ABRATES

Journal of Seed Science
ISSN 2317-1545
www.abrates.org.br/revista

REVIEW

Journal of Seed Science, v.45, e202345006, 2023

http://dx.doi.org/10.1590/2317-1545v45268562

Importance of the lignin content in the pod wall and seed coat on soybean seed physiological and health performances

Francisco Carlos Krzyzanowski[1]*, José de Barros França-Neto[1], Fernando Augusto Henning[1]

ABSTRACT: The effects of lignin on soybean seed quality have been studied since the early 1990s. Initially, higher lignin content in the seed coat was associated with greater resistance to mechanical damage by the seeds. Later, positive relationships of lignin content in the seed coat were associated with tolerance to stresses caused by weathering in pre-harvest, resulting in improved seed quality. Additional studies have shown that higher lignin content in the pod wall of soybean is also related to production of seeds with high physiological and sanitary qualities. Colored soybean seeds (black or brown) are known to have higher seed quality due to higher lignin content in the seed coat; in addition, the presence of anthocyanin in the seed coat of colored seeds also contributes to improve seed quality. Finally, the effects of boron on lignin synthesis are also highlighted in this review article. As demonstrated in this review article, lignin content in the seed coat and pod wall of soybean plays an important role in relation to physical, health, and physiological seed qualities. These parameters should be considered in studies related to evaluation of the quality of soybean seeds as affected by genetic factors. These parameters should also be strongly considered for inclusion in breeding programs to improve soybean seed and grain quality.

Index terms: mechanical damage, pod wall, seed coat, seed quality, weathering.

RESUMO: Os efeitos da lignina na qualidade da semente de soja vêm sendo estudados desde os primeiros anos da década de 1990. Inicialmente, verificou-se que maiores teores de lignina no tegumento da semente estavam associados a maior resistência a danos mecânicos pelas sementes. Posteriormente, relações positivas do teor de lignina no tegumento das sementes foram associadas à tolerância aos estresses causados pelo intemperismo na fase de pré-colheita, resultando em melhor qualidade das sementes. Estudos adicionais mostraram que maiores teores de lignina na parede da vagem da soja também estão relacionados à produção de sementes com altas qualidades fisiológicas e sanitárias. Sementes de soja coloridas (pretas ou marrons) são conhecidas por possuírem sementes de maior qualidade devido ao maior teor de lignina no tegumento; além disso, a presença de antocianina nos tegumentos das sementes coloridas também contribui para a melhoria da qualidade das sementes. Finalmente, os efeitos do boro na síntese de lignina também são destacados neste artigo de revisão. Conforme demonstrado neste artigo de revisão, o teor de lignina no tegumento e na parede da vagem da soja desempenha um papel importante relacionado às qualidades físicas, sanitárias e fisiológicas das sementes. Esses parâmetros devem ser considerados em estudos relacionados à avaliação da qualidade de sementes de soja, afetadas por fatores genéticos, pois também devem ser fortemente considerados para serem incluídos em programas de melhoramento, para melhorar a qualidade de sementes e grãos de soja.

Termos para indexação: dano mecânico, vagem, tegumento, qualidade de semente, intemperismo.

***Corresponding author**
E-mail: francisco.krzyzanowski@embrapa.br

Received: 10/10/2022.
Accepted: 11/17/2022.

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

CONCEPTUALIZATION

Lignin is one of the main phenolic compounds in the tissues of angiosperms and gymnosperms. It occurs in plant vascular tissues, and the name comes from the Latin "*lignum*", meaning wood (Fengel and Wegener, 1984). It was discovered by Anselme Payen in 1833 (McCarthy and Islam, 1999).

Lignin is the generic term for a large group of aromatic polymers resulting from the oxidative combinatorial coupling of 4-hydroxyphenylpropanoids (Boerjan et al., 2003; Ralph et al., 2004; Vanholme et al., 2010). It is therefore a complex heteropolymer that primarily consists of *p*-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) units formed by oxidative coupling of the monolignols *p*-coumaryl alcohol and sinapyl alcohol, which are products of the phenylpropanoid pathway (Vanholme et al., 2010; Moreira-Vilar et al., 2014; Marchiosi et al., 2020) (Figure 1).

These polymers are predominantly on the secondary cell walls and thicken them. Lignin promotes coating for cellulose and hemicellulose microfibrils, resulting in greater rigidity, strength, and impermeability for lignified tissues. Lignin protects cell wall polysaccharides from microbial degradation, thus imparting resistance to decay (Vanholme et al., 2010). It is the second most abundant polymer in nature, after cellulose (Polle et al., 1994), the third largest component of the cell wall, and the main component of intracellular substances (Cowling and Kirk, 1976). When the lignification process is complete, it usually coincides with cell death, forming what is called a resistance tissue; hence, it can be concluded that lignin is an end product of plant metabolism (Klock, 2014).

METHODOLOGIES FOR LIGNIN DETERMINATION

Several analytical methodologies for determination of lignin are described in the literature. The sulfuric acid method, described by Bailey (1967), modified by Vidaure (1991), and adapted for lignin determination in the soybean seed coat by Alvarez et al. (1997), is used in studies of seed resistance to mechanical damage and requires 24 hours to complete. The potassium permanganate method (Van Soest and Wine, 1968) is somewhat simpler than the first and requires only six hours to perform. This methodology was used by Panobianco et al. (1999) in evaluation of the relationship between the lignin content and the electrical conductivity of the soybean seed soaking solution.

A comparison between these two gravimetric methods for determination of lignin content in the soybean seed coat was performed by Krzyzanowski et al. (2001), who concluded that the sulfuric acid method had greater accuracy and sensitivity in the results. This method ranked 12 cultivars in five distinct groups of lignin content, whereas the potassium permanganate method classified them in only three groups.

In search of greater accuracy in the results of lignin content, the methods for determination evolved to LTGA – Lignothioglycolic acid, as described by Capeleti et al. (2004). Huth et al. (2016), in studies on the relationship between weathering damage and lignin content in soybean seeds, used the Acetylbromide method, as described by Moreira-Vilar et al. (2014). In both methods, the cell wall is removed to determine the lignin content, which is found between cellulose and hemicellulose. The last method shows better lignin recovery in different herbaceous tissues than the other existing methods; it is quick and simple to perform and accurate in the results provided. Lignin is solubilized and subsequently quantified by spectrophotometry, using a UV spectrophotometer with a 280 nm wavelength optical density, obtaining the absorbance of the samples. This absorbance is compared with the predetermined standard curve, in order to obtain the lignin content of the tissue under analysis. Brezezinski et al. (2022) used this methodology in studying pre-harvest field deterioration of soybean seeds.

IMPORTANCE OF LIGNIN FOR SEED HEALTH AND QUALITY

Water impermeable soybean seeds were reported to have a higher percentage of seed coat lignin content than permeable ones, which could be a characteristic responsible for the higher quality present by the impermeable seed

