Ciências Agrárias
SEMINA

DOI: 10.5433/1679-0359.2022v43n3p1127

Antioxidant and physiological responses of seeds of soybean cultivars to delayed harvest

Respostas antioxidantes e fisiológicas de sementes de cultivares de soja ao atraso de colheita

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Highlights

Seed deterioration was evaluated in different soybean cultivars.
Soybean seed germination and vigor were reduced with delayed harvest.
The cultivars differed in terms of physiological and antioxidant responses.

Abstract

Soybean seeds are susceptible to field deterioration after reaching physiological maturity, which usually occurs at the R7 stage, with harvest at R8. This study aimed to evaluate the biochemical and physiological changes in seeds of different soybean cultivars submitted to delayed harvest in the field. Seeds of the cultivars NS 5959, BMX Potência, and TMG 1175 were harvested at four times (0, 10, 20, and 30 days after reaching R8). Moisture, germination, first count, accelerated aging, emergence, and tetrazolium tests were performed in each harvest period. The activities of the antioxidant enzymes catalase (CAT), peroxidase (POX), and ascorbate peroxidase (APX) were also evaluated. The experiment was carried out in a completely randomized design in a 3×4 factorial scheme (three cultivars and four harvest times). A reduction in germination and vigor of seeds of different soybean cultivars was found at R8 + 20 and R8 + 30 days related to field deterioration due to delayed harvest. The seeds of the cultivars did not differ in terms of viability under delayed harvest but differed in terms of antioxidant and physiological responses. Better and worse seed performances were found for the cultivars BMX Potência and TMG 1175, respectively, at R8 and in R8 + 10 days.

Key words: *Glycine max* (L.) Merrill. Delayed harvest. Enzymatic activity.

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Received: June 15, 2021 - Approved: Feb. 25, 2022

Resumo

Sementes de soja são suscetíveis à deterioração em campo após atingirem a maturidade fisiológica, que em geral, ocorre no estádio R7 sendo a colheita realizada em R8. Objetivou-se com esse trabalho avaliar as alterações bioquímicas e fisiológicas em sementes de diferentes cultivares de soja submetidas ao atraso de colheita no campo. Sementes das cultivares NS 5959, BMX Potência e TMG 1175 foram colhidas em quatro épocas (0, 10, 20 e 30 dias após atingirem R8). Em cada período de colheita, foram realizados os testes de umidade, germinação, primeira contagem, envelhecimento acelerado, emergência e tetrazólio. Também foram avaliadas as atividades das enzimas antioxidantes catalase (CAT), peroxidase (POX) e ascorbato peroxidase (APX). O experimento foi conduzido em DIC, em esquema fatorial 3 x 4 (três cultivares e quatro épocas de colheita). Em R8 + 20 e R8 + 30 dias, foi encontrada redução na germinação e vigor das sementes das diferentes cultivares de soja devido à deterioração em campo pelo atraso de colheita. Sob atraso de colheita, as sementes das cultivares não diferiram quanto à viabilidade, mas diferiram quanto às respostas antioxidantes e fisiológicas. No estádio R8 e em R8 + 10 dias foi possível observar melhor desempenho das sementes da cv. 'BMX Potência' e pior para a cv. 'TMG 1175'.

Palavras-chave: *Glycine max* (L.) Merrill. Retardamento de colheita. Atividade enzimática.

Introduction

Recently, Brazil has reached the position of the world's largest soybean (*Glycine max* L.) producer, with a planted area of 38.2 million hectares and production of 135 million tons (Companhia Nacional de Abastecimento [CONAB], 2020). The use of high-quality seeds is essential due to this great relevance and demand. Seed quality encompasses physical, physiological, genetic, and sanitary attributes, which together allow the seeds to germinate to obtain a vigorous, uniform, and productive cultivations in the field (Krzyzanowski, França, & Henning, 2018).

The physiological quality of soybean seeds is directly influenced by the genotype, being maximum at physiological maturity, when the plants reach the R7 stage. However, the high water content of the seeds at this stage makes harvesting at the R8 stage recommendable, when 95% of the pods present the typical coloration of mature pods. This point is also called harvest maturity, in which the seeds present moisture between 12 and 15% (Fehr & Caviness, 1977). The interval between physiological maturity and harvest of soybean seeds can vary from a few days to several weeks due to situations such as unsuitable weather conditions for mechanized harvesting or the insufficient number of harvesters for the field size (Zuffo, Zuffo, Carvalho, Steiner, & Zambiazi, 2017). Adverse climate conditions, such as rains at the pre-harvest stage, delay harvest and increase the period of exposure of seeds to the environment, which contributes to a reduction in quality, especially in tropical regions (Castro, Oliveira, Lima, Santos, & Barbosa, 2016; Mathias et al., 2017; Pinheiro et al., 2021).

Delayed harvest reduces germination and vigor of soybean seeds through deterioration. However, the level of these responses is highly dependent on the analyzed genotype (Diniz et al., 2013; Lima et al., 2007; Mathias et al., 2017; Xavier et al., 2015; Zanatta et al., 2018; Zuffo et al., 2017). Vieira et al. (2013) harvested soybean seeds at physiological maturity (R7), R7 + 7 days, and R7 + 15 days and observed a reduction in vigor in this last harvest and structural damage to the seed coat. Soybean seeds harvested at the R8 stage had greater physiological potential than those harvested at 7, 14, 21, and 28 days after R8, and the longer the harvest delay, the greater the deterioration (Xavier et al., 2015). Diniz et al. (2013) observed that delaying the harvest for 15 days caused a significant reduction in seed vigor of three soybean cultivars, while the reduction in germination was observed at 30 days after R8.

