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Physiological and sanitary quality of soybean seeds in response to harvest delay[1]

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ABSTRACT - Delays in soybean harvesting can limit seed viability and vigor. In this study, soybean cultivar seeds' physiological and sanitary quality was evaluated in response to harvest delay. The experimental design used was completely randomized blocks, arranged in a split-plot scheme, with three replicates. The plots were represented by the cultivation of two soybean cultivars (BMX Bônus IPRO and BMX Olimpo IPRO), while the subplots were represented by three harvest times [0, 7, 14 days after the seed's full maturity (stage R_8)]. The following characteristics of the physiological and sanitary quality of the seeds were measured: water content, plant emergence, total dry matter, germination, accelerated aging, electrical conductivity, and seed health. The delay in the soybean harvest affects the seeds' physiological and sanitary quality. Seeds harvested seven days after full maturity have a higher incidence of *Aspergillus flavus* and *Macrophomina* sp. In comparison, seeds harvested 14 days after full maturity have a higher incidence of *Phomopsis* sp. and *Fusarium* sp.

Key words: Germination. Pathogens. Seed vigor. Seed viability.

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INTRODUCTION

Soybean [*Glycine max* (L.) Merrill.] is the agricultural crop that has most expanded its cultivation area in Brazil in recent decades. The state of Maranhão, the second largest soybean producer in the Northeast region of Brazil, saw a 71% increase in soybean agricultural production, cultivating 1.25 million hectares in the 2023/2024 season (Companhia Nacional de Abastecimento, 2023), and has been driven by the combination of a series of factors, including the use of new technologies, improved crop management, the genetic potential of the cultivar, and the use of seeds with physiological and sanitary quality. High-quality seeds are essential for obtaining an appropriate stand of soybean plants in the field. Therefore, the use of high-quality seeds is crucial to obtaining high grain yields and being successful in agricultural production (França-Neto; Krzyzanowski, 2019).

Seed vigor and viability are considered the main characteristics of physiological quality. These characteristics depend on environmental conditions during the seed formation phases in agricultural production fields. Under unfavorable environmental and field conditions, lower seed vigor and viability can have a direct negative impact on the uniformity of the germination rate, emergence, and initial growth of plants (Peske; Barros; Schuch, 2019). Therefore, seed vigor and viability are directly related to changes in water content, enzymatic activity, mobilization of nutrients, and reserve substances (Chen *et al*., 2020, 2021; Zhu *et al*., 2016).

The fungi *Aspergillus* sp., *Penicillium* sp., and *Cercospora kikuchii* are pathogens with high incidence in soybean seeds. The occurrence of these fungi can occur from the field to storage, especially when environmental humidity conditions are compromised, and storage time is prolonged. Therefore, the use of certified seeds with high sanitary and physiological quality can increase soybean grain yield. Therefore, the use of certified seeds with high sanitary and physiological quality can increase the yield of soybean crops, especially because these seeds are not sources of pathogen inoculum for soybean cultivation (Tonello *et al*., 2019). However, the physiological and sanitary quality of soybean seeds can be affected by delays in harvesting (Zuffo *et al*., 2017a, b).

Late harvesting of soybean crops has negative impacts on the initial physiological quality of the seeds and can cause damage associated with changes in moisture content and loss of sanitary quality of the seeds during the storage period (Vergara *et al*., 2019). Campos *et al.* (2023) state that delaying soybean harvesting for more than 20 days can limit seed quality. Zuffo *et al*. (2017a) reported that when soybeans are harvested after the R_8 stage, there is an increase in damage caused by stink bugs and fluctuations in moisture content. These authors also concluded that delaying soybean harvesting for more than 10 days after the R_8 stage results in lower seed vigor, while delaying it for more than 15 days results in lower seed viability. Similarly, Zuffo *et al*. (2017b) showed that a 10-day delay in soybean harvesting after the R_8 stage harmed seed vigor and viability, as well as increased the incidence of pathogens.

