PAB
Pesquisa Agropecuária Brasileira

ISSN 1678-3921

Journal homepage: www.embrapa.br/pab

For manuscript submission and journal contents, access: www.scielo.br/pab

Seed Technology/ Original Article

Structural analysis of soybean pods and seeds subjected to weathering deterioration in pre-harvest

Cristian Rafael Brzezinski[(1)],
Julia Abati[(2✉)],
Claudemir Zucareli[(2)],
Cristiano Medri[(2)],
Liliane Marcia Mertz-Henning[(3)],
Francisco Carlos Krzyzanowski[(3)],
José de Barros França-Neto[(3)] and
Fernando Augusto Henning[(3)]

[(1)] GDM Genética do Brasil S.A., Loteamento Córrego Prata, Zona Rural, CEP 77500-000 Porto Nacional, TO, Brazil. E-mail: cbrzezinski@gdmseeds.com

[(2)] Universidade Estadual de Londrina, Rodovia Celso Garcia Cid, PR 445, Km 380, CEP 86057-970 Londrina, PR, Brazil. E-mail: juliaabati@yahoo.com.br, claudemircca@uel.br, cristianomedri@uel.br

[(3)] Embrapa Soja, Rodovia Carlos João Strass, s/nº, Acesso Orlando Amaral, Distrito de Warta, Caixa Postal 4006, CEP 86085-981 Londrina, PR, Brazil. E-mail: liliane.henning@embrapa.br, francisco.krzyzanowski@embrapa.br, jose.franca@embrapa.br, fernando.henning@embrapa.br

✉ Corresponding author

Received
September 17, 2021

Accepted
January 10, 2022

How to cite
BRZEZINSKI, C.R.; ABATI, J.; ZUCARELI, C.; MEDRI, C.; MERTZ-HENNING, L.M.; KRZYZANOWSKI, F.C.; FRANÇA-NETO, J. de B.; HENNING, F.A. Structural analysis of soybean pods and seeds subjected to weathering deterioration in pre-harvest. **Pesquisa Agropecuária Brasileira**, v.57, e02697, 2022. DOI: https://doi.org/10.1590/S1678-3921.pab2022.v57.02697.

Abstract – The objective of this work was to analyze structurally the pods and seed coats of soybean (*Glycine max*) cultivars, as well as to determine the quality of seeds when subjected to deterioration by weathering in pre-harvest. A 7×3 factorial arrangement was used – seven cultivars and three volumes of simulated rainfall (0, 54, and 162 mm). Exposure to rainfall was simulated in the R8 phenological stage. Then, the plants were taken to a greenhouse, where they were kept until the time of collection of pods and seeds. After collection, the following evaluations were carried out: tetrazolium test, lignin content in pods and seed coats, and structural analysis of pods (exocarp, mesocarp, and endocarp thickness) and seed coats (epidermis, hypodermis, and parenchymal-cell thickness). Pre-harvest rainfall of 54 and 162 mm reduces the quality of soybean seeds; however, the response to deterioration by weathering differs according to the cultivar. The tolerance to all simulated rainfall was greater for BRSMT Pintado, BRS Jiripoca, and M8210IPRO and lower for BRS 1010IPRO. Pods with a greater thickness of the exocarp, mesocarp, and endocarp and a high lignin content show greater resistance to weathering deterioration and seeds with greater vigor and viability. The thickness of the hypodermis of the testa is related to resistance to weathering deterioration and to the obtainment of high-quality seeds.

Index terms: *Glycine max*, endocarp, exocarp, hypodermis of the testa, lignin content, vigor.

Análise estrutural de vagens e sementes de soja submetidas à deterioração por umidade em pré-colheita

Resumo – O objetivo deste trabalho foi analisar estruturalmente as vagens e os tegumentos de cultivares de soja (*Glycine max*), bem com determinar a qualidade das sementes quando submetidas à deterioração por umidade em pré-colheita. Utilizou-se arranjo fatorial 7×3 – sete cultivares e três volumes de precipitações pluviais simuladas (0, 54 e 162 mm). A exposição à chuva foi simulada no estádio fenológico R8. Em seguida, as plantas foram levadas à casa de vegetação, onde foram mantidas até a coleta das vagens e das sementes. Após a coleta, realizaram-se as seguintes avaliações: teste de tetrazólio, teor de lignina nas vagens e nos tegumentos, e análise estrutural de vagens (espessura do exocarpo, do mesocarpo e do endocarpo) e tegumentos (espessura da epiderme, da hipoderme e das células parenquimatosas). Precipitações de 54 e 162 mm em pré-colheita reduzem a qualidade das sementes de soja; entretanto, a resposta à deterioração por umidade difere de acordo com a cultivar. A tolerância a todas as precipitações pluviais simuladas foi maior para BRSMT Pintado, BRS Jiripoca e M8210IPRO e menor para BRS 1010IPRO. Vagens com maior espessura do exocarpo, do mesocarpo e do endocarpo e elevados teores de lignina apresentam maior tolerância à deterioração por umidade e sementes com maior vigor e viabilidade. A espessura da hipoderme da testa de tegumentos está relacionada à tolerância à deterioração por umidade e à obtenção de sementes de elevada qualidade.

