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Assessment of the tolerance of soybean seeds to weathering deterioration in the pre-harvest phase by multivariate analysis

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ABSTRACT: Weathering deterioration in the pre-harvest phase impairs high-quality soybean seed production. The performance of several tests to infer seed quality is common in genotype selection, and multivariate statistics can assist in the interpretations. This study aimed to assess the efficiency of the principal component analysis (PCA) and canonical discriminant analysis (CDA) multivariate statistical methods in assessing the tolerance of seeds of different soybean cultivars to weathering deterioration in the pre-harvest phase under greenhouse conditions. Seeds of six soybean cultivars (DM 6563, BMX Apolo, BMX Potência, NA 5909, NS 5959, and TMG 1175) were produced. Different simulated precipitation levels (0, 60, 120, and 180 mm) were applied in the pre-harvest phase. The seeds were collected and assessed for physiological, physical, and biochemical analyses and the data were analyzed by PCA and CDA techniques. The results showed that PCA and CDA are efficient for assessing the tolerance to weathering deterioration in soybean seeds. PCA and CDA assisted in the recommendation of the tests first germination count, accelerated aging, tetrazolium, percentage of seeds with seed coat wrinkling, protein content, and protease activity in the pre-selection of genotypes for weathering deterioration. PCA and CDA also helped to identify the cultivars DM 6563 and BMX Potência as more susceptible and NA 5909 and TMG 1175 as more tolerant to weathering deterioration in the pre-harvest phase.

Index terms: *Glycine max* L., seed quality, statistical analysis.

RESUMO: A deterioração por umidade na fase de pré-colheita prejudica a obtenção de sementes de soja de alta qualidade. Em seleção de genótipos, é comum a realização de vários testes para inferir sobre qualidade de sementes, sendo que a estatística multivariada pode auxiliar nas interpretações. O objetivo do trabalho foi avaliar a eficiência dos métodos de estatística multivariada de componentes principais (PCA) e discriminantes canônicos (CDA) na avaliação da tolerância de sementes de diferentes cultivares de soja à deterioração por umidade na fase de pré-colheita, em condições de casa de vegetação. Foram produzidas sementes de seis cultivares de soja (DM 6563, BMX Apolo, BMX Potência, NA 5909, NS 5959 e TMG 1175). Na fase de pré-colheita, foram aplicados diferentes níveis de precipitação simulada (0, 60, 120 e 180 mm). As sementes foram colhidas e avaliadas quanto a análises fisiológicas, físicas e bioquímicas e os dados obtidos foram analisados pelas técnicas PCA e CDA. Os resultados mostraram que a PCA e a CDA são eficientes para avaliação da tolerância à deterioração por umidade em sementes de soja. Na pré-seleção de genótipos para essa característica, a PCA e a CDA auxiliaram na recomendação dos testes de primeira contagem de germinação, envelhecimento acelerado, tetrazólio, porcentagem de sementes com enrugamento no tegumento, teor de proteínas e atividade da protease. A PCA e a CDA também auxiliaram na identificação das cultivares DM 6563 e BMX Potência como mais suscetíveis e as cultivares NA 5909 e TMG 1175 como mais tolerantes à deterioração por umidade na fase de pré-colheita.

Termos para indexação: *Glycine max* L., qualidade de sementes, análise estatística.

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INTRODUCTION

Brazil has recently become the world's largest soybean producer, with a total production of 141.3 million tons (CONAB, 2021). Seeds are constantly exposed to unfavorable situations in the field, such as high humidity and high temperatures. It reinforces the importance of using high-quality seeds regarding physical, physiological, genetic, and sanitary aspects, which, together with proper crop management, would ensure the establishment of uniform and productive crops (Krzyzanowski et al., 2018; Otranto, 2020).

Weathering deterioration in the pre-harvest phase has been one of the main factors affecting high-quality soybean seed production, especially in tropical regions with high precipitation levels (França-Neto et al., 2016). Successive cycles of water absorption and loss after physiological maturity cause weathering deterioration and negatively impact soybean seed quality. Forti et al. (2013) and Pinheiro et al. (2021) observed that weathering deterioration disrupted seed coat cells, exposing the internal seed tissues and reducing physiological quality. Castro et al. (2016), Huth et al. (2016), and Brzezinski et al. (2022) observed a direct association between lignin levels in the seed coat and the effects of weathering deterioration in different soybean cultivars. The deterioration effects in soybean seeds are also directly related to oxidative stress and seed protein metabolism (Fialho et al., 2022).

The performance of several tests to infer seed quality is common in genotype selection regarding tolerance to biotic or abiotic factors. It implies the need to analyze multiple variables to understand patterns and trends that will help in decision-making. In this sense, the study of multivariate statistical analysis methods that treat the variables together to extract valuable and accurate information about the obtained results has become important. Johnson and Wichern (1992) mentioned that multivariate analysis methods aim at reducing the volume of data, creating groups of similar variables, as well as investigating the interdependence and relationship between variables.