Seed deterioration process mechanisms involve a series of degenerative changes initiated from the formation of reactive oxygen species (ROS) (Zhang, Zhang, Sun, Meng, & Tao, 2021). ROS are generated by the partial reduction of molecular oxygen, causing deleterious effects on cells at high levels, such as inactivation of enzymes, protein degradation, disruption of the cell membrane system, DNA molecule integrity loss, among others (Ebone, Caverzan, & Chavarria, 2019; Kurek, Plitta-Michalak, & Ratajczak, 2019). Cells have a complex antioxidant defense system, which involves the action of enzymes such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POX), and ascorbate peroxidase (APX) (Sharma, Jha, Dubey, & Pessarakli, 2012).

Antioxidant enzymes have been used as biochemical markers related to the oxidative process that causes seed deterioration. Usha and Dadlani (2016) observed that high vigor soybean seeds showed higher SOD and CAT activity compared to medium and low vigor. Soybean seeds from genotypes with higher lignin contents subjected to weathering deterioration showed lower oxidative stress, indicated by the lower activity of SOD and POX enzymes (Huth et al., 2016). Zuffo et al. (2017) found that soybean seeds harvested after the R8 stage showed lower germination, vigor, and expression of SOD, malate dehydrogenase, alcohol dehydrogenase, esterase, and isocitrate lyase enzymes.

Thus, monitoring the activity of antioxidant enzymes and their association with germination and vigor can generate interesting information for use in breeding programs that seek to select lines with seed characteristics of high quality or more tolerant to deterioration in the field. Considering that delayed harvest can be used as a strategy for this purpose, this study aimed to evaluate the antioxidant and physiological responses of seeds of three soybean cultivars submitted to delayed harvest in the field.

Material and Methods

Soybean plants of the cultivars NS 5959 IPRO, BMX Potência, and TMG 1175 RR from the relative maturation groups 5.9, 6.7, and 7.5, respectively, were grown for seed production in the experimental area of the Department of Agronomy at the Federal University of Viçosa, located in the district of São José do Triunfo, Viçosa, Minas Gerais, Brazil. The climate data recorded in the pre-harvest and seed harvest phases are shown in Figure 1 (A and B).

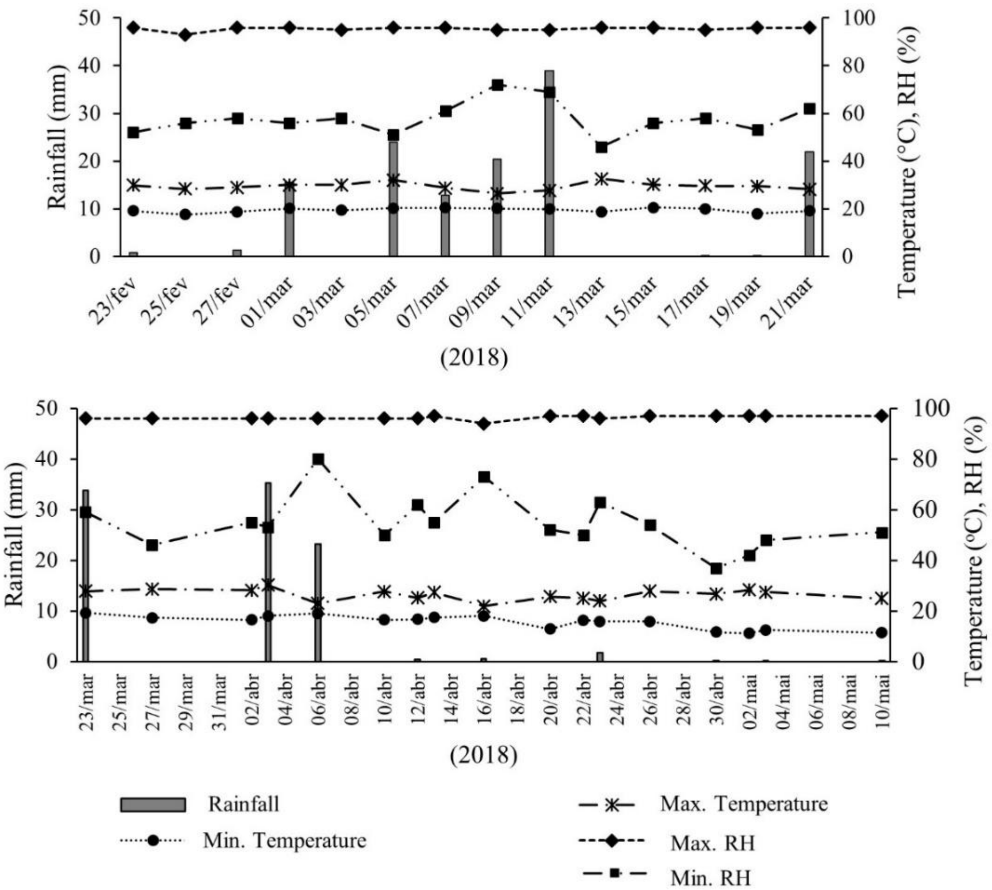

Figure 1. Meteorological data for the municipality of Viçosa, MG, Brazil, during the pre-harvest (February 23 to March 21, 2018) (A) and harvest periods (March 23 to May 10, 2018) (B). Daily total precipitation (mm), daily maximum and minimum temperature (°C), and daily maximum and minimum relative humidity (RH%). Source: Meteorological Station 86824 located in Viçosa, MG, Brazil. INMET (2018).