The occurrence of rainfall and variations in relative air humidity and temperature during the stages between physiological seed maturity and mechanized harvesting can accelerate seed deterioration and the incidence of pathogens (Vergara *et al*., 2019). According to Lee *et al*. (2020), the incidence of rain during the harvest period can reduce the seed yield and quality. Therefore, delays in soybean harvesting can be a limiting factor in obtaining high-quality seeds.

This study was conducted to evaluate the physiological and sanitary quality of soybean cultivar seeds in response to harvest delay.

MATERIAL AND METHODS

Experimental area description

The field experiment was conducted at the Accert PCA Experimental Station located in the municipality of Balsas – MA (07°31’57’’ S, 46°02’08’’ W and altitude of 283 m), during the 2022/2023 agricultural season. The municipality of Balsas is located in the southern region of Maranhão, integrating the Gerais de Balsas microregion (Passos; Zambrzycki; Pereira, 2017). The region’s climate, according to Köppen’s classification, is hot and humid tropical (Aw), with rainy summers and dry winters (Maranhão, 2002). Annual rainfall is approximately 1175 mm (Passos; Zambrzycki; Pereira, 2017). Rainfall data during soybean crop development are shown in Figure 1.

The soil in the experimental area was classified as sandy clay loam Oxisol (Latossolo Vermelho-Amarelo in the Brazilian classification) (Santos *et al*., 2018). Before starting the experiment, the soil was sampled for chemical and particle size analysis down to 0.40 m (Table 1).

Experimental design and treatments

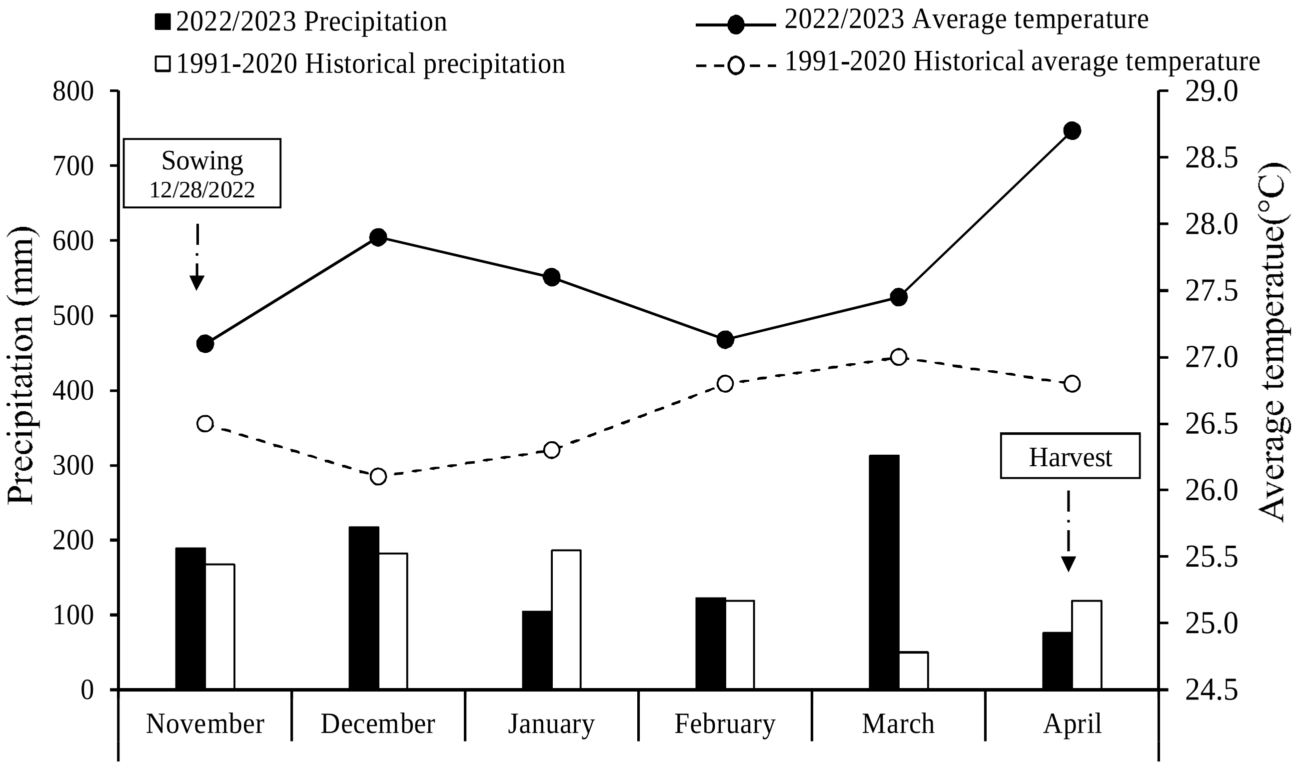
The experimental design used was completely randomized blocks and the treatments were arranged in a split-plot scheme with three replicates. The whole plots were represented by the cultivation of two soybean cultivars (BMX Bônus IPRO and BMX Olimpo IPRO), while the subplots were represented by three harvest times [0, 7, 14 days after the seed’s full maturity (stage R_8)].