Principal components analysis (PCA) and canonical discriminant analysis (CDA) stand out among the most used techniques in multivariate analysis. PCA aims to find linear combinations between a set of response variables to produce latent variables that are uncorrelated to each other, thereby reducing the dimensionality of the data and facilitating the interpretation of results (Jolliffe, 2022). Similar to PCA, CDA seeks to find linear combinations of a large number of response variables to obtain groups of smaller dimensions (canonical variables) (Cruz-Castillo et al., 1994). However, unlike PCA, CDA is used in analyses carried out from experiments with randomized designs with replications, as usually used in seed research.

PCA has been widely used in seed research work due to its high efficiency in identifying relationships between variables (Medeiros et al., 2018). This analysis proved to be effective in studies on the adequacy of methodology for analyzing the viability of *Jatropha curcas* seeds by the tetrazolium test (Araújo et al., 2019), discrimination of sunflower seed lots with different deterioration levels (Morais et al., 2021), assessment of the physiological quality of wheat seeds produced under different irrigation depths and leaf silicon levels (Gama et al., 2021), and assessment of the effect of osmotic conditioning in sunflower seeds (Barros et al., 2021), among others. In addition, it has been adopted as a strategy in the pre-processing of data used in machine learning (Fan et al., 2020; Medeiros et al., 2020; Shi et al., 2020) and exploratory data analysis (Ribeiro et al., 2021; Wang et al., 2021).

On the other hand, CDA has been less explored in seed analysis compared to PCA. Some studies have confirmed the importance of this analysis in assessing the seed quality of different *Brassica* genotypes (Niemann et al., 2018) and obtaining patterns for the differentiation of *Jatropha curcas* seed lots (Bianchini et al., 2021).

In this context, this study aimed to assess the tolerance of seeds of different soybean cultivars to weathering deterioration in the pre-harvest phase using multivariate statistical methods.

MATERIAL AND METHODS

This study was carried out at the Agronomy Department of the *Universidade Federal de Viçosa*, Minas Gerais, Brazil. Six soybean cultivars with indeterminate growth and different maturation groups (MG) were used, as follows: DM 6563 (MG = 6.3), BMX Apolo (MG = 5.5), BMX Potência (MG = 6.7), NA 5909 (MG = 6.2), NS 5959 (MG = 5.9), and TMG 1175 (MG = 7.5).

The seeds of the different cultivars were produced in a greenhouse. A completely randomized design in a 6×4 factorial scheme with six cultivars and four simulated precipitation levels was used. Sowing was performed in 3.5 dm^{-3} pots, spaced 0.5 m apart, and filled with sandy clay soil (0.398, 0.091, 0.087, and 0.424 $kg.kg^{-1}$ of coarse sand, fine sand, silt, and clay, respectively). Sowing fertilization was carried out after soil chemical analysis (200, 350, 200, 40, 0.81, 1.33, 1.55, 3.66, 0.15, and 4.0 $mg.dm^{-3}$ of N, P, K, S, B, Cu, Fe, Mn, Mo, and Zn, respectively), whereas topdressing fertilization was conducted at 45 days after sowing (240 and 100 $kg.ha^{-1}$ of N and K, respectively).

Initially, six seeds were sown per pot, which remained on benches. Only the two most vigorous seedlings were maintained when they reached the V1 stage. The light was uniformly focused on the plants of the different cultivars, as they were randomly distributed on the benches. Irrigation was performed daily until the R8 stage, aiming to maintain soil moisture close to field capacity. All other cultural practices were carried out according to soybean cultivation recommendations (Borém et al., 2022).

An Agrojet NA1 (40-micron droplets, flow of 7.14 $L.h^{-1}$, working pressure of 10-50 mWC, and hourly precipitation of 3.5 mm) micro-sprinkler system was installed on two benches for the application of the simulated precipitations. The spacing between the micro-sprinklers was 0.5 m, totaling 12 per bench (total sprinkler area of 36 m^2). The water used in the application of precipitation was kept in a reservoir and the system (pipe + micro-sprinklers) was fed using a SOMAR SHP-35 pump, with 0.5 hp, maximum pressure of 35 mWC, a maximum flow rate of 2.1 $m^3.h^{-1}$, and rotation of 3,400 rpm.

A preliminary test was carried out with the system running for 30 minutes, with water collection in recipients randomly distributed on the benches. These data allowed calculating the Christiansen's uniformity coefficient (CUC) of 90.1%. The time of 15 minutes of operation of the system for applying 20 mm of precipitation was determined from the collected volumes.

