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ARTICLE

Quality and chemical composition of soybean seeds with different lignin contents in the pod and seed coat subjected to weathering deterioration in pre-harvest

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Cristian Rafael Brzezinski[1], Julia Abati[2]*, Claudemir Zucareli[2], Francisco Carlos Krzyzanowski[3], Ademir Assis Henning[3], Fernando Augusto Henning[3]

ABSTRACT: Soybean seeds may show different responses to weathering deterioration depending on the intrinsic characteristics of the genetic material and the intensity and duration of the rainfall period. This study aimed to evaluate the effect of pre-harvest weathering deterioration on the quality and chemical composition of soybean seeds with different lignin contents in the pod and seed coat. The percentage of weathering damage was evaluated by the tetrazolium test, X-ray test, lignin content in the pod and seed coat, germination, accelerated aging, seedling emergence, seed health test, and oil, protein, and chlorophyll contents. The experimental design was completely randomized in a 7×3 factorial scheme, that is, seven cultivars and three rainfall volumes (0, 54, and 162 mm), simulated when 95% of the pods had a mature color. The physiological and sanitary quality of soybean seeds reduces with an increase in pre-harvest rainfall. Soybean seeds from cultivars with higher lignin contents in the pod present a higher tolerance to pre-harvest weathering deterioration and provide seeds of better physiological quality. Seed oil and protein content is reduced in some cultivars as rainfall increases. Plants with higher lignin contents in the pods produce seeds with a lower incidence of the fungus *Cercospora kikuchii* and lower chlorophyll content when associated with pre-harvest rainfall.

Index terms: *Glycine max* (L.) Merrill, germination, oil content, sanity, vigor.

RESUMO: Sementes de soja podem apresentar respostas diferenciadas à deterioração por umidade, em função das características intrínsecas do material genético e da intensidade e duração do período de precipitação pluvial. O objetivo foi avaliar o efeito da deterioração por umidade em pré-colheita sobre a qualidade e composição química de sementes de soja com diferentes teores de lignina na vagem e no tegumento. Foram avaliados a porcentagem de danos por umidade pelo teste de tetrazólio, teste de raios-X, teor de lignina na vagem e no tegumento, germinação, envelhecimento acelerado, emergência de plântulas, análise sanitária, teores de óleo, proteína e de clorofila. O delineamento experimental foi inteiramente casualizado, em esquema fatorial 7×3 (sete cultivares e três volumes de precipitações pluviais: 0, 54 e 162 mm; simuladas quando 95% das vagens apresentavam coloração madura). A qualidade fisiológica e sanitária das sementes reduz com o aumento das precipitações pluviais em pré-colheita. Sementes oriundas de cultivares com maiores teores de lignina na vagem apresentam maior tolerância à deterioração por umidade em pré-colheita e proporcionam sementes de melhor qualidade fisiológica. O teor de óleo e proteína das sementes é reduzido conforme se aumenta as precipitações pluviais, em algumas cultivares. Plantas com maiores teores de lignina nas vagens produzem sementes com menor incidência do fungo *Cercospora kikuchii* e menor teor de clorofila quando associado a precipitações pluviais em pré-colheita.

Termos para indexação: *Glycine max* (L.) Merrill, germinação, teor de óleo, sanidade, vigor.

***Corresponding author**
E-mail: juliaabati@yahoo.com.br

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[1]Departamento de Pesquisa, GDM Genética do Brasil S.A., 775000-000 – Porto Nacional, TO, Brasil.

[2]Departamento de Agronomia, Universidade Estadual de Londrina, Caixa Postal 6001, 86051-990 – Londrina, PR, Brasil.

[3]Embrapa Soja, Caixa Postal 231, 86001-970 – Londrina, PR, Brasil.

INTRODUCTION

Excess rainfall and humidity fluctuations associated with high temperatures during soybean pre-harvest phase cause physical changes in its seeds due to successive expansions and contractions of their volume, resulting in the formation of wrinkling of cotyledons and seed coat in the region opposite the hilum (Marcos-Filho, 2015; França-Neto et al., 2016). These changes can cause the rupture of the seed coat and embryonic tissues, compromising the control of membrane permeability and affecting organelles, with less energy production required for germination (Marcos-Filho, 2015).

The presence of weathering damage makes it difficult to produce quality seeds (Shu et al., 2020; Pinheiro et al., 2021), as the physical, sanitary, and physiological attributes of soybean seeds are susceptible to this type of damage. In this sense, Forti et al. (2013) observed a reduction in the physiological potential of soybean seeds associated with weathering deterioration. Pinheiro et al. (2021) evaluated the physiological performance of soybean seed cultivars after the application of simulated rainfall in volumes of 0 (control), 60, 120, and 180 mm during the pre-harvest phase and observed reductions in seed germination and vigor as a function of weathering deterioration, especially at higher rainfall levels. Furthermore, the seeds are exposed to the attack of a significant number of pathogenic fungi, such as *Phomopsis* spp., *Fusarium* spp., and *Colletotrichum truncatum*, which intensify the deterioration process and contribute to reducing vigor and germination by infecting the seed (França-Neto et al., 2016).