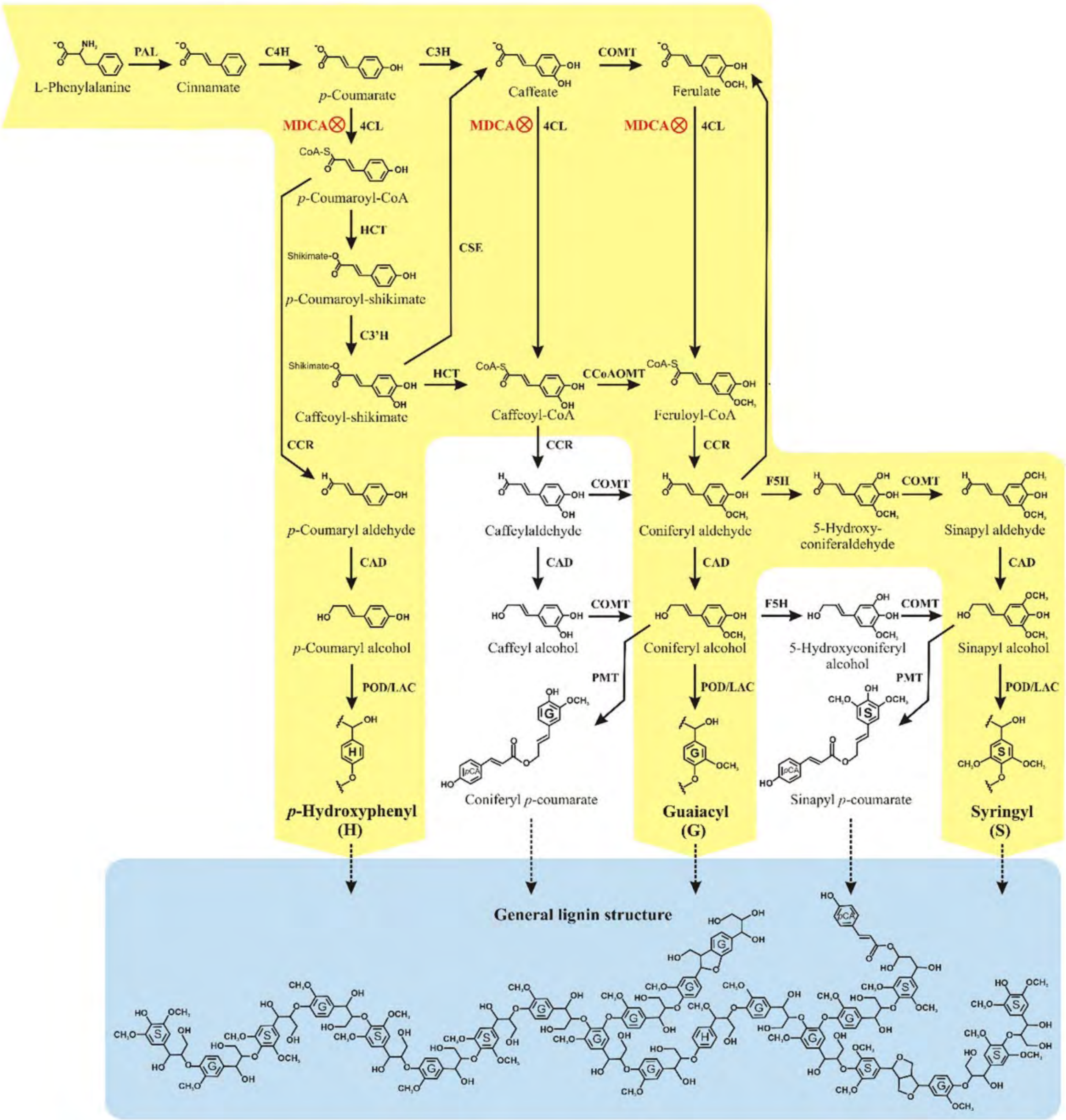

Figure 1. The main biosynthetic route toward the monolignols *p*-coumaryl, coniferyl, and sinapyl, which are the precursors of the monomeric units of *p*-hydroxyphenyl (H), guaiacyl (G) and syringyl (S). PAL: phenylalanine ammonia-lyase; C4H: cinnamate 4-hydroxylase; 4CL: 4-coumarate-CoA ligase; HCT: quinate/shikimate *p*-hydroxycinnamoyl transferase; C3H: 4-coumarate 3-hydroxylase; CSE: caffeoyl shikimate esterase; COMT: cinnamyl (caffeate) O-methyltransferase; CCoAOMT: caffeoyl-CoA 3-0-methyl transferase; CCR: cinnamoyl-CoA reductase; F5H: ferulate (coniferil aldehyde/alcohol) 5-hydroxylase; CAD: cinnamyl alcohol dehydrogenase; POD: peroxidase; LAC: laccase. (Source: Marchiosi et al., 2020).

type (Tavares et al., 1987). However, as reported by Potts et al. (1978), the impermeability of soybean seeds to water may also be due to the deposition of a continuous layer of suberin in the palisade cells of the seed coat.

Pods with high lignin content have a lower rate and speed of water absorption. Soybean pods with greater exocarp, endocarp, and mesocarp thickness and high lignin content have greater tolerance to pre-harvest weathering damage and provide seeds with greater viability and vigor (Table 1). Plants with higher lignin content in pods produce seeds with a lower incidence of the fungus *Cercospora kikuchi* and lower chlorophyll content, especially when associated with pre-harvest rainfall (Brzezinski et al., 2022).

Studying the relationship of the level of phenolic compounds (phenol, lignin, and isoflavones) in soybean cultivars and their resistance response to pod rot by *Phomopsis*, Bellaloui et al. (2012) observed that these compounds occurred in higher concentrations in the cultivars classified as moderately resistant and resistant to this disease than in susceptible cultivars indicating a possible association of these phenolic compounds with the defense mechanism of this disease.

Regarding the physiological quality of soybean seeds, it is widely known that quality declines during storage, with higher rates of reduction in an uncontrolled environment. The phenylpropanoid pathway metabolites, especially lignin, interfere with seed storage potential. Cultivars with higher levels of lignin in the seed coat (average values of 14.23%) and with black seed coat had greater storage potential, mainly in an uncontrolled environment, in relation to yellow seed coat genotypes, which had average lignin content of 4.00% (Abati et al., 2021).

Many factors contribute to seed deterioration, but physical damage due to improper harvesting and handling and its effect on seed coat integrity is a leading cause (McDonald, 1985). Mechanical damage was the major factor responsible for decreasing soybean seed germination and vigor in Brazil in four crop years, from 2014/15 to 2018/19, as reported by França-Neto (2016) and França-Neto et al. (2017; 2018; 2019).

As lignin is the third major component of the cell wall and the main component of intracellular substances, it is primarily responsible for maintaining the integrity and structural cohesion of plant fibers (Butler and Bailey, 1973; Cowling and Kirk, 1976). It is of considerable importance in resistance to mechanical damage in soybean seeds, which is one of the main factors that affect soybean physical and physiological qualities (Alvarez et al., 1997). The deposition of lignin in integumentary tissue is important, as it provides mechanical strength and protects the cell wall against microorganisms (Rijo and Vasconcelos, 1983). In addition, due to its impermeability/semi-permeability characteristic, lignin also protects soybean seeds against the negative effects of weathering conditions that occur during the pre-harvest period (França-Neto et al., 2016). Several studies indicate that the soybean seed coat is very thin and has low lignin content, providing little protection to the embryonic axis, which is in a vulnerable position under the seed coat (Gupta et al., 1973; Agrawal and Menon, 1974; França-Neto and Henning, 1984).

White lima bean seeds (*Phaseolus lunatus* L.) are highly susceptible to mechanical damage because of their lignin content, which is one per cent of the weight of the seed coat. However, lignin content is 15 per cent of seed coat weight in seeds of dark color (Kannenberg and Allard, 1964). Working with soybean seeds, Agrawal and Menon (1974) found a relationship between seed coat thickness and lignin content and difference in susceptibility to mechanical damage between Clark 63 and Adelpina cultivars.

Mechanical damage (Figure 2) is one of the causes of great loss of quality in soybean seeds in tropical and subtropical environments (França-Neto et al., 2019). The development of cultivars that are less prone to mechanical damage is an important contribution of breeders to soybean growers to overcome this limitation. In addition to improving the grain and seed quality, introducing this characteristic to soybean genotypes will reduce the amount of splits, cracks and micro-cracks in the seed coat, reducing the degree of acidity of the grain and improving the grain organoleptic qualities, and changing the behavior of the seed during the storage process, as these seed coat cracks are openings for the entry of moisture that will promote the deterioration process (Krzyzanowski et al., 2019).

Black-coated soybean seeds are higher in quality as compared to yellow-coated seeds (França-Neto and Krzyzanowski, 2000). One of the reasons for this difference is due to the higher lignin content in the seed coats of black-coated genotypes (12.18 %) as compared to the yellow types (4.75 %).

Carbonell and Krzyzanowski (1995) developed the pendulum test for identifying lines with seeds resistant to mechanical damage. The test evaluates the resistance of seeds to mechanical damage, establishing rates of mechanical

Table 1. Weathering damage evaluated by the tetrazolium test, germination, and seedling emergence in sand substrate of soybean seed cultivars with different levels of lignin in the pod and in the seed coat, produced under different volumes of simulated rainfall in pre-harvest (Source: Brzezinski et al., 2022).

	Weathering damage 1-8 (%)				Weathering damage 6-8 (%)		
	Precipitation			Cultivar	Precipitation		
Lignin – pod (%)	0 mm	54 mm	162 mm		0 mm	54 mm	162 mm
13.46 D	22 Aa	41 Bb	66 Dc	BRS 1010 IPRO	8 Ca	22 Db	28 Dc
14.10 C	31 Ba	52 Cb	64 Dc	BRS 284	5 Ba	14 Cb	20 Cc
15.34 B	20 Aa	36 Bb	53 Cc	NA 5909 RR	2 Aa	8 Bb	19 Cc
16.13 A	16 Aa	20 Ab	22 Ab	BRS MG 752 S	2 Aa	3 Aa	5 Ba
16.19 A	12 Aa	17 Ab	22 Ab	BRS Pintado	2 Aa	2 Aa	2 Aa
18.56 A	17 Aa	18 Aa	20 Aa	BRS Jiripoca	1 Aa	4 Ba	4 Ba
15.18 B	15 Aa	31 Bb	40 Bb	M 8210 IPRO	3 Aa	7 Bb	9 Bb
	Germination (%)				Seedling emergence (%)		
Lignin – seed coat (%)	0 mm	54 mm	162 mm	Cultivar	0 mm	54 mm	162 mm
4.27 B	89 Aa	83 Bb	76 Cc	BRS 1010 IPRO	89 Aa	80 Bb	75 Bb
4.20 B	85 Ba	79 Bb	72 Dc	BRS 284	85 Ba	64 Cb	55 Cc
3.60 C	94 Aa	87 Bb	81 Cb	NA 5909 RR	93 Aa	87 Ab	84 Ab
4.58 A	83 Ba	82 Ba	82 Ca	BRS MG 752 S	81 Ba	80 Ba	77 Ba
4.47 A	91 Aa	93 Aa	92 Aa	BRS Pintado	88 Aa	89 Aa	87 Aa
4.26 B	91 Aa	88 Aa	87 Ba	BRS Jiripoca	83 Ba	80 Ba	82 Aa
4.35 B	91 Aa	88 Aa	86 Ba	M 8210 IPRO	82 Ba	80 Ba	83 Aa

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

Figure 2. Soybean seeds with mechanical damage. (Photo: José de Barros França-Neto).

damage; the higher the index number, the better is the seed physiological quality. Based on this test, 12 field grown soybean cultivars were classified as resistant, moderately resistant, and susceptible. In the same line of research, Alvarez et al. (1997) found that the resistance to mechanical damage of these cultivars was directly related to the amount of lignin content in the seed coat (Table 2 and Figure 3).