Three precipitation levels applied to the plants were defined to moisten the seeds and induce weathering deterioration, being divided into two applications with intervals of 72 hours between them: 60 mm (30 + 30), 120 mm (60 + 60), and 180 mm (90 + 90). The benches where the precipitations were applied were physically distant from where the other plants were and, in addition, they were isolated by a plastic curtain. All treatments (precipitation levels) were applied when the plants reached the R8 stage (95% dry pods). The control treatment (0 mm) consisted of plants not subjected to precipitation simulation.

Harvest was carried out 72 h after the application of the precipitation levels for all treatments, except the control treatment (0 mm), which was harvested when the seeds reached approximately 15% moisture at the R8 stage. The pods of all plants (two per pot) were harvested manually and separated from the seeds. During this period, the average minimum and maximum temperatures in the greenhouse were 22.4 and 41.2 °C, respectively. The minimum and maximum relative humidities were 28.6 and 87%, respectively. Subsequently, the seeds were placed in the shade in a laboratory environment until reaching hygroscopic equilibrium (around 12% moisture). Subsequently, the seeds were submitted to physiological, physical, and biochemical analyses, as described below:

Physiological analyses

Germination: carried out with four replications of 50 seeds in paper towel rolls moistened 2.5 times the weight of the dry paper and kept in a germinator at 25 °C. The average percentage of normal seedlings was assessed on the eighth day after the beginning of the test (Brasil, 2009).

Electrical conductivity: assessed using four replications of 50 seeds, which were weighed and placed in plastic cups with 75 mL of deionized water for 24 hours at 25 °C (Vieira and Marcos-Filho, 2020). Then, the conductivity was read in a conductivity meter. The results were expressed in $\mu S.cm^{-1}.g^{-1}$ of seed.

Accelerated aging: conducted using four replications of 50 seeds placed in a single layer on a screen coupled in gerbox boxes with 40 mL of water and kept in a BOD at 41 °C for 48 hours. After this period, the seeds were placed to germinate at 25 °C, as described for the first germination count test, and the assessment was performed on the fifth day (Marcos-Filho, 2020).

Tetrazolium test: conducted using four replications of 50 seeds pre-conditioned on paper towels moistened with distilled water for 16 hours in a germinator at 25 °C. Then, the seeds were placed in plastic cups and submerged in 50 mL of 0.075% tetrazolium solution (2, 3, 5-triphenyltetrazolium chloride) and placed in an incubator at 40 °C for 3.5 h in the absence of light. The seeds were washed with running water after the staining process and individually classified as to their viability, according to the criteria proposed by França-Neto and Krzyzanowski (2020).

Seedling emergence: conducted using four replications of 25 seeds sown in trays filled with soil and sand at a 3:1 ratio in a greenhouse. Irrigations were performed whenever necessary to keep the soil close to field capacity. Daily counts of normal seedlings emerged until stabilization (approximately 14 days) were performed, and the percentage of emergence (Krzyzanowski et al., 2020) and the emergence speed index (ESI) (Maguire, 1962) were calculated.

Seedling development: four replications of 20 seeds were distributed in a row arranged in the upper third of two sheets of paper for germination and covered with a third sheet. The paper was previously moistened with an amount of water equivalent to 2.5 its dry weight. The rolls were kept in a germinator at 25 °C for three days when the images of the seedlings were acquired by means of a 200 dpi resolution scanner (HP, Scanjet 200). The images were inserted into the ImageJ® software and the hypocotyl and the root part of each seedling were individually marked after scale adjustments. Root lengths (RL) and shoot lengths (SL) were generated for each replicate from these data, with the results expressed in $cm.seedling^{-1}$ (Krzyzanowski et al., 2020). In addition, the corrected vigor index (CVI) was calculated with the results expressed in dimensionless values, ranging from 1 to 1000; the higher the value, the better the performance for this trait (Medeiros and Pereira, 2018).

Physical analyses (X-ray)

Five replications of 20 seeds were neatly fixed on adhesive plastic. Then, radiographic images were generated using a Faxitron MX-20 (Faxitron X-ray Corp. Wheeling, IL, U.S.A), submitting the seeds to radiation for 5 seconds, 23 kV, and a focal length of 41.6 cm. The generated digital images were saved in a computer and analyzed in a semi-automated way by the ImageJ® software to obtain the following variables:

Weathering damage: seeds with or without seed coat wrinkling in the region opposite the hilum were quantified in percentage.

Integrated density: sum of pixel values in the image or selection, which is equivalent to the product of the seed area and the average gray value of the selection pixels. The results were expressed in $gray.pixel^{-1}.mm^{-2}$, and the higher the pixel values in the image, the higher the seed density.