In addition, pre-harvest weathering deterioration can also affect seed chemical composition. Oil and protein contents of soybean seeds are genetic material dependent but strongly influenced by the environment (Bellaloui et al., 2015; Patil et al., 2017; Assefa et al., 2018; Faria et al., 2018). Soybean is sown throughout the Brazilian territory and significant variations in these contents are expected due to exposure to different cultural practices and weather conditions, especially rainfall and temperature fluctuations, especially during the grain filling period.

In addition, soybean seeds may have different responses to weathering deterioration depending on cultivar characteristics and the intensity and duration of the rainfall period. Therefore, the identification of characteristics that can be exploited to increase seed tolerance to weathering deterioration in the pre-harvest phase becomes essential. Pod and seed coat permeability influenced by the lignin content stands out among these characteristics. According to Oliveira et al. (2014) and Krzyzanowski and França-Neto (2021), the study on the lignin content can be of great value for genetic breeding programs, as seed coats and pods with higher lignin contents may have lower permeability to water, which results in less seed deterioration in the field and obtaining better quality seeds.

In this context, this study aimed to evaluate the effect of pre-harvest weathering deterioration on the quality and chemical composition of soybean seeds with different lignin contents in the pod and seed coat.

MATERIAL AND METHODS

The experiment was carried out at the Brazilian Agricultural Research Corporation (Embrapa Soybean), Londrina, PR, Brazil, at the Laboratories of Physiology, Technology, Pathology, and Chemistry of Seeds and Physicochemical and Chromatographic Analysis, and Luiz de Queiroz College of Agriculture of the University of São Paulo (ESALQ/USP), Piracicaba, SP, Brazil, at the Laboratory of Image Analysis of the Department of Plant Production.

Seeds from different cultivars (Table 1) were produced under greenhouse conditions (Van der Hoeven® model), with partial control of temperature and relative humidity. The conditions were monitored using a Data Logger (Figure 1).

Plants were grown in nine-liter pots, with soil classified as a clay-textured eutroferric Red Latosol (Oxisol), duly corrected according to the crop requirements. The seeds were inoculated, and sowing was carried out at a depth of 3 to 5 cm on 10/27/2015. The inoculation was performed using the commercial liquid inoculant BIAGRO NG® with the bacterium *Bradyrhizobium japonicum* strains SEMIA 5079 and 5080 ($5x10^9$ viable cells.mL^{-1}) at a dose of 100 mL of the commercial product for each 50 kg of seeds. Seed treatment was carried out with the commercial fungicide Derosal Plus® (carbendazim 15% + thiram 35%) at a dose of 200 mL.100 kg^{-1} seeds. Four seeds were sown per pot and the two most vigorous seedlings were kept after emergence.

Table 1. List of soybean cultivars used in the experiment and their respective characteristics.

Cultivar	Type[1]	Habit[2]	Cycle	Group	Pubescence
BRS 1010 IPRO	I	Ind.	Early	6.1	Grey
BRS 284	C	Ind.	Early	6.3 a 7.1	Grey
NA 5909 RR	RR	Ind.	Early	5.9	Grey
BRSMG 752S	C	Ind.	Semi-early	7.5	Brown
BRSMT Pintado	C	Det.	Intermediate	8.7	Grey
BRS Jiripoca	C	Det.	Intermediate	8.4	Grey
M8210 IPRO	I	Det.	Early	8.2	Brown

[1]Type of technology: I: Intacta; C: conventional and RR: Roundup Ready®.

[2]Growth habit: Ind.: indeterminate and Det.: determinate.

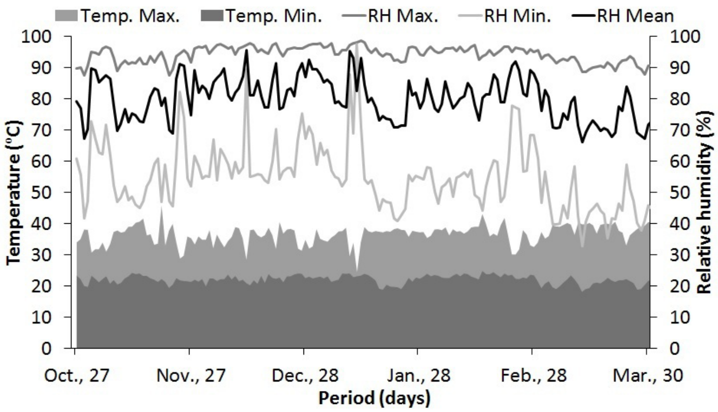

Figure 1. Maximum and minimum daily temperature (°C) and maximum, minimum, and mean daily relative humidity (%) for the development of soybean grown under greenhouse conditions.

Four replications of five pots with two plants each were used for each treatment, totaling 40 plants. Daily drip irrigation was performed. The cultural management (insecticide and fungicide applications) was carried out according to the crop requirements and recommendations.