The seed coat lignin content was found to be high in the cultivars with high index for resistance to mechanical damage, and vice-versa. The same fact was observed for snap bean (Bay et al., 1995). A lignin content above five per cent in the seed coat is proposed as a reasonable indicator of resistance to mechanical damage for soybean seed (Alvarez et al., 1997). Based on knowledge of seed coat lignin content, it is possible to set up a methodology for screening soybean genotypes for resistance to mechanical damage in a breeding program for seed quality (Alvarez et al., 1997). Lignin content, quantified by the acetyl bromide method, explain the resistance of seeds of the Doko and IAS-5 cultivars and the susceptibility of the Savana cultivar to mechanical damage. The levels of monomers G (guaiacyl) and S (syringyl) were inverse in the Doko and Savana cultivars, suggesting that the monomeric composition of lignin varies significantly between cultivars that are resistant and susceptible to mechanical damage. Negative linear correlations between lignin levels and S monomers and S/G ratios can be used as markers of resistance or susceptibility of soybean seeds to mechanical damage (Menino, 2022) (Figure 4).

The lignin content in the seed coat also influences the seed electrical conductivity of different soybean cultivars. The higher the lignin content, the lower the leakage of sugars and amino acids into the seed soaking solution. High lignin content is a desirable genetic characteristic to improve the physical, health, and physiological qualities of the soybean seed. Lower electrical conductivity values of the soybean seed soaking solution are directly related to higher lignin content in the seed coat (Mertz-Henning et al., 2015). Panobianco et al. (1999) analyzed lignin content using the potassium permanganate method, which provides higher values than the sulfuric method, and they related high lignin content with high quality of soybean seeds based on their low indices of electrical conductivity (Table 3 and Figure 5).

In a study conducted by Castro et al. (2016), in which five soybean cultivars were evaluated, it was observed that the cultivar AS 7307 RR had a higher lignin content in the seed coat and a lower percentage of weathering damage evaluated by the tetrazolium test at all harvest times. It therefore showed higher physiological performance, as

Table 2. Means of the seed coat lignin content and the index of resistance to mechanical damage as determined by the pendulum test for seeds of 12 soybean cultivars. (Source: Alvarez et al., 1997).

Cultivar	Lignin Content (%)	Mechanical Damage Index
Doko	6.203 a[1]	494 c[1]
FT-2	6.195 a	575 b
Santa Rosa	5.733 ab	456 d
IAC-8	5.722 ab	413 e
IAS-5	5.717 ab	594 a
Paraná	5.555 ab	505 c
FT-10	5.283 bc	400 e
Bossier	5.278 bc	401 e
Paranagoiana	4.785 cd	374 f
Davis	4.620 cd	373 f
Savana	4.358 d	349 g
IAC-2	4.210 d	266 h

[1]Means not sharing a letter in common differ significantly at the 0.05 level of probability, as determined by Tukey’s test.

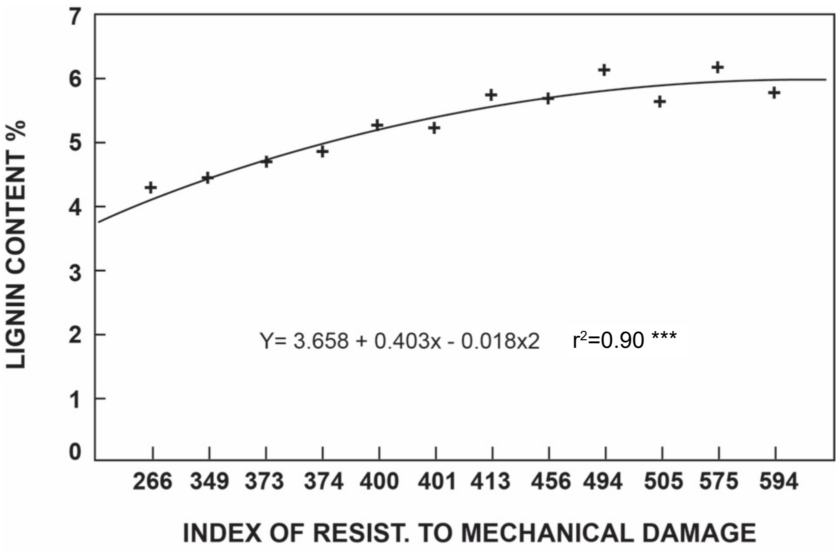

Figure 3. Regression analysis of seed coat lignin content and the index of resistance to mechanical damage as determined by the pendulum test for seeds of 12 soybean cultivars. (Source: Alvarez et al., 1997).

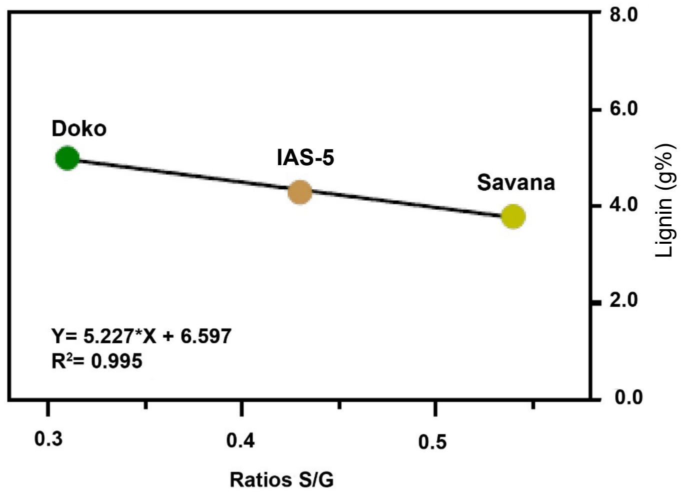

Figure 4. Correlations between lignin content in seed coats and S/G ratios of the soybean cultivars Doko, IAS-5, and Savana. (Source: Menino, 2022).

evaluated by germination and vigor tests. The cultivars NK 7059 RR and SYN 1163 RR presented lower levels of lignin in the seed coat and higher percentages of weathering damage evaluated by the tetrazolium test at all harvest times.

Susceptibility to weathering damage and oxidative stress in soybean seeds with different lignin content in the seed coat was evaluated by Huth et al. (2016). It was reported that seeds with high lignin content were less susceptible to weathering damage, as demonstrated by the standard germination, accelerated aging, and tetrazolium tests, and they exhibited lower oxidative stress due to low activities of superoxide dismutase, guaiacol peroxidase, and lipid peroxidation.

The timing of harvest is a major factor affecting seed quality in soybean, particularly when rainfall during the harvest period is common. Bellaloui et al. (2017) evaluated the effect of the timing of harvest on soybean seed quality

Table 3. Effects of cultivar on electrical conductivity and the seed coat lignin content of a single seed lot of nine soybean cultivars. (Source: Panobianco et al., 1999).

Cultivar	Conductivity (µmhos $cm^{-1}.g^{-1}$)	Lignin Content (%)
Santa Rosa	66 a[1]	7.74 d[1]
FT 10	57 b	7.95 d
Savana	50 c	7.69 d
Bossier	49 c	8.47 c
IAC-8	47 c	8.03 d
IAS-5	41 d	8.57 bc
Doko	41 d	9.28 a
FT2	38 d	9.05 a
Paraná	38 d	8.96 ab

[1]Means not sharing a letter in common differ significantly at the 0.05 level of probability, as determined by Tukey's test.