Biochemical analyses

Antioxidant enzymes: the seeds were imbibed in water for 24 hours at 25 °C. Subsequently, the embryos (cotyledons + embryonic axis) were frozen in liquid nitrogen. The activity of superoxide dismutase (SOD) (Beauchamp and Fridovich, 1971; Giannopolitis and Ries, 1977), catalase (CAT) (Anderson et al., 1995; Havir and McHale, 1987), ascorbate peroxidase (APX) (Nakano and Asada, 1981), and peroxidase enzymes (POX) (Chance and Maehly, 1955; Kar and Mishra, 1976) were determined by obtaining crude extracts through the maceration of 0.2 g of the embryos in liquid nitrogen, followed by the addition of 2 mL of extraction medium, potassium phosphate buffer (0.1 M, pH 6.8), containing ethylenediaminetetraacetic acid (EDTA) (0.1 mM), phenylmethylsulfonyl fluoride (PMSF) (1.0 mM), and 1% (w/v) polyvinylpolypyrrolidone (PVPP) (Peixoto et al., 1999). The homogenate was centrifuged at 19,000 *g* for 15 min at 4 °C.

Total proteins: the same extract used in the enzymatic assessments was used, with bovine serum albumin (BSA) as a standard (Bradford, 1976).

Protease: 0.2 g of embryos (cotyledons and embryonic axis) were macerated in 2 mL of Tris-HCl buffer (pH 6.8) followed by centrifugation (14,000 rpm) for 15 minutes. Subsequently, the extract was added to 400 µL of BSA (2%) and 1500 µL of Tris-HCl buffer (pH 6.8). The test tubes were vortexed and incubated in a water bath for 20 min at 37 °C. The reaction was then stopped by adding 1 mL of 10% trichloroacetic acid (TCA) and another centrifugation was performed. The supernatant was collected, and the absorbance was performed in a spectrophotometer at 280 nm (adapted from Pilon et al. (2006)). Protease activity was calculated based on a calibration curve and the results were expressed as U of protease per mL, with U corresponding to the amount of enzyme required to release 1 µmol of tyrosine per minute.

Hydrogen peroxide (H_2O_2): 0.2 g of embryos (cotyledons and embryonic axis) were ground in liquid N and homogenized in 2.0 mL of 50 mM potassium phosphate buffer, pH 6.5, containing 1 mM hydroxylamine, followed by centrifugation at 14,000 *g* for 15 min at 4 °C and supernatant collection (Kuo and Kao, 2003). Aliquots of the supernatant were added to 250 µM ferrous ammonium sulfate, 25 mM sulfuric acid, 250 µM xylenol orange, and 100 mM sorbitol, in a final volume of 2 mL (Gay and Gebicki, 2000), homogenized, and kept in the dark for 30 min. Absorbance determination at 560 nm and H_2O_2 quantification were performed based on a calibration curve.

Lipid peroxidation: determined through the assessment of malonaldehyde (MDA) content. 0.2 g of embryos (cotyledons and embryonic axis) macerated in trichloroacetic acid (0.1% TCA, w/v) were used. After centrifugation (19,000 *g*, 15 min, 4 °C), 500 µL of the supernatant was collected and added to 1.5 mL of thiobarbituric acid solution (0.5% TBA in 20% TCA). Instead of the sample, 500 µL of 0.1% TCA was added to the blank. The samples and the blank were incubated for 30 min at 90 °C in a water bath with stirring. The reaction was stopped on ice after 30 minutes and another centrifugation was performed (19,000 *g*, 15 min, 4 °C). Readings were taken in a spectrophotometer at 532 nm. The MDA concentration was calculated using the molar extinction coefficient of 155 $mM^{-1}.cm^{-1}$. The results were expressed in nmol g^{-1} of fresh matter (Cakmak and Horst, 1991).

Statistical analysis

The data were subjected to multivariate analyses after confirming the normal distribution of errors by the Shapiro-Wilk test. An n x p matrix was obtained to perform the principal component analysis (PCA), where n corresponds to the number of treatments (n = 24) and p is the number of analyzed variables (p = 20). The eigenvalues and eigenvectors were calculated from the covariance matrices and plotted on two-dimensional graphs (category sorting diagram and correlation circle), generated using the Factoextra package. The canonical discriminant analysis (CDA) was performed similarly to the PCA, that is, for the data set of the n x p matrix. However, in this analysis, the eigenvalues and eigenvectors were obtained from the matrix of treatments and residuals $W^{-1}B$ of MANOVA (multivariate analysis variance), where B and W are the matrix of treatments and residuals, respectively. The R software (R Core Team, 2020) was used in all statistical analyses.

