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Impact of harvest delay on the physiological and sanitary quality of *Sorghum* sp. seeds[1]

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ABSTRACT - Harvest delay can alter the physiological and sanitary quality of sorghum seeds due to the longer exposure time of seeds to adverse environmental conditions in the field. In this study, we investigated the impact of delayed *Sorghum* sp. harvest on the physiological and sanitary quality of seeds in four hybrids grown in the tropical soil of the Brazilian Cerrado region. Plants from four *Sorghum* sp. hybrids (IPA SUDAN 4202, BRS 658, BRS 810, and BRS 373) were grown and submitted to four seed harvest periods (0, 7, 14, and 21 days after stage 9 – seed physiological maturity). The treatments were arranged in a completely randomized block design in a 4 × 4 factorial, with four replicates. At each harvest period, the thousand-seed weight, water content, first count of the germination test, germination, seedling emergence, emergence speed index, seedling dry matter, accelerated aging, electrical conductivity, and sanitary quality of seeds were determined. Seeds of higher physiological and sanitary quality of the IPA SUDAN 4202 and BRS 658 sorghum hybrids can be obtained when the harvest is carried out up to 7 days after the physiological maturity stage. However, the delay in seed harvesting at 14 and 21 days after physiological maturity results in a higher incidence of *Colletotrichum* sp., *Curvularia* sp., *Rhizoctonia* sp., and *Alternaria* sp. on the seeds of these hybrids. Delaying the harvest of sorghum seeds from 7 days after the physiological maturity stage compromises seed vigor and viability and increases the incidence of pathogens.

Key words: Seed vigor. Seed viability. *Sorghum bicolor. Sorghum sudanense*.

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INTRODUCTION

Sorghum [*Sorghum bicolor* (L.) Moench.] and sudan grass (*Sorghum sudanense* (Piper.) Stapf.) are two of the most important cereal crops in the world, especially due to their multiple uses in human food, animal feed, forage production, ethanol production, and energy generation from biomass burning (ABDELHALIM *et al*., 2019; ESPITIA-HERNÁNDEZ *et al.*, 2022). The crop is a C4 species with high photosynthetic capacity and a high potential for grain and biomass production (BORÉM *et al*., 2014; MENEZES, 2021). Its high degree of tolerance to drought and high temperatures and its adaptation to low-fertility soils make it an alternative for cultivation in the tropical and semiarid regions of the Brazilian Cerrado (CARMO *et al.*, 2020; OLIVEIRA *et al.*, 2020; SODRÉ-FILHO *et al*., 2021). However, seed quality is a key factor for the expansion of *Sorghum* sp. cultivation areas in tropical and semiarid regions of Brazil.

The sorghum seed production process is important for obtaining high physical, physiological, and sanitary genetic material. Among the factors that can affect seed quality, harvest delay is one of the most important. According to Zuffo *et al*. (2017), such a delay can result in quantitative and qualitative losses, resulting in seeds with lower germination potential and a higher incidence of pathogens. Sorghum seeds reach their maximum dry matter accumulation and maximum physiological quality at stage 9 (physiological maturity) (MENEZES, 2021) when they must be harvested. However, at this stage of crop development, seed moisture content typically varies between 25% to 35% (ALMEIDA *et al*., 2016; MENEZES, 2021), depending on the genotype and growing conditions, which has often made mechanized harvesting unfeasible due to the high seed moisture content. In general, mechanized sorghum harvesting has been carried out when the seeds have moisture between 18% and 20% (BORÉM *et al*., 2014) to avoid mechanical damage to the seeds.

In some situations, however, the ideal harvesting period has been exceeded due to extensive cultivation areas and insufficient harvesters or high rainfall rates during the harvesting season (ZUFFO *et al*., 2017). When exposed to long periods in the field, the seeds are subject to adverse conditions, such as climate variations, insects, and pathogens (CASTRO *et al*., 2016), which can compromise their vigor and physiological quality.

Delaying soybean harvest from 10 days after physiological maturity resulted in lower vigor, lower viability, and higher incidence of pathogens in seeds Zuffo *et al*. (2017). Similarly, Scariot *et al*. (2017) and Vergara *et al*. (2019) observed that the vigor and viability of wheat and soybean seeds were progressively reduced due to harvest delay.