The determination of the phenological stage of plants was based on the scale of Ritchie et al. (1997). The rainfall simulation was performed at the levels of 54 and 162 mm. The control treatment consisted of not applying simulated rainfall (0 mm). These maximum and minimum rainfall volumes corresponded to the historical annual means observed in the pre-harvest period for the main soybean-producing regions in Brazil. The treatments were applied at the crop development stage R8 (full maturity with 95% of the pods with mature color). The simulation occurred daily for three days to obtain the mean daily and cumulative rainfall (Table 2), that is, three applications were carried out for each rainfall level, with an interval of 24 h between each application. The water depth was measured with rain gauges distributed throughout the experimental area of the simulation room.

The rainfall simulations were performed using specially designed equipment in a closed room. This equipment has a spray bar responsible for the rainfall simulation system, which moves through a useful area of 15 m^2 in the direction of the equipment length. The spray boom was equipped with seven high-flow hollow cone nozzles model TKSS20 spaced 0.50 m from each other and placed to provide better rainfall uniformity in the applied area. These specifications allowed the production of artificial rainfall drops with a median volumetric diameter of approximately 1,140 microns, according to the manufacturer's information (Spraying Systems). The working pressure was 0.81 $kgf.cm^{-2}$, the boom height was 1.45 m relative to the surface of the experimental units, and the displacement speed was 0.050 $m.s^{-1}$.

Table 2. Mean daily and cumulative rainfall (mm) in soybean cultivars at the phenological stage of crop development (R8).

	Day 1	Day 2	Day 3	Cumulative
Rainfall 0 (control)	0	0	0	0
Rainfall 54	18	18	18	54
Rainfall 162	54	54	54	162

The plants were taken to the greenhouse after the hydration and dehydration cycles and kept until the moment of seed collection (harvest point) within three to four days. After collection, the seeds were sent to the laboratory for analysis, according to the following methodologies:

Weathering damage by the tetrazolium test: conducted with two subsamples of 50 seeds per replication, pre-conditioned on germitest paper moistened with water for a period of 16 hours in a germinator with a temperature of 25 °C. Subsequently, the seeds were submerged in a tetrazolium solution (2,3,5-triphenyl tetrazolium chloride) at a 0.075% concentration and kept at a temperature of 40 °C for approximately 150 min in the absence of light. The seeds were then individually evaluated for weathering damage, following the criteria proposed by França-Neto and Krzyzanowski (2018). The results were expressed as a percentage.

X-ray test: performed for qualitative-visual characterization of the evaluated treatments and to evaluate the effects of simulated rainfall and weathering damage on the internal structure of soybean seeds, without statistical analysis. The seeds of each treatment were placed on an acrylic container specially developed for conducting the analysis. The acrylic plate with seeds was placed directly on an X-ray film (Kodak MIN-R EV 2000, 18 x 24 cm) at 57 cm from the X-ray source to obtain the radiography. The radiographs were taken using a device called FAXITRON X-Ray model MX-20, with an intensity of 25 kV and an exposure time of 40 s (Pinto et al., 2007). The film processing was carried out in a Hope X-Ray processor model 319 Micromax. Subsequently, the images of the X-ray films were captured by a Umax Scanner model PowerLook 1100 for magnification and computer visualization.

Lignin content in the pod and seed coat: determined using four replications of 100 seeds and 50 pods for each treatment. The seeds, separated from the pods, were immersed in water for 12 h to separate the seed coat from the cotyledons. After this procedure, the seed coats and the separated pods were dried in an oven at 105 °C for 24 h. The obtained dry matter was crushed and homogenized. Subsequently, 0.3 g were weighed for the step of extracting the proteins bound to the cell wall. Lignin was quantified after obtaining the protein-free material using the acetyl bromide method (Moreira-Vilar et al., 2014).

Germination: carried out with two subsamples of 50 seeds per replication, totaling 400 seeds per treatment. The seeds were placed on germitest paper towel rolls moistened with distilled water at a proportion of 2.5 times the paper weight. After assembly, the rolls were taken to a germinator at a temperature of 25 °C. Assessments were performed at eight days, with results expressed as a percentage (Brasil, 2009a).

Accelerated aging: conducted in plastic boxes (gerbox) with a screen, containing 40 mL of water at the bottom (Marcos-Filho, 2020). The seeds were arranged in a uniform layer on the surface of the inner screen and then taken to an incubator at 41 °C for 48 h. The incubator consisted of a water jacketed incubator. Two subsamples of 50 seeds per replication were submitted to the germination test after the aging period. The evaluation was performed on the fifth day after sowing, following the recommendations of the Rules for Seed Testing (Brasil, 2009a). The results were expressed as a percentage.

Seedling emergence in the sand: performed with four replications of 100 seeds for each treatment. Sowing was carried out in plastic trays containing previously washed and sterilized sand. The test was conducted under greenhouse conditions and moisture was maintained with daily irrigations. The final evaluation of the number of emerged normal seedlings was counted on the twelfth day, and the results expressed as a percentage.

