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Artificial aging for predicting the storability of soybean seeds via GGE biplot

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ABSTRACT: Creation, adjustments and adoption of tests and tools that help in the prediction of seed storability have been highly demanded. Therefore, this work aimed to analyze the efficiency of different artificial aging times in predicting the performance of soybean seeds after storage, using the GGE biplot method. Seeds of six genotypes were subjected to storage, under refrigerated and non-refrigerated conditions, and artificial aging, being artificially aged for periods of 0, 48, 96 and 144 hours. Seeds freshly harvested and after natural and artificial aging were subjected to germination and vigor tests. The experiments were analyzed separately, using means test, regression analysis and model identity test, and together, using the GGE biplot method. Artificial aging at a temperature of 41 °C for 96 hours has the potential to be used to predict the performance of soybean seeds after eight months of storage. The GGE biplot is a method that can be used as a tool to analyze the relationships between aging environments and visualize the ranking of genotypes regarding the performance of seeds subjected to natural and artificial aging.

Index terms: *Glycine max* L. Merrill, longevity, physiological quality of seeds, seed deterioration, seed vigor.

RESUMO: Tem sido altamente requerida a elaboração, ajustes e adoção de testes e ferramentas que auxiliem na predição da armazenabilidade de sementes. Diante disso, este trabalho teve por objetivo analisar a eficiência de diferentes tempos de envelhecimento artificial na predição do desempenho das sementes de soja após o armazenamento, utilizando-se o método GGE biplot. Sementes de seis genótipos foram submetidas ao armazenamento, sob condição refrigerada e não refrigerada, e envelhecimento artificial, sendo envelhecidas artificialmente pelos períodos de 0, 48, 96 e 144 horas. As sementes recém-colhidas e após o envelhecimento natural e artificial foram submetidas a testes de germinação e vigor. Os experimentos foram analisados separadamente, por meio de teste de médias, análise de regressão e teste de identidade de modelos, e em conjunto, utilizando-se o método GGE biplot. O envelhecimento artificial, à temperatura de 41 °C por 96 horas apresenta potencial para ser utilizado na predição do desempenho de sementes de soja após oito meses de armazenamento. O GGE biplot é um método que pode ser utilizado como ferramenta para analisar as relações entre os ambientes de envelhecimento e visualizar o ranqueamento dos genótipos quanto ao desempenho das sementes submetidas ao envelhecimento natural e artificial.

Termos para indexação: *Glycine max* L. Merrill, longevidade, qualidade fisiológica de sementes, deterioração de sementes, vigor de sementes.

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INTRODUCTION

Physiological quality of seeds is one of the factors responsible for the success of soybean plantations. However, this quality is gradually lost after physiological maturity, either in the field or during storage (Shelar et al., 2008). Under these conditions, seeds are prone to deterioration, with reduced vigor and, ultimately, loss of viability. As a consequence, after sowing, there are failures in the stand and emergence, as well as uneven development of plants, which compromises yield (Ebone et al., 2020).

Deterioration is a progressive and irreversible process (Bewley et al., 2013). However, the environment to which the seeds are exposed exerts a lot of influence on the intensity and speed of this process (Singh et al., 2017). In addition, there are differences between species, cultivars, and lots regarding tolerance to deterioration (Tripathi and Khare, 2016; Sano et al., 2016; Naik et al., 2019), which result in rapid or slow reduction in seed vigor during storage.

The rapid decline in seed vigor during storage implies losses for growers and companies. Thus, identifying seed lots with high and low tolerance to deterioration in storage emerges as a necessity, and it is essential to develop and adopt rapid and assertive tests that can predict the storage potential in newly harvested seeds.

Artificial aging techniques have been reported in studies on the mechanisms linked to natural seed aging (Delouche and Baskin, 1973; Balešević-Tubić et al., 2010). Artificial aging causes stress to seeds due to the exposure to high temperatures and relative humidity, which leads to rapid deterioration (Delouche and Baskin, 1973). The temperature, humidity, exposure time, and efficacy of the artificial aging method as a predictor of seed storability depend on several factors, including the species and cultivar (Fantazzini et al., 2018).

The accelerated aging test indicated for soybean seeds, using a temperature of 41 °C for 48 hours (Mcdonald and Phaneendranath, 1978), is routinely used by companies to evaluate seed vigor. The performance of the seeds after this test is highly correlated with their storage potential (Delouche and Baskin, 1973; Tian et al., 2008; Balešević-Tubić et al. 2010), which can indicate which lots have the highest and lowest tolerance to deterioration. However, adjustments of the binomial time and temperature may be necessary, especially for seeds of transgenic cultivars recently released on the market.

New techniques have been used for data analysis in which the effect of genotype and environment can be investigated. Among these techniques, the GGE biplot method stands out. This method was developed to graphically represent results of principal component analysis or decomposition of singular values, in which the relationship between genotypes and environments can be visualized by the product of vectors and the cosine of the angle between two vectors (Yan and Kang, 2003). The GGE biplot allows the evaluation of the performance of cultivars in different environments, analyzing the additive effect of genotype (G) to the multiplicative effect of genotype-environment (GE) interaction and subjecting them to principal component analysis (Yan, 2001). It is a technique widely used in breeding studies involving soybean crop (Dalló et al., 2019; Woyann et al., 2020), especially when the intent is to select representative and discriminatory environments and indicate stable genotypes adapted to specific environments (Yan and Holland, 2010). This method was applied to assess the environmental and genotypic influence on micronutrient levels in common bean seeds (Philipo et al., 2020) and to assess the adaptability and stability of soybean cultivars for seed production and quality (Silva et al., 2017). Despite still being little explored in studies in the area of seeds, it is a tool with potential to be used, as it allows the analysis of the association between genotypes, between environments and the interaction between them (Yan and Holland, 2010). Thus, this analysis can help in studies that aim to analyze the relationships between natural and artificial aging environments and compare the performance of seeds of genotypes in these environments.

