Water uptake of soybean pods and seeds with different lignin contents[1]

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ABSTRACT

The identification of pod- and seed-related traits of soybean genotypes and their interaction with water uptake can be applied in breeding programs with a view to establishing cultivars developed for the production and maintenance of high-quality seeds. The objective was to evaluate the water uptake of pods and seeds of soybean cultivars with different lignin contents after different soaking periods. The experimental was arranged in a completely randomized, 7×10 factorial design, with four replications. The factors consisted of seven soybean cultivars and 10 soaking periods (1, 2, 3, 4, 5, 6, 7, 8, 24, 48 hours). The pods and seeds were grown in a greenhouse and harvested at the phenological growth stage R8 (at full maturation, when 95% of the pods had mature color). The following traits were evaluated: lignin content in pod and seed coat, moisture content, and rate of water uptake of pods and seeds. Soybean cultivars with pods with high lignin contents have a lower rate of water uptake. The lignin contents of the seed coats, within the chosen limits, did not affect the seed water uptake.

Keywords: *Glycine max* (L.) Merrill; hydration; permeability; seed coat.

INTRODUCTION

Soybean represents the main oilseed grown and consumed worldwide. However, yield increases are still necessary and possible, e.g., by means of planting top-quality seed. High-quality seed has adequate sanitary, physical, genetic and physiological traits. These characteristics are relevant for the field performance of the seeds, and contribute to the ideal establishment of the plant population and to high yields (Marcos-Filho, 2015; Finch-Savage & Bassel, 2016; Bagateli *et al*., 2019).

The seed quality is the final result of the entire production process. In the case of soybean, which is highly sensitive to environmental conditions, the phase in which the seed is maintained in the field is critical and promotes the most frequent and significant losses in quality. Among the climatic factors, especially in pre-harvest, excess water is detrimental to the soybean seed quality, for causing weathering deterioration (Giurizatto *et al*., 2003; França-Neto *et al*., 2016; Pinheiro *et al*., 2021). This damage consists of wrinkling and cracks in the seed coat, due to seed coat expansion and contraction when exposed to alternating cycles of relative humidity, especially associated with high temperatures (Forti *et al*., 2013; França-Neto *et al*., 2016).

Soybean seeds are rather susceptible to weathering damage, due to the proper morphological seed traits, since the vital parts of the embryo, e.g., radicle, hypocotyl and plumule, are covered by only a thin coat, leaving them practically unprotected (França-Neto & Henning, 1984). Therefore, the identification of genotypic traits related to weathering damage tolerance of pre-harvest seeds is essential for breeding programs focused on seed quality. A possible tolerance to deterioration may be associated with the permeability of pods and seed coats and their relationship with water uptake.

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According to Ma *et al*. (2004), the soybean seed coat can be permeable or semi-permeable to water. Permeable seed coats facilitate imbibition, whereas semipermeable ones restrict it to varying degrees. Several authors have reported that seed coat permeability is related to the presence of compounds such as the suberin layer, the cuticle and others and to their structure (Ma *et al*., 2004; Mertz *et al*., 2009; Vu *et al*., 2014). The lignin content can also be decisive for water permeability, and the control of seed deterioration in the field (Oliveira *et al*., 2014).

Several authors concluded that the lignin content in seed coats differs between soybean genotypes (Menezes *et al*., 2009; Gris *et al*., 2010; Huth *et al*., 2016). Furthermore, higher lignin levels raise the resistance to seed damage and may influence the tolerance to weathering deterioration, by reducing the rate and speed of seed imbibition, which is favorable for yields with higher-quality seeds (Santos *et al*., 2007).

In soybean seeds of cultivar CD 215, with a high seed coat lignin content, Moreira *et al*. (2012) observed a higher germination percentage. This may be explained by the semi-permeability of the seed coat, which, aside from protecting the seed, slowed down the water entry into the embryo. In soybean seeds, Santos *et al*. (2007) also found a lower imbibition rate, due to a higher lignin content. In soybean pods