Sowing (14 seeds m^{-1}) was carried out on November 10, 2017, after soil analysis and tillage and planting fertilization. The seeds were treated with Derosal Plus fungicide (carbendazim + thiram) at a dose of 200 mL 100 kg^{-1} seeds. The total experimental area was 79.5 m2, with four rows of 53 m in length, spaced 0.5 m from each other. Each plot consisted of two central rows of 15 m in length, two rows corresponding to the border of the plot. Three plots were used for each cultivar, totaling 15 m per cultivar within each row. The borders of the experimental field consisted of soybean plants spaced 0.5 m from each side of the area. Cultural treatments were carried out when necessary, following instructions for the soybean crop management (Sediyama, Silva, & Borém, 2015).

All plants were harvested manually at four times: I) R8 stage (plants with 95% of pods showing brown color, typical of mature pods); II) R8 + 10 days; III) R8 + 20 days, and; IV) R8 + 30 days. The harvest dates for each treatment and seed moisture content collected according to each cultivar are shown in Table 1.

Table 1
Harvest dates and seed moisture content of three soybean cultivars at 0, 10, 20 and, 30 days after R8

Cultivar	R8	Moisture (%)	R8 + 10	Moisture (%)	R8 + 20	Moisture (%)	R8 + 30	Moisture (%)
NS 5959	Mar. 23	28.2	Apr. 2	18.3	Apr. 12	17.3	Apr. 22	17.0
			Apr. 6	31.2	Apr. 16	19.0	Apr. 26	16.9
BMX Potência	Mar. 27	23.9	Apr. 6	24.8	Apr. 16	18.3	Apr. 26	16.3
	Apr. 3	23.2	Apr. 13	17.8	Apr. 23	16.9	May 3	12.9
			Apr. 20	14.9	Apr. 30	15.0	May 10	14.8
TMG 1175	Apr. 2	22.5	Apr. 12	20.1	Apr. 22	17.9	May 2	12.6
	Apr. 10	27.2	Apr. 20	14.7	Apr. 30	14.5	May 10	15.0

After harvesting, the plants were maintained in a shed under shade until the threshing was completed. The seeds of each treatment were classified in circular opening sieves to standardize the size, eliminating those with a diameter of less than 5 mm. After the classification by size, the seeds of each treatment were homogenized and placed in open trays for approximately 10 days under shade in a laboratory environment (average temperature of 22 °C and average relative humidity of 58%) until they reached a moisture content of approximately 12%. Then, the following tests were performed:

Moisture content

Three replications of 25 seeds were used. Moisture content was determined by the oven method at 105 ± 3 °C for 24 h, with results expressed as a percentage (wet basis) (Ministério da Agricultura, Pecuária e Abastecimento [MAPA], 2009).

Germination

Four replications of 50 seeds were sown on paper towels (Germitest®) moistened with a water volume equivalent to 2.5 times the dry paper weight. Rolls were made and maintained in a germinator at 25 °C and an 8-h photoperiod. Evaluations were carried out on the eighth day after the test was set up and the results were expressed in percentage of normal seedlings (MAPA, 2009).

First germination count

Performed together with the germination test, with the percentage of normal seedlings obtained on the fifth day after the test was set up (MAPA, 2009).

Accelerated aging

Conducted with four replications of 50 seeds, which were arranged in a single layer on stainless steel screens inserted inside plastic “gerbox” boxes with 40 mL of water. The boxes were placed in BOD at 41 °C for 48 h. After this period, the seeds were submitted to the germination test, with the percentage of normal seedlings obtained on the fifth day after sowing (Marcos-Filho, 2020).

Seedling emergence in the field

Conducted with four replications of 50 seeds for each treatment, which were sown in furrows 1 m long, 0.03 m deep, and spaced at 0.3 m. Daily sprinkler irrigation was performed. The number of normal seedlings that emerged with the cotyledons above the soil surface was counted daily (Krzyzanowski, França, Gomes, & Nakagawa, 2020).

Tetrazolium

Conducted with four replications of 50 seeds, which were pre-conditioned on moistened paper towels for 16 h at 25 °C. Then, they were immersed in a 0.075% 2,3,5-triphenyl-tetrazolium chloride solution for 3 h at 40 °C in the dark. After washing in running water, they were analyzed individually and the results were expressed as a percentage of viability (classes 1–5) and vigor (classes 1–3), according to criteria established by França & Krzyzanowski (2020).

Antioxidant enzyme activity

Initially, four replications of 25 seeds were soaked for 24 h in paper towels moistened with water. Then, the seed coat was removed, and the embryos were frozen in liquid nitrogen (N). Subsequently, 0.2 g of embryos were macerated in liquid nitrogen (N) and 2 mL of the following homogenization medium were added: 0.1 M potassium phosphate buffer at pH 6.8, 0.1 mM ethylenediaminetetraacetic acid (EDTA), 1 mM phenylmethylsulfonyl fluoride (PMSF), and 1% (w/v) polyvinylpolypyrrolidone (PVPP) (Peixoto, Cambraia, Sant’Anna, Mosquim, & Moreira, 1999). The extract was then centrifuged for 15 min at 14,000 RPM and the supernatant was removed. Catalase (CAT) (Anderson, Prasad, & Stewart, 1995), peroxidase (POX) (Kar & Mishra, 1976), and ascorbate peroxidase (APX) (Nakano & Asada, 1981) enzyme activities were determined.