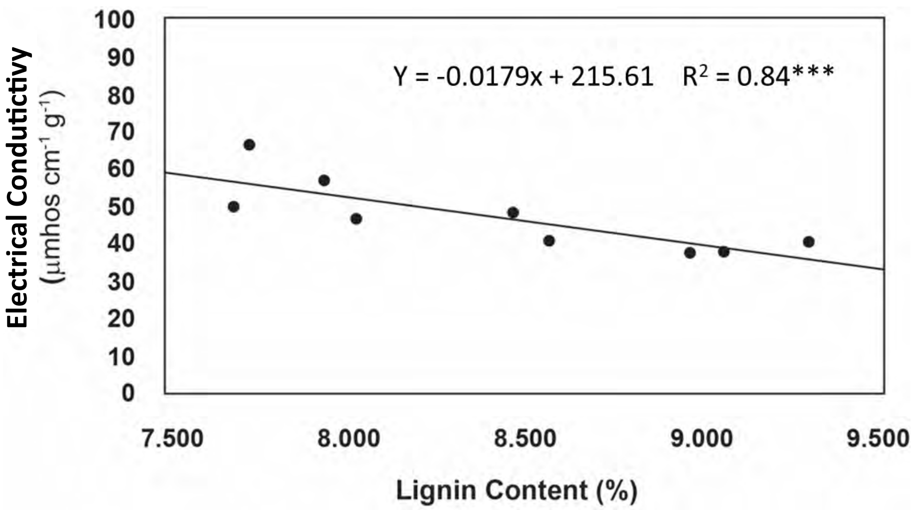

Figure 5. Relationship between seed electrical conductivity (µmhos.$cm^{-1}.g^{-1}$) and the percent of seed coat lignin content for nine soybean cultivars. (Source: Panobianco et al., 1999).

(seed composition, germination, seed coat boron, and lignin) in high germinability (HG) breeding lines (50% exotic) developed under high heat. Results showed that at 28 days after harvest maturity (delayed harvest), the content of seed protein, oleic acid, sugars, seed coat boron, and seed coat lignin were higher in some of the exotic HG lines than in compared with the checks, indicating a possible involvement of these seed constituents, especially seed coat boron and seed coat lignin, in maintaining seed coat integrity and protecting seed coat against physical damage. Highly significant positive correlations were found between germination, seed protein, oleic acid, sugars, seed coat boron with seed coat lignin. These results should suggest to breeders that there is some advantage in selecting for high seed coat boron and lignin content (Bellaloui et al., 2017).

Soybean seeds with dark seed coats are recognized as having better physiological and health qualities than seeds with yellow seed coats. Black soybean seeds have high levels of lignin and anthocyanin in their seed coats, as reported by Abati et al. (2021). Anthocyanin has antioxidant action (Ávila et al., 2012; Zabala and Vodkin, 2014; Choi et al., 2020) and acts to protect cells by preventing the formation of free radicals or by promoting the sequestration or degradation of these molecules.

Six near-isogenic soybean lines, visually differing in terms of seed coat color, black or yellow (Figure 6), were introduced from the University of Florida (USA) and evaluated for physiological and health performance and for lignin content, as reported by França-Neto et al. (1998; 1999). The dark seeds presented average lignin content of 12.18% and the yellow seeds had 4.75% (Table 4). These seeds were deteriorated under extreme conditions in an accelerated aging chamber by exposure to conditions of 41°C and 100% RH for 96 hours. After exposure to this process, the black seed coated seeds had an average of 47% more seedling emergence in sand substrate in relation to those with a yellow seed coat. Seeds of the F 84-7-30 line showed 69.3% emergence for black seeds, and only 23% for yellow seeds (Table 5). Regarding seed health quality, the black seed coated lines averaged less than half the infection rates by *Aspergillus flavus* observed for the yellow seeds; line F 84-7-30 showed 20% infection in black seeds and 55% in yellow seeds. Mertz et al. (2009) also concluded that soybean seeds with black seed coats showed superior physiological quality as compared to seeds with yellow seed coats.

Histological studies using scanning electron and optical microscopy showed that anatomically the seed coats of these yellow and black strains do not differ (Figure 7). The only difference is in regard to the presence of dark coloration, probably due to anthocyanin, present in the palisade cell layer of the black seeds (Figure 7B).

Brown-colored segregating seeds of the soybean cultivars Embrapa 48, BRS 156, and BRS 133 were evaluated by Santos et al. (2007) for imbibition rate at 3-hour intervals in a 24-hour period. Germination, vigor by the accelerated aging and tetrazolium tests, and lignin and protein concentration were also evaluated. It was observed that expression of the brown color in the seed coat of the same soybean cultivar, due to its higher concentration of lignin, positively affects the imbibition speed and the physiological quality of its seeds (Tables 6, 7 and 8).

Studies carried out by Menezes et al. (2009) on the chemical and structural aspects of the physiological quality of soybean seeds observed positive correlations at 5% probability between the lignin content and the percentage of normal seedlings in the accelerated aging test on the 5th and 11th days of evaluation after sowing. This fact implies that the lignin constituted a protection for the seeds and led to less deterioration. The correlation between germination

Figure 6. Soybean seeds with yellow and black seed coats; lines introduced from the University of Florida (USA) (Photo: José de Barros França-Neto).

Table 4. Lignin content (%) in the seed coat of soybeans from six lines with black and yellow seed coats. (Source: França-Neto et al., 1998).

Line	Seed Coat Lignin Content (%)		
	Black Seed Coat	Yellow Seed Coat	Mean
F 84-7-11	12.92 aA[1]	5.52 aB	9.22 a
F 84-7-13	13.00 aA	5.34 aB	9.17 a
F 84-7-26	12.56 abA	5.38 aB	8.97 ab
F 84-7-30	12.22 abA	4.46 aB	8.34 abc
F 84-7-24	11.52 abA	4.04 aB	7.78 bc
F 84-7-14	10.86 bA	3.78 aB	7.32 c
Mean	12.18 A	4.75 B	8.47

[1]Means followed by the same lowercase letter vertically and uppercase letter horizontally do not differ from each other by Tukey's test at 5% probability.

Table 5. Seedling emergence (%) in sand substrate of six soybean lines with black and yellow seed coats after exposure to accelerated aging conditions (41 °C and 100% RH) for 96 hours. (Source: França-Neto et al., 1998).

Line	Seedling Emergence (%)		
	Black Seed Coat	Yellow Seed Coat	Mean
F 84-7-30	69.3 aA[1]	23.0 aB	46.2 a
F 84-7-13	62.7 abA	4.7 bB	33.7 bc
F 84-7-24	61.0 abA	7.3 bB	34.2 bc
F 84-7-26	58.7 bA	13.7 bB	36.2 b
F 84-7-14	54.0 bA	6.7 bB	30.3 bc
F 84-7-11	43.7 cA	13.0 bB	28.3 c
Mean	12.18 A	4.75 B	8.47

[1]Means followed by the same lowercase letter vertically and uppercase letter horizontally do not differ from each other by Tukey's test at 5% probability.

speed and lignin content was negative; that is, the higher the lignin content, the shorter the germination time. The authors observed that the faster repair of the membrane systems of less deteriorated seeds would lead to faster emergence of the seedlings.

According to Peske and Pereira (1983), the seed coat of soybean is composed of three main layers, characterized from the surface towards the cotyledons in the following manner: palisade cell layer, composed of very compact cells, practically without intercellular spaces; the osteosclereid layer, or hourglass cells, also called columnar cells, with wide intercellular spaces; and the parenchyma, composed of parenchymal cells, which is in contact with the surface of the cotyledon cells. A clear illustration of these three layers is shown in Figure 7C.

Scanning microscopy and light microscopy studies performed by Menezes et al. (2009) showed that lignin is present in the testa of soybean seeds, is deposited in greater thickness in the cell walls of the palisade cells, and is also found in the cell walls of hourglass cells, as illustrated in Figure 8.

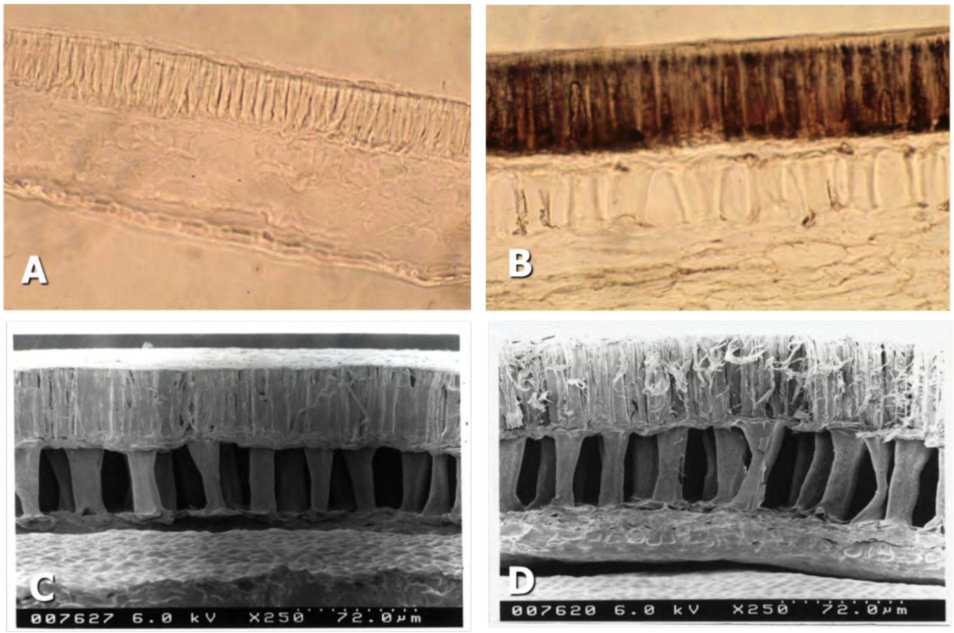

Figure 7. Histological sections of the soybean seed coat: A - yellow seed coat, B - black seed coat; and soybeans under a scanning electron microscope: C - yellow seed, D - black seed (Photos: José de Barros França-Neto).

