Looking at Lignin

A structural component of the cell wall of the soybean seed coat that has great importance on its physiological performance

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LIGNIN IS A COMPLEX HETEROPOLYMER that primarily consists of p-hydroxyphenil (H), guaiacyl (G), and syringyl (S) units formed by oxidative coupling of p-coumaryl, and sinapyl alcohols, respectively, which are products of the phenylpropanoid pathway (Moreira-Vilar et al., 2014; Vanholme et al., 2010). Lignin is the generic term for a large group of aromatic polymers resulting from the oxidative combinatorial coupling of 4-hydroxyphenylpropanoids (Vanholme et al., 2010; Boerjan et al., 2003; Ralph et al., 2004j) (figure 1).

These polymers are deposited predominantly in the walls of secondarily thickened cells, making them rigid and impervious. Lignin protects cell wall polysaccharides from microbial degradation, thus imparting decay resistance (Vanholme et al., 2010).

Water impermeable soybean seeds were reported to have 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 type (Tavares et al., 1987).

Many factors contribute to seed deterioration, but physical damage due to improper harvesting and handling and its consequent effect on seed coat integrity is a leading cause (McDonald, 1985). Mechanical damage was the major factor responsible for decreasing soybean [*Glycine max (L.)*] seed germination and vigour in Brazil in the 2014/15 and 2015/16 seasons, as reported by França-Neto (2016) and França-Neto et al. (2017). The soybean seed coat is very thin and low in lignin content, and provides little protection to the fragile radicle, which lies in a vulnerable position beneath the seed coat (Agrawal and Menon, 1974; Gupta et al., 1973; França-Neto and Henning, 1984). The deposition of lignin in the seed coat tissue is important since it provides mechanical resistance and also protects the cell wall against micro-organisms (Rijo and Vasconcelos, 1983). Additionally, due to its impermeability/semi-permeability feature, lignin may also protect soybean seeds against the negative effects of weathering conditions that occur during the pre-harvest period (França-Neto et al., 2016). Lignin is the third major component of the cell wall and the main component of intracellular substance, responsible for the maintenance of integrity and structural cohesion of plant fibre (Cowling and Kirk, 1976).

The cause of high susceptibility to mechanical damage of white lima bean seeds (*Phaseolus lunatus L.*) can be related to its lignin content, which is one per cent of the weight of the seed coat, when compared with seeds of dark color, where the occurrence of lignin is 15 per cent of seed coat weight (Kannenberg and Allard, 1964). Agrawal and Menon (1974) related the thickness of the seed coat and the lignin content to the difference in susceptibility to mechanical damage between soybean cultivars Clark 63 and Adelpina.

Black coated soybean seeds are higher in quality as compared to yellow coated ones (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 seeded genotypes (12.18 per cent) as compared to the yellow types (4.75 per cent).

Mechanical damage is one of the causes of great loss of soybean seed quality in tropical and subtropical environments. Developing cultivars that are less prone to mechanical damage is an important contribution from the breeders to the soybean growers for overcoming this limitation – in addition to improving grain quality by reducing the amount of splits and cracks. Carbonell and Krzyzanowski (1993) developed the pendulum test for identifying lines with seeds resistant to mechanical damage. Based on this test, 12 field grown soybean cultivars were classified as resistant, moderately resistant and susceptible categories; the higher the index number the better is the seed physiological quality. In the same line of research, Alvarez et al. (1997) were able to explain that the mechanical damage resistance of these cultivars was directly related to the amount of lignin content in the seed coat (Table 1 and Figure 2).

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

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 in the seed coat above five per cent is proposed to be a reasonable indicator of resistance to mechanical damage for soybean seed. Based on the 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).

The lignin content in the seed coat also influences the seed electrical conductivity of the soybean cultivars. The higher the lignin content the lower the leakage into the soaking solution of the seeds. High lignin content is a desirable genetic characteristic for improving soybean seed quality, because based on the electrical conductivity test – it is an indicative of high vigour seed (Panobianco et al,, 1999) (Table 2, Figure 3).

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

Susceptibility to weathering damage and oxidative stress on 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, measured by the standard germination, accelerated ageing and tetrazolium tests, and presented lower oxidative stress due to low activities of the superoxide dismutase, guaiacol peroxidase and lipid peroxidation.

The timing of harvest is a major factor affecting seed quality in soybean, particularly when rain during harvest period is common. The effect of time of harvest on soybean seed quality (seed composition, germination, seed coat boron, and lignin) in high germinability (HG) breeding lines (50 per cent exotic) developed under high heat was evaluated. 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 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 correlation were found between germination and seed protein, oleic acid, sugars, and seed coat boron and seed coat lignin. These results should suggest to breeders of some advantage of selecting for high seed coat boron and lignin content (Bellaloui et al., 2017).