Termos para indexação: *Glycine max*, endocarpo, exocarpo, hipoderme da testa, teor de lignina, vigor.

Introduction

Seeds are the basic and vital input – including technologies introduced by breeding – for a sustainable increase in yield and agricultural production (Sharma et al., 2015). Therefore, breeding programs should consider the seed quality factor.

The quality of seeds depends on the seed production process, adequate sowing dates, and suitable areas for production. To meet crop requirements, seed maturity should occur under milder temperatures and low rainfall volumes and the most critical phenological phases should coincide with periods of favorable meteorological conditions (Wang et al., 2012; França-Neto et al., 2016). However, these conditions are not always present, especially in tropical regions where seed placement at sowing, even in seed-producing areas, is usually done to make the cultivation of the second crop viable.

In the case of soybean [*Glycine max* (L.) Merr.] development, high volumes of rainfall in certain periods, mainly in pre-harvest, can be harmful to seed quality because of weathering deterioration (Pinheiro et al., 2021). This damage causes wrinkling and splitting in the seed coat due to the expansion and contraction of this tissue when exposed to alternating cycles of temperature and relative humidity (Forti et al., 2013; França-Neto et al., 2016; Monteiro et al., 2021).

The susceptibility to weathering deterioration is related to the morphological characteristics of the soybean seeds, since the vital parts of the embryo are situated under a seed coat that is not very thick and that practically does not provide any protection (França-Neto & Henning, 1984). In addition, with successive hydrations, the seed coat begins to change physiologically and structurally (França-Neto et al., 2016). Therefore, anatomical and physiological studies related to the integrity of the pod and seed layers can assist in understanding the response of genotypes to weathering damage, as well as in identifying characteristics with potential for use in breeding programs.

Permeability is a conditioning factor in resistance to weathering damage and is associated with seed coat structure. According to Ma et al. (2004), the cuticle of the palisade layer is determining for seed coat permeability, which is also dependent on the thickness of the epidermal and hypodermal layers, presence of pigments, and cell shape and organization (Mertz et al., 2009).

Weathering deterioration may cause physiological disturbances as reported by Forti et al. (2013). The authors found that the layers of palisade cells, hourglass cells, and of the parenchyma of soybean seed coats gradually decreased in size in the region opposite the hilum, where the wrinkling caused by weathering deterioration usually occurs. The hourglass cells also had a twisted, folded, and flattened appearance.

Regarding resistance to weathering deterioration, semi-permeability related to lignin content is an important trait. According to Oliveira et al. (2014), the study of pod lignin content may be of great value for seed technology since pods with more lignin may show less permeability to water, leading to a lower deterioration of seeds in the field. Several authors confirmed this result both for pods and seed coats, concluding that lignin content differs among genotypes (Oliveira et al., 2014; Huth et al., 2016; Bellaloui et al., 2017) and may be related to weathering deterioration (Castro et al., 2016; Huth et al., 2016) just as other characteristics linked to permeability.

The objective of this work was to analyze structurally pods and seed coats of soybean cultivars, as well as to determine the quality of seeds when subjected to deterioration by weathering in pre-harvest.

Materials and Methods

The experiment was carried out at the Seed Physiology, Technology, and Chemistry Laboratories, in the Seed and Grain Technology Center of Embrapa Soja, as well as in the Plant Anatomy Laboratory of Universidade Estadual de Londrina, both located in the municipality of Londrina, in the state of Paraná, Brazil.

The experimental design was completely randomized, in a 7×3 factorial arrangement, with four replicates. The factors consisted of seven soybean cultivars (BRS 1010IPRO, BRS 284, NA 5909 RG, BRSMG 752S, BRSMT Pintado, BRS Jiripoca, and M8210IPRO) and three simulations of rainfall volumes (0, 54, and 162 mm) (Table 1).

Seeds of the used cultivars were produced under greenhouse conditions, with partial control of temperature and relative humidity, which were monitored during the experiment using the data logger device (Figure 1).