A total of 24 plots, 2.0 m wide × 3.0 m long, comprised the entire study area.

Soybean seed production

Soybean cultivars were mechanically sown on December 28th, 2022, in rows 0.50 m apart at a density of 15 seeds m^{-1}. The seeds were previously treated with pyraclostrobin + methyl thiophanate + fipronil (Standak Top®) at a dose of 2 mL kg^{-1} of seed and then inoculated with the SEMIA 5079 and SEMIA 5080 strains of *Bradyrhizobium japonicum* using 3 mL kg^{-1} of the liquid inoculant Simbiose Nod Soja® (Simbiose: Biological Agrotechnology) with a minimum concentration of 7.2 x 10^9 viable cells per mL. Mineral fertilization consisted of 50 kg ha^{-1} of P_2O_5 applied in the sowing furrow using simple superphosphate. After 30 days of plant emergence, 90 kg ha^{-1} of K_2O was applied top dressing using potassium chloride.

Figure 1 - Monthly total rainfall (bars) and monthly average temperature (lines) in Balsas, Maranhão, Brazil during the soybean crop development in the 2022/2023 season and 30-year historical average data (1991 to 2020)



Source: Accert CPA (2023) and National Meteorological Institute (2023)

Table 1 - Soil chemical properties and particle size at 0.0 – 0.20 and 0.20 – 0.40 m depth at the beginning of the experiment

Depth	pH	O. M.	$P_{Mehlich-1}$	H + Al	Ca	Mg	K	CEC	V
(m)		g kg^{-1}	mg dm^{-3}	cmol$_c$ dm^{-3}					%
0.0 – 0.20	6.0	12.9	55.0	1.20	2.15	0.71	0.35	4.41	73
0.20 – 0.40	4.7	2.3	20.7	1.80	0.95	0.30	0.18	3.23	44

Depth	S - SO_4	Micronutrient					Particle size		
		B	Cu	Fe	Mn	Zn	Clay	Silt	Sand
(m)	mg dm^{-3}						g kg^{-1}		
0.0 – 0.20	6.30	0.22	0.44	113.21	14.28	0.73	242	93	665
0.20 – 0.40	12.60	0.23	0.40	81.98	4.25	0.37	–	–	–

pH in water. O.M.: organic matter. CEC: cation exchange capacity at pH 7.0. V: soil base saturation. Source: Research data

The management of weeds, pests, and diseases during the development of the soybean crop was carried out using the following products: glyphosate, haloxyfope-p-methyl, pyraclostrobin + epoxiconazole, picoxystrobin + benzovindiflupyr, mancozeb, azoxystrobin + cyproconazole, teflubenzuron, chlorpyrifos, cypermethrin and imidacloprid + beta-cyfluthrin.