Since lignin determines the rate of water absorption throughout the seed coat, its occurance may have an effect on soybean seed deterioration during storage. Marwanto and Marlinda (2003) observed that the seed coat lignin content was significantly and negatively related to membrane deterioration 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 the lignin content in the seed coat is one screening parameters for this trait. Considering that many breeding lines will be evaluated in each growing season, a long period is required for the evaluation of the whole breeding program. This time limitation could influence lignin content assessment, if lignin is degraded during storage. Research conducted with 12 soybean seed cultivars stored for one year in a controlled environment (10oC temperature and 50 per cent air relative humidity) reported no differences between the lignin content of each cultivar when compared the results obtained at harvest time and after one year of storage. This fact indicates that the lignin determination in soybean seed coat can be performed over a long period without any bias due to change in its content (Krzyzanowski et al., 2008).

In the studies of the relationship between soybean seed physiological quality and the lignin content of the seed coat, authors used several lignin determination methodologies. The sulfuric acid method in the studies of resistance to mechanical damage (Alvarez, et al., 1997). The potassium permanganate in the relationships between the lignin content and the electrical conductivity of the seed imbibition solution (Panobianco et al., 1999). The LTGA linothioglycolic acid method in the studies of weathering damage and lignin content in soybean seeds (Huth et al., 2016) and currently the methodology in use is acetyl bromide because it is faster, simpler and presents best recovery of lignin in different herbaceous tissues than Klason and thioglycolic acid methods (Moreira-Vilar et al., 2014).

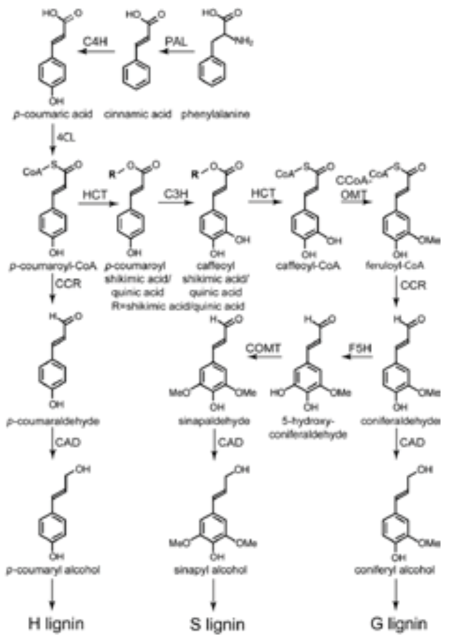

Figure 1. The main biosynthetic route toward the monolignois p-coumaryl, coniferyl, and sinapyl alcohol (Boerjan et al., 2003; Vanholme et al., 2010).

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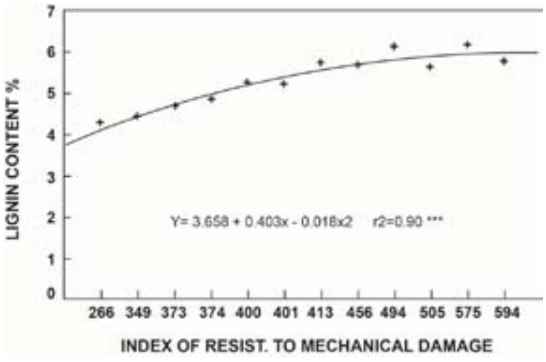

Figure 2. Regression analysis of seed coat lignin content and the index of resistance to mechanical damage determined by the pendulum test, for seeds of 12 soybean cultivars (Alvarez, et al., 1997).

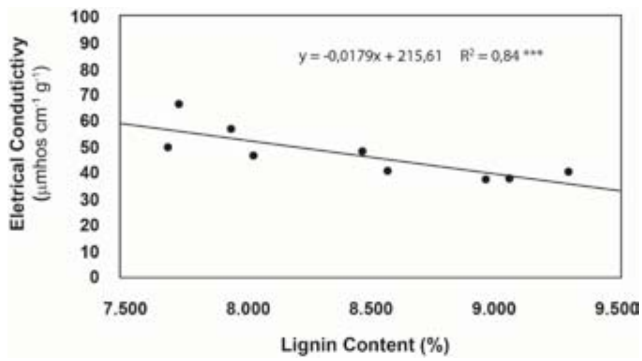

Figure 3. Relationship between seed electrical conductivity (Qmhos cm-1 g-1) and the per cent seed coat lignin content for nine soybean cultivars (Panobianco et al., 1999).

Cultivar	Conductivity1 Qmhos cm-1g-1	Lignin Content1 (%)
Santa Rosa	66 a	7.74 d
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
FT 2	38 d	9.05 a
Paraná	38 d	8.96 ab
Means not sharing a letter in common differ significantly at the 0.05 level of probability, as determined by the Tukey test.		

Table 1. Means of the seed coat lignin content and the index of resistance to mechanical damage determined by the pendulum test for seeds of 12 soybean cultivars (Alvarez et al., 1997).

Cultivar	Lignin Content (%)	Mechanical Damage Index
Doko	6.203 a	494 c
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
Means not sharing a letter in common differ significantly at the 0.05 level of probability, as determined by the Tukey test.		

Table 2. Effects of cultivar on the electrical conductivity and the seed coat lignin content of a single seed lot of nine soybean cultivars (Panobianco et al., 1999).

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