Seeds were sown on 10/27/2015, in 9.0 L pots, at a depth of 3.0 to 5.0 cm, in a soil classified as a Latossolo Vermelho Eutroférrico (Santos et al., 2018) of clayey texture, i.e., an Oxisol, with soil amendments according to crop needs (Tecnologias…, 2013). Hours before sowing, the seeds were inoculated with the BIAGRO NG commercial liquid inoculant (Bayer S.A., Cambé, PR, Brazil), containing the SEMIA 5079 and SEMIA 5080 *Bradyrhizobium japonicum* bacterial strains (5×10^9 viable cells per milliliter), at a rate of 100 mL of product for each 50 kg^{-1} of seeds. Seeds were treated with the Derosal Plus commercial fungicide (Bayer

Table 1. Soybean (*Glycine max*) cultivars used in the experiment and their respective characteristics, as well as mean daily rainfall and accumulated rainfall in the R8 phenological stage of the crop in 2016, in the municipality of Londrina, in the state of Paraná, Brazil.

Cultivar	Type[(1)]	Habit	Cycle	Group	Pubescence
BRS 1010IPRO	I	Indeterminate	Early	6.1	Gray
BRS 284	C	Indeterminate	Early	6.3–7.1	Gray
NA 5909 RG	RR	Indeterminate	Early	5.9	Gray
BRSMG 752S	C	Indeterminate	Semi-early	7.5	Brown
BRSMT Pintado	C	Determinate	Medium	8.7	Gray
BRS Jiripoca	C	Determinate	Medium	8.4	Gray
M8210IPRO	I	Determinate	Early	8.2	Brown
		Mean daily rainfall and accumulated rainfall (mm)			
		Day 1	Day 2	Day 3	Accumulated
Rainfall 0		0	0	0	0
Rainfall 54		18	18	18	54
Rainfall 162		54	54	54	162

[(1)]Type of technology: I, intact; C, conventional; and RR, roundup ready. Source: based on information available at the cultivar registry of Ministério da Agricultura, Pecuária e Abastecimento (Brasil, 2022).

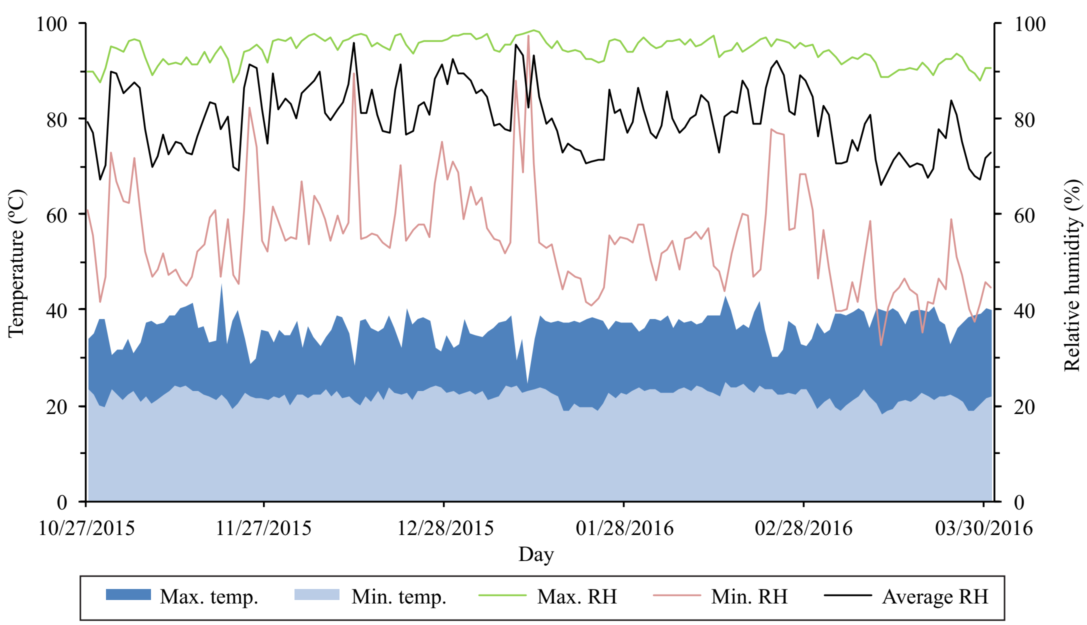

Figure 1. Maximum and minimum daily temperature and maximum, minimum, and mean relative humidity (RH) monitored using the data logger device during the development period of soybean (*Glycine max*) cultivars grown under greenhouse conditions in the 2016 season, in the municipality of Londrina, in the state of Paraná, Brazil.

S.A., Belford Roxo, RJ, Brazil), containing the active ingredients carbendazim + thiram, at a rate of 200 mL 100 kg[1] of seed. Initially, four seeds were planted per pot and, after seedling emergence, plants were thinned out to two.