Protein content

Standard curve constructed with bovine serum albumin (BSA) was used as a reference (Bradford, 1976). Aliquots of 25 µL of the diluted solution (10x) of enzyme extract, 1 mL of Bradford reagent, and 0.975 mL of distilled water were added to the same extract used for the quantification of enzyme activity, followed by stirring. After 20 minutes, the absorbance of the sample was read in a spectrophotometer at 595 nm.

Experimental design and statistical analysis

The randomized block design in a split-plot design was used to set up the trial in the field. Physiological and biochemical analyses were carried out in the laboratory using a completely randomized design in a 3×4 double factorial scheme (cultivars × harvest times), with four replications. The data were subjected to analysis of variance. Data normality and homoscedasticity were tested using the Shapiro-Wilk and Bartlett tests. The means were compared using Tukey's test at a 5% probability (p ≤ 0.05). Multivariate principal component analysis (PCA) was also performed. The R software (R Core Team [R], 2020) was used for all statistical analyses.

Results and Discussion

The climate data obtained in the pre-harvest period (Figure 1A) allowed observing that the relative air humidity ranged from 46 to 96% and the temperature varied from 17.6 to 32.6 °C. The maximum rainfall of the period was 39 mm on March 11, 2018 (Figure 1A). Regarding the harvest period (Figure 1B), the relative air humidity varied between 37 and 97% and the temperatures ranged from 11.2 to 30.2 °C. The maximum rainfall of the period was 35.2 mm on April 3, 2018 (Figure 1B). This range observed in temperature and humidity, especially in the pre-harvest phase, directly contributes to a reduction in seed germination and vigor due to the deterioration process (Shu et al., 2020; Pinheiro et al., 2021). Therefore, these data are important because climate conditions in the pre-harvest phase directly influence the quality of soybean seeds, especially when submitted to delayed harvest. High altitude locations (above 700 m) and mild temperatures (approximately 20 °C) during the night are preferred for producing better quality soybean seeds, ensuring that the seeds remain in the field without suffering interference and reducing their quality (França et al., 2016). However, delayed harvest can reduce vigor even in regions with favorable characteristics for seed production, such as lower temperatures, dry weather, and low relative humidity during the harvest period (Mathias et al., 2017).

Plants of the cultivar NS 5959 reached the R8 stage on March 23, 2018, while those of the cultivars BMX Potência and TMG 1175 reached R8 on March 27 and April 3, 2018, and April 2 and 10, 2018 (Table 1), respectively. The plants reached the R8 stage on different dates because the evaluated cultivars have different maturation groups, requiring more than one harvest. In this context, seed moisture content was defined at each harvest (Table 1), allowing obtaining the means of each treatment (Figure 2).

Seed moisture content showed a difference among cultivars at all harvest times (Figure 2). Seed moisture content at R8 was lower for the cultivar BMX Potência (23.5%) and higher for the cultivar NS 5959 (28.2%). These results occurred because the cultivar BMX Potência had more harvests on dates when there was no rainfall (March 27 and April 2, 2018). The seeds of the cultivar NS 5959 were exposed to rainfall on March 21, 2018 (Table 1 and Figure 1). The lowest moisture content at R8 + 10 days was 17.2% (TMG 1175) and the highest was 24.7% (NS 5959), corresponding to the seeds of cultivars less and more exposed to rainfall, respectively. A decrease in moisture content was observed at 20 days after R8 for the cultivars NS 5959 (18.1%) and BMX Potência (16.7%), while the moisture content of the cultivar TMG 1175 remained similar to that obtained at R8 + 10 days. In general, lower values were obtained at 30 days after R8, ranging from 16.9 to 13.8% for the cultivars NS 5959 and TMG 1175, respectively (Figure 2). Thus, in general, there was a gradual decrease in moisture content with the delayed harvest of seeds of all evaluated cultivars, as seeds harvested at stages R8 + 20 and R8 + 30 were not exposed to rainfall in the pre-harvest phase (Table 1 and Figure 1).

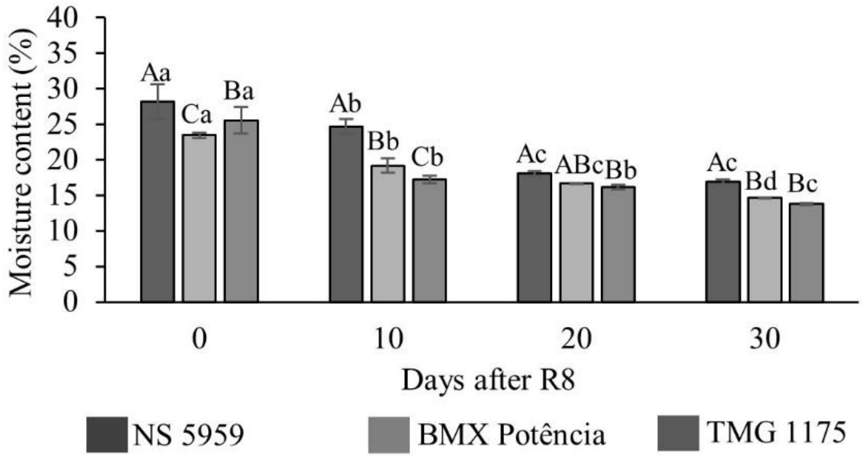

Figure 2. Moisture content of soybean seeds harvested at 0, 10, 20, and 30 days after R8. Uppercase letters compare cultivars at each harvest time and lowercase letters compare harvest times for each cultivar by Tukey's test at a 5% probability.