RESULTS AND DISCUSSION

The principal component analysis (PCA) showed that components 1 (PC1) and 2 (PC2) explained 43.2 and 16.3% of the total variability of the data, respectively (Figure 1A and B). The variability or dispersion can be described mainly through the range, variance, and standard deviation of the obtained data. In general, variability refers to the distribution of a set of data, and the higher the value, the less homogeneous and consistent these data are. In this context, as they explain about 60% of this total data variability by the two principal components, PCA can be considered efficient (Jolliffe, 2022).

According to Medeiros et al. (2018), PCA can be used in the interpretation of variables related to seed quality. In this sense, the sorting diagram (Figure 1A) shows that all cultivars had a trend to group the treatments control (0 mm) and the lowest precipitation level (60 mm) in the positive scores of PC1, where the physiological variables such as germination

(G), first germination count (FGC), accelerated aging (AA), tetrazolium (TZ), emergence (EMERG), emergence speed index (ESI), shoot length (SL), root length (RL), and corrected vigor index (CVI), as well as the biochemical variables superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), peroxidase (POX), and proteins (PROT), were also concentrated in the correlation circle (Figure 2B). In contrast, the clustering of the treatments 120 and 180 mm in the negative scores of PC1 (Figure 2A) corresponded to the location of the variables electrical conductivity (EC), integrated density (IntDens), weathering damage (WD), hydrogen peroxide (PEROXIDE), protease (PROASE), and malonaldehyde (MDA) in the correlation circle (Figure 1B).

Overall, the clusters of the data obtained with the seeds of the cultivars NA 5909, NS 5959, and TMG 1175 were more centralized in the PC1 axis, in addition to being close to the vectors related to physiological quality and antioxidant enzyme activity, as well as far from the vectors EC, WD, PEROXIDE, PROASE, and MDA, when considering the distribution of cultivars in the sorting diagram (Figure 2A). On the other hand, the cultivars BMX Apolo, DM 6563, and BMX Potência had a more dispersed clustering in the correlation diagram, in addition to being closer to the vectors EC, WD, PEROXIDE, PROASE, and MDA and far from the variables of physiological quality and antioxidant enzyme activity (Figure 1A). In general, these observations indicate a higher susceptibility, especially of the cultivars DM 6563 and BMX Potência, to weathering deterioration in the pre-harvest phase.

The analysis of the loadings of PC1 shows that the variables G, FGC, AA, TZ, EMERG, WD, CAT, PROT, and PROASE contributed more than 70% to the total variability of the data (Figure 1C). Therefore, PCA shows an indication that these variables contribute more than the others in the responses of soybean seeds under weathering deterioration conditions.

In general, the results obtained by canonical discriminant analysis (CDA) (Figure 2) were similar to those observed by PCA (Figure 1). Canonical variables 1 (Can 1) and 2 (Can 2) explained 32.8 and 28.8% of the total variability of the data, respectively. The vectors of the physiological variables G, FGC, AA, TZ, EMERG, ESI, SL, RL, and CVI, as well as the biochemical variables SOD, CAT, APX, POX, and PROT, were concentrated in the negative scores of Can 1. Overall, a higher concentration of these variables was observed close to the treatments control (■0 mm) and the lowest level of applied precipitation (●60 mm). On the other hand, the vectors EC, WD, IntDens, PEROXIDE, MDA, and PROASE were concentrated in the positive scores of Can 1, as opposed to the variables mentioned above, which are related to higher seed quality. A higher concentration of these variables was also observed close to the highest levels of applied precipitation (▲120 mm and +180 mm) (Figure 2A).

Similar to PCA, clusters of seed data of the cultivars TMG 1175 and NA 5909 were found closer to vectors related to physiological quality, protein content, and antioxidant enzyme activity. In contrast, the data obtained from the cultivars DM 6563 and BMX Potência were concentrated close to variables whose results are inversely proportional to seed quality, such as EC, WD, PEROXIDE, PROASE, and MDA. The cultivars BMX Apolo and NS 5959 had, in general, an intermediate behavior compared to the others. Ebone et al. (2019) suggested that the deterioration process of orthodox seeds occurs in three phases: slight reduction in vigor caused by the reactions of reducing sugars with antioxidant enzymes and genetic material; cellular oxidative damage, causing lipid peroxidation and solute leaching; and cell collapse with the deconstruction of the mitochondrial membrane and high accumulation of ROS, MDA, and reducing sugars. Therefore, our results show an advanced process of seed deterioration of the different cultivars, especially when subjected to the highest levels of precipitation (120 and 180 mm).