Four replicates of five pots with two plants, totaling 40 plants, were used for each treatment. Plants were irrigated daily using drip nozzles. Insecticides and fungicides were applied according to the needs of the crop and to the recommendations for its cultivation (Tecnologias…, 2013).

Rain simulation was performed in the R8 crop development stage (full maturity with 95% of the pods with mature color) during three consecutive days in order to obtain the mean daily rainfall and accumulated rainfall (Table 1); water depth was measured using rainfall gauges distributed across the entire experimental area. The phenological stage of the plants was determined based on the scale of Ritchie et al. (1997).

Rainfall was simulated, at different depths, in a closed room, using a projected device with flat jet spray nozzles to produce large droplets. The device consisted of a 3.0 m high metallic structure to which a suspended "wheeled support" can be connected at a height of 2.5 m. This support has a spray bar for the rain simulation system to move across an area of 15 m^2 in the direction of the length of the device. The bar of the device was pulled by chains and gears with the assistance of an electric motor adjusted by a frequency modulator, allowing a previously determined constant speed.

A hydraulic pump, with constant pressure and automatic activation, was used to pump stored water from a 3,000 L capacity tank to the spray bar equipped with the nozzles responsible for forming the simulated raindrops. A total of seven TKSS20 high-flow conical nozzles (Spraying Systems do Brasil, São Bernardo do Campo, SP, Brazil), spaced at 0.50 m, were used to provide a greater rainfall uniformity over the area. Operating pressure was 0.81 kgf cm^{-2}, the height of the bar was 1.45 m in relation to the surface of the experimental units, and the displacement speed was 0.050 m s^{-1}. Therefore, for each total displacement of the bar, 0.9 mm of simulated rainfall was applied. These specifications led to the production of artificial raindrops with a mean volumetric diameter of approximately 1,140 micra, according to information from the manufacturer of the nozzles (Spraying Systems do Brasil, São Bernardo do Campo, SP, Brazil).

After the hydration and dehydration cycles, the plants were taken back to the greenhouse, where they were kept until the time of collection of pods and seeds (time of harvest), within three to four days. Pods and seeds were collected from the upper, middle, and lower third of the plant in equal proportion. After collected, the pods and seeds were sent to the laboratory for analyses, which included the tetrazolium test, structural analysis of pods and seed coats, and determination of lignin content of pods and seed coats.

For the tetrazolium test, two subsamples of 50 seeds per replicate were used, being pre-conditioned on germination test paper that was moistened with distilled water for 16 hours in a seed germinator at 25ºC. After this period, seeds were totally submerged in tetrazolium solution (2,3,5-triphenyl tetrazolium chloride) at a concentration of 0.075% and kept at 40°C for approximately 150 min in a germination chamber in the absence of light. Then, the seeds were classified according to the criteria – viability, vigor, weathering damage (classes 1–8 and 6–8) – proposed by França-Neto & Krzyzanowski (2018). The results were expressed in percentage.

For the structural analysis of the pods and of the seed coats, the samples were initially fixed in FAA 50 medium, consisting of formaldehyde, glacial acetic acid, and 50% ethyl alcohol at a ratio of 1:1:8 (Johansen, 1940). To visualize the structural differences between pods and seed coats, the following steps were taken. The pod and seed coat fragments were fixed in paraffin. The EM UC7 ultramicrotome (Leica Microsystems GmbH, Wetzlar, HE, Germany) was used to slice cross sections with 1.0 µm thickness in the region opposite the connection to the funiculus of the pod and opposite the seed hilum. The plant tissue was then stained with 1.0% Astra Blue and 1.0% fuchsine and visualized in the BX 51 OLYMPUS optical microscope (Olympus Corporation of the Americas, Center Valley, PA, USA), at 40x magnification. The images were digitalized by a video camera adapted to the microscope and processed by a microcomputer using the Motic Images Plus 3.0 software (MoticEurope, S.L.U., Barcelona, Spain). The results for thickness of layers of the pods (exocarp, mesocarp, and endocarp) and seed coats (epidermis of the testa, hypodermis of the testa, and parenchymal cells) were presented in µm.

To determine the lignin content of the pods and seed coats, four replicates of 100 seeds and 50 pods were used for each treatment. Initially, the seeds were separated from the pods and then immersed in water for 12 hours to separate seed coats from cotyledons. After this procedure, the seed coats and the pods were dried in a laboratory oven, at 105°C, for 24 hours. The dry matter obtained was ground and homogenized. After that, 0.3 g was weighed for the extraction of proteins connected to the cell wall. After the material free of proteins was obtained, the amount of lignin was quantified by the acetyl bromide method (Moreira-Vilar et al., 2014).