Table 6. Average values (%) of lignin in relation to the total weight of the seed coat. (Source: Santos et al., 2007).

Cultivar	Seed coat color	
	Yellow	Brown
BRS 48	2.5	5.2
BRS 156	2.3	4.4
BRS 133	2.9	6.0
Mean	2.6	5.2

Table 7. Water absorbed (%) by the seeds of two soybean cultivars subjected to eight soaking periods at three-hour intervals in relation to the initial weight. Source: (Santos et al., 2007).

Cultivar	Seed coat	Soaking times							
		3 h	6 h	9 h	12 h	15 h	16 h	21 h	24 h
Embrapa 48	Yellow	15.65 A[1]	27.84 A	38.12 A	46.53 A	62.16 A	74.18 A	80.52 A	87.01 A
Embrapa 48	Brown	14.40 B	26.99 B	36.44 B	44.83 B	60.36 B	67.97 B	75.05 B	84.99 B
BRS 133	Yellow	12.44 A[1]	26.90 A	34.15 A	45.37 A	59.35 A	75.37 A	75.17 A	89.52 A
BRS 133	Brown	12.42 A	26.05 A	33.81 A	42.12 B	53.31 B	65.35 B	71.61 B	80.51 B

[1]Means followed by the same uppercase letter horizontally do not differ from each other by Tukey's test at 5% probability.

Table 8. Mean values (%) of vigor by the tetrazolium test of seeds of three soybean cultivars as a function of seed coat characteristics. (Source: Santos et al., 2007).

Cultivar	Seed coat color		Mean
	Yellow	Brown	
BRS 156	78[1]	85	81.5 A
Embrapa 48	68	81	74.5 A
BRS 133	69	76	72.5 A
Mean	71.7 b	80.7 a	

[1]Means followed by the same lowercase letter horizontally and uppercase letter vertically do not differ from each other by Tukey's test at 5% probability.

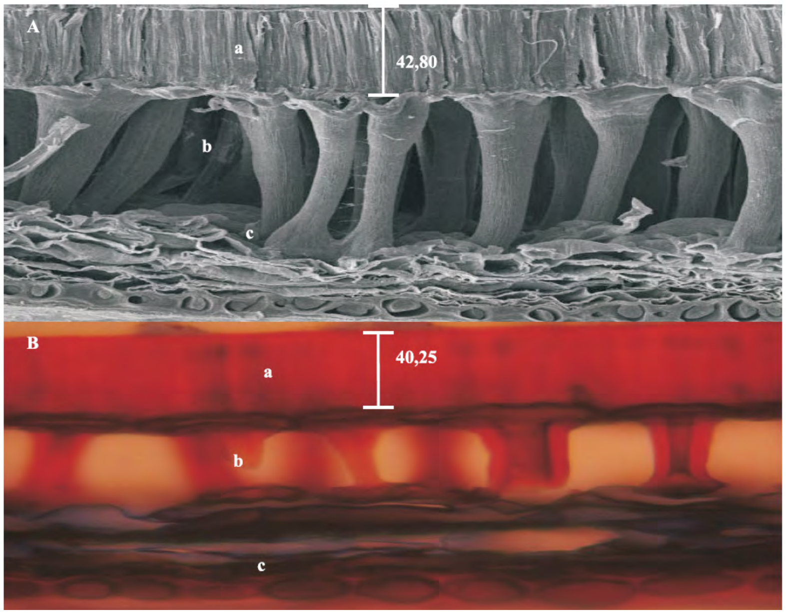

Figure 8. Comparison of the thickness (µm) of the palisade cell layers (A) of soybean seeds, obtained by scanning electron microscopy, with the thickness of lignin (B), obtained by light microscopy of the hybrid B x 1. a: palisade cell layer; b: hourglass cell layer; and c: spongy parenchyma. (Source: Menezes et al., 2009).

Regarding the physiological quality of soybean seed, deterioration from weathering is a result of exposure of soybean seeds to unfavorable weather conditions in pre-harvest, due to alternating cycles of wet and dry environmental conditions in the final stage of maturation (Figure 9). Such damage is of large magnitude if it occurs in warm environments, typical of tropical and subtropical regions (França-Neto and Krzyzanowski, 2018). Seeds with weathering deterioration show characteristic wrinkles in the cotyledons in the region opposite the hilum (Figure 10), or on the embryonic axis. One of the reasons why this wrinkling occurs with greater intensity in this region is due to the variability in the thickness of the hourglass cell layer (Pereira and Andrews, 1985; Forti et al., 2013). Its thickness is maximum in nearby regions to the hilum, decreasing progressively as they move away from this region, reaching rudiments in the region opposite the hilum (Figure 11). This condition favors the occurrence of this type of intense wrinkling in this region, because when

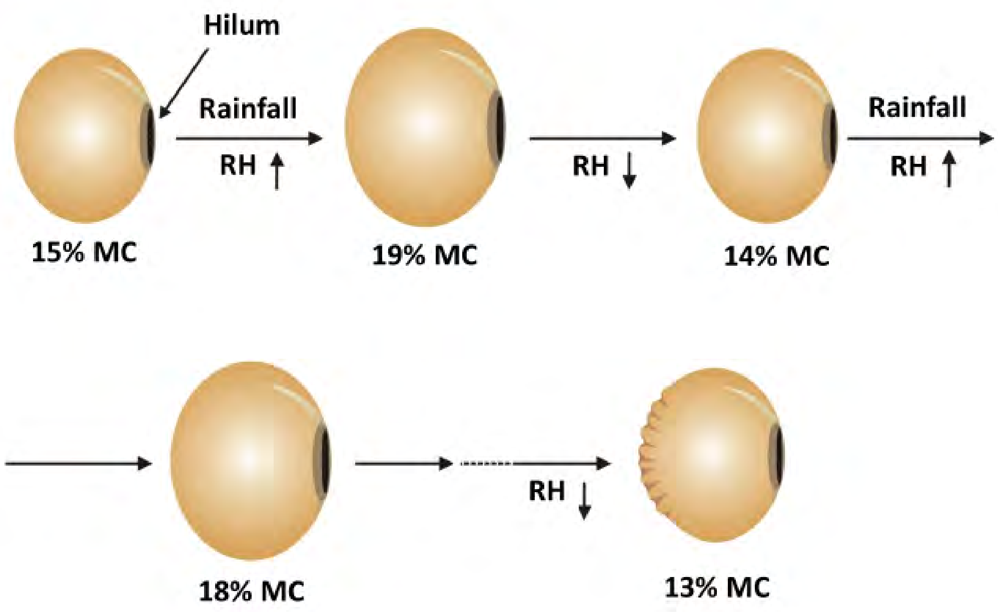

Figure 9. Process of physical alterations, due to oscillation in the moisture content (MC) of the soybean seed as a function of environmental moisture conditions, resulting in the appearance of wrinkling in the soybean seed, which is characteristic of moisture/weathering deterioration. (Source: França-Neto et al., 2016).

Figure 10. Soybean seeds with typical symptoms of moisture/weathering deterioration; on the left: dry seeds with wrinkling due to this type of damage; on the right: soybean seeds with typical symptoms of moisture/weathering deterioration, after staining with tetrazolium salt solution. (Source: França-Neto and Krzyzanowski, 2018).

the hourglass cells have greater thickness, they function as "dampers", resulting in lower rates of wrinkling; when the hourglass cells have less thickness, the opposite is true. With higher lignin content in the seed coat, specifically in the palisade cell layer, the seed coat becomes more rigid and, consequently, the possibility of more intense wrinkling of the seed coat decreases, providing a lower incidence of damage caused by weathering, thus preserving the physiological quality of the seeds.

Since lignin determines the rate of water absorption throughout the seed coat, the presence of lignin may have an effect on soybean seed deterioration during storage. Marwanto and Marlinda (2003) observed that seed coat lignin content was significantly and negatively related to membrane deterioration that is associated with a decline in soybean seed quality after storage.