Seed health test: the blotter test was used (Brasil, 2009b). A total of 200 seeds were used per treatment, divided into 10 transparent plastic gerbox boxes, with 20 seeds each. The seeds were distributed on four sheets of blotting paper previously moistened with distilled and autoclaved water. The seeds remained incubated for seven days in a chamber with a controlled temperature of 20 ± 2 °C under white fluorescent light. Subsequently, the fungi were identified using a stereoscopic and optical microscope, and their incidence was expressed as a percentage.

Oil and protein content: percentage contents were determined in intact soybean seeds using the near-infrared reflectance (NIR) technique (Heil, 2010). The seeds of each sample were submitted to readings in triplicate with a Thermo Antaris II equipped with an integrating sphere with a resolution of 4 cm^{-1}, mean of 32 scans, and background at each reading. Mathematical models developed by Embrapa Soybean in 2011/12 were used for predicting protein [180 standards, correlation coefficient (r) = 0.97, standard error of calibration (RMSEC) = 0.64] and oil contents [170 standards, correlation coefficient (r) = 0.98, standard error of calibration (RMSEC) = 0.452].

Total chlorophyll content: determined using the method described by Arnon (1949) with adaptations by Pádua et al. (2007). A sample of 3 g of finely ground soybean was added to 15 mL of an 80% acetone solution in water in plastic tubes covered with aluminum foil to avoid the incidence of light. The sample was subjected to homogenization in a vortex mixer for 1 h with stirring every 15 min. Subsequently, the material in the tubes was filtered, and the filtrate was placed in a dark container until reading in a UV-VIS absorption spectrophotometer at wavelengths of 645 and 663 nm. The results were expressed in mg chlorophyll.kg^{-1} sample.

The experimental design was completely randomized in a 7 (cultivars) × 3 (rainfall levels) factorial scheme, with four replications. The data were analyzed for normality and homoscedasticity, using the Shapiro-Wilk and Hartley tests, respectively, which indicated the need to transform the sanitary quality data into a square root of x+0.5. No data transformation was required for the other variables. Analysis of variance was performed, and the means were compared using the Scott-Knott test at a 5% probability. The analyses were performed using the computer program System for Analysis of Variance – SISVAR (Ferreira, 2011).

RESULTS AND DISCUSSION

The evaluation of weathering damage by the tetrazolium test showed that rainfall simulations were efficient to distinguish the cultivars (Table 3). Those volumes of simulated rainfall allowed the characterization of cultivars as a function of weathering damage at different intensities, allowing determining susceptible and tolerant genotypes to pre-harvest deterioration. Similarly, the effect of the studied factors on the physiological and sanitary attributes and the chemical composition of soybean seeds could be verified.

The X-ray images showed the effect of rainfall volumes on the seeds, characterized by wrinkling in the region opposite the hilum (Figure 2). The control treatment (0 mm) had a low incidence of wrinkling, which did not change the structure of seed reserve tissues. These wrinkles, even in the control treatment, occurred due to natural temperature and humidity fluctuations inside the greenhouse (Figure 1). An increase in the incidence of wrinkling in the region opposite the hilum and embryonic axis was observed as the simulated rainfall volumes increased to 54 and 162 mm (Figure 2), providing a considerable decrease in the mass and tissues of seed reserves.

Importantly, the used rainfall corresponded to the historical annual means found in the pre-harvest period for the main soybean-producing regions in Brazil. This result demonstrates the similarity between the tested variables and the reality observed by seed producers in the field. Huth et al. (2016) worked with the induction of weathering deterioration through simulated rainfall and reproduced a similar situation with weathering deterioration that occurs in the field, through the determination of weathering damage by the tetrazolium test.

The germination test showed that the cultivars BRS 284 and BRSMG 752S had the lowest percentages of germination in the control treatment (0 mm), with values of 85 and 83%, respectively (Table 3). This result showed that the cultivars responded differently to production sites even when produced under the same environmental conditions. It allowed estimating the response of cultivars to different rainfall events, depending on their genetic character and interaction between genotype and environment.

Table 3. Weathering damage assessed by the tetrazolium test, germination, accelerated aging, and seedling emergence in the sand of soybean cultivars produced under different simulated rainfall volumes in the pre-harvest.

