., 2020).

Furthermore, considering the seed alive does not imply that it will become a complete seedling capable of establishing itself under a wide range of environmental or stressful conditions. Therefore, these DNA integrity profiles represent samples of seeds with similar germination characteristics but different vigor criteria. The study on seed vigor and DNA alterations raised intriguing issues that deserve future in-depth investigation. These include the potential relationship between DNA damage and molecular processes and their contribution to seed vigor. The results of this study indicate that maintaining DNA integrity is related to the ability to develop essential seedling structures, even after seed exposure to factors such as high temperature and relative humidity.

This hypothesis posits that the DNA degradation observed in the agarose gel indicates non-programmed events of cell death or necrosis (Pereira and Masetto, 2021). Moreover, the generated fragments by the comet assay confirm the occurrence of DNA damage in seed lots with low physiological potential, thereby corroborating the DNA electrophoretic profile. However, even in a representative sample, seeds may exhibit varying molecular statuses despite showing similar physiological manifestations (Mira et al., 2020).

The seeds eventually lose quality and die, and the decrease of seed quality, regardless of the condition before or after harvest, from seed vigor to DNA stability, represents a significant impediment to proper plant establishment and efficient use of field resources.

It is well-documented that DNA damage accumulates in seeds even in the absence of external stresses due to the inherent instability of DNA in the cellular environment (Schumacher et al., 2021). In the present study, the seed lots belong to the same genotype, originated from the same crop season, and have not been previously stored. Thus, the observed differences in germination and vigor can be attributed to genuine factors between seed lots.

Our findings reinforce the hypothesis that soybean seeds high vigor is achieved through specific mechanisms pertaining to the maintenance of DNA integrity. We

propose that DNA disassembly contributes to low soybean seed physiological performance. Consequently, germination assays may underestimate the number of viable seeds. Therefore, we suggest that additional tests, such as molecular markers for the prediction of seed vigor, should be employed in the evaluation of soybean seed lots. The findings of this study provide new insights into the germination and vigor loss processes, which are influenced by the degradation of DNA. This research contributes to a better understanding of the DNA integrity of soybean seeds. In conclusion, soybean seeds with low physiological quality are more susceptible to high temperatures and relative humidity. Additionally, seeds with low physiological quality present slow seedling emergence under field conditions and low seedling growth. The genomic DNA of seeds with high germination and vigor demonstrated high stability, verified by the absence of degradation in the electrophoretic profile. Conversely, soybean seeds with low germination and vigor exhibited severe loss of DNA integrity. The low physiological quality of soybean seeds is associated with genotoxicity, which is defined as damage to the DNA structure.

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Authors' Contributions

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