Breeding soybean for high quality seed is an important approach for developing cultivars for tropical regions, and lignin content in the seed coat is one screening parameters for this characteristic. Considering that many breeding lines will be evaluated in each growing season, a long period is required for evaluation of the whole breeding program. This time limitation could influence lignin content assessment, if lignin degrades during storage. Research conducted on 12 soybean seed cultivars stored for one year in a controlled environment (10 °C temperature and 50% relative humidity) reported no differences in the lignin content of each cultivar comparing the results obtained at harvest time and after one year of storage. This fact indicates that lignin determination in the soybean seed coat can be performed over a long period, without any limitation due to change in lignin content (Krzyzanowski et al., 2008).

BORON CONTENT AND LIGNIN CONTENT IN THE SEED COAT

The main functions of boron (B) in plants are in the translocation of sugars and in the formation of the cell wall (Moraes et al., 2002). Dameto et al. (2022) evaluated the effect of boron doses from two sources, boric acid and ulexite, on the lignin content of the seed coat. Regardless of the boron source, there was an inverse relationship between doses and lignin content (Figure 12).

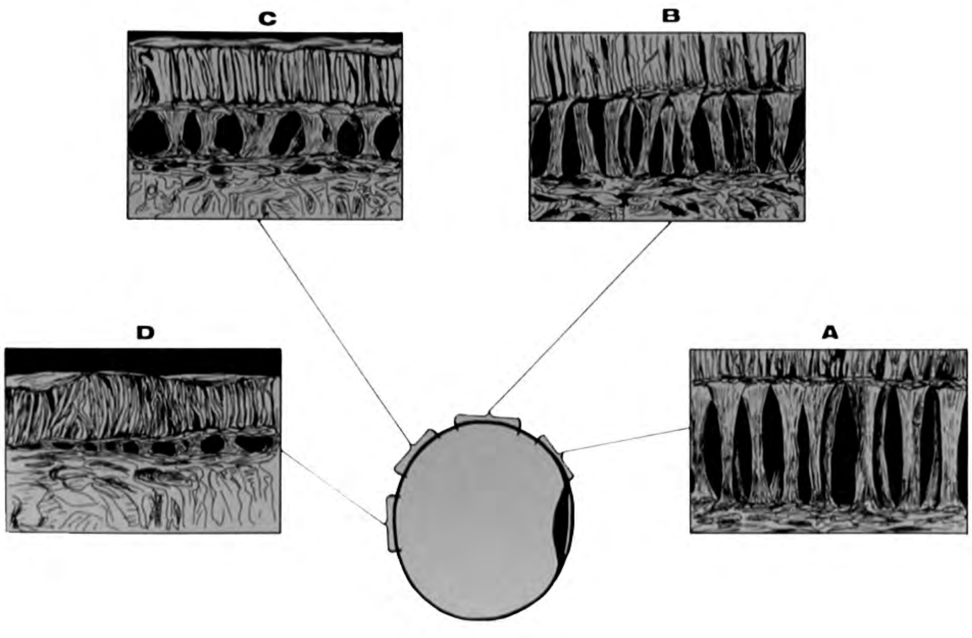

Figure 11. Illustration of the histological section of the soybean seed coat in four regions of the seed – A: close to the hilum; B: in an intermediate region between the hilum and the region opposite the hilum; C: in the region close to the side opposite the hilum; and D: in the region opposite the hilum. Adapted from Pereira and Andrews (1985) and Forti et al. (2013). Original photos taken by José de Barros França-Neto using a scanning electron microscope. (Illustrations: Danilo Estevão).

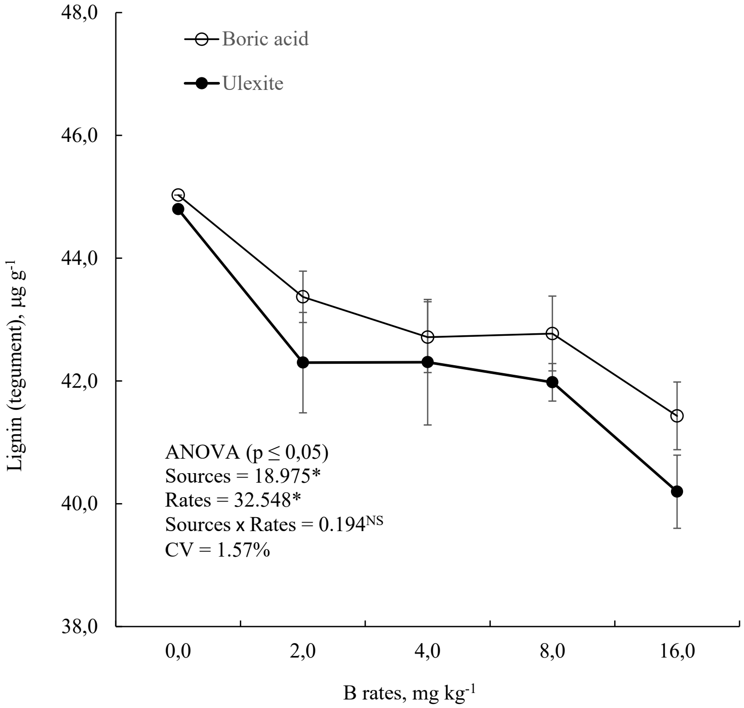

Figure 12. Lignin content in the soybean seed coat in response to application of boron sources and doses. * – significant at 5% probability; NS – non-significant at 5% probability. (Source: Dameto et al., 2022).

FINAL REMARKS

As demonstrated in this review article, lignin content in the seed coat and pod wall of soybean plays an important role related to seed physical, health, and physiological qualities. These parameters should be considered in studies related to evaluating the quality of soybean seeds as affected by genetic factors. They should also be strongly considered for inclusion in breeding programs for improving soybean seed and grain quality.

REFERENCES

ABATI, J.; ZUCARELI, C.; BRZEZINSKI, C.R.; KRZYZANOWSKI, F.C.; FRANÇA-NETO, J.B.; HENNING, F.A. Metabolites of the phenylpropanoid pathway and physiological quality of soybean seeds in storage. *Journal of Seed Science*, v.43, e202143033, 2021. http://dx.doi.org/10.1590/2317-1545v43253585.

AGRAWAL, P.K.; MENON, K. Lignin content and seed coat thickness in relation to seed coat cracking. *Seed Research*, v.2, p.64-66, 1974.

ALVAREZ, P.J.C.; KRZYZANOWSKI, F.C.; MANDARINO, J.M.G.; FRANÇA-NETO, J.B. Relationship between soybean seed coat lignin content and resistance to mechanical damage. *Seed Science and Technology*, v.25, p.209-214, 1997. https://www.bdpa.cnptia.embrapa.br/consulta/busca?b=ad&id=466869&biblioteca=vazio&busca

ÁVILA, M.R.; BRACCINI, A.L.; SOUZA, C.G.M.; MANDARINO, J.M.G.; BAZO, G.L.; CABRAL, Y.C.F. Physiological quality, content and activity of antioxidants in soybean seeds artificially aged. *Revista Brasileira de Sementes*, v.34, n.3, p.387-407, 2012. https://www.scielo.br/pdf/rbs/v34n3/06.pdf.

BAILEY, R.W. Quantitative studies of ruminant digestion II. Loss of ingested carbohydrates from the reticule rumen. *New Zealand Journal of the Agriculture Research,* v.10, n.1, p.15-32, 1967. https://doi.org/10.1080/00288233.1967.10423074

BAY, A.P.M.; TAYLOR, A.G.; BOURNE, M.C. The influence of water activity on three genotypes of snap bean (*Phaseolus vulgaris* L.) in relation to mechanical damage resistance. *Seed Science and Technology*, v.23, p.583-593, 1995. https://agris.fao.org/agris-search/search.do?recordID=CH19960068115

BELLALOUI, N; MENGISTU, A.; ZOBIOLE, L.H.S. *Phomopsis* seed infection effects on soybean seed phenol, lignin and isoflavones in maturity group V genotypes differing in *Phomopsis* resistance. *Journal of Crop Improvement,* v.26, n.5, p.26693-710, 2012. https://doi.org/10.1080/15427528.2012.671236

BELLALOUI, N.; SMITH, J.R.; MENGISTU, A. Seed nutrition and quality, seed coat boron and lignin are influenced by delayed harvest in exotically-derived soybean breeding lines under high heat. *Frontiers in Plant Science*, v.8, p.1-16, 2017. https://doi.org/10.3389/fpls.2017.01563

BOERJAN, W.; RALPH, J., BAUCHER, M. Lignin biosynthesis. *Annual Review Plant Biology*, v.54, p.519-546, 2003. https://doi.org/10.1146/annurev.arplant.54.031902.134938.