Cultivar	Weathering damage 1–8 (%)			Weathering damage 6–8 (%)		
	------------Rainfall------------			------------Rainfall------------		
	0 mm	54 mm	162 mm	0 mm	54 mm	162 mm
BRS 1010 IPRO	22 Aa	41 Bb	66 Dc	8 Ca	22 Db	28 Dc
BRS 284	31 Ba	52 Cb	64 Dc	5 Ba	14 Cb	20 Cc
NA 5909 RR	20 Aa	36 Bb	53 Cc	2 Aa	8 Bb	19 Cc
BRSMG 752S	16 Aa	20 Ab	22 Ab	2 Aa	3 Aa	5 Ba
BRSMT Pintado	12 Aa	17 Ab	22 Ab	2 Aa	2 Aa	2 Aa
BRS Jiripoca	17 Aa	18 Aa	20 Aa	1 Aa	4 Ba	4 Ba
M8210 IPRO	15 Aa	31 Bb	40 Bb	3 Aa	7 Bb	9 Bb

Cultivar	Germination (%)			Accelerated aging (%)			Seedling emergence (%)		
	0 mm	54 mm	162 mm	0 mm	54 mm	162 mm	0 mm	54 mm	162 mm
BRS 1010 IPRO	89 Aa	83 Bb	76 Cc	82 Aa	76 Ba	65 Ab	89 Aa	80 Bb	75 Bb
BRS 284	85 Ba	79 Bb	72 Dc	81 Aa	67 Cb	63 Ab	85 Ba	64 Cb	55 Cc
NA 5909 RR	94 Aa	87 Bb	81 Cb	84 Aa	71 Ba	66 Ab	93 Aa	87 Ab	84 Ab
BRSMG 752S	83 Ba	82 Ba	82 Ca	78 Aa	74 Ba	71 Aa	81 Ba	80 Ba	77 Ba
BRSMT Pintado	91 Aa	93 Aa	92 Aa	85 Aa	83 Aa	77 Aa	88 Aa	89 Aa	87 Aa
BRS Jiripoca	91 Aa	88 Aa	87 Ba	80 Aa	73 Ba	73 Aa	83 Ba	80 Ba	82 Aa
M8210 IPRO	91 Aa	88 Aa	86 Ba	80 Aa	73 Ba	70 Aa	82 Ba	80 Ba	83 Aa

Means followed by the same lowercase in the row and uppercase letter in the column do not differ from each other by the Scott-Knott test at a 5% probability.

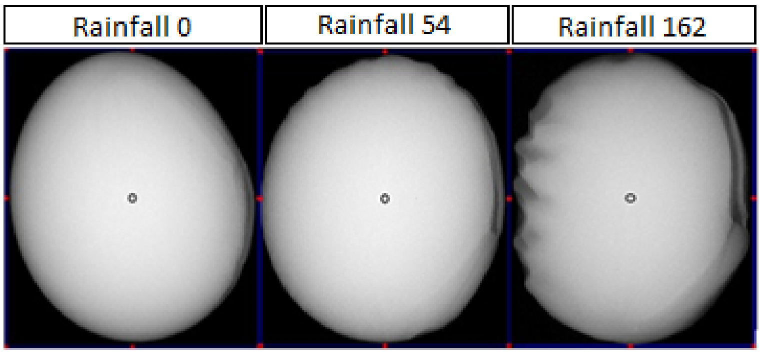

Figure 2. Weathering damage through X-ray images in soybean seeds of the cultivar BRS 1010 IPRO produced under different simulated rainfall volumes (0, 54, and 162 mm) in the pre-harvest.

The cultivars BRSMT Pintado, BRS Jiripoca, and M8210 IPRO showed the highest germination values with the application of a rainfall depth of 54 mm (Table 3). Thus, pre-harvest rainfall events with mean volumes of 54 mm are harmful to seed quality, given the reduction of its germination potential. The cultivar BRSMT Pintado showed the highest germination under 162 mm, followed by the cultivars BRS Jiripoca and M8210 IPRO. The association of these results with the tetrazolium test shows that the genotypes had lower percentages of weathering damage after the application of pre-harvest water depths (Table 3). Consequently, these cultivars showed higher tolerance to rainfall events and weathering deterioration. Pinheiro et al. (2021) also observed reductions in seed germination of soybean cultivars subjected to pre-harvest rainfall at volumes of 60, 120, and 180 mm, mainly at higher volumes. Furthermore, similarly to the present study, the authors found differences in the tolerance of soybean cultivars to weathering deterioration in the pre-harvest phase.

Lignin content is one of the factors that may be related to the resistance of cultivars in the field to pre-harvest rainfall events. The cultivars BRSMG 752S, BRSMT Pintado, and BRS Jiripoca presented higher lignin content in the pods (Table 4). This factor may be associated with the permeability and water absorption of these tissues since the pod is considered to protect the seeds in the field against external factors. This process occurs because lignin has a hydrophobic character (Kang et al., 2019) and, thus, the passage of water through the tissues becomes slow, especially when associated with other waxy substances, such as suberin.

Only the cultivar BRSMT Pintado showed a relationship between lignin contents in the seed coat and the germination data, while the other cultivars did not show this relationship, within the evaluated contrast limits (Tables 3 and 4). Carvalho et al. (2014) used cultivars with lignin contents between 3 and 5% in the seed coat and found that seeds of soybean cultivars with higher lignin contents in the seed coat did not necessarily present better physiological quality.