Given the need to develop or adapt rapid tests that can be predictive for seed storage potential and the importance of associating analyses to identify this correlation, this study aimed to evaluate the efficiency of different artificial aging times in predicting the storability of soybean seeds using the GGE biplot method.

MATERIAL AND METHODS

Experiment location and overview: Soybean seeds from pre-commercial lines, called GEN1 (maturity group 5.9, with RR technology), GEN2 (maturity group 5.6, with RR technology), GEN3 (maturity group 5.8, with IPRO technology), GEN4 (maturity group 5.5, with IPRO technology), GEN5 (maturity group 6.8, with IPRO technology) and GEN6 (maturity group 5.5, with IPRO technology) were used in the experiment.

The seeds of the six genotypes were produced under the same cultivation conditions in the municipality of Passo Fundo, RS, Brazil. The seeds were analyzed at the Seed Research Laboratory of the *Universidade Federal de Viçosa*, MG, Brazil, when freshly harvested, and then subjected to storage (natural aging) and artificial aging.

Natural aging (Storage): The freshly harvested seeds of the six genotypes were placed in Multi-sheet Kraft paper bags (properly sealed and identified) and stored for a period of eight months, under refrigerated conditions, refrigerated environment (17 ± 3 °C and 70 ± 5% relative humidity (RH)) and, non-refrigerated, shed environment (13.8 - 30.7 °C and 50 - 92% RH), in the city of Passo Fundo, RS, Brazil.

Seeds freshly harvested and after storage under refrigerated (8m-Refri) and non-refrigerated (8m-Shed) conditions were subjected to the following tests and determinations:

Moisture Content (MC) - Moisture content was determined immediately before the tests, by the oven method at 105 °C for 24 h, using four replicates of 50 seeds per treatment (Brasil, 2009).

Germination (G): Four replications of 50 seeds per treatment were used. The seeds were sown 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 performed with recording of the percentage of normal seedlings on the 5th and 8th days after sowing (Brasil, 2009).

Accelerated Aging (AA): Four replications of 50 seeds per treatment were used. The seeds were distributed in a single layer on a wire mesh tray attached to a Gerbox-type box containing 40 mL of distilled water at the bottom. The boxes were closed to obtain approximately 100% RH inside and kept in a BOD chamber at 41 °C for 48 hours (Mcdonald and Phaneendranath, 1978). After this period, the seeds were subjected to the germination test, and the percentage of normal seedlings was evaluated on the 5th day after the beginning of the test (Brasil, 2009).

Seedling Growth: Four replications of 20 seeds per treatment were used. These seeds were sown equidistantly between three sheets of Germitest paper, moistened with distilled water in a volume equivalent to 2.5 times the weight of the dry paper (Nakagawa, 1999). Subsequently, rolls were made and placed in a germinator at 25 °C, where they were kept for three days. After this period, the seedlings were scanned and Vigor-S® software was used to measure root length and shoot length. Length data were used to calculate vigor indices (VI) (Medeiros and Pereira, 2018). The same seedlings used to measure the length were used to obtain seedling dry mass (SDM). Dry mass was obtained after drying the seedlings in an oven with forced air circulation at 70 °C for 72 hours. The material was weighed on an analytical balance with precision of 0.001 g and the results were expressed in milligrams per seedling (mg seedling^{-1}).

Artificial Aging: For artificial aging, freshly harvested seeds were distributed on wire mesh trays attached to Gerbox-type boxes, containing 40 mL of distilled water at the bottom (Delouche and Baskin, 1973). The seeds were kept under relative humidity of approximately 100% at 41 °C for periods of 48, 96 and 144 hours. For the control treatment, seeds without exposure to artificial aging were used. This treatment was called time zero.

After aging, the seeds were left on a bench, in a laboratory environment, for natural drying until they reached the initial moisture (approximately 12%), and then the tests of moisture content, germination, accelerated aging and seedling growth were performed (according to the methodology described for the natural aging experiment). In addition to these, the electrical conductivity and emergence velocity index tests were also carried out as described below:

Electrical Conductivity (EC): Four replications of 50 seeds per treatment were weighed and placed in plastic cups containing 75 mL of distilled water. The set was then kept in a BOD chamber at 25 °C for 24 hours (Vieira and Carvalho,

1994). After this period, the electrical conductivity of the solution was determined using a conductivity meter.

Emergence Speed Index (ESI): Four replications of 50 seeds per treatment were sown in polystyrene trays containing two liters of sand as substrate. The substrate was initially moistened until it reached 60% of the water holding capacity and irrigated daily. Daily counts of the number of seedlings emerged were performed up to the 12th day after sowing. The data from the counts were used to obtain the ESI, as proposed by Maguire (1962).

The selection of variables MC, G, AA, SDM and VI for the natural aging experiment and MC, G, AA, SDM, VI, EC and ESI for the artificial aging experiment was obtained using the test of Singh (1981). For both experiments, 14 variables were collected. However, these variables were the most important to distinguish the treatments. The other variables showed importance lower than 1% and were, therefore, not used in the present study.

*Natural vs. Artificial Aging via GGE Biplot***:** The seeds of the six genotypes were analyzed in the five aging environments: refrigerated storage, non-refrigerated storage, and under artificial aging for periods of 48, 96 and 144 hours. The relationships between genotypes and environments were analyzed using GGE biplot.

Statistical Analysis: For natural aging (storage), a completely randomized experimental design was adopted, in a 6 x 3 factorial scheme, with four replicates. Treatments consisted of six genotypes and three types of environments (freshly harvested seeds; after eight months of storage under refrigerated conditions, 8m-Refri, and after eight months of storage under non-refrigerated conditions, 8m-Shed). The significance of the sources of variation was examined by the F statistic ($p < 0.05$) and the means were compared by Tukey test, at 5% probability level. The analyses were carried out using R Software (R Core Team, 2020).