BRZEZINSKI, C.R.; ABATI, J.; ZUCARELLI, C.; MEDRI, C.; MERTZ-HENNING, L.M.; KRZYZANOWSKI, F.C.; FRANÇA-NETO, J.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. https://doi.org/10.1590/S1678-3921.pab2022.v57.02697.

BUTLER, G.W.; BAILEY, R.W. *Chemistry and biochemistry of herbage*. London and New York: Academic Press, 1973. 416p.

CAPELETI, I.; FERRARESE, M.L.L.; KRZYZANOWSKI, F.C.; FERRARESE-FILHO, O. A new procedure for quantification of lignin in soybean (*Glycine max* (L.) Merrill) seed coat and their relationship with the resistance to mechanical damage. *Seed Science and Technology*, v.33, p.511-515, 2004. https://doi.org/10.15258/sst.2005.33.2.25.

CARBONELL, S.A.M.; KRZYZANOWSKI, F.C. The pendulum test for screening soybean genotypes for seeds resistant to mechanical damage. *Seed Science and Technology*, v.23, p.331-339, 1995.

CASTRO, E.M.; OLIVEIRA, J.A.; LIMA, A.E., SANTOS, H.O.; BARBOSA, J.I.L. Physiological quality of soybean seeds produced under artificial rain in the pre-harvesting period. *Journal of Seed Science*, v.38, n.1, p.14-21, 2016. https://doi.org/10.1590/2317-1545v38n1154236.

CHOI, Y.M.; YOON, H.; LEE, S.; KO, H.C.; SHIN, M.J.; LEE, M.C.; HUR, O.S.; RO, N.Y.; DESTA, K.T. Isoflavones, anthocyanins, phenolic content, and antioxidant activities of black soybeans (*Glycine max* (L.) Merrill) as affected by seed weight. *Scientific Reports*, v.10, n.19960, p.1-13, 2020. https://www.nature.com/articles/s41598-020-76985-4.pdf

COWLING, E.B.; KIRK, T.K. Properties of cellulose and lignocellulose materials as substrates for enzymatic conversion processes. *Biotechnology Bioengineering Symp,* v.6, p.95-123, 1976.

DAMETO, L.S.; MORAES, L.A.C.; KRZYZANOWSKI, F.C.; MOREIRA. *Fontes e doses de boro nos componentes de rendimento, volume de raízes e qualidade de sementes de soja*. Master Dissertation in Agronomy. Londrina State University: Londrina, 2022. 71p.

FENGEL, D.; WEGENER, G. *Wood, chemistry, ultrastructure, reactions*. New York: Waster & Grugter, 1984. 613p. https://doi.org/10.1002/pol.1985.130231112.

FORTI, V.A.; CARVALHO, C.; TANAKA, F.A.O.; CICERO, S.M. Weathering damage in soybean seeds: assessment, seed anatomy and seed physiological potential. *Seed Technology*, v.35, n.2, p.213-224, 2013. https://www.jstor.org/stable/24642271

FRANÇA-NETO, J.B. Características fisiológicas da semente: germinação, vigor, viabilidade, danos mecânicos tetrazólio, deterioração por umidade tetrazólio e dano por percevejo tetrazólio. In: LORINI, I. (Ed.). *Qualidade de sementes e grãos comerciais de soja no Brasil – safra 2014/15.* Londrina: Embrapa Soja, 2016. p.31-47. http://www.alice.cnptia.embrapa.br/alice/handle/doc/1098335

FRANÇA-NETO, J.B.; HENNING, A.A. *Qualidades fisiológica e sanitária de sementes de soja*. Londrina: EMBRAPA - Centro Nacional de Pesquisa de Soja, 1984. 39p.

FRANÇA-NETO, J.B.; KRZYZANOWSKI, F.C. *Metodologia do teste de tetrazólio em sementes de soja*. Londrina: Embrapa Soja, 2018. 94p. https://ainfo.cnptia.embrapa.br/digital/bitstream/item/193315/1/Doc-406-OL.pdf

FRANÇA-NETO, J.B.; KRZYZANOWSKI, F.C. Tecnologia de sementes e o melhoramento de plantas – Biowork III. In: BORÉM, A. (Ed.). *Anais do Workshop em Biotecnologia e Produção de Sementes*. Viçosa: UFV, 2000. p.75-101.

FRANÇA-NETO, J.B.; KRZYZANOWSKI, F.C.; HENNING, A.A.; COSTA, N.P.; WEST, S.H. Comparação da qualidade da semente de soja com tegumento preto e amarelo após exposição a condições de envelhecimento acelerado. In: EMBRAPA: Centro Nacional de Pesquisa de Soja. *Resultados de Pesquisa de Soja 1997.* Londrina, 1998. p.173-174. https://www.embrapa.br/busca-de-publicacoes/-/publicacao/461153/comparacao-da-qualidade-de-sementes-de-soja-com-tegumento-preto-e-amarelo.

FRANÇA-NETO, J.B.; KRZYZANOWSKI, F.C.; HENNING, A.A.; PÁDUA, G.P.; LORINI, I.; HENNING, F.A. *Tecnologia da produção de semente de soja de alta qualidade*. Londrina: Embrapa Soja, 2016. 82p. https://ainfo.cnptia.embrapa.br/digital/bitstream/item/151223/1/Documentos-380-OL1.pdf

FRANÇA-NETO, J.B.; KRZYZANOWSKI, F.C.; PÁDUA, G.P. Características fisiológicas da semente: vigor, viabilidade, germinação, danos mecânicos tetrazólio, deterioração por umidade tetrazólio, dano por percevejo tetrazólio e sementes verdes In: LORINI, I. (Ed.). *Qualidade de sementes e grãos comerciais de soja no Brasil – safra 2015/16.* Londrina: Embrapa Soja, 2017. p.35-61. https://www.embrapa.br/busca-de-publicacoes/-/publicacao/1076672/qualidade-de-sementes-e-graos-comerciais-de-soja-no-brasil---safra-201516.

FRANÇA-NETO, J.B.; KRZYZANOWSKI, F.C.; PÁDUA, G.P.; LORINI, I. Características fisiológicas da semente: vigor, viabilidade, germinação, danos mecânicos tetrazólio, deterioração por umidade tetrazólio, dano por percevejo tetrazólio e sementes verdes. In: LORINI, I. (Ed.). *Qualidade de sementes e grãos comerciais de soja no Brasil – safra 2016/17*. Londrina: Embrapa Soja, 2018. p.31-59. https://www.embrapa.br/busca-de-publicacoes/-/publicacao/1097658/qualidade-de-sementes-e-graos-comerciais-de-soja-no-brasil---safra-201617.

FRANÇA-NETO, J.B.; KRZYZANOWSKI, F.C.; PÁDUA, G.P.; LORINI, I. Características fisiológicas da semente: vigor, viabilidade, germinação, danos mecânicos tetrazólio, deterioração por umidade tetrazólio, dano por percevejo tetrazólio e sementes verdes. In: LORINI, I. (Ed.). *Qualidade de sementes e grãos comerciais de soja no Brasil – safra 2017/18*. Londrina: Embrapa Soja, 2019. p.33-61. https://www.embrapa.br/busca-de-publicacoes/-/publicacao/1118960/qualidade-de-sementes-e-graos-comerciais-de-soja-no-brasil---safra-201718.

FRANÇA-NETO, J.B.; KRZYZANOWSKI, F.C.; WEST, S.H.; HENNING, A.A.; COSTA, N.P. Determinação do conteúdo de lignina nos tegumentos de sementes de soja com tegumento preto e amarelo In: *Reunião de Pesquisa de soja da região central do Brasil, 1999*. Anais... Londrina: Embrapa Soja, 1999. 247p. https://www.embrapa.br/busca-de-publicacoes/-/publicacao/461153/comparacao-da-qualidade-de-sementes-de-soja-com-tegumento-preto-e-amarelo.

GUPTA, P.C.; MILLER, D.A.; HITTLE, C.N. Note on the effect of threshing on seed damage, seed vigor and germination in two soybean varieties. *Indian Journal of Agricultural Science*, v.43, p.617-618, 1973.

HUTH, C.; MERTZ-HENNING, L.M.; LOPES, S.J.; TABALDI, L.A.; ROSSATO, L.V.; KRZYZANOWSKI, F.C.; HENNING, F.A. Susceptibility to weathering damage and oxidative stress on soybean seed with different lignin content in the seed coat. *Journal of Seed Science*, v.38, n.4, p.296-304, 2016. https://doi.org/10.1590/2317-1545v38n4162115.

KANNENBERG, L.M.; ALLARD, R.W. An association between pigment and lignin formation in the seed coat of the Lima Bean. *Crop Science*, v.4, p.621-622, 1964.

KLOCK, U. *Química da madeira lignina*, 2014. 76p. http://www.madeira.ufpr.br/disciplinasklock/quimicadamadeira/lignina20132.pdf.