Importantly, the physiological variables FGC, AA, and TZ, the physical variable WD, and the biochemical variables PEROXIDE, MDA, PROT, and PROASE contributed the most to the total variability of the data, with vector loads higher than 70% when considering the main canonical variable (Can 1) (Figure 2B). Therefore, the physiological variables FGC, AA, and TZ, the physical variable WD, and the biochemical variables PROT and PROASE contributed the most to explaining the total variability of the data when considering both PCA (Figure 1C) and CDA (Figure 2B). The first germination count test is based on the principle that more vigorous lots will have a higher percentage of seedlings on the date of the first germination test count, indicating faster germination (Krzyzanowski et al., 2020). Thus, the proximity of the FGC vector in both PCA (Figure 1) and CDA (Figure 2) reinforces faster germination of seeds produced under 0 and 60 mm of precipitation, mainly for seeds of the cultivars NA 5909 and TMG 1175. The high contribution of this variable in the total variability of the data (Figures 1C and 2B) indicates that the first germination count, performed at five days, can be used in pre-selections of genotypes resistant to weathering deterioration in internal seed quality control programs, as it provides faster information than the germination test, which is performed with eight days for soybean (Brasil, 2009).

The accelerated aging test is considered one of the most important for assessing soybean seed vigor, consisting of evaluating the performance of seeds subjected to high temperature and humidity (Marcos-Filho, 2020). In this context and similarly to what was observed for FGC, PCA (Figure 1) and CDA (Figure 2) analyses reinforce the lower vigor of seeds produced under the highest precipitation levels (120 and 180 mm), mainly of the cultivars DM 6563 and BMX Potência.

The tetrazolium test is one of the most outstanding tests for the assessment of the physiological quality of soybean seeds, providing the achievement of viability and vigor indices, in addition to diagnosing problems in seed quality (França-Neto and Krzyzanowski, 2019). Similar to the FGC and AA tests, weathering deterioration induced by the application of the highest precipitation levels (120 and 180 mm) caused significant reductions in the viability of soybean seeds. It is evidenced by the position of the TZ vector both in PCA (Figure 1) and in CDA (Figure 2), close to the other physiological variables and far from the treatments 120 and 180 mm of precipitation. Similarly, Pinheiro et al. (2021) observed the efficiency of the tetrazolium test to detect weathering damage in the pre-harvest phase of soybean seeds.

Interestingly, among the vigor tests that most contributed to the total variability of the data in PCA (Figure 1) and CDA (Figure 2) multivariate analyses, there is a test related to seedling performance (FGC), a test related to stress (AA), and a biochemical test (TZ). As already mentioned, the lower the variability of the data in an experiment, the better. Therefore, obtaining significant contributions by multivariate analyses in vigor tests with different principles helps to further support the results found in this study.

The negative correlation of the occurrence of weathering damage (WD) with the physiological seed quality and antioxidant mechanisms was evident by both PCA (Figure 1) and CDA (Figure 2) due to the position of its vector. The correlation was positive with an increase in hydrogen peroxide, lipid peroxidation, and proteolytic activity in the seeds, indicating that weathering damage contributes to increase oxidative stress in cells. The seed coat wrinkling of soybean seeds exposed to moisture cycles is due to the rupture of layers of parenchyma cells, which exposes the embryo's tissues to the environment. Thus, the seed deterioration process is accelerated, with a reduction in germination and vigor and the inactivation of antioxidant mechanisms in the seeds (Forti et al., 2013; Pinheiro et al., 2021; Fialho et al., 2022). Therefore, the results indicate that the previous assessment of the percentage of seeds with weathering damage, evidenced by the wrinkling opposite the hilum, becomes a simple, fast, and efficient alternative in the definition of the tolerance of soybean seeds of different cultivars to weathering deterioration in the pre-harvest phase.

Vector positions in both PCA (Figure 1) and CDA (Figure 2) showed the direct relationship of biochemical assessments related to protein metabolism of seeds with weathering deterioration. In this context, protein content (PROT) and protease activity (PROASE) were negatively correlated with each other, indicating higher protein breakdown by protease, mainly at higher precipitation levels (120 and 180 mm). The seed deterioration process is known to affect the protein structure of membranes and important pathways, impairing the energy supply to the embryo and the germination process (Lv et al., 2018). It is mainly related to the reactive oxygen species (ROS) excess produced in the deterioration process. It is also related to increased protease activity, as already reported in soybean seeds (Min et al., 2017).

In summary, the results obtained in this study highlight some more accurate tests for use in soybean breeding programs, aiming at the selection of genotypes tolerant to weathering deterioration in the pre-harvest phase. In addition, the tests can be a valuable tool for internal seed quality control, with the pre-selection of tolerant materials in a faster and more objective way. In terms of seed quality control, the tests can help speed up internal decision-making on lot management, among others.