In general, the harvest delay related to the variation in relative air humidity (humid and dry) can cause cracks and wrinkles in the seed coat, which aggravates the seed deterioration process due to the greater ease of penetration of pathogens and greater exposure from the embryonic tissue to the environment (MARCANDALLI *et al*., 2011). According to Ali *et al*. (2018), the loss of viability and vigor of crop seeds caused by the delay in harvesting is directly associated with increased pathogen infection, especially *Phomopsis* sp.

This study investigated the impact of delayed *Sorghum* sp. harvest on the physiological and sanitary quality of seeds in four hybrids.

MATERIAL AND METHODS

The field experiment was conducted in the agricultural experimental area of the State University of Mato Grosso do Sul, in Balsas, Maranhão, Brazil (07°31’59” S, 46°02’06” W with an altitude of 283 m), during the 2022 growing season. The region has a tropical savanna climate (Aw), according to the Köppen–Geiger climate classification, with hot and humid summers and dry winters, with a dry season between May and October. The average annual temperature is 27.1 °C, and the annual rainfall is 1.1750 mm (PASSOS *et al.*, 2017). The climatic conditions recorded during the experiment are shown in Figure 1. The total rainfall during the sorghum growing season was 490 mm.

The soil of the agricultural experimental area was classified as Rhodic Hapludox (SOIL SURVEY STAFF, 2014) or “Latossolo Vermelho-Amarelo” in the Brazilian soil classification (SANTOS *et al*., 2018). The occurrence of Rhodic Hapludox in the southern region of Maranhão state is common, and this class of soil has no restrictions concerning agricultural use and management (SANTOS *et al*., 2018). Before starting the experiments, soil samples were collected in the 0-0.20 m layer, and the main chemical properties of the soil are shown in Table 1. Limestone (CaO 42%, MgO 4%, and effective calcium carbonate equivalent 97%) was applied at a 4.0 Mg ha^{-1} rate to raise the soil base saturation to 60%. After liming, the field soil was plowed and harrowed to a depth of 0.20 m and divided into plots before sorghum sowing. The sorghum hybrids were sown 60 days after liming.

The experiment was arranged in a completely randomized block design in a 4 × 4 factorial, with four replicates. Treatments included four *Sorghum* sp. hybrids [IPA SUDAN 4202, BRS 658, BRS 810, and BRS 373] and four seed harvest periods (0, 7, 14, and 21 days after stage 9 – seed physiological maturity). Some of the characteristics and agricultural uses of the *Sorghum* sp. hybrids used in this study are shown in Table 2.

Figure 1 - Monthly total rainfall (bars) and monthly average temperature (lines) in Balsas, Maranhão, Brazil, during sorghum cropping in the 2022 season and 30-yr historical average data (1990 to 2020). Data were accessed through the Brazilian National Meteorological Institute database