For artificial aging, a completely randomized experimental design was adopted, in a 6 x 4 factorial scheme, with four replicates. Treatments consisted of six soybean cultivars and four aging times (0, 48, 96 and 144 hours). The data were subjected to analysis of variance and the means of the aging times for each cultivar were subjected to regression analysis. The curves obtained were subjected to the model identity test, using the GENES program (Cruz, 2016).

GGE biplot analysis was performed to investigate the behavior of the seeds of the cultivars in the different aging environments and to check which of the artificial aging environments has the greatest correlation with the natural aging environments. For this, the data from both natural and artificial aging trials were used, except for the data from the control treatment (non-aged seeds).

Initially, the data of the variables were standardized by the equation: $X_i = (X - \mu) / \sigma$, where X_i is the mean of the standardized replicate, X is the mean of the replicate, μ is the overall mean of the variable, and σ is the overall standard deviation of the variable. By means of the product of the standardized values of all the variables selected by the Singh's (1981) method, a single variable called Performance Index was obtained for each treatment. The electrical conductivity variable, for being inversely proportional to seed vigor, was multiplied by 1/EC.

The interaction between the six cultivars and the five aging environments (F-test, $p < 0.05$) was tested and graphs were generated using the GGE biplot method. Analyses of discrimination and representativeness of environments, performance and stability of the cultivars in relation to the mean of the environments were performed and the performance of seeds of the cultivars in each aging environment was ranked, according to the methodology proposed by Yan (2001).

RESULTS AND DISCUSSION

The moisture content of the seeds, in the initial condition, after storage and after artificial aging, at the time of the tests, was 11.69 ± 0.62%.

Considering the natural aging, which occurs during storage, there was a reduction in the germination and vigor of the seeds of the cultivars, in different proportions between the storage environments (Figure 1).

Initially, seeds of the cultivars GEN4 and GEN5 showed the lowest percentage of germination and the lowest vigor index. There was lower vigor in seeds of GEN6 and GEN4 after the AA test. After storage, there was a reduction in the

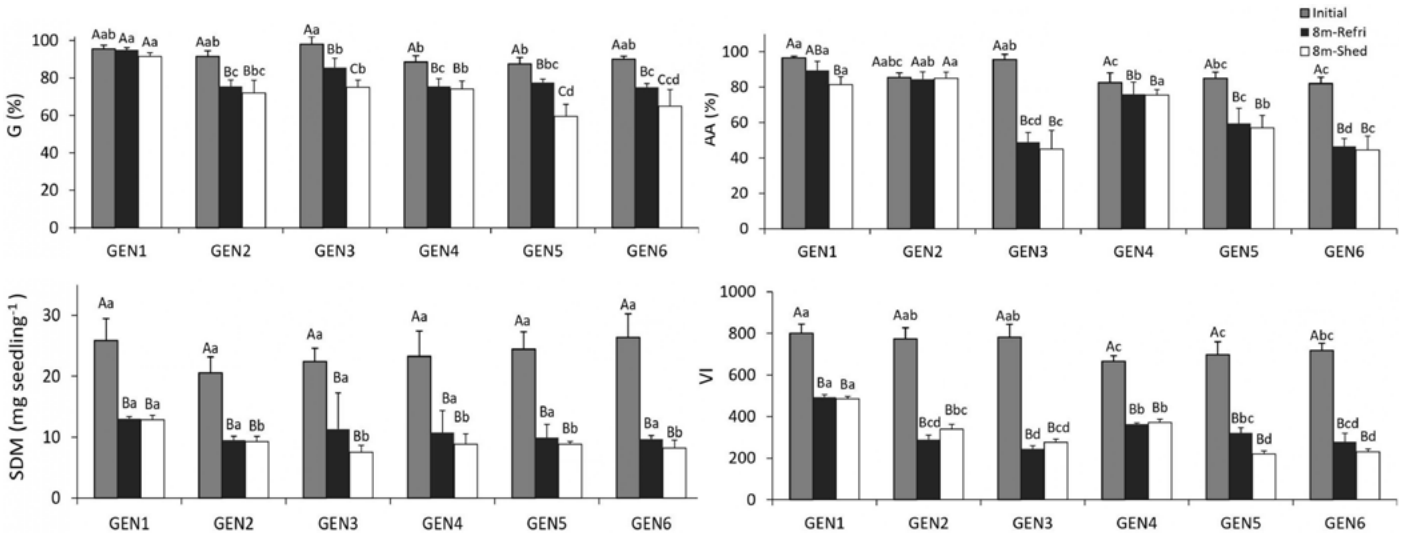

Figure 1. Germination (G), accelerated aging (AA), seedling dry mass (SDM) and vigor index (VI) of soybean seeds freshly harvested (Initial) and after eight months of storage under refrigerated (8m-Refri) and non-refrigerated (8m-Shed) conditions. *** Means followed by the same uppercase letter, comparing the initial period of storage and after 8 months of storage, 8m-Refri and 8m-Shed, and lowercase letters, comparing the genotypes in each environment, did not differ from each other by Tukey test at 5% probability level.

performance of seedlings of all genotypes, as evidenced by the decrease in VI and SDM (Figure 1). With the G, AA and VI tests, it was possible to observe that seeds of the GEN1, GEN4 and GEN2 genotypes maintained high physiological quality after natural aging, regardless of the storage environment. On the other hand, seeds of the cultivar GEN3 obtained germination above the standard for commercialization after storage (86%), but showed a drastic drop in vigor, evidenced by the AA (49%) and VI (244) tests. GEN6 seeds showed the lowest germination and vigor after storage under both refrigerated and non-refrigerated conditions, which may be a consequence of the lower initial vigor detected by AA (82%) (Figure 1). On the other hand, the cultivar GEN1, which had the highest initial physiological quality (germination of 96%, AA of 95% and VI of 800), was the one with the highest tolerance to deterioration during storage.