The obtained data were analyzed for normality and homoscedasticity using the Shapiro-Wilk and Hartley tests, respectively, which indicated that data transformation was not necessary. The analysis of variance was performed, and means were compared by the Scott-Knott test, at 5% probability. Analyses were carried out using the SISVAR computer software (Ferreira, 2011).

Results and Discussion

The summary of the analysis of variance for the effects of cultivars and simulated rainfall volumes for the evaluated variables, as well as the mean squares for the isolated effect of cultivar for lignin content, are presented in Table 2.

Data on weathering damage determined by the tetrazolium test showed that rainfall simulations were effective in differentiating the cultivars using the three volumes initially proposed (Table 3). This made it possible to determine the genotypes susceptible or resistant to deterioration in pre-harvest, as well as to verify the effect of these factors on the structural composition of pods and seed coats. It is important to highlight that the maximum and minimum simulated rainfall volumes corresponded to the annual historical mean values found for the pre-harvest period of the main soybean-producing regions of Brazil (Inpe, 2017). For this reason, the obtained results show the similarity between the tested variables and the reality observed by the seed grower in the field.

The structural differences among the pods and seed coats of the tested cultivars were also clearly visualized by morphological characterization, though optical microscopy. It was possible to identify the structures of the pericarp of the pods, for example (Figure 2 A). According to Appezzato-da-Glória & Carmello-

Table 2. Summary of the table of analysis of variance for the data obtained both for the structural analysis and lignin content of pods and seed coats and for the quality of seeds of soybean (*Glycine max*) cultivars under different volumes of simulated rainfall (0, 54, and 162 mm), in pre-harvest, in 2016, in the municipality of Londrina, in the state of Paraná, Brazil[(1)].

SV	DF	Mean square					
		EXO	MESO	ENDO	EP	HP	PC
Cultivar	6	65.19**	1,638.7**	65.19**	31.38^{ns}	77.47**	31.39^{ns}
Rainfall	2	3.38^{ns}	59.82^{ns}	3.38^{ns}	10.77^{ns}	180.54**	0.86^{ns}
Cultivar×Rainfall	12	3.46^{ns}	5.11^{ns}	3.46^{ns}	5.19^{ns}	14.12**	9.67^{ns}
Error	63	8.67	40.02	8.67	15.16	4.43	17.01
Mean	-	54.59	112.91	32.44	42.87	18.02	37.27
CV (%)	-	5.40	5.60	9.08	9.08	11.68	11.07
SV	DF	PL	SCL	TZ V	TZ VIA	W 1–8	W 6–8
Cultivar	6	10.965**	0.3951**	2,298.85**	1,738.98**	1,506.56**	243.40**
Rainfall	2	-	-	692.76**	549.33**	1,567.00**	172.15**
Cultivar×Rainfall	12	-	-	214.09**	142.88**	253.30**	58.96**
Error	21[(2)]-63	0.090	0.0290	19.87	15.74	69.54	7.88
Mean	-	15.57	4.25	77.38	82.76	39.57	7.01
CV (%)	-	1.93	4.01	5.76	4.79	21.07	40.05

[(1)]SV, source of variation; DF, degrees of freedom; EXO, pod exocarp thickness; MESO, pod mesocarp thickness; ENDO, pod endocarp thickness; EP, thickness of the epidermis of the testa; HP, thickness of the hypodermis of the testa; PC, thickness of parenchymal cells; PL, pod lignin content; SCL, seed coat lignin content; TZ V, vigor by the tetrazolium test; TZ VIA, viability by the tetrazolium test; W 1–8, class 1–8 weathering damage, determined by the tetrazolium test; W 6–8, class 6–8 weathering damage, determined by the tetrazolium test; and CV, coefficient of variation. [(2)]Error degree of freedom only for the variable lignin contents of pod and seed coat. **Significant by the F-test, at 1% probability. ^{ns}Nonsignificant.

DOI: 10.1590/S1678-3921.pab2022.v57.02697

Guerreiro (2006), the pericarp can be analyzed structurally by delimitating its regions, usually denominated exocarp, mesocarp, and endocarp.

For seed coats, different thicknesses were verified for the layers epidermis of the testa, hypodermis of the testa or hourglass cells, and parenchymal cells (Figure 2 B).

Table 3. Vigor, viability, and weathering damage determined by the tetrazolium test carried out on seeds of soybean (*Glycine max*) cultivars under different simulated rainfall volumes (0, 54 and 162 mm), in pre-harvest, in 2016, in the municipality of Londrina, in the state of Paraná, Brazil[(1)].