Still for the germination test, the cultivars BRSMG 752S, BRSMT Pintado, BRS Jiripoca, and M8210 IPRO showed no reductions in the germination potential due to an increase in rainfall (Table 3). Seed germination reduced for the other cultivars as the rainfall volumes increased, which is related to an increase in weathering damage. Castro et al. (2016) worked with the soybean cultivars AS 7307 RR and SYN 1283 RR and observed that the stress caused by harvest delay associated with excess moisture caused an increase in the percentage of weathering damage. Forti et al. (2013) studied two lots of soybean seeds of the cultivar TMG 115 RR and also found that weathering damage interferes with seed quality depending on its extension and location.

In addition, the cultivars BRS 1010 IPRO and BRS 284, which have the lowest lignin contents in the pod (Table 4), showed a higher reduction in germination relative to the other cultivars with the maximum precipitation (162 mm) (Table 3). Thus, the seed lots of these cultivars could not be marketed after a pre-harvest rainfall of 162 mm, as they did not present the minimum standard of 80% germination (MAPA, 2013).

The accelerated aging test allowed observing differences between cultivars from 54 mm of simulated rainfall, in which the cultivar BRS 284 presented the lowest vigor and the cultivar BRSMT Pintado the highest vigor (Table 3). The seeds of the cultivars BRS 1010 IPRO, BRS 284, and NA 5909 RR showed a reduction in vigor as the rainfall volumes increased. The other cultivars showed no significant reduction in vigor with the increased rainfall volumes. This difference in seed vigor is related to the seed weathering stage due to the high rates of weathering damage (Table 3), as lower quality seeds deteriorate faster than more vigorous ones when exposed to the adverse conditions of high temperature and relative humidity that the test provides.

Table 4. Lignin contents in pods and seed coats and total chlorophyll contents in seeds of soybean cultivars, considering the general mean of the simulated rainfall.

Cultivar	Lignin – pod (%)	Lignin – seed coat (%)	Chlorophyll (mg.kg^{-1})
BRS 1010 IPRO	13.46 D	4.27 B	0.74 C
BRS 284	14.10 C	4.20 B	0.64 C
NA 5909 RR	15.34 B	3.60 C	0.55 B
BRSMG 752S	16.13 A	4.58 A	0.51 B
BRSMT Pintado	16.19 A	4.47 A	0.43 B
BRS Jiripoca	18.56 A	4.26 B	0.19 A
M8210 IPRO	15.18 B	4.35 B	0.35 A

Means followed by the same letter in the column do not differ from each other by the Scott-Knott test at a 5% probability.

The cultivars BRS 1010 IPRO, NA 5909 RR, and BRSMT Pintado showed higher percentages of seedlings that emerged without rainfall (Table 3). The cultivar BRS 284 showed the lowest values under 54 mm. The cultivar BRS 284 also showed the lowest percentages of emergence under the simulated rainfall of 162 mm, followed by BRS 1010 IPRO and BRSMG 752S. The increase in rainfall volumes caused significant reductions in seedling emergence of the cultivars BRS 1010 IPRO, BRS 284, and NA 5909 RR, while the other cultivars showed no decrease in the percentage of emergence as the rainfall volumes increased, standing out the cultivar BRSMT Pintado with the highest values for this variable.

Regarding the chlorophyll content data, which is related to the number of greenish seeds, the cultivars BRS 1010 IPRO and BRS 284, which have the lowest lignin contents in the pod, had the highest levels of chlorophyll in the mean of seeds produced under different pre-harvest rainfall volumes (Table 4). Thus, plants with higher lignin contents in the pod can become an indication for the reduction in the chlorophyll content and number of greenish seeds in the field, providing better seed quality. According to Pádua et al. (2007) and Teixeira et al. (2020), the higher the percentage of greenish soybean seeds in a lot, the lower is its physiological quality.

The cultivars BRS 284 and NA 5909 RR had higher percentages of oil in the control treatment (0 mm) (Table 5). Seeds of soybean cultivars may vary in their chemical composition due to exposure to different environmental conditions during cultivation. This fact may influence qualitatively and quantitatively the availability of compounds that can be readily used by the embryo and affect the germination process and seed quality (Delarmelino-Ferraresi et al., 2014).

Seeds of the cultivars BRS 1010 IPRO, BRSMT Pintado, and M8210 IPRO submitted to a rainfall volume of 54 mm showed the lowest oil contents (Table 5). The cultivars BRS 1010 IPRO, BRS 284, and BRSMT Pintado showed higher oil contents in the seeds for the rainfall volume of 162 mm than those observed for the other cultivars. In addition, the increase in rainfall reduced the percentage of oil in the seeds of the cultivar NA 5909 RR.

The cultivars BRS 1010 IPRO and NA 5909 RR had lower percentages of protein in the seeds, regardless of the rainfall to which they were subjected (Table 5). This result can directly influence the physiological seed quality. According to Bortolotto et al. (2008), proteins are peptides used in the formation of new tissues at points of embryo growth, which is related to metabolism efficiency and seedling vigor and can be associated with the physiological seed potential. Bellaloui et al. (2017) found positive correlations between germination and protein content in seeds. The cultivars BRS 1010 IPRO and BRSMG 752S showed decreases in the protein content of seeds under 162 mm, while the other cultivars showed no significant effect (Table 5).