According to Shelar et al. (2008), the maintenance of viability and vigor during storage is strictly associated with the initial quality of the seeds. Vergara et al. (2019) proved that the performance of soybean seed lots stored for 180 days had a high correlation with their initial quality. However, although the initial vigor of the seeds is decisive for greater longevity during storage, the GEN3 genotype, whose seeds initially had high germination and vigor, showed a drastic reduction in vigor with storage (Figure 1). On the other hand, the GEN4 genotype, which initially had lower physiological quality (89% of G, 83% of AA, 665 of VI), showed a lower reduction in vigor compared to most genotypes (Figure 1). These results lead to inferences about different levels of tolerance to seed deterioration among the genotypes studied.

The genetic makeup of each cultivar can lead to increased tolerance or susceptibility to deterioration, which affects the longevity of seeds during storage (Naik et al., 2016). The level of tolerance to deterioration is a genetic attribute (Shelar et al., 2008), which depends on the ability of seeds to resist degradation, as well as the protective mechanisms they have (Balešević-Tubić et al., 2010).

The acquisition of longevity occurs during the maturation process, starting at the end of the grain filling stage and extending until the seeds reach the dry state (Lima et al., 2017). Some studies suggest that seeds acquire greater longevity during late maturation (Lima et al., 2017; Basso et al., 2018). It is likely that there is a post-abscission transcriptional program, which is associated with the expression of genes encoding chaperones, such as heat shock proteins, with the repression of chloroplast genes, with the increase in the proportion of oligosaccharides of the raffinose family, and

with abscisic acid signaling (Lima et al., 2017). In addition, longevity is associated with physical characteristics (Tripathi and Khare, 2016) and chemical properties of seeds (Singh et al., 2017), as well as the conditions to which they remain exposed during storage (Nagel et al., 2014).

In this study, seeds of all genotypes showed equal or higher physiological quality after storage in the refrigerated environment, compared to the shed condition (Figure 1). Seeds that were stored in a shed were subjected to greater thermal and hygroscopic amplitude and were exposed to high temperature and relative humidity, especially in the last two months of storage.

Storage under high temperature and/or high relative humidity can cause an increase in seed mass temperature, increase in acidity, intensification of respiration, degradation of reserves, alteration of fatty acid fractions, decomposition of membranes, peroxidation of lipids, among other factors, which culminate in loss of vigor and germination capacity (Šimić et al., 2007; Singh et al., 2017). Loss of viability usually occurs last, and seed vigor is the first parameter affected by deterioration during storage (Bewley et al., 2013), as shown in Figure 1. This reduction in vigor affects the performance of the seedlings, since the deterioration compromises the mobilization of storage tissue reserves to the embryonic axis (Mohammadi et al., 2011), which leads to reduced growth, reducing seedling dry mass and vigor (Figure 1). However, regardless of storage conditions, the genetic diversity that exists among soybean genotypes is notorious in terms of seed longevity (Naik et al., 2016), as observed in Figure 1.

In artificial aging, seeds exposed to 41 °C for 0 (control), 48, 96 and 144 hours showed a reduction in vigor with increasing time of exposure to aging (Figure 2). By means of the model identity test, it was possible to observe that the seeds of all cultivars showed identical responses, with reductions in G, AA and VI as aging time increased. Therefore, a single curve was obtained with data from all genotypes for these variables (Figure 2). Among these variables, a more pronounced reduction in VI values was observed as aging time increased. However, there was a difference between the genotypes regarding SDM, EC and ESI (Figure 2). Seeds of the cultivar GEN1 showed a less marked reduction in SDM, while for GEN6, there was a marked increase in the electrical conductivity of the seeds.

ESI was the index that showed the greatest difference in seed vigor between genotypes when subjected to artificial aging (Figure 2). Seeds of the GEN1 genotype had a smaller reduction in vigor, while seeds of GEN6 showed a greater reduction in physiological quality with artificial aging. The other genotypes had intermediate behavior between GEN1 and GEN6. These results are similar to those observed after storage, in which GEN1 seeds had higher physiological quality and GEN6 seeds had lower physiological quality (Figure 1). Thus, it is evident that artificial aging can be used as a test to predict the longevity of seeds during storage. However, it is necessary to know which exposure time best indicates the storage potential of the seeds. In this context, the adoption of methods that allow the analysis of the correlation between natural aging and the times of exposure to artificial aging arises as a necessity. Thus, the performance of the seeds of the six soybean genotypes after artificial aging - for 48, 96 and 144 hours - and natural aging - storage for eight months under refrigerated and shed conditions - was jointly analyzed by means of the GGE biplot (Figure 3).

It was found that more than 95% of the total variation in the data was explained by the first two principal components (Figure 3), which shows that this analysis was precise and accurate. Figure 3A presents the analysis of the discrimination capacity and representativeness of the environments, as well as the stability and performance of the seeds of the genotypes as a function of the aging environments. The ATC (average tester coordinate) axis represents the mean axis of the environments, defined by the means of PC1 and PC2 related to all environments tested. The ATCx axis passes through the origin of the biplot, and the arrow represented over it is set as the average environment marker. The shorter the distance of the projection of the genotype onto the marker, the better the performance of the genotype. Thus, seeds of GEN1 had the best performance, while seeds of GEN6 showed the worst performance, when considering all aging environments. The ATCy axis, which also passes through the origin and is perpendicular to the ATCx, in addition to representing the fine line to define the genotypes with above-average performance (to the left of the line) and below-average performance (to the right of the line), is also a representative coordinate for the stability of the genotypes. The closer to the origin in relation to ATCy, the greater the stability of the genotype in these