The comparison among harvest times for each cultivar shows that the harvest carried out at 20 days after R8 led to a reduction in the percentage of germination of the cultivar NS5959 by approximately 10 percentage points (pp) (Figure 3A). However, the seeds of this cultivar were not affected in terms of vigor according to the harvest times, evaluated by the first germination count, which also did not differ among harvest times (Figure 3B). The cultivar BMX Potência showed significant reductions in the percentage of germination and first count from the harvest carried out at 20 days after R8. These reductions reached approximately 13 and 10 pp, respectively, for this cultivar. A different behavior was observed for the cultivar TMG 1175, whose germination and first germination count at R8 + 10 days were higher than the others (Figure 3A and B).

Overall, the delayed harvest for 20 and 30 days, associated with variations in temperature, relative air humidity, and rainfall that occurred at R8 and R8 + 10 days (Figure 1), led to a reduction in seed germination of the cultivars NS 5959 and BMX Potência. TMG 1175 reached the R8 stage later because it is a late cultivar, causing the plants to not be exposed to the same environmental conditions as the other cultivars. Therefore, the reduction in seed germination of this cultivar occurred mainly at 30 days after R8 (Figure 3A). The occurrence of rainfall and variations in relative humidity, especially when associated with high temperatures, result in seed deterioration and contribute to a reduction in seed quality as harvest is delayed (Mathias et al., 2017; Shu et al., 2020). Weathering damage is characterized by the disruption of cell layers and coat wrinkling, causing increased exposure of the embryo's tissues to the environment (Forti, Carvalho, Tanaka, & Cicero, 2013; Pinheiro et al., 2021).

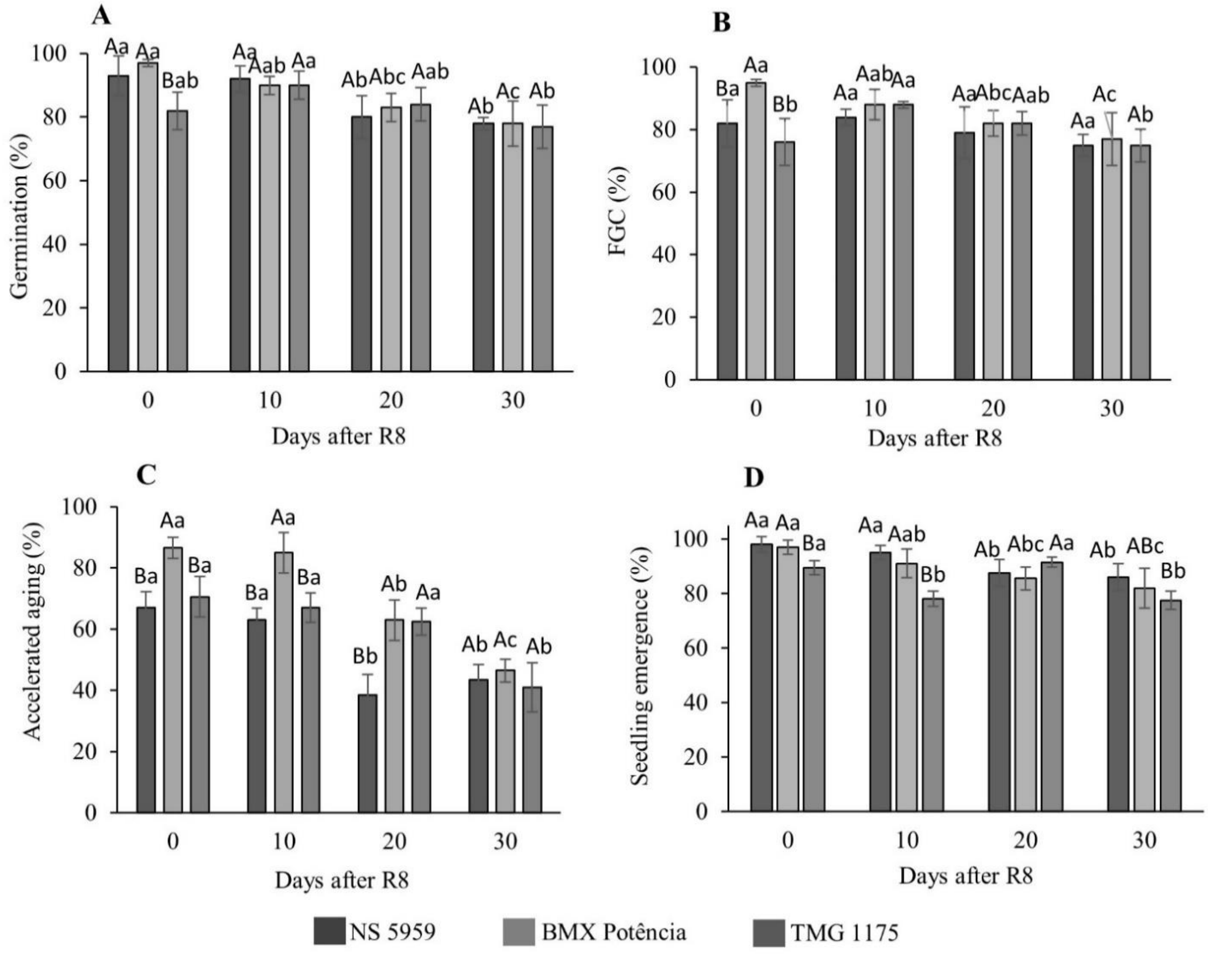

Figure 3. Germination (A), first germination count (FGC) (B), accelerated aging (C), and seedling emergence (D) of soybean seeds harvested at 0, 10, 20, and 30 days after R8. Uppercase letters compare cultivars at each harvest time and lowercase letters compare harvest times for each cultivar by Tukey's test at a 5% probability.