Soybean seeds were harvested manually at the R_8 stage (full maturity) and, subsequently, with a delay of 7 and 14 days. The harvested plants were mechanically threshed, and the seed water content was standardized to 11% through slow drying in the shade. After standardizing the moisture content, the seeds from each of the treatments were homogenized and used to determine the physiological and sanitary quality traits according to the following methods described below.

Measurement of seed physiological quality

Water content: after slow drying in the shade at room temperature, the seed water content was determined using the oven drying method at 105 ºC, for 24 h (Brasil, 2009).

Emergence: the seedling emergence rate was evaluated in a substrate composed of soil and sand (2:1; v:v) previously moistened to 70% of the water retention capacity and placed in 8-L plastic boxes. Four replicates of 50 seeds were used. The plastic boxes were kept in a greenhouse with a constant temperature of 25 ºC. The emergence of soybean seedlings was evaluated daily until 14 days, and, with the values recorded, the emergence percentage (E) and the emergence speed index (IVE) were calculated as described by Maguire (1962).

Total dry biomass: all seedlings that emerged up to 14 days after sowing were collected, washed in running water, packed in paper bags, and dried in an oven at 60 ºC for 72 h. The total dry biomass was constituted by the dry mass of all seedlings determined on an analytical balance (0.0001 g), dividing the value obtained by the number of seeds sown.

Germination: the seeds were distributed on three sheets of germination paper previously moistened with distilled water in an amount equivalent to 2.5 times the dry mass of the germination paper. The sheets were then turned into rolls and placed in germination chamber under a constant temperature of 25 ℃ and a 12-hour photoperiod. Four replicates of 50 seeds were used. The measurement of germinated seedlings was carried out on the 5 th (first count of the germination test) and the 8 th day after sowing (final germination) according to the criteria established in the Rules for Seed Analysis (Brasil, 2009).

Accelerated aging: seeds were placed on a stainless-steel screen placed in Gerbox-type plastic boxes, containing 40 mL of water (Krzyzanowski; Vieira; Marcos-Filho, 1999). The plastic boxes were closed and kept in an incubation chamber with a temperature regulated at 41 ℃ for 96 h. Then, the seeds were subjected to the germination test, as previously described. Germinated seedlings were counted on the 5 th day after sowing according to the methodology described by Brasil (2009).

Electrical conductivity: 50 previously weighed seeds were placed in plastic cups containing 75 mL of deionized water and kept at a temperature of 25 ºC for 24 hours. After this period, the plastic cups were gently shaken, and the solution's electrical conductivity reading was measured using a digital conductivity meter (MS TECNOPON® – model mCA150). The seed's electrical conductivity was determined using the mass conductivity method, as proposed by Krzyzanowski, Vieira and Marcos-Filho (1999).

Measurement of seed sanitary quality

Seed health: the seed sanitary quality was measured using the Blotter-test method. Five replicates of 40 seeds were used. The Petri dishes containing the seeds were kept in an incubation chamber at 20 ºC and a 12-hour photoperiod for 7 days (Brasil, 2009). The identification of the morphological structures of the fungal species was carried out using an optical microscope. The assessments consisted of the presence or absence of pathogens associated with the seeds as proposed by Barnett and Hunter (1998).