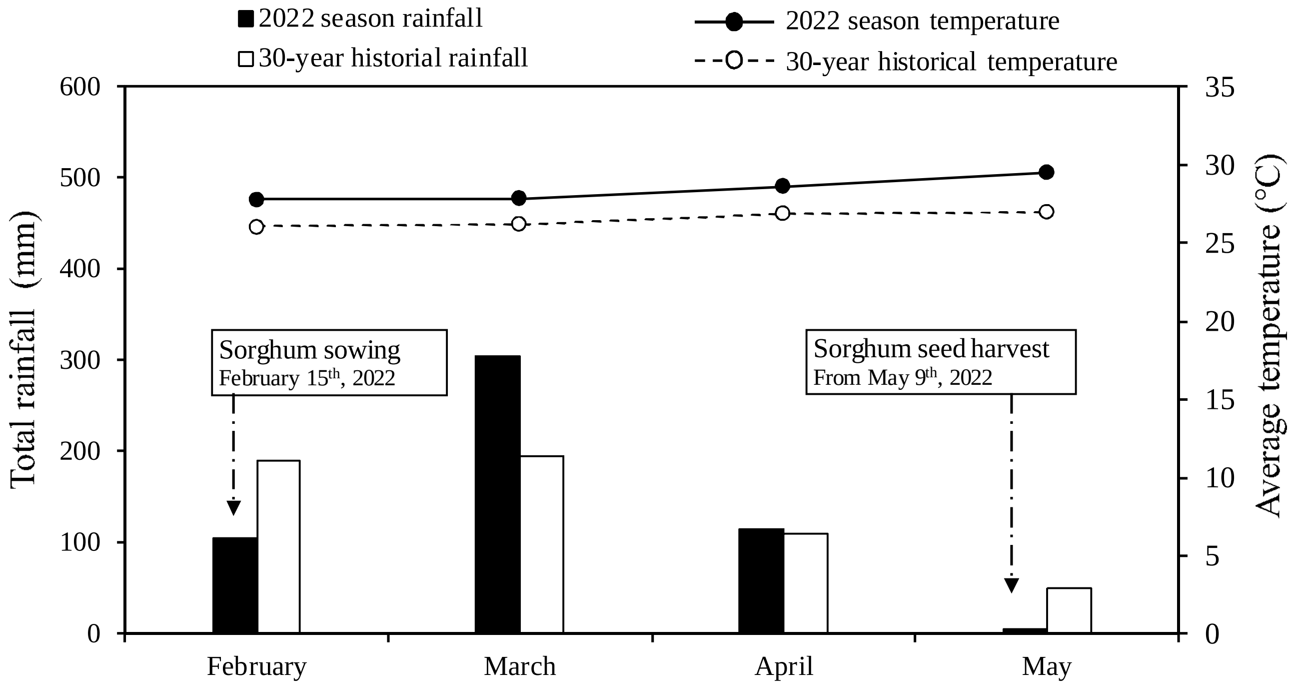


Table 1 - Soil chemical properties in the agricultural experimental area subjected to the cultivation of *Sorghum* sp. hybrids

pH	$P_{Mehlich-1}$	Al^{3+}	Ca^{2+}	Mg^{2+}	K^{+}	CEC	V
H_2O	mg dm^{-3}		------------------ cmol$_c$ dm^{-3} --------------------				%
4.6	3.0	1.0	0.5	0.3	0.1	7.9	11.4

CEC: cation exchange capacity at pH 7.0. V: Soil base saturation

Table 2 - Characteristics and agricultural aptitude of the four sorghum hybrids used in this study

Sorghum cultivar species	Common name	Hybrid	Aptitude or agricultural use
Sorghum sudanense (Piper) Stapf.	Sudan grass	IPA SUDAN 4202	Biomass/Cutting/Grazing
Sorghum bicolor (L.) Moench.	Forage sorghum	BRS 658	Silage
Sorghum bicolor (L.) Moench. × *Sorghum sudanense* (Piper) Stapf	Forage sorghum	BRS 810	Cutting/Grazing
Sorghum bicolor (L.) Moench.	Grain sorghum	BRS 373	Grain production

Source: Menezes (2021)

Seeds of four sorghum hybrids, two commercial hybrids of *Sorghum bicolor* (L.) Moench. (BRS 658 and BRS 373), one commercial hybrid of *Sorghum sudanense* (Piper) Stapf. (IPA SUDAN 4202), and a commercial hybrid from the crossing of *Sorghum bicolor* (L.) Moench. and *Sorghum sudanense* (Piper) Stapf. (BRS 810) were manually sown on 15 May 2022 at a rate of 12 seeds m^{-1} under row spacings of 0.50 m. Fertilization was performed by applying 300 kg ha^{-1} of 04-14-08 NPK formulation at the sowing furrow, according to technical recommendations for sorghum crops (BORÉM *et al*., 2014). The experimental units consisted of four 3.0 m-long sorghum rows, with 0.50-m between-row spacing, and the useful area was the two central rows of each plot, disregarding 0.5 m of each edge.

During the sorghum growing season, the control and management of weeds, pests, and diseases were carried out by applying chemical products recommended for the sorghum crop, as described by Borém *et al.* (2014). All the chemicals used in the present investigation were of analytical grade.