Cultivar	Rainfall volume		
	0 mm	54 mm	162 mm
	Vigor (%)		
BRS 1010IPRO	82Ba	70Bb	64Cb
BRS 284	83Ba	77Ba	72Bb
NA 5909 RG	96Aa	88Aa	75Bb
BRSMG 752S	79Ba	72Bb	70Bb
BRSMT Pintado	84Ba	82Aa	86Aa
BRS Jiripoca	86Ba	81Aa	79Aa
M8210IPRO	90Aa	86Aa	85Aa
	Viability (%)		
BRS 1010IPRO	82Ca	76Cb	72Db
BRS 284	89Ba	84Bb	78Cc
NA 5909 RG	96Aa	90Ab	79Cc
BRSMG 752S	87Ba	84Ba	80Cb
BRSMT Pintado	88Ba	86Ba	87Ba
BRS Jiripoca	89Ba	86Ba	85Ba
M8210IPRO	95Aa	91Aa	91Aa
	Weathering damage – classes 1–8 (%)		
BRS 1010IPRO	22Aa	41Bb	66Dc
BRS 284	31Ba	52Cb	64Dc
NA 5909 RG	20Aa	36Bb	53Cc
BRSMG 752S	16Aa	20Ab	22Ab
BRSMT Pintado	12Aa	17Ab	22Ab
BRS Jiripoca	17Aa	18Aa	20Aa
M8210IPRO	15Aa	31Bb	40Bb
	Weathering damage – classes 6–8 (%)		
BRS 1010IPRO	8Ca	22Db	28Dc
BRS 284	5Ba	14Cb	20Cc
NA 5909 RG	2Aa	8Bb	19Cc
BRSMG 752S	2Aa	3Aa	5Ba
BRSMT Pintado	2Aa	2Aa	2Aa
BRS Jiripoca	1Aa	4Ba	4Ba
M8210IPRO	3Aa	7Bb	9Bb

[(1)]Mean values followed by equal letters, lowercase in the rows and uppercase in the columns, do not differ from each other by the Scott-Knott test, at 5% probability.

However, it was not possible to evaluate the cuticle layer associated with the epidermis of the testa. When characterizing seeds from soybean cultivars, Mertz et al. (2009) observed those same structures, as well as contrasting permeability for seed coats; however, the authors were also not able to analyze the cuticle layer. Ma et al. (2004) concluded that the cuticle layer is related to seed coat permeability, which may be weaker or stronger, when showing or not cracks, respectively. This information is important since the cuticle, a fine external layer of the seed coat made up of polymers, such as cutin and cuticular waxes (Appezzato-da-Glória & Carmello-Guerreiro, 2006; Smýkal et al., 2014; Vu et al., 2014), is the first barrier to water imbibition, showing a varying structure according to genetic, physiological, and environmental factors. Therefore, knowledge of the cells that compose pods and seed coats is essential because it is through these layers that water can reach the embryo of the soybean seed.

For the cultivar effect, the characterization of the thickness of the pod layers showed that the exocarp from the BRS 1010IPRO and BRS 284 cultivars was not as thick as the other ones (Table 4). Originating from the outer epidermis of the ovary, the exocarp is the outermost layer of the pod and may have a cuticle layer, stomata, and trichomes; it may also have one or more layers of lignified sclerenchymatous cells, conferring it rigidity (Appezzato-da-Glória & Carmello-Guerreiro, 2006), which directly affects water uptake. Therefore, cultivars with pods with a thinner exocarp may be less tolerant to moisture fluctuations in the environment since water would be rapidly absorbed when crossing a shorter distance, i.e., a thin cell layer. This result was confirmed indirectly by the greater percentage of weathering damage, within classes 1–8 and 6–8, in the seeds from pods with a more limited exocarp thickness (Table 3).

Another factor that may be directly related to water uptake through the exocarp is lignin content (Table 4). According to the obtained results, the pods of cultivars BRS 1010IPRO and BRS 284 contain a lower lignin content than all others. Lignin is formed through the oxidative coupling of monomers of hydroxycinnamyl alcohols, called monolignols, and, besides affecting the degree of resistance to mechanical damage, may also affect the process of water absorption (Zhao & Dixon, 2011; Kuchlan et al., 2018; Liu et al., 2018). This effect can be attributed to the hydrophobic character of lignin, which slows the passage of water through tissues, especially when associated with other waxy substances, such as suberin (Vishwanath et al., 2014; Abati et al., 2022).