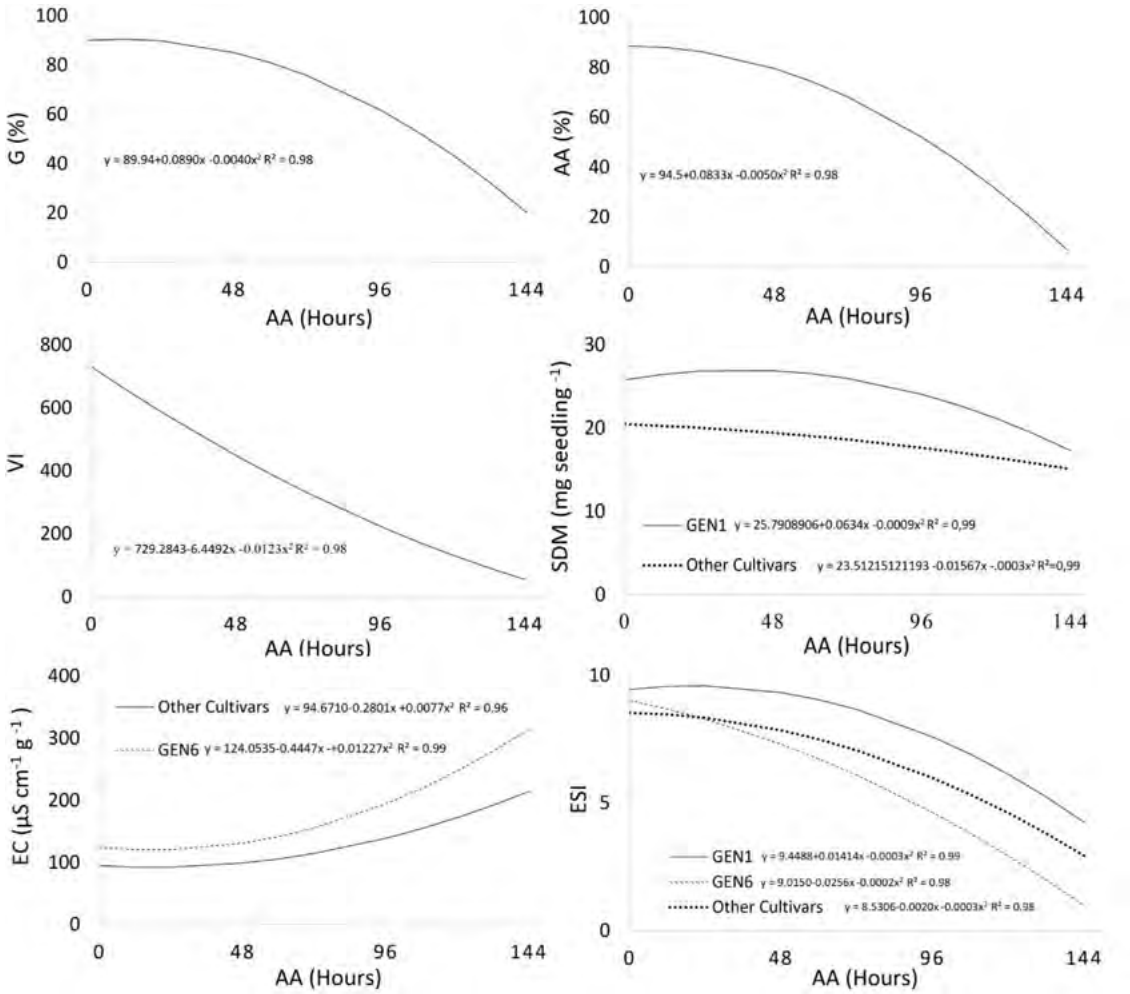

Figure 2. Germination (G), accelerated aging (AA), vigor index (VI), seedling dry mass (SDM), electrical conductivity (EC) and emergence speed index (ESI) of seeds of different soybean genotypes subjected to different artificial aging times (AA – Hours).

environments. Thus, GEN6 was the most stable cultivar and GEN3 was the one with the lowest stability. This means that GEN6 seeds always showed low performance, regardless of the environment, while GEN3 seeds showed higher or lower performance, depending on the environment to which they were subjected. In addition, the angle between the vectors of the environments shows the relationship between them, so the smaller the angle, the more associated they are. Thus, it was possible to observe that the artificial aging of seeds for 96 hours was more associated with seed storage for eight months, in both refrigerated and shed environments (Figure 3A).

When analyzing the performance of the genotypes in each aging environment, considering storage under the refrigerated condition (Figure 3B), storage under the shed condition (Figure 3C) and after artificial aging for 48 hours (Figure 3D), 96 hours (Figure 3E) and 144 hours (Figure 3F), it was observed that the ranking of the cultivars was different in all environments. After seed storage for eight months under refrigerated conditions, cultivars GEN1 and GEN4 showed the highest performance index, followed by cultivars GEN2, GEN5 and GEN3, which did not differ from each other, and cultivar GEN6, whose seeds showed the worst performance (Figure 3B). In the storage of seeds in the shed environment, cultivars GEN1 and GEN4 showed above-average performance, followed by GEN2, which did not differ from GEN5, and cultivars GEN3 and GEN6, respectively (Figure 3C). When artificial aging was applied for 48 hours to the seeds of the cultivars GEN1 and GEN3, the performance was above average, followed by GEN5, GEN4, GEN2 and GEN6, respectively (Figure 3D). In the environment with 96 hours of AA, seeds of the cultivars GEN1 and GEN4 were