The sorghum harvest was carried out manually at four different periods: Stage 9 (when seeds were at physiological maturity) and 7, 14, and 21 days after this stage (MENEZES, 2021). After harvesting, the seeds were manually threshed, and then the thousand-seed weight and water content were determined as described in the seed analysis rules (BRASIL, 2009). Afterward, the seeds were subjected to the following physiological and sanitary tests:

Germination test: four replicates of 50 sorghum seeds were distributed between three sheets of germitest paper towel, previously moistened with a volume of distilled water equivalent to 2.5 times the dry mass of the substrate. The sheets were then turned into rolls and packaged into plastic bags to prevent evaporation and maintain a relative humidity close to 100%. Afterward, the rolls were taken to a germination chamber with alternating temperatures of 20 °C (night) and 30 °C (day). Germinated seeds were recorded at five days (first germination test count) and ten days (total germination percentage) after starting the test (BRASIL, 2009).

Emergence test: seedling emergence was carried out in plastic trays (42 cm × 28 cm × 6 cm) filled with the commercial substrate ‘Carolina Soil’ composed of peat and vermiculite in the proportion of 7:3 (v:v), using four replicates of 50 seeds. The moisture content of the substrate was maintained at 70% of the field capacity with daily irrigation. The trays were maintained under greenhouse conditions at a temperature of 30 °C. The count of emerged seedlings was performed daily until emergence stabilization (14 days). The percent seedling emergence (E) and emergence speed index (ESI) were calculated at 14 days, as described by Maguire (1962).

Accelerated aging: sorghum seeds were placed on a stainless-steel screen inside plastic boxes (11.0 cm × 11.0 cm × 3.0 cm) containing 40 mL of distilled water (KRZYZANOWSKI *et al*., 1999). The plastic boxes were kept in a BOD incubation chamber at 41 °C for 96 h. After this period, four replicates of 50 seeds for each treatment were submitted to the germination test, as previously described above. The evaluation was assessed on the 5 th day after sowing, and the percentage of normal seedlings was recorded (BRASIL, 2009).

Electrical conductivity: four replicates of 50 sorghum seeds were placed in 300 mL plastic cups and weighed on an analytical scale (0.001 g accuracy). Then, 75 mL of distilled water was added to each container. The containers were placed in a BOD incubator at a constant temperature of 25 °C for 24 h (KRZYZANOWSKI *et al*., 1999). After this period, the seeds were gently agitated for homogenization of the solution, and the electrical conductivity was measured using a conductivity meter (MS TECNOPON® - mCA150).