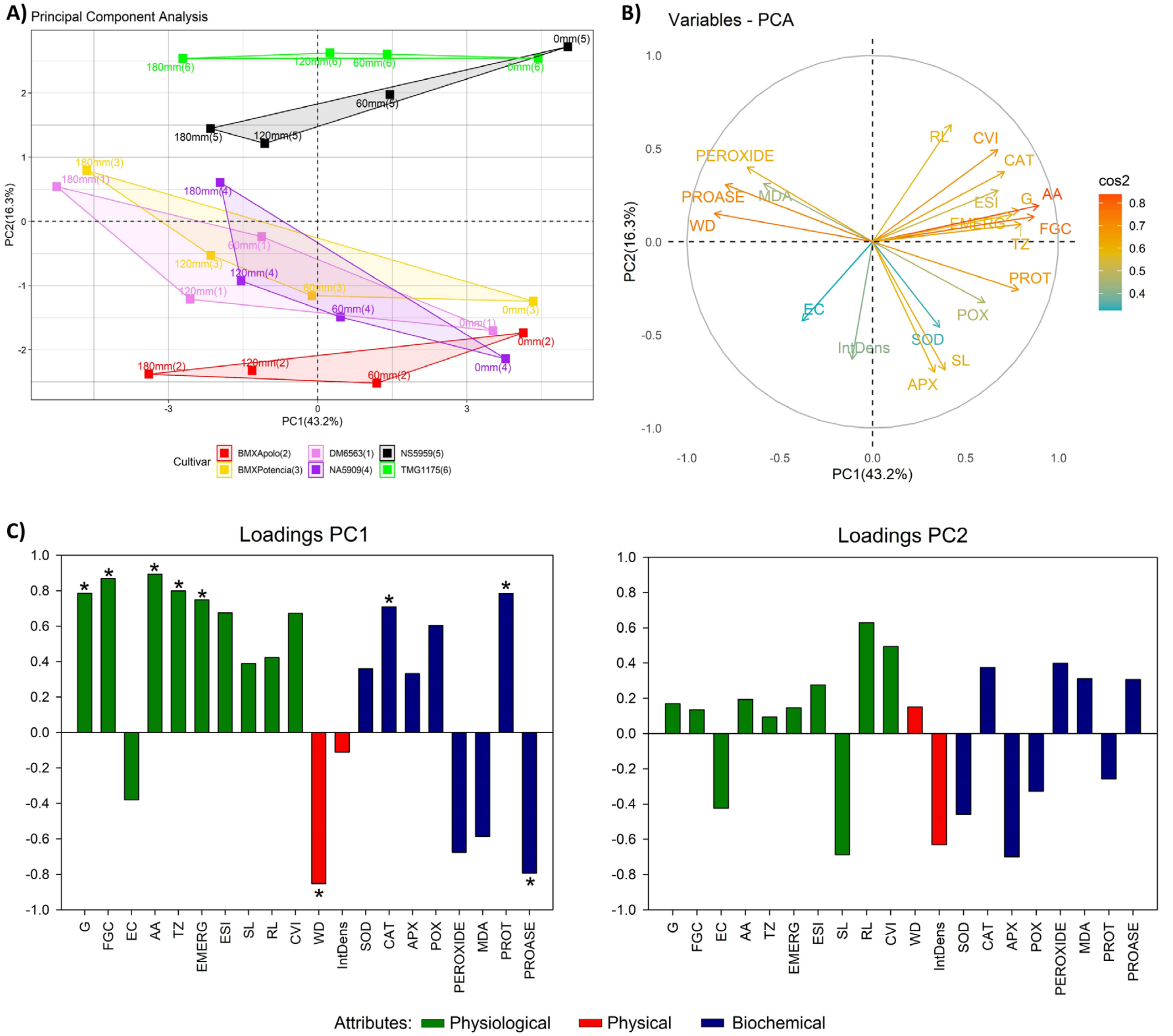

Figure 1. Principal component analysis (PCA). Sorting diagram (A); Correlation circle (B); Vector loads of principal components 1 and 2 (C). Principal component 1 (PC1); Principal component 2 (PC2); Germination (G); First germination count (FGC); Electrical conductivity (EC); Accelerated aging (AA); Viability by tetrazolium (TZ); Emergence (EMERG); Emergence speed index (ESI); Shoot length (SL); Root length (RL); Corrected vigor index (CVI); Weathering damage (WD); Integrated Density (IntDens); Superoxide dismutase (SOD); Catalase (CAT); Ascorbate peroxidase (APX); Peroxidase (POX); Hydrogen peroxide (PEROXIDE); Malonaldehyde (MDA); Proteins (PROT); Protease (PROASE).