Table 5. Oil and protein content of seeds of soybean cultivars produced under three simulated rainfall volumes in the pre-harvest.

Cultivar	Oil content (%)			Protein content (%)		
	------------Rainfall------------			------------Rainfall------------		
	0 mm	54 mm	162 mm	0 mm	54 mm	162 mm
BRS 1010 IPRO	23.03 Ba	22.23 Ba	23.56 Aa	37.8 Ba	36.7 Ba	34.7 Bb
BRS 284	24.23 Aa	24.46 Aa	24.00 Aa	38.9 Aa	38.2 Aa	38.6 Aa
NA 5909 RR	23.90 Aa	24.16 Aa	22.46 Bb	37.0 Ba	36.2 Ba	36.3 Ba
BRSMG 752S	21.73 Bb	23.50 Aa	22.70 Ba	39.2 Aa	38.9 Aa	38.3 Ab
BRSMT Pintado	22.40 Bb	21.86 Bb	23.70 Aa	39.5 Aa	39.8 Aa	38.6 Aa
BRS Jiripoca	22.80 Ba	23.43 Aa	22.23 Ba	40.1 Aa	40.0 Aa	38.0 Aa
M8210 IPRO	22.66 Ba	22.60 Ba	22.20 Ba	39.5 Aa	39.6 Aa	40.0 Aa

Means followed by the same lowercase letter in the row and uppercase letter in the column do not differ from each other by the Scott-Knott test at a 5% probability.

The analysis of the sanitary quality of soybean seeds showed low incidence of many genera of fungi, most of them saprophytes or contaminants, except *Macrophomina phaseolina* and *Corynespora cassiicola*. The other fungi, *Alternaria* spp., *Botryodiplodia* spp., *Cladosporium* spp., *Rhizopus* spp., *Penicillium* sp., *Trichoderma* spp., and *Chaetomium* sp. and bacteria were saprophytes. For this reason, no effect was observed on seed quality. Moreover, no significant effect was observed for the tested factors. In addition, *Aspergillus flavus* was also observed (Table 6). The cultivars BRS 1010 IPRO, NA 5909 RR, and M8210 IPRO showed higher percentages of *Aspergillus flavus* in seeds from plants produced under a rainfall of 162 mm. Moreover, the increase in rainfall volume for these cultivars led to an increase in the incidence of *Aspergillus flavus*.

No relationship was observed between weathering damage and lignin content in the pod and seed coat with the incidence of this fungus in soybean seeds. According to Dantas et al. (2012), there is no interference of the lignin content of the seed coat on the resistance to infection by the fungus *Aspergillus flavus*. Carvalho et al. (2015) evaluated the effect of lignin and Mn leaf application on the incidence of fungi and found that the cultivar BRS Celeste had the lowest lignin content and was among those with the lowest incidence of *Aspergillus flavus*, inferring that the highest lignin content in the seed coat of soybean seeds do not result in increased resistance to infection.

The incidence of the fungus *Cercospora kikuchii* was observed in all cultivars (Table 6). The cultivars BRS 1010 IPRO and NA 5909 RR showed higher percentages for rainfall events of 0 (control) and 54 mm, while the cultivars BRS 1010 IPRO, BRS 284, and NA 5909 RR presented higher incidences under the rainfall of 162 mm. The effect of rainfall events on the cultivars showed that the increase in pre-harvest rainfall volumes provided higher incidences of this pathogen

Table 6. Sanitary quality of seeds of soybean cultivars produced under different simulated rainfall volumes in the pre-harvest.

Cultivar	*Aspergillus flavus* (%)			*Cercospora kikuchii* (%)		
	------------Rainfall------------			------------Rainfall------------		
	0 mm	54 mm	162 mm	0 mm	54 mm	162 mm
BRS 1010 IPRO	0.0 Aa	0.5 Bb	1.6 Cc	19.5 Ca	21.2 Cb	25.7 Dc
BRS 284	0.0 Aa	0.0 Aa	0.0 Aa	15.6 Ba	17.8 Bb	22.7 Dc
NA 5909 RR	0.5 Ba	0.5 Ba	1.0 Bb	20.2 Ca	23.2 Cb	24.5 Dc
BRSMG 752S	0.0 Aa	0.0 Aa	0.0 Aa	11.7 Ba	10.5 Ba	13.1 Ca
BRSMT Pintado	0.0 Aa	0.0 Aa	0.0 Aa	1.7 Aa	1.8 Aa	1.6 Aa
BRS Jiripoca	0.0 Aa	0.0 Aa	0.0 Aa	1.6 Aa	2.3 Aa	3.2 Aa
M8210 IPRO	0.0 Aa	0.6 Bb	1.0 Bc	14.0 Bb	9.5 Ba	7.3 Ba
	Fusarium spp. (%)			*Phomopsis* spp. (%)		
Cultivar	0 mm	54 mm	162 mm	0 mm	54 mm	162 mm
BRS 1010 IPRO	0.0 Aa	1.0 Ba	0.5 Aa	3.1 Aa	12.2 Db	14.5 Dc
BRS 284	2.0 Ba	3.5 Bb	4.5 Db	12.7 Ca	11.2 Da	14.5 Db
NA 5909 RR	3.7 Ca	5.8 Cb	5.0 Eb	5.7 Ba	5.5 Ba	9.0 Cb
BRSMG 752S	2.3 Ba	2.0 Ba	2.5 Ca	6.5 Ba	7.5 Ca	9.0 Ca
BRSMT Pintado	0.0 Aa	0.0 Aa	0.0 Aa	1.5 Aa	0.5 Aa	0.0 Aa
BRS Jiripoca	0.0 Aa	1.0 Bb	0.0 Aa	3.8 Aa	2.5 Aa	2.5 Ba
M8210 IPRO	0.7 Aa	0.5 Aa	1.5 Ba	4.5 Ba	4.5 Ba	4.0 Ba