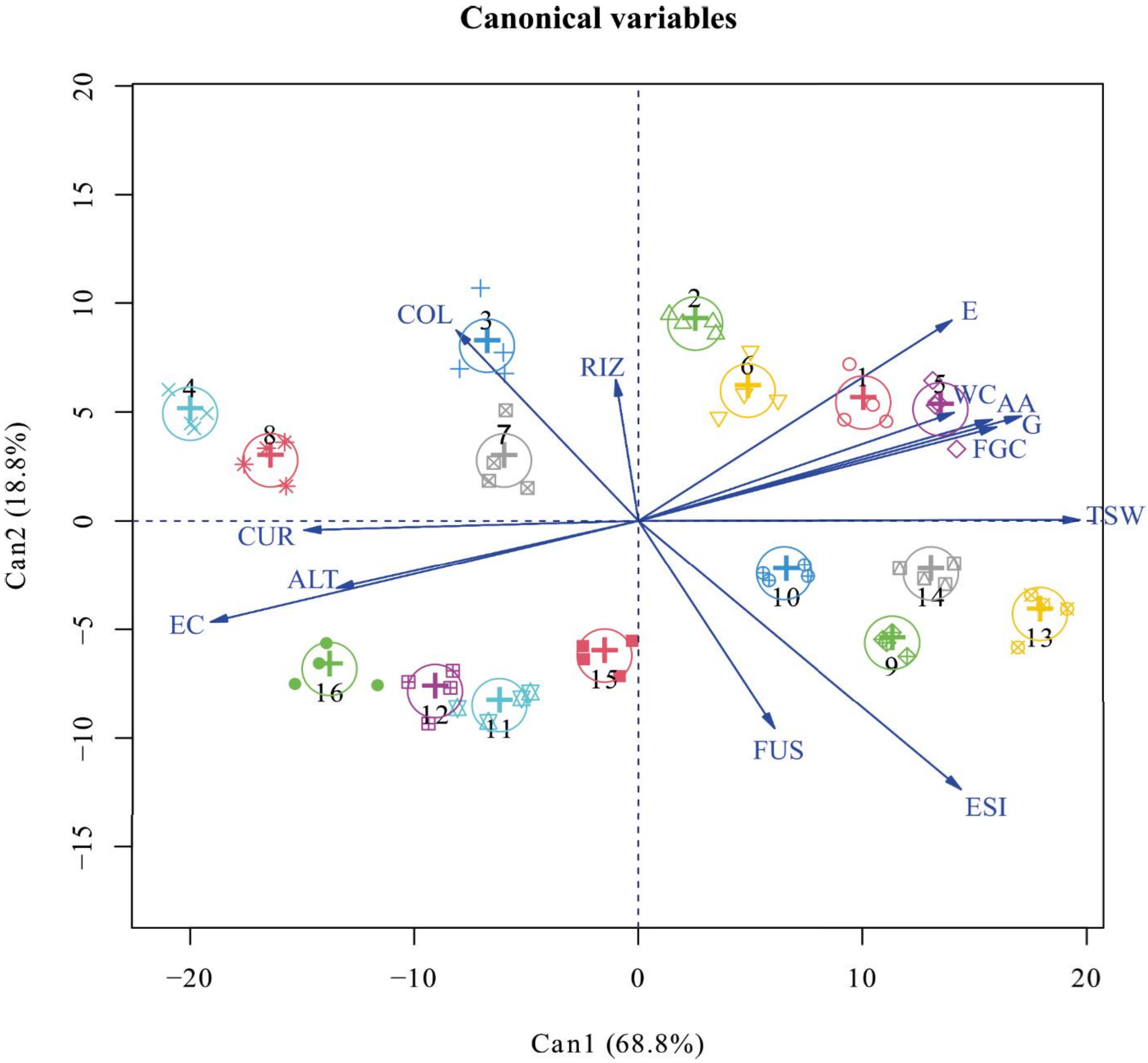
Seed health: the sanity test was performed using the Blotter-test method, with five replicates of 40 seeds. The plates were kept in an incubation room at 20 °C and a photoperiod of 12 h for 7 days (BRASIL, 2009). The evaluation and identification of the presence of pathogens associated with seeds were performed as described by Barnett and Hunter (1998). The morphological structures of the pathogenic fungi were identified using an optical microscope.

Canonical correlation analysis (CCA) was used to study the interrelationships between sets (vectors) of independent variables (sorghum hybrids and seed harvest period) and dependent variables (seed quality characteristics). These analyses were performed using Rbio software version 140 for Windows (Rbio Software, UFV, Viçosa, MG, Brazil). Statistical correlations based on Pearson’s correlation networks (threshold set at 0.60, $p < 0.05$) were performed between the seeds’ physiological and sanitary quality characteristics for the four sorghum hybrids harvested at different times. A correlation network was used to graphically illustrate Pearson’s correlation analyses, in which the proximity between the nodes is proportional to the absolute correlation values between the seed quality characteristics. The bands’ relative thickness and color density indicate the strength of Pearson’s correlation coefficients, and the color of each band indicates a positive or negative correlation (red for negative and green for positive).

RESULTS AND DISCUSSION

Canonical correlation analysis was used to verify the contribution of each dependent variable related to the seed’s physiological and sanitary quality characteristics and how these characteristics were affected by the sorghum hybrids and delay periods in seed harvest (Figure 2). For the scores to be represented in a two-dimensional graph, the percentage of variance retained must be greater than 80% (MINGOTI, 2005). In this study, the accumulated variances in the two main canonical variables were 87.6% (Figure 2), allowing a precise interpretation.