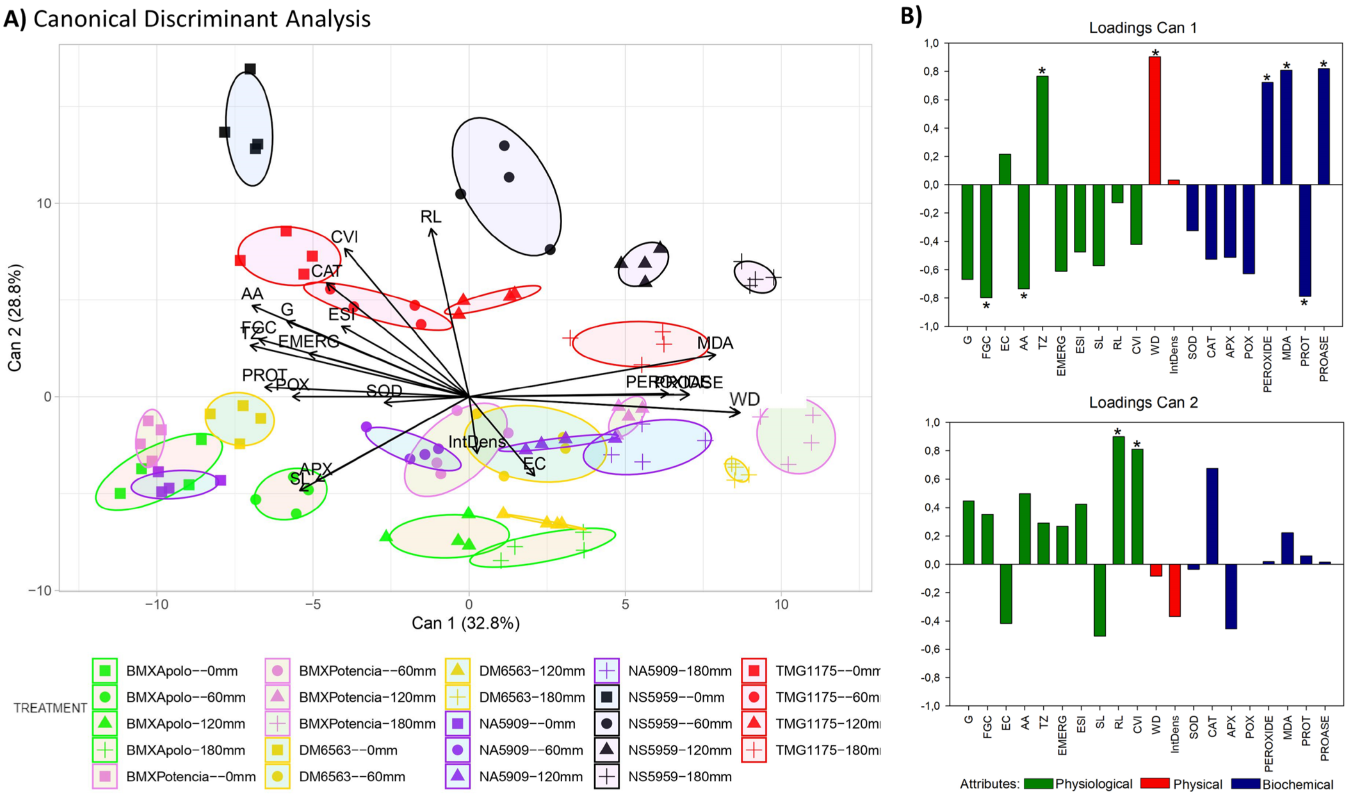

Figure 2. Canonical discriminant analysis (CDA). Biplot (A); Vector loads of canonical variables (B). Canonical variable 1 (Can1); Canonical variable 2 (Can2); Germination (G); First germination count (FGC); Electrical conductivity (EC); Accelerated aging (AA); Viability by tetrazolium (TZ); Emergence (EMERG); Emergence speed index (ESI); Shoot length (SL); Root length (RL); Corrected vigor index (CVI); Weathering damage (WD); Integrated density (IntDens); Superoxide dismutase (SOD); Catalase (CAT); Ascorbate peroxidase (APX); Peroxidase (POX); Hydrogen peroxide (PEROXIDE); Malonaldehyde (MDA); Proteins (PROT); Protease (PROASE).

CONCLUSIONS

The PCA and CDA multivariate statistical analysis methods are efficient to assess the tolerance to weathering deterioration in soybean seeds in the pre-harvest phase.

The tests accelerated aging, tetrazolium, first germination count, percentage of seeds with weathering damage (coat seed wrinkling), protein content, and protease activity were efficient in terms of optimizing the selection of soybean genotypes resistant to weathering deterioration in the pre-harvest phase.

The PCA and CDA multivariate analyses helped to identify the soybean cultivars DM 6563 and BMX Potência as being more susceptible and the cultivars NA 5909 and TMG 1175 as more tolerant to weathering deterioration in the pre-harvest phase.

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