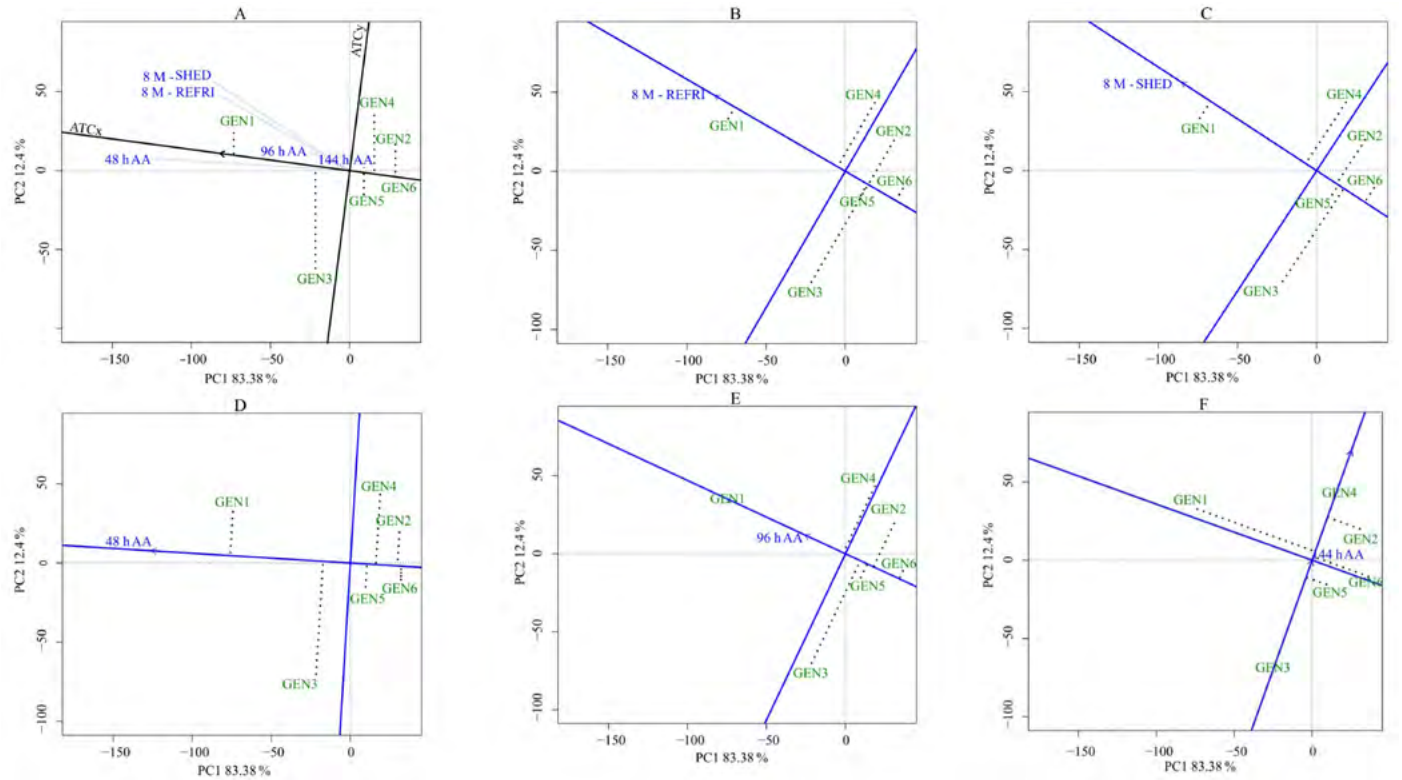

Figure 3. GGE biplot analysis of the physiological quality data obtained for the six soybean genotypes after storage (natural aging) and different artificial aging times. 8M-Shed – stored for eight months under non-refrigerated condition, shed; 8M-Refri – stored for eight months under refrigerated condition; 48 h AA – 48 hours of artificial aging; 96 h AA – 96 hours of artificial aging; and 144 h AA – 144 hours of artificial aging. A – analysis of the ability to discriminate and representativeness of the aging environments, considering natural and artificial aging; B – analysis of genotypes in relation to the environment 8 M – REFRI; C – analysis of genotypes in relation to the environment 8 M – SHED; D - analysis of genotypes in relation to the environment 48 h AA; E – analysis of genotypes in relation to the environment 96 h AA; F - analysis of genotypes in relation to the environment 144 h AA.

the ones with the highest indices, followed by those of GEN3, GEN5, GEN2 and GEN6 (Figure 3E). After artificial aging for 144 hours, seeds of the cultivars GEN4, GEN2, GEN1 and GEN6 showed above-average performance and only seeds of the cultivars GEN5 and GEN3 showed below-average performance (Figure 3F). Although none of the environments ranked the genotypes identically, the ones that had the most similar ranking were the refrigerated storage and shed environments and artificial aging for 96 hours, which were also the ones that showed the highest correlation with each other (Figure 3A).

Thus, the analysis of the GGE biplot, using the data from both experiments, ratified what was found individually for each experiment, in addition to allowing highlighting the correlation between the environments and ranking the performance of the genotypes in each environment. The great advantage of the GGE method is that it allows the visualization of the relationship between genotypes and the interaction between genotypes and environments, by means of an easy-to-interpret biplot graph (Silva and Benin, 2012). There are numerous analyses that can be performed with the GGE biplot and, according to the objective of the research, studies with a certain focus can be explored, such as: assessing the performance of different cultivars in a given environment; analyzing the adaptation of a cultivar in different environments; comparing two or more cultivars; identifying the best cultivar in each environment; assessing the average performance and stability of cultivars and the representativeness and capacity to discriminate environments;

ranking cultivars based on average performance and stability or categorizing the environment based on its ability to discriminate and representativeness, among others (Yan, 2001).

Thus, the GGE biplot is a very useful analysis tool used in soybean breeding (Dalló et al., 2019; Woyann et al., 2020) and has also been addressed in some studies related to seed quality, with an emphasis on breeding (Silva et al., 2017; Philipo et al., 2020). However, to the best of our knowledge, this is the first study to evaluate the physiological quality of seeds under aging environments.

Although the analysis of GGE biplot is not yet widely adopted in studies involving the evaluation of the performance of seeds of cultivars under different environments, in this study we explored the associations between seeds of different genotypes under different aging conditions, highlighting the relationships between environments and genotypes. It is understood that this analysis has the potential to be applied in studies in the area of physiological quality of seeds.