Figure 2 - Canonical correlation analysis (CCA) between the seed physiological and sanitary quality characteristics of sorghum hybrids harvested at different periods after physiological maturity. The blue lines show the canonical correlation between the centroids of the first pair of canonical variates and the linear tendency line



Abbreviations: TSW: thousand-seed weight; WC: water content; E: seedling emergence; ESI: emergence speed index; FGC: first germination test count; G: seed germination; AA: accelerated aging; EC: electrical conductivity; ALT: *Alternaria* sp.; COL: *Colletotrichum* sp.; CUR: *Curvularia* sp.; FUS: *Fusarium* sp.; RIZ: *Rhizoctonia* sp. 1, 2, 3 and 4: IPA SUDAN 4202 hybrid seeds harvested at 0, 7, 14 and 21 days after physiological maturity, respectively. 5, 6, 7, and 8: BRS 658 hybrid seeds harvested at 0, 7, 14, and 21 days after physiological maturity, respectively. 9, 10, 11, and 12: BRS 810 hybrid seeds harvested at 0, 7, 14, and 21 days after physiological maturity, respectively. 13, 14, 15, and 16: BRS 373 hybrid seeds harvested at 0, 7, 14, and 21 days after physiological maturity, respectively

An angle (between vectors) of less than 90° indicates a positive correlation between the dependent variables (seed quality characteristics) and independent variables (sorghum hybrids and seed harvest period). The seeds of the sorghum hybrids IPA SUDAN 4202 and BRS 658 harvested at 0 and 7 days after physiological maturity had higher thousand-seed weight, water content, percentage of seedling emergence, accelerated aging, first germination count, and final germination (Figure 2). Therefore, the seeds of the IPA SUDAN 4202 and BRS 658 hybrids harvested with up to 7 days of delay have a greater thousand-seed weight. This greater amount of seed reserve improved seed physiological quality and initial seedling growth. The higher physiological quality of the seeds of these two sorghum hybrids may be related to their intrinsic characteristics, genetic composition, and adaptation to the edaphoclimatic conditions of the growing region. Maximum seed quality is reached at the physiological maturity stage with greater dry matter (reserves) accumulation, vigor, and germination potential (CARVALHO; NAKAGAWA, 2012). However, the prolonged permanence of seeds in the field after physiological maturity causes deterioration and even loss of seed vigor (ZUFFO *et al*., 2017; SCARIOT *et al*., 2017; VERGARA *et al*., 2019; ZUFFO *et al*., 2020).

A greater emergence speed index and greater incidence of the pathogen *Fusarium* sp. were observed in the seeds of BRS 810 and BRS 373 hybrids harvested at 0 and 7 days after physiological maturity (Figure 2). According to Souza *et al*. (2007), *Fusarium* sp. can produce mycotoxins responsible for reducing the seed germination potential, especially for causing several biochemical transformations in the seeds. In addition, this fungus can cause wilting, rotting, and damping off of seedlings.

The delayed harvest of the IPA SUDAN 4202 and BRS 658 hybrids by 14 and 21 days after the physiological maturity of the seeds resulted in a higher incidence of the pathogens *Colletotrichum* sp., *Curvularia* sp. and *Rhizoctonia* sp. (Figure 2). Flávio *et al*. (2014) also reported a high incidence rate of fungi of the genus *Penicillium* sp. (12.8%), *Curvularia* sp. (42%), *Fusarium* sp. (29.2%) and *Aspergillus* sp. (14.3%) on the seeds of the sorghum hybrid BR 310. Similar results were shown by Pinto (2004), who reported that the main fungi affecting sorghum seed sanitary quality are *Penicillium* sp., *Aspergillus* sp., *Rhizopus* spp., A*lternari*a sp., *Curvularia* sp., and *Fusarium* sp. The incidence of these fungi on sorghum seeds can cause qualitative and quantitative losses, reducing the physiological quality, size, and seed reserve quantity (ZUFFO *et al*., 2017).

The delay in seed harvest of the IPA SUDAN 4202 and BRS 658 hybrids by 14 and 21 days after physiological maturity resulted in a higher incidence of *Alternaria* sp. and a higher electrical conductivity value. A high incidence rate (45.5%) of *Alternaria* sp. was reported in wheat seeds by Danelli *et al.* (2012); however, this high incidence of *Alternaria* sp. did not interfere with the germination of wheat seeds. This fungus is easy to identify due to its conidial morphology and characteristics belonging to its colonies (CASA *et al*., 2012).