The mesocarp of the pods of BRS 1010IPRO, BRS 284, and NA 5909 RG was not as thick as the other ones (Table 4), as observed for the endocarp of the first two cultivars. The obtained results are indicative that the pod layers were not affected by the volumes of simulated rainfall and that the differences found among cultivars are related to the genetic characteristics of each material. Therefore, cultivars with thicker pods can be used in breeding programs as an indicator for selection of materials resistant to weathering deterioration in pre-harvest both due to the phenotypic stability of their pods and to their qualitative character, resulting in a low genotype by environment interaction.

Seed coat measurements showed an interaction between cultivars and the simulated rainfall volumes for the hypodermis of the testa, but there was no

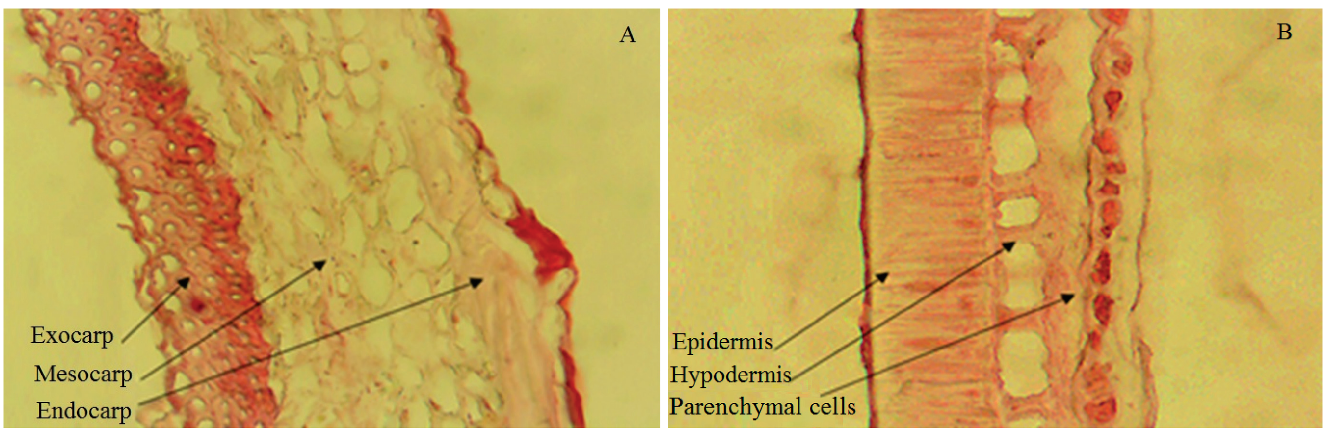

Figure 2. Cross section of the layers of soybean (*Glycine max*) pods (A) and seed coats (B) sliced with a ultramicrotome, stained with Astra Blue and fucsine, and visualized under an optical microscope with 40x magnification, showing the differences in the structures of the pods (exocarp, mesocarp, and endocarp) and seed coats (epidermis of the testa, hypodermis of the testa, and parenchymal cells) under greenhouse conditions, without rainfall simulation, in the 2015/2016 season, in the municipality of Londrina, in the state of Paraná, Brazil.

Table 4. Thickness of pods (exocarp, mesocarp, and endocarp) considering the overall mean of the simulated rainfall volumes, thickness of the hypodermis of the testa of seed coats under different simulated rainfall volumes, and lignin content in pods and seed coats of soybean (*Glycine max*) cultivars, in pre-harvest, in the 2016 season, in the municipality of Londrina, in the state of Paraná, Brazil(1).

Cultivar	Thickness of pods (µm)(2)			Thickness of the hypodermis of the testa (µm)			Lignin content (%)	
	Exocarp	Mesocarp	Endocarp	0 mm	54 mm	162 mm	In pods	In seed coats
BRS 1010IPRO	52.1C	102.6D	30.0C	21.0Aa	16.0Bb	12.1Cc	13.46D	4.27B
BRS 284	52.3C	105.0D	30.2C	19.0Ba	15.3Bb	13.6Cb	14.10C	4.20B
NA 5909 RG	58.3A	102.2D	34.2A	18.6Ba	16.7Ba	13.8Cb	15.34B	3.60C
BRSMG 752S	54.3B	114.4C	33.2A	20.3Aa	18.0Ba	16.7Ba	16.13A	4.58A
BRSMT Pintado	53.9B	124.9A	31.8B	19.2Ba	17.6Ba	19.1Aa	16.19A	4.47A
BRS Jiripoca	59.7A	121.3B	35.6A	21.9Aa	22.0Aa	21.5Aa	18.56A	4.26B
M8210IPRO	58.1A	119.7B	33.9A	21.2Aa	18.4Bb	15.6Bc	15.18B	4.35B

(1)Mean values followed by equal letters, lowercase in the rows and uppercase in the columns, do not differ from each other by the Scott-Knott test, at 5% probability. (2)Isolated effect of cultivar, considering the mean of the simulated rainfall volumes (0, 54, and 162 mm).

significant effect of the evaluated factors on the epidermis of the testa and parenchymal cells (Table 2). Cavariani et al. (2009) obtained a similar result when analyzing the thickness of the palisade layer, thickness of the hypodermis, and total thickness of the seed coat of soybean seeds, finding that the applied treatments did not differ significantly in any cultivar × location combination.