It was found that 96 hours was the artificial aging time whose effect on seed quality was more similar to the storage of eight months under refrigerated and shed conditions, due to the proximity of the vectors of these environments (Figure 3A). In the GGE biplot methodology, the angle between two vectors can be used as a measure of association, and the cosine of the angle between two environments corresponds to the correlation between them (Silva and Benin, 2012). In addition, the ranking of genotypes in these environments was also similar (Figure 3). On the other hand, the performance index obtained with aging for 48 hours, often described as a predictor of seed longevity (Delouche; Baskin, 1973), was not the one that showed the greatest association with the results obtained for the genotypes in storage (Figure 3). Other authors have also found that 48 hours of artificial aging was not the best aging time for predicting the performance of seeds in storage (Freitas et al., 2006; Patil et al., 2017; Fantazzini et al., 2018), which reinforces the evidence of the need to adjust the artificial aging time to predict the storability of soybean seeds with greater reliability. It is also believed that the aging time may need to be adjusted according to the genotypes used, as reported in the studies conducted by Patil et al. (2017) and Fantazzini et al. (2018).

CONCLUSIONS

Artificial aging, at a temperature of 41 °C for 96 hours, has the potential to be used to predict the tolerance of soybean seeds to deterioration during storage.

The GGE biplot is a method that can be used as a tool to analyze the relationships between aging environments and visualize the ranking of genotypes regarding the performance of seeds subjected to natural and artificial aging.

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REFERENCES

BALEŠEVIĆ-TUBIĆ, S.; TATIĆ, M.; ĐORĐEVIĆ, V.; NIKOLIĆ, Z.; ĐUKIĆ, V. Seed viability of oil crops depending on storage conditions. *Helia*, v.33, p.153-160, 2010. https://doi.org/10.2298/hel1052153b

BASSO, D.P.; HOSHINO-BEZERRA, A.K.; SARTORI, M.M.P.; BUITINK, J.; LEPRINCE, O.; SILVA, E.A.A. Late seed maturation improves the preservation of seedling emergence during storage in soybean. *Journal of Seed Science*, v.40, p.185-192, 2018. https://doi.org/10.1590/2317-1545v40n2191893

BEWLEY, J.D.; BRADFORD, K.J.; HILHORST, H.W.M.; NONOGAKI, H. *Seeds: physiology of development, germination and dormancy*. New York: Springer, 2013. 392p.

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento (MAPA). *Regras para Análise de Sementes*. Ministério da Agricultura, Pecuária e Abastecimento. Secretaria de Defesa Agropecuária. Brasília: Mapa/ACS, 2009. 399p. https://www.gov.br/agricultura/pt-br/assuntos/insumos-agropecuarios/arquivos-publicacoes-insumos/2946_regras_analise__sementes.pdf

CRUZ, C.D. Genes Software – extended and integrated with the R, Matlab and Selegen. *Acta Scientiarum*, v.38, p.547-552, 2016.

DALLÓ, S.C.; ZDZIARSKI, A.D.; WOYANN, L.G.; MILIOLI, A.S.; ZANELLA, R.; CONTE, J.; BENIN, G. Across year and year-by-year GGE biplot analysis to evaluate soybean performance and stability in multienvironment trials. *Euphytica*, v.215, n.113, p.1-12, 2019. https://doi.org/10.1007/s10681-019-2438-x

DELOUCHE, J.C.; BASKIN, C.C. Accelerated aging techniques for predicting the relative storability of seed lots. *Seed Technology Papers*, v.1, p.427-452, 1973. https://scholarsjunction.msstate.edu/seedtechpapers/10

EBONE, L.A.; CAVERZAN, A.; TAGLIARI, A.; CHIOMENTO, L.T.; SILVEIRA, D.C.; CHAVARRIA, G. Soybean seed vigor: uniformity and growth as key factors to improve yield. *Agronomy*, v.10, p.1-15, 2020. https://doi.org/10.3390/agronomy10040545

FANTAZZINI, T.B.; ROSA, D.V.F.; PEREIRA, C.C.; PEREIRA, D.S.; CIRILLO, M.A.; OSSANI, P.C. Association between the artificial aging test and the natural storage of coffee seeds. *Journal of Seed Science*, v.40, p.164-172, 2018. https://doi.org/10.1590/2317-1545v40n2188120

FREITAS, R.A.; DIAS, D.C.F.S.; OLIVEIRA, M.G.A.; DIAS, L.A. S.; JOSÉ, I C. Physiological and biochemical changes in naturally and artificially aged cotton seeds. *Seed Science & Technology*, v.34, p.253-264, 2006. https://doi.org/10.15258/sst.2006.34.2.01

LIMA, J.J.P.; BUITINK, J.; LALANNE, D.; ROSSI, R.F.; PELLETIER, S.; SILVA, E.A.A.; LEPRINCE, O. Molecular characterization of the acquisition of longevity during seed maturation in soybean. *PLoS One*, v.12, p.1-25, 2017. https://doi.org/10.1371/journal.pone.0180282

MAGUIRE, J. D. Speed of germination-aid selection and evaluation for seedling emergence and vigor. *Crop Science*, v.2, p.176-177, 1962.

MCDONALD, M.B.; PHANEENDRANATH, B.R. A modified accelerated aging seed vigor test for soybeans. *Journal of Seed Technology*, v.3, p.27-37, 1978. https://www.jstor.org/stable/23432640

MEDEIROS, A.D.; PEREIRA, M.D. SAPL®: a free software for determining the physiological potential in soybean seeds. *Pesquisa Agropecuária Tropical,* v.48, p.222–228, 2018. https://doi.org/10.1590/1983-40632018v4852340

MOHAMMADI, H.; SOLTANI, A.; SADEGHIPOUR, H.R.; ZEINALI, E. Effects of seed aging on subsequent seed reserve utilization and seedling growth in soybean. *International Journal of Plant Production*, v.5, p.65-70, 2011.