The delay in harvesting sorghum seeds for 14 and 21 days after physiological maturity may have caused damage to cell membranes and structures, as reported by Zuffo *et al*. (2017), Scariot *et al.* (2017), and Zuffo *et al.* (2020). This damage caused to cell membranes facilitates the contact of the enzyme polyphenoloxidase, present only in plastids, with phenolic compounds stored in vacuoles, promoting the oxidation of phenols that are transformed into quinones and can react with proteins (ALMEIDA *et al*., 2016). In addition, it should be noted that fungi belonging to the genera *Fusarium* sp., *Colletotrichum* sp., and *Alternaria* sp. are known as soil fungi. Their occurrence is accentuated when the seeds remain in the field for long periods after physiological maturity and are exposed to the oscillations of the hot and humid environment (CARVALHO; NAKAGAWA, 2012; CASTRO *et al*., 2016).

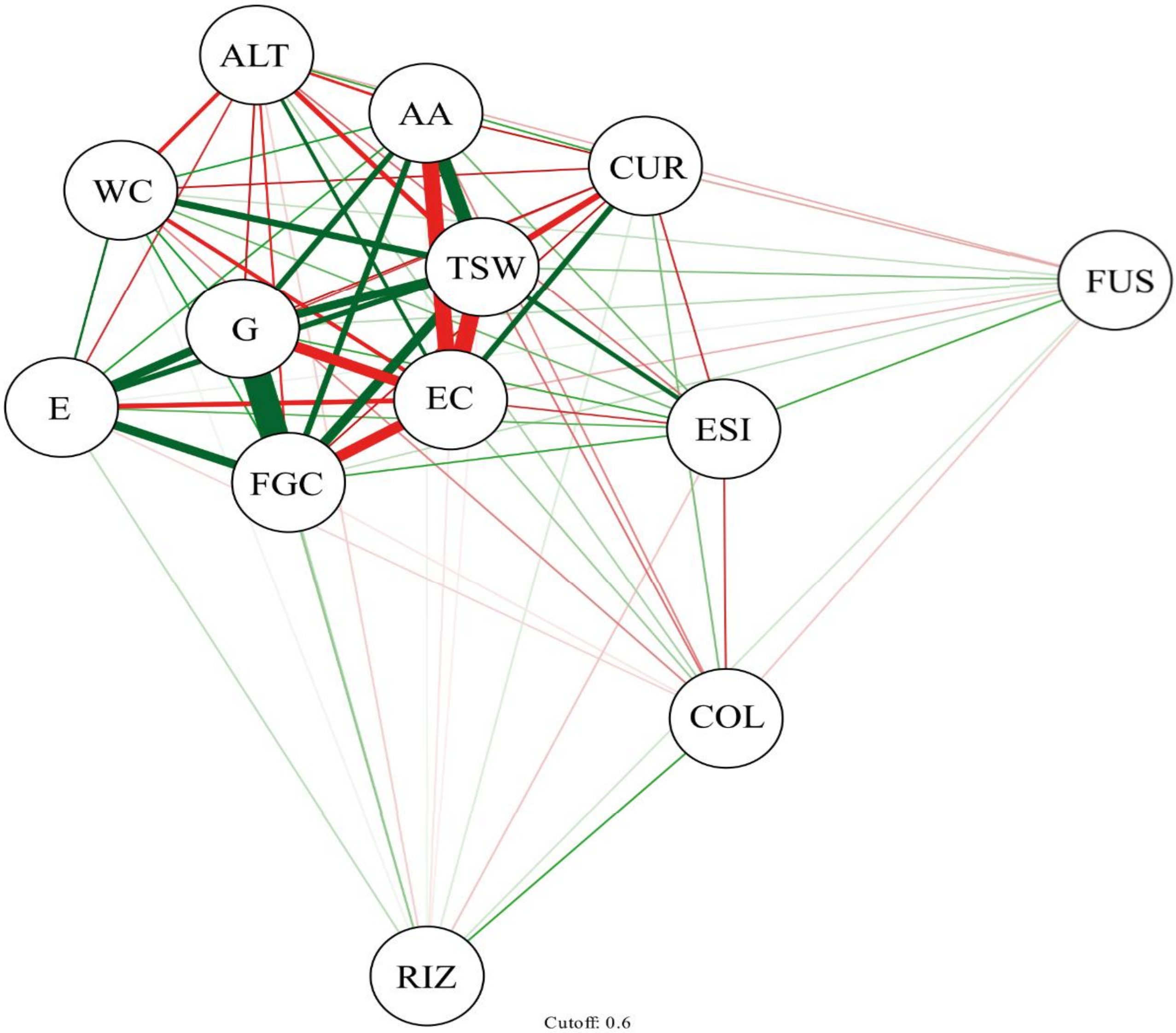
Based on all sorghum seed physiological and sanitary quality characteristics and their respective correlations, a network diagram was constructed (Figure 3). All interactions between seed quality characteristics can be observed from the correlation network. Pearson's linear correlation coefficient was used to determine the most adequate characteristics to represent the physiological and sanitary quality of sorghum seeds harvested at different times after physiological maturity (Figure 3). Correlation analysis reported a positive and highly significant association between the first germination count (FGC) and final germination (G), seedling emergence (SE), accelerated aging (AA), and thousand-seed weight (TSW); between the thousand-seed weight and the water content (WC) and emergence speed index (ESI); and between the presence of *Curvularia* sp. and electrical conductivity (EC).