For the hypodermis of the testa, the increase in simulated rainfall led to a reduction in the thickness of the hourglass cells (Table 4). Forti et al. (2013) highlighted that the wrinkling caused by weathering damage led to a reduction in the thickness of these cells, which showed a twisted, folded, and flattened appearance.

Regarding the hypodermal layer, a greater thickness was observed for BRS 1010IPRO, BRSMG 752S, BRS Jiripoca, and M8210IPRO in the treatment without rainfall (Table 4), indicating genetic differences between cultivars. For cultivar BRS Jiripoca, at 54 mm of rainfall, the layer maintained its structure under excess weathering, showing a greater tolerance to rainfall than all others. For the maximum simulated rainfall of 162 mm, cultivars BRSMT Pintado and BRS Jiripoca, with a genetic trait of tolerance to rainfall in pre-harvest, showed the best results and BRS 1010IPRO, BRS 284, and NA 5909 RG, the worst ones. This result confirms those obtained in the evaluation of pod structure (exocarp, mesocarp, and endocarp).

No relationship was observed between the hypodermis of the testa and lignin content in the seed coat (Table 4). According to Bahry et al. (2015), water absorption in seeds depends on the conditions in which they were formed, varying among genotypes, regardless of the color of the seed coat and its concentration of lignin.

For vigor, cultivars NA 5909 RG and M8210IPRO exhibited higher values in the treatment without any application of water, i.e., without simulated rainfall (Table 3). At a rainfall volume of 54 mm, the cultivars whose seeds showed the greatest vigor were NA 5909 RG, BRSMT Pintado, BRS Jiripoca, and M8210IPRO. However, at 162 mm, the last three cultivars had a better performance than the first one, with the lowest seed vigor. Since there was no statistical difference for lignin content in the seed coat among cultivars BRS 1010IPRO, BRS 284, BRS Jiripoca, and M8210IPRO, it was not possible to verify the relationship between lignin content in the seed coat and seed vigor, within the limits of contrasts evaluated in the present work.

Overall, increased rainfall volumes reduced the vigor of the tested seeds, except for cultivars BRSMT Pintado, BRS Jiripoca, and M8210IPRO (Table 3). Maintenance of vigor with the increase in rainfall in the pre-harvest period may be a parameter for studies aiming to determine resistance factors and seed quality under weathering deterioration. This attribute is important since high vigor seeds result in a greater percentage, speed, and uniformity of seedling emergence in the field, especially under unfavorable conditions; moreover, high vigor seeds contribute positively to crop yield performance (Tavares et al., 2013; Marcos-Filho, 2015; Bagateli et al., 2019).

The results for viability confirm those obtained for the vigor test. With the maximum rainfall of 162 mm, the cultivars that showed the best results were BRSMT Pintado, BRS Jiripoca, and M8210IPRO, whereas BRS 1010IPRO had the lowest viability for all simulated rainfall volumes (Table 3).

Considering the obtained results, a greater tolerance to fluctuations in ambient moisture was observed for seeds from pods with thicker walls, particularly when associated with a high lignin content, leading to a lower percentage of weathering damage and a greater vigor and viability of seeds. The thickness of the hypodermis layer is also directly related to resistance to weathering damage and to vigor.

Conclusions

1. The simulated pre-harvest rainfall of 54 and 162 mm reduces the quality of soybean (*Glycine max*) seeds, which differs according to the responses of the used cultivars to deterioration by weathering.
2. The BRSMT Pintado, BRS Jiripoca, and M8210IPRO cultivars have greater tolerance to weathering deterioration in pre-harvest, while BRS 1010IPRO has the lowest seed vigor for all simulated rainfall.
3. Soybean pods with a greater thickness of the exocarp, mesocarp, and endocarp, as well as a high lignin content, have a greater resistance to weathering deterioration and seeds with greater vigor and viability.
4. The thickness of the hypodermis of the testa of soybean seed coats is related to resistance to

weathering deterioration and to the obtainment of high-quality seeds.

Acknowledgments

To Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes), for financing, in part, this study (Finance Code 001); to Universidade Estadual de Londrina (UEL) and to Embrapa Soja, for the used structures and financial support.

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