Importantly, the germination values obtained in the four harvest seasons were above 80%, which is the minimum standard established for seed commercialization in Brazil (MAPA, 2013). Zanatta et al. (2018) obtained similar results for the cultivars ATIVA RR and 5909 RG, which had germinations higher than 80% even with permanence in the field for 10 and 15 days after R8, respectively. The comparison of behavior among cultivars for germination showed significant differences for harvest at R8, with lower performance for TMG 1175 seeds compared to the others, which did not differ from each other. The plants of cultivar TMG 1175 reached the R8 stage on April 2 and 10, 2018 (Table 1) and were exposed to rainfall on April 3 and 6, 2018 (Figure 1), which may explain the lower seed quality of this cultivar relative to the others, reinforcing the influence of the environment on seed quality. The other harvest times showed no significant difference for seed germination among the three cultivars. Lima et al. (2007) observed no differences for germination among different soybean genotypes when harvested at R8, but this difference was significant at R8 + 15 and R8 + 30 days. Diniz et al. (2013) reported that a reduction in seed germination of eight soybean varieties was only observed at 30 days after R8.

The highest seed vigor, obtained by the accelerated aging test, was observed in the cultivar BMX Potência at R8 and R8 + 10 days compared to other times, being up to 40 pp higher. The seeds of the cultivar NS 5959 showed significant reductions in vigor through this test at R8 + 20 and R8 + 30 days. Moreover, the seeds of the cultivar TMG 1175 showed a significant reduction in vigor through the accelerated aging test only at R8 + 30 days. Delaying the harvest for 30 days did not allow observing differences in seed vigor among the three cultivars (Figure 3C). According to Diniz et al. (2013) and Zanatta et al. (2018), the delay in harvesting for 15 days after R8 was sufficient to differentiate soybean cultivars by the accelerated aging test.

In general, the seedling emergence test showed differences among cultivars with seeds harvested at R8, R8 + 10, and R8 + 30 days. The cultivars NS 5959 and BMX Potência showed similar behavior, with significant reductions at R8 + 20 and R8 + 30 days. However, the lowest percentages of normal seedlings by the emergence test were observed for the cultivar TMG 1175 (Figure 3D). In general, these results agree with those observed for the germination test (Figure 3A).

The tetrazolium test showed no difference in the viability among cultivars (data not shown due to non-significant interaction). However, regarding harvest times, higher viability was observed for seeds harvested at R8 and R8 + 10 days compared to R8 + 20 and R8 + 30 days, which did not differ from each other (Figure 4A). Vigor by the tetrazolium test (Figure 4B) did not differ among cultivars, except for harvest at 30 days after R8, with lower vigor for seeds of the cultivar TMG 1175 compared to the others. The comparison among harvest times showed, in general, a reduction in vigor from R8 + 20 days (around 25 pp), except for TMG 1175, in which the reduction was only observed at 30 days after R8 (Figure 4B).

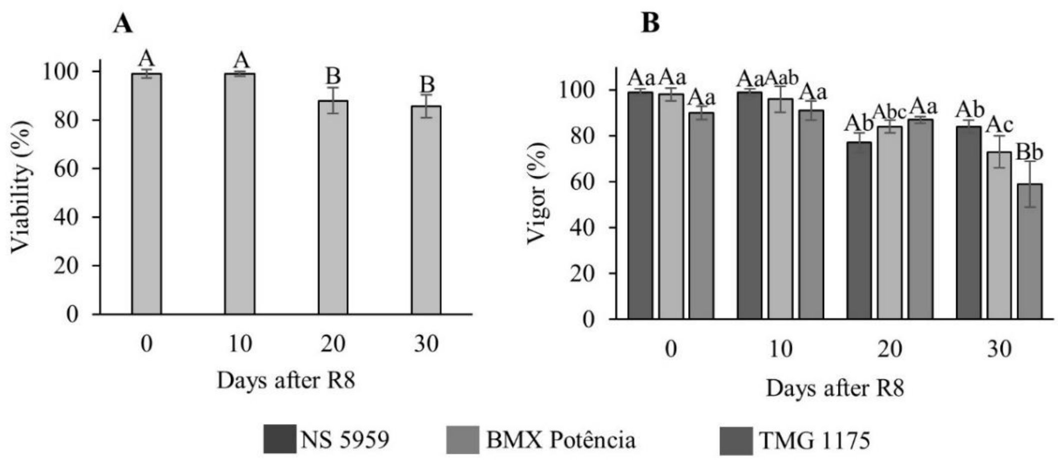

Figure 4. Viability (A) and vigor (B) by the tetrazolium test in soybean seeds harvested at 0, 10, 20, and 30 days after R8. Uppercase letters compare cultivars at each harvest time and lowercase letters compare harvest times for each cultivar by Tukey’s test at a 5% probability.