The positive correlation between the first germination count and the final germination can be explained because the greater number of seeds germinated on the 5th day resulted in a greater percentage of final germination at 12 days. The accelerated aging process can facilitate the seed germination process since it influences the consumption of some substances that can act in the seed dormancy process, such as the presence of tannins in sorghum seeds. Furthermore, the occurrence of dormancy in sorghum seeds is higher when the seeds are not subjected to drying (ALMEIDA *et al*., 2016). The accelerated aging process can also facilitate seed imbibition, resulting in increased seed mass and water content. In turn, this increase in seed water content can provide favorable conditions for the occurrence of pathogens such as *Curvularia* spp. (ULLMANN *et al*., 2015) and result in higher values of electrical conductivity of the seeds.

Negative and significant correlations were detected between accelerated aging (AA) and electrical conductivity (EC); between the thousand-seed weight (TSW) and the incidence of *Curvularia* sp. and electrical conductivity (EC); between electrical conductivity (EC) and first germination count (FGC), final germination (G) and water content (WC); and between the thousand-seed weight (TMW) and the incidence of *Alternaria* sp. (Figure 3).

The electrical conductivity test is capable of measuring the degree of deterioration of the seed cellular structures and membranes through the exudation of electrolytes from the seeds into the solution (distilled and deionized water) in which the seeds are immersed (KRZYZANOWSKI *et al*., 1999). This explains the existence of a negative correlation between electrical conductivity and the first germination count, final germination, and water content since the highest electrical conductivity values are obtained in seeds that have suffered disturbances in their structure or cell membranes, which impairs the seed germination process.

Figure 3 - Correlation networks illustrate the most significant Pearson correlations between physiological and sanitary quality characteristics of seeds of sorghum hybrids harvested at different periods after physiological maturity. Thicker green lines represent the highest positive correlations (threshold set at 0.6 and p values < 0.05). Thicker and red lines represent the highest negative correlations (threshold set at 0.6 and p values < 0.05)



Abbreviations: TSW: thousand-seed weight; WC: water content; E: seedling emergence; ESI: emergence speed index; FGC: first germination test count; G: seed germination; AA: accelerated aging; EC: electrical conductivity; ALT: *Alternaria* sp.; COL: *Colletotrichum* sp.; CUR: *Curvularia* sp.; FUS: *Fusarium* sp.; and RIZ: *Rhizoctonia* sp

CONCLUSIONS

1. Seeds of higher physiological and sanitary quality of the IPA SUDAN 4202 and BRS 658 sorghum hybrids can be obtained when the harvest is carried out up to 7 days after the physiological maturity stage. However, the delay in seed harvesting at 14 and 21 days after physiological maturity results in a higher incidence of *Colletotrichum* sp., *Curvularia* sp., *Rhizoctonia* sp., and *Alternaria* sp. on the seeds of these hybrids;
2. The seeds of the BRS 810 and BRS 373 sorghum hybrids harvested up to 7 days after the physiological maturity stage had a higher emergence speed index and a higher incidence of *Fusarium* sp;
3. The delay in harvesting sorghum seeds from 7 days after the physiological maturity stage compromises the vigor and viability of the seeds, in addition to increasing the incidence of pathogens.

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