Statistical analyzes

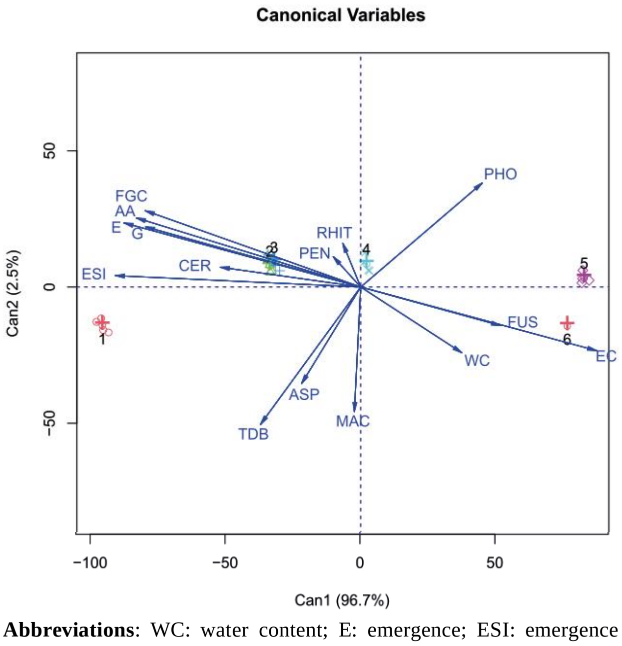
Canonical variable analysis was used to study the interrelationship between sets (vectors) of independent variables (soybean cultivar and harvest time) and dependent variables (physiological and sanitary quality of the seeds). Correlation analysis using Pearson's coefficient was carried out between the physiological and health quality traits of soybean seeds considering a set threshold of 0.60 and a significance level of 0.05. The result of this analysis was represented using a correlation network. In this correlation network, the nodes represent the physiological and sanitary quality traits of the seeds, while the distance between the nodes represents the absolute values of the correlation between these characteristics, based on Pearson's correlation coefficient. In turn, the width of the bands and the intensity of the color of the bands indicate the magnitude of the Pearson correlation coefficients. Furthermore, the color of the bands identifies whether the correlation is positive (green) or negative (red). All analyses were performed using the Rbio software version 140 for Windows (Bhering, 2017).

RESULTS AND DISCUSSION

Canonical variable analysis was used to evaluate the contribution of each dependent variable to the physiological and sanitary quality of the seeds, and how these variables were influenced by the combinations of soybean cultivars and seed harvest times (Figure 2). In this study, the accumulated variances in the two main canonical variables were 99.2% (Figure 2), having a precise interpretation as indicated by Mingoti (2005). An angle between vectors less than 90° indicates a positive correlation between seed quality traits (dependent variables) and combinations of soybean cultivars and seed harvest times (independent variables). The seeds of the BMX Bônus IPRO and BMX Olimpo IPRO cultivars harvested at the R_8 stage are associated with the variable's emergence (E), emergence speed index (ESI), first germination count (FGC), germination (G), *Rhizoctonia* sp. (RHIT) and *Penicillium* sp. (PEN).

These results indicate that soybean seeds harvested at the R_8 stage have greater viability and vigor

Figure 2 - Analysis of canonical variables to illustrate the interrelationship between the physiological and sanitary quality traits of seeds from soybean cultivars harvested at different times. The blue lines illustrate the canonical correlation between the centroids of the first set of canonical variables, along with the linear trend line



Abbreviations: WC: water content; E: emergence; ESI: emergence speed index; EC: electrical conductivity; FGC: first germination count; G: germination; TDB: total dry biomass; AA: accelerated aging; CER: *Cercospora* sp.; PHO: *Phomopsis* sp., FUS: *Fusarium* sp., RHIT: *Rhizoctonia* sp., ASP: *Aspergillus flavus*; MAC: *Macrophomina* sp., PEN: *Penicillium* sp.; 1: BMX Bônus IPRO soybean cultivar harvested at stage R_8; 2: BMX Olimpo IPRO soybean cultivar harvested at stage R_8; 3: BMX Bonus IPRO harvested 7 days after stage R_8; 4: BMX Olimpo IPRO harvested 7 days after stage R_8; 5: BMX Bônus IPRO harvested 14 days after stage R_8; 6: BMX Olimpo IPRO harvested 14 days after stage R_8. Symbols in different colors represent the arrangement of individual data associated with treatments

when compared to a delay of 7 and 14 days of soybean harvest. Similar results were reported by Vergara *et al*. (2019), which obtained lower germination and vigor of soybean cultivar seeds due to seed deterioration in the field caused by a 10-day harvest delay after the R_8 stage. A delay in soybean harvesting can significantly compromise the physiological and sanitary quality of the seeds, resulting in lower germination and vigor due to prolonged exposure to adverse environmental conditions, such as humidity and high temperatures (Zuffo *et al*., 2017a, b). Furthermore, delaying harvesting can increase the incidence of pathogenic fungi and seed deterioration, negatively influencing the physiological and sanitary quality of the seeds.