NAGEL, M.; KRANNER, I.; NEUMANN, K.; ROLLETSCHECK, H.; SEAL, C.E.; COUVILLE, L.; FERNANDEZ-MARÍN, B.; BORNER, A. Genome-wide association mapping and biochemical markers reveal that seed ageing and longevity are intricately affected by genetic background and developmental and environmental conditions in barley. *Plant, Cell and Environment*, v.38, p.1011–1022, 2014. https://doi.org/10.1111/pce.12474

NAIK, S.M.; MADHUSUDAN, K.; MOTAGI, B.M.; MUGALI, S.; NADAF, H.L. Molecular characterization of seed longevity and associated characters using SSR markers in soybean [*Glycine max* (L.) Merrill]. *Journal of Pharmacognosy and Phytochemistry*, v.8, p.2357-2360, 2019.

NAIK, S.M.; MADHUSUDAN, K.; MOTAGI, B.N.; NADAF, H.L.; THIMMARAJU. Diversity in soybean (*Glycine max*) accessions based on morphological characterization and seed longevity characteristics. *Progressive Research – An International Journal Society for Scientific Development,* v.11, p.377-381, 2016. http://oar.icrisat.org/id/eprint/9856

NAKAGAWA, J. Teste de vigor baseados no desempenho das plântulas. In: KRZYZANOWSKI, F.C.; VIEIRA, R.D.E; FRANÇA-NETO, J.B. (Eds.) *Vigor de sementes: conceito e testes*. ABRATES, Londrina, 2.1-2.6, 1999.

PATIL, S.S.; DODDAGOUDAR, S.R.; KURNALLIKER, V.K.; MATHAD, R.C.; PATIL, R.P. Prediction of storability in soybean seeds through accelerated ageing technique [*Glycine max* (L.) Merrill]. *Legume Research*, v.40, p.1-6, 2017. http://dx.doi.org/10.18805/LR-3760

PHILIPO, M.; NDAKIDEMI, P.A.; MBEGA, E.R. Environmental and genotypes influence on seed iron and zinc levels of landraces and improved varieties of common bean (*Phaseolus vulgaris L*.) in Tanzania. *Ecological Genetics and Genomics*, v.15, n.100056, p.1-10, 2020. https://doi.org/10.1016/j.egg.2020.100056

R CORE TEAM. *R: A language and environment for statistical computing.* R Foundation for Statistical Computing, Vienna, Austria. 2020. https://www.R-project.org/

SANO, N.; RAJJOU, L.; NORTH, H.M.; DEBEAUJON, I.; MARION-POLL, A.; SEO, M. Staying alive: molecular aspects of seed longevity. *Plant & Cell Physiology*, v.57, p.660-674, 2016. https://doi.org/10.1093/pcp/pcv186

SHELAR, V.R.; SHAIKH, R.S.; NIKAM, A.S. Soybean seed quality during storage: a review. *Agricutural Reviews*, v.29, p.125 – 131, 2008.

SILVA, K.B.; BRUZI, A.T.; ZAMBIAZZI, E.V.; SOARES, I.O.; PEREIRA J.L.A.R.; CARVALHO, M.L.M. Adaptability and stability of soybean cultivars for grain yield and seed quality. *Genetics and Molecular Research*, v.16, gmr16029646, 2017. http://dx.doi.org/10.4238/gmr16029646

SILVA, R.R.; BENIN, G. Biplot analysis: concepts, interpretations and uses. *Ciência Rural*, v.42, p.1404-1412, 2012.

ŠIMIĆ, B.; POPOVIĆ, R.; SUDARIĆ, A.; ROZMAN, V.; KALINOVIĆ, I.; ĆOSIĆ, J. Influence of storage condition on seed oil content of maize, soybean and sunflower. *Agriculturae Conspectus Scientificus,* v.72, p.211-213, 2007. https://hrcak.srce.hr/17093

SINGH, D. The relative importance of characters affecting genetic divergence. *The Indian Journal of Genetic and Plant Breeding*, v.41, p.237-245, 1981.

SINGH, J.; PAROHA, S.; MISHRA, R.P. Factors affecting oilseed quality during storage with special reference to soybean (*Glycine max*) and niger (*Guizotia abyssinica*) seeds. *International Journal of Current Microbiology and Applied Sciences*, v.6, p.2215-2226, 2017.

TIAN, X.; SONG, S.; LEI, Y. Cell death and reactive oxygen species metabolism during accelerated ageing of soybean axes. *Russian Journal of Plant Physiology*, v.55, p.33-40, 2008.

TRIPATHI, N.; KHARE, D. Molecular approaches for genetic improvement of seed quality and characterization of genetic diversity in soybean: a critical review. *Biotechnology Letters*, v.38, p.1645-1654, 2016. https://doi.org/10.1007/s10529-016-2154-8

VERGARA, R.O.; GAZOLLA-NETO, A.; GADOTTI, G.I. Space distribution of soybean seed storage potential. *Revista Caatinga*, v.32, p.399-410, 2019. https://doi.org/10.1590/1983-21252019v32n213rc

VIEIRA, R.D.; CARVALHO, N.M. *Testes de vigor em sementes*. Jaboticabal: FUNEP, 1994. 164p.

YAN, W.; HOLLAND, J.B.A heritability-adjusted GGE biplot for test environment evaluation. *Euphytica*, v.171, p.355-369, 2010. https://doi.org/10.1007/s10681-009-0030-5

YAN, W. GGE biplot - A windows application for graphical analysis of multienvironment trial data and other types of two-way data. *Agronomy Journal*, v.93, p.1111–1118, 2001. https://doi.org/10.2134/agronj2001.9351111x

YAN, W.; KANG, M.S. *GGE biplot analysis: a graphical tool for breeders, geneticists, and agronomists*. Flórida: Boca Raton, 2003. 286p. https://doi.org/10.1201/9781420040371

WOYANN, L. G.; MATEI, G.; ZDZIARSKI, A. D.; DALLACORTE, L. V.; MADELLA, L. A.; BENIN, G. Selection indexes based on linear-bilinear models applied to soybean breeding. *Agronomy Journal*, v.112, p.175-182, 2020. https://doi.org/10.1002/agj2.20044