Means followed by the same lowercase letter in the row and uppercase letter in the column do not differ from each other by the Scott-Knott test at a 5% probability.

in the cultivars BRS 1010 IPRO, BRS 284, and NA 5909 RR. Moreover, seeds from cultivars with higher incidences of the fungus *Cercospora kikuchii* (Table 6) showed higher percentages of weathering damage by the tetrazolium test (Table 3) and lower lignin contents in the pods (Table 4). Furthermore, the cultivar NA 5909 RR presented the lowest lignin content in the seed coat (3.6%). Thus, the use of cultivars with characteristics that provide higher tolerance to environmental humidity fluctuations, such as high lignin contents in the pods, is essential for reducing weathering deterioration and incidence of the fungus *Cercospora kikuchii* in the field.

The fungus *Fusarium* spp. had the highest incidence in the cultivars BRS 284, NA 5909 RR, and BRSMG 752S compared to the others for the control treatment (0 mm) (Table 6). The cultivar NA 5909 RR presented the highest percentages under 54 mm, followed by the cultivars BRS 1010 IPRO, BRS 284, BRSMG 752S, and BRS Jiripoca. Moreover, the cultivars BRS 284, NA 5909 RR, BRSMG 752S, and M8210 IPRO showed the highest incidence of this fungus under the rainfall of 162 mm. The comparison between rainfall volumes showed that 54 and 162 mm caused an increase in the incidence of *Fusarium* spp. for seeds of the cultivars BRS 284 and NA 5909 RR. This fact may be associated with seed weathering deterioration through excessive pre-harvest rainfall and seed exposure due to the high wrinkling rate in the seed coats, becoming a gateway to fungal attack. In this context, according to the morpho-anatomical study carried out by Pinheiro et al. (2021), weathering damage is observed not only by the wrinkling of the seed coat but also by the compaction and rupture of its cells, especially in the layers of the hourglass and parenchyma cells.

The fungus *Phomopsis* spp. was also observed in the blotter test (Table 6). The cultivar BRS 284 showed higher percentages of this fungus in the evaluated seeds for the control (0 mm), followed by the cultivars NA 5909 RR, BRSMG 752S, and M8210 IPRO. The cultivars BRS 1010 IPRO and BRS 284, which have lower lignin content in the pod, showed higher incidences under rainfall events of 54 and 162 mm. The increased rainfall volumes caused an increase in the incidence of *Phomopsis* spp. in the seeds of the cultivar BRS 1010 IPRO. The highest percentages in the cultivars BRS 284 and NA 5909 RR were observed under the rainfall of 162 mm. This fungus often reduces the quality of soybean seeds, especially when there are rainy periods associated with high temperatures during the maturation stage until harvest, as these conditions favor its growth (Gillen et al., 2012; Li and Chen, 2013; Li et al., 2017). In addition, this fungus can negatively interfere with the evaluation of the seed germination test (in the paper roll) (França-Neto et al., 2016).

Therefore, the evaluated cultivars presented different responses to pre-harvest weathering deterioration. Soybean seeds produced from cultivars with the highest lignin contents in the pods showed lower percentages of weathering damage, in addition to higher physiological and sanitary quality. The lignin contents in the seed coat showed no evidence of a relationship with the evaluated factors, except for the cultivar BRSMT Pintado. This fact may be related to a reduction in the percentage of this compound in the seeds over the years within breeding programs due to the focus on grain yield. Thus, lignin has generally appeared in seeds at low contents to the point of not causing a significant change in water absorption and quality of soybean seeds.

CONCLUSIONS

The physiological and sanitary quality of soybean seeds is reduced with the increase in pre-harvest rainfall. Soybean seeds from cultivars with higher lignin contents in the pod present higher tolerance to pre-harvest weathering deterioration and provide seeds of better physiological quality. The oil and protein content of soybean seeds is reduced as pre-harvest rainfall increases in some cultivars. Soybean plants with higher lignin contents in the pods produce seeds with a lower incidence of the fungus *Cercospora kikuchii* and lower chlorophyll content when associated with pre-harvest rainfall events.

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