Seed of the BMX Bônus IPRO soybean cultivar harvested 7 days after the R_8 stage had higher total dry biomass (TDB) and a higher incidence of the pathogens *Aspergillus flavus* (ASP) and *Macrophomina* sp. (MAC) (Figure 2). The 14-day delay after the R_8 stage in the harvest of the BMX Bônus IPRO soybean cultivar resulted in a higher incidence of *Phomopsis* sp. (Figure 2). In turn, the seeds of the BMX Olimpo IPRO soybean cultivar harvested 14 days after the R_8 stage have higher water content, electrical conductivity, and incidence of the pathogen *Fusarium* sp. These findings confirm the results reported by Zuffo *et al.* (2017b), which showed that soybean seeds harvested 15 days after the R_8 stage have a significant increase in the incidence of the pathogens *Aspergillus flavus*, *Phomopsis* sp., and *Fusarium* sp.

The fungus of the genus *Aspergillus* belongs to the group of pathogens associated with seed storage (Torres *et al*., 2022). However, this fungus has the potential to cause damage before harvest and during harvesting, transport, storage, and processing of seeds under conditions of high temperature and high relative humidity that are suitable for the growth and multiplication of the fungus (Londoño-Cifuentes; Martínez-Miranda, 2017). The growth of the fungus *Macrophomina* sp. is strongly associated with conditions of high temperature and low relative air and soil humidity (i.e., hot and dry periods) (Doubledee *et al*., 2018). Therefore, when storage conditions are inadequate or harvest is delayed, degraded seeds facilitate the action of these two pathogenic fungi.

The fungus of the genus *Phomopsis* sp. can reduce the quality of soybean seeds, and the pods can be infected at any stage after their formation and the highest incidence of this fungus occurs in conditions of delayed harvest (Tsukahara *et al*., 2016). Delayed harvesting contributes to the deterioration of soybean seeds by intensifying the action of fungi such as *Fusarium* sp. and *Phomopsis* sp. on seeds (Diniz *et al*., 2013). Other factors that contribute to the incidence of this fungus are adverse field conditions, such as deterioration caused by humidity, damage caused by bedbugs, and mechanical damage (Zuffo *et al*., 2017b). In this context, the BMX Bônus IPRO cultivar has a longer maturation cycle when compared to the BMX Olimpo IPRO cultivar, and when this cultivar was subjected to harvest delay, the longer period of permanence of its seeds in the field, resulted in greater exposure to the adverse environmental conditions and greater possibility of losses and damages caused by *Phomopsis* sp. Each cultivar has intrinsic characteristics and adverse environmental conditions can influence the agronomic and productive characteristics of soybeans (Zuffo *et al*., 2016).

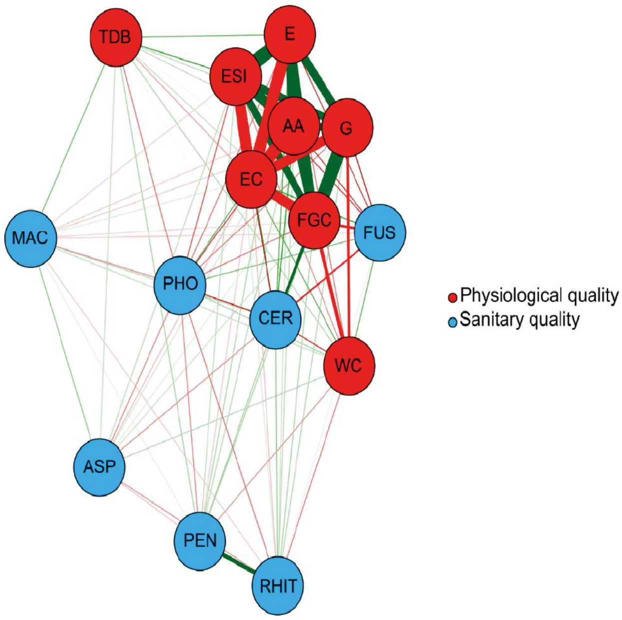
Delayed harvesting can impact seed vigor and viability due to several physiological and biochemical factors (Zuffo *et al.*, 2017a). Seeds harvested late remain exposed for longer periods to adverse environmental conditions, such as humidity and temperature, increasing the risk of physiological and biochemical deterioration (Zuffo *et al*., 2017b). This delay accelerates the natural aging process of the seeds, reducing their vigor and compromising their germination and initial growth capacity. Furthermore, prolonged exposure in the field favors the accumulation of reactive oxygen species (ROS), causing oxidative damage to lipids, proteins, and nucleic acids, which diminishes seed viability. Additionally, seeds that remain in the field for extended periods are more susceptible to infestation by fungi and bacteria, degrading seed quality and increasing the incidence of diseases, further impairing their germination capacity and plant establishment.