França and Krzyzanowski (2020) considered soybean seed lots with a value equal to or higher than 85% in classes 1–3 in the tetrazolium test as vigorous. In general, only seeds harvested at R8 and R8 + 10 days were above this value. Zuffo et al. (2017) and Vergara et al. (2019) found that viability and vigor by the tetrazolium test were negatively affected when harvesting was performed after R8. These authors also found that seeds harvested more than 10 days after R8 showed a predominance of weathering damage due to rainfall. Forti et al. (2013) and Pinheiro et al. (2021) evaluated weathering deterioration in soybean seeds and reported a direct relationship between weathering damage and reduced physiological seed quality.

The interaction between the factors genotype and harvest time was not significant when determining seed protein content and CAT activity, with no significant difference among cultivars (data not shown). Protein content was similarly reduced when seeds were subjected to delayed harvest (Figure 5A). It may be related to the seed deterioration process, in which there is an increase in the oxidation of proteins and an induction of loss of their functional properties (Rajjou & Debeaujon, 2008). More than 40 proteins sensitive to the deterioration process are reported in soybean seeds (Wang, Ma, Song, Shu, & Gu, 2012), which are mainly affected due to ROS accumulation (Min et al., 2017). Lower CAT activity was observed in seeds harvested at 30 days after R8 (Figure 5B). The delay in harvesting for 30 days was harmful to the physiological seed quality of the three evaluated cultivars, as shown by the vigor tests (Figures 3 and 4).

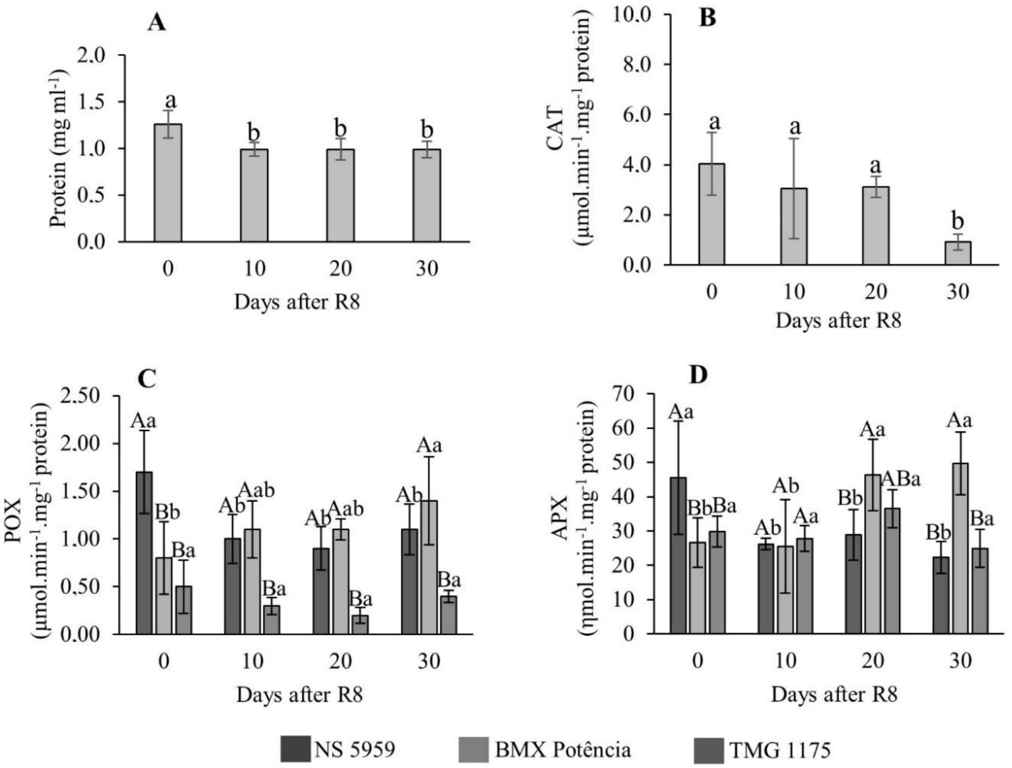

Figure 5. Protein content (A), catalase activity (CAT) (B), peroxidase activity (POX) (C), and ascorbate peroxidase activity (APX) (D) in soybean seeds harvested at 0, 10, 20, and 30 days after R8. Means followed by the same letter do not differ from each other by Tukey's test at a 5% probability.

The POX activity in seeds of the cultivar TMG 1175 was similar to that of the cultivar BMX Potência at R8, but lower in harvests carried out at R8 + 10, R8 + 20, and R8 + 30 days relative to the other cultivars (Figure 5C). The comparison among harvest times for each cultivar showed a reduction in POX activity for NS 5959 with the delayed harvest from R8, while BMX Potência had an increase in the activity at R8 compared to R8 + 30 days. In general, BMX Potência seeds had less vigor reduction with the delayed harvest. No significant difference was found among harvest times for TMG 1175 for both POX (Figure 5C) and APX (Figure 5D) activities.

APX activity was higher in seeds of the cultivar NS 5959 at R8 and BMX Potência at R8 + 20 and R8 + 30 days. Among harvest times, NS 5959 had a decrease in the activity of this enzyme in harvests performed after R8. The cultivar BMX Potência showed higher activity in seeds harvested at R8 + 20 and R8 + 30 days relative to the others. NS 5959 had a decrease in the activity of this enzyme in harvests performed after R8 (Figure 5D). No significant difference was found among harvest times of the cultivar TMG 1175 for APX activity. Similarly, deteriorated sunflower seeds also showed a significant reduction in the activity of antioxidant enzymes (Bailly et al., 2004). The increase in enzyme activity is mainly related to the antioxidant defense mechanism and reduction in the excessive increase in seed respiratory rates and excessive ROS accumulation, which cause enzyme inhibition and other deleterious effects (Kurek et al., 2019) and contribute to reducing the intensity of seed deterioration.