KRZYZANOWSKI, F.C.; FRANÇA-NETO, J.B.; LORINI, I. Características físicas do grão: dano mecânico não aparente, dano mecânico pelo teste de tetrazólio e grãos partidos In: LORINI, I. (Ed.). *Qualidade de sementes e grãos comerciais de soja no Brasil – safra 2017/18*. Londrina: Embrapa Soja, 2019. p.129-142. https://www.embrapa.br/busca-de-publicacoes/-/publicacao/1118960/qualidade-de-sementes-e-graos-comerciais-de-soja-no-brasil---safra-201718.

KRZYZANOWSKI, F.C.; FRANÇA-NETO, J.B.; MANDARINO, J.M.G.; KASTER, M. Comparison between two gravimetric methods to determine the lignin content in soybean seed coat. *Seed Science and Technology*, v.29, p.619-624, 2001. https://worldveg.tind.io/record/4670/

KRZYZANOWSKI, F.C.; FRANÇA-NETO, J.B.; MANDARINO, J.M.G.; KASTER, M. Evaluation of lignin content of soybean seed coat stored in a controlled environment. *Revista Brasileira de Sementes*, v.30, p.220-223, 2008. https://doi.org/10.1590/S0101-31222008000200028.

MARCHIOSI, R.; SANTOS, W.D.; CONSTANTIN, R.P.; LIMA, R.B.; SOARES, A.R.; FINGER-TEIXEIRA, A.; MOTA, T.R.; OLIVEIRA, D.M.; FOLETTO-FELIPE, M.P.; ABRAHÃO, J.; FERRARESE-FILHO, O. Biosynthesis and metabolic actions of simple phenolic acids in plants. *Phytochemistry Reviews*, v.19, p.865–906, 2020. https://link.springer.com/article/10.1007/s11101-020-09689-2.

MARWANTO, M.; MARLINDA, M. The relationship between seed coat lignin content and seed quality of soybean seed during storage. *Jurnal Ilmu-Ilmu Pertanian Indonesia*, v.5, p.12-17, 2003. http://repository.unib.ac.id/210/1/12.pdf

MCCARTHY, J.L.; ISLAM, A. Lignin chemistry, technology, and utilization: a brief history. In: GLASSER, W.G.; NORTHEY, R.A.; SCHULTZ, T.P. (Eds.). *Lignin: Historical, Biological, and Materials Perspectives*. Washington DC: ACS, 1999. p.2-99. https://pubs.acs.org/doi/10.1021/bk-2000-0742.

MCDONALD, M.B.JR. Physical seed quality of soybean. *Seed Science and Technology*, v.13, p.601-609, 1985.

MENEZES, M.; VON-PINHO, E.V.R.; JOSÉ, S.C.B.R.; BALDONI, A.; MENDES, F.F. Aspectos químicos e estruturais da qualidade fisiológica de sementes de soja. *Pesquisa Agropecuária Brasileira*, v.44, p.1716-1723, 2009. https://doi.org/10.1590/S0100-204X2009001200022.

MENINO, T.V.R. *Lignina e composição monomérica em tegumentos de sementes de soja* [*Glycine max* (L.) Merrill] *resistente e susceptível aos danos mecânicos.* Master Dissertation. Maringá: Maringá State University, 2022. 25p.

MERTZ, L.M.; HENNING, F.A.; CRUZ, H.L.; MENEGHELLO, G.E.; FERRARI, C.S.; ZIMMER, P.D. Diferenças estruturais entre tegumentos de sementes de soja com permeabilidade contrastante. *Revista Brasileira de Sementes*, v.31, n.1, p.23-29, 2009. https://doi.org/10.1590/S0101-31222009000100003.

MERTZ-HENNING, L.M.; NAGASHIMA, A.I.; KRZYZANOWSKI, F.C.; BINNECK, E.; HENNING, F.A. Relative quantification of gene expression levels associated with lignin biosynthesis in soybean seed coat. *Seed Science and Technology*, v.43, p.445-455. 2015. https://doi.org/10.15258/sst.2015.43.3.13

MORAES, L.A.C.; MORAES, V.H.F; MOREIRA. Relationship between stem flexibility of rubber tree and boron deficiency. *Pesquisa Agropecuária Brasileira*, v.37, n.10, p.1431–1436, 2002. https://doi.org/10.1590/S0100-204X2002001000011

MOREIRA-VILAR, F.C.; SIQUEIRA-SOARES, R.C.; FINGER-TEIXEIRA, A.; OLIVEIRA, D.M.; FERRO, A.P., ROCHA, G.J.; FERRARESE, M.L.L.; SANTOS, W.D.; FERRARESE-FILHO, O. The acetyl bromide method is faster, simpler and presents best recovery of lignin in different herbaceous tissues than klason and tioglycolic acid methods. *Plos One*, v.9, p.1-7, 2014. https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0110000

PANOBIANCO, M.; VIEIRA, R.D.; KRZYZANOWSKI, F.C.; FRANÇA-NETO, J.B. Electrical conductivity of soybean seed and correlation with seed coat lignin content. *Seed Science and Technology*, v.27, p.945-949, 1999.

PEREIRA, L.A.G.; ANDREWS, C.H. Comparison of non-wrinkled and wrinkled soybean seedcoats by scanning electron microscopy. *Seed Science and Technology*, v.13, n.3, p.853-860, 1985.

PESKE, S.T.; PEREIRA, L.A.G. Tegumento da semente de soja. *Tecnologia de Sementes*, v.6, p.23-34, 1983.

POLLE, A.; OTTER, T.; SEIFERT, F. Apoplastic peroxidases and lignification in needles of Norway spruce (*Picea abies* L.). *Plant Physiology*, v.106, p.53-60, 1994. https://doi.org/10.1104/pp.106.1.53.

POTTS, H.C.; DUANGPATRA, J.; HAIRSTON, W.G.; DELOUCHE, J.C. Some influences of hardseededness on soybean seed quality. *Crop Science*, v.18, n.2, p.221-224, 1978. https://doi.org/10.2135/cropsci1978.0011183X001800020006x

RALPH, L.K.; BRUNOV, G.; LU, F.; KIM, H.; SCHATZ, P.F.; MARITA, J.M.; HATFIELD, R.D.; RALPH, S.A.; CHRISTENSEN, J.H. Lignins: natural polymers from oxidative coupling of 4-hydroxyphenylpropanoids. *Phytochemistry Review*, v.3, p.29-60, 2004. https://link.springer.com/article/10.1023/B:PHYT.0000047809.65444.a4.

RIJO, L.; VASCONCELOS, I. Formação de calose e de lignina em combinações incompatíveis *Coffea sp*. *H. vastatrix* (Callose and lignin formation in incompatible combinations *Coffea sp*. *H. vastatrix*). Simpósio sobre ferrugem do cafeeiro. *Oeiras*, p.269-281, 1983.

SANTOS, E.L.; POLA, J.N.; BARROS, A.S.R.; PRETE, C.E.C. Qualidade fisiológica e composição química das sementes de soja com variação na cor do tegumento. *Revista Brasileira de Sementes,* v.29, p.20-26, 2007. https://www.scielo.br/j/rbs/a/nLjBHP9NkrqgCF7V9ZYNv9x/?lang=pt&format=pdf

TAVARES, D.M.; MIRANDA, M.A.C.; UMINO, C.Y.; DIAS, G.M. Características estruturais do tegumento de sementes de linhagens de soja permeável e impermeável. *Revista Brasileira de Botânica*, v.10, p.147-153, 1987. https://www.infraestruturameioambiente.sp.gov.br/institutodebotanica/1987/01/caracteristicas-estruturais-do-tegumento-de-sementes-permeaveis-e-impermeaveis-de-linhagens-de-soja-glycine-max-l-merril/

VANHOLME, R.; DEMEDTS, B.; MORREEL, K.; RALPH, J.; BOERJAN, W. Lignin biosynthesis and structure. *Plant Physiology*, v.153, p.895-905, 2010. https://doi.org/10.1104/pp.110.155119

VAN SOEST, P.J.; WINE, R.H. Determination of lignin and cellulose in acid-detergent fiber with permanganate. *Journal of the Association of Official Analytical Chemists*, v.51, p.780-785, 1968. https://doi.org/10.1093/jaoac/51.4.780

VIDAURE, J.C. *Otimização do processo de pré-tratamento do bagaço de cana- de-açúcar com peróxido de hidrogênio alcalino e sua hidrólise por enzimas celulolíticas*. Master Thesis. Maringá: State University of Londrina, 1991. 130p.

ZABALA, G.; VODKIN, L.O. Methylation affects transposition and splicing of a large CACTA transposon from a MYB transcription factor regulating anthocyanin synthase genes in soybean seed coats. *Plos One*, v.9, n.11, p.1-20, 2014. https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0111959