The correlation between the physiological and health quality traits of soybean seeds showed that there was a positive and significant correlation between emergence (E) and the emergence speed index (ESI), first germination count (FGC), and germination (G) (Figure 3). A significant and positive correlation was reported between the emergence speed index (ESI) and first germination count (FGC), accelerated aging (AA), and germination (G). There was also a significant and positive correlation between germination (G) and the first germination count (FGC) and incidence of *Cercospora* sp. (CER) (Figure 3). Similar results were reported by Martins *et al*. (2024), which showed that there was a positive correlation between several traits of the physiological quality of the seeds. The positive correlation between the first germination count and final germination can be explained because the greater number of seeds germinated on the 5th day resulted in greater final germination at 8 days. The accelerated aging test can facilitate seed imbibition, resulting in an increase in seed mass and water content (Martins *et al*., 2024).

Significant and positive correlations were reported between the incidence of *Penicillium* sp. (PEN) and *Rhizoctonia* sp. (RHIT) (Figure 3). The fungi *Penicillium* sp. and *Rhizoctonia solani* are pathogens present in the soil and are saprophytic. The positive correlation between these pathogens may occur through the individual action of each fungus; therefore, seed degradation caused by the incidence of *Rhizoctonia solani* may facilitate the incidence of *Penicillium* sp. in the seeds.

Significant and negative correlations were reported between emergence (E) and electrical conductivity (EC); between emergency speed index (ESI) and electrical conductivity (EC); between electrical conductivity (EC) and first germination count (FGC); and, between water content (WC) and first germination count (FGC) and final germination (G) (Figure 3). Soybean seeds with low viability and vigor have greater electrolyte leakage, which results in greater electrical conductivity of the solution (Krzyzanowski; Vieira; Marcos-Filho, 1999). Therefore, a higher value of electrical conductivity results in lower seed viability (less germination) due to damage caused to cell membrane structures, which limits the seed germination process.

Figure 3 - Network of the most significant Pearson correlation coefficients between the physiological and health quality traits of soybean seeds. Thick green lines represent the highest positive correlations (threshold set at 0.60; p-values < 0.05). Thick red lines represent the highest negative correlations (threshold set at 0.60; p-values < 0.05)



Abbreviations: WC: water content; E: emergence; ESI: emergence speed index; EC: electrical conductivity; FGC: first germination count; G: germination; TDB: total dry biomass; AA: accelerated aging; CER: *Cercospora* sp.; PHO: *Phomopsis* sp., FUS: *Fusarium* sp., RHIT: *Rhizoctonia* sp., ASP: *Aspergillus flavus*; MAC: *Macrophomina* sp., PEN: *Penicillium* sp

CONCLUSIONS

1. The delay in the soybean harvest affects the physiological and sanitary quality of the seeds;
2. Seeds harvested seven days after full maturity have a higher incidence of *Aspergillus flavus* and *Macrophomina* sp, while seeds harvested 14 days after full maturity have a higher incidence of *Phomopsis* sp. and *Fusarium* sp.

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