The multivariate principal component analysis (PCA) explained 73.5% of the total variability of the data through two components (PC1 and PC2) and helped to summarize the previous results. The blue and red vectors in the biplot represent the physiological and biochemical variables, respectively. The concentration of treatments R8 + 30 days of all cultivars can be observed in the negative scores of principal component 2 (PC2-), as opposed to the vectors of physiological attributes and catalase (CAT) (Figure 6). These observations reinforce the effect of delayed harvest, especially for 30 days, on reducing the physiological quality of soybean seeds due to their higher exposure to the environment. On the other hand, treatments in which there was no delayed harvest (R8) and a delay for 10 days (R8 + 10 days) were concentrated closer to the physiological quality vectors, reinforcing the benefits of harvesting as close as possible to R8.

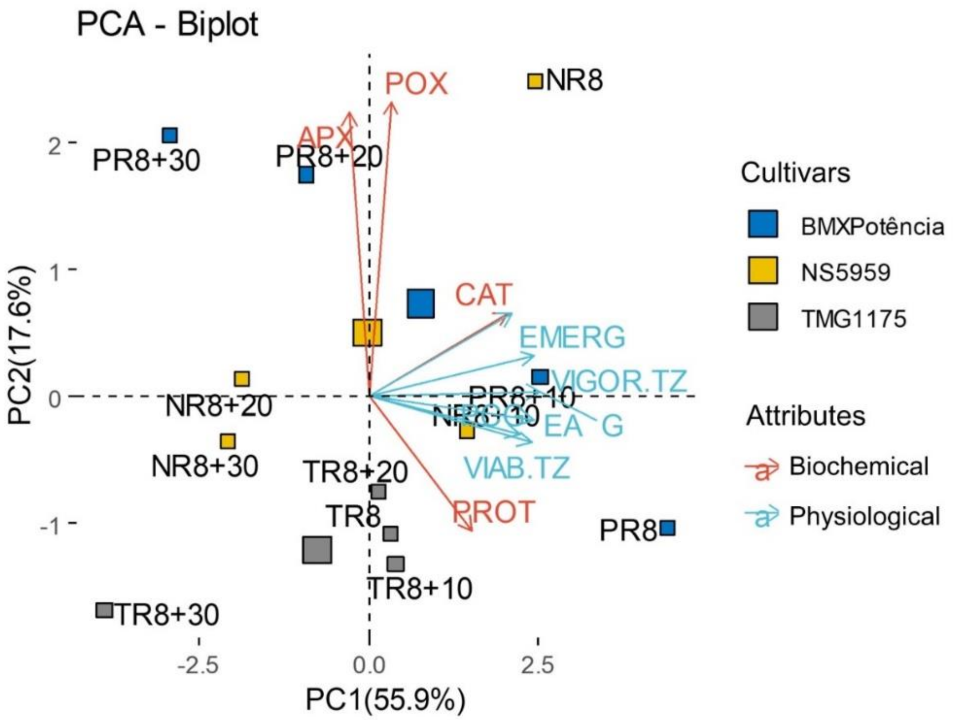

Figure 6. Biplot obtained by the linear combination of variables related to the physiological and biochemical characteristics of soybean seeds harvested at 0, 10, 20, and 30 days after R8. Germination (G), first germination count (FGC), accelerated aging (AA), seedling emergence (EMERG), viability by the tetrazolium test (VIAB.TZ), vigor by the tetrazolium test (VIGOR.TZ), protein content (PROT), catalase (CAT), peroxidase (POX), and ascorbate peroxidase (APX).

Among the analyzed cultivars, a higher seed quality was observed for the cultivar BMX Potência (except for R8 + 30 days) due to the higher proximity to the physiological quality vectors. On the other hand, treatments related to the cultivar TMG 1775 were concentrated further away from these vectors, reinforcing the most significant reduction in their seed quality due to delayed harvest. In general, the cultivar NS 5959 showed an intermediate behavior compared to the cultivar BMX Potência and TMG 1175 (Figure 6).

Genotype has a significant influence on soybean seed responses to deterioration (Shu et al., 2020), but humidity and temperature conditions are also preponderant for these responses. In this context, seeds of all cultivars were exposed to rainfall at the R8 stage and variations in relative humidity and temperature during the harvest time. Thus, environmental conditions may be related to the better performance of the seeds of the cultivar BMX Potência, which were the least exposed to these conditions at the R8 stage relative to the others (Table 1 and Figure 1). The lower physiological seed quality of the cultivar TMG 1175 may be related not only to genetic factors but also to the fact that it is a late material, and its seeds were exposed to the cultivation environment for a longer time.

Conclusions

Soybean seed deterioration in the field due to delayed harvest causes a decrease in germination and vigor of soybean seeds, especially when harvested at R8 + 20 and R8 + 30 days. The seeds of the cultivars did not differ in terms of viability under delayed harvest but differed in terms of antioxidant and physiological responses.

Seeds of the cultivars BMX Potência and TMG 1175 had the best and worst performance, respectively, at the R8 stage and R8 + 10 days.

Acknowledgments

To the National Council for Scientific and Technological Development (CNPq), the Coordination for the Improvement of Higher Education Personnel (CAPES, Financing Code 001), and the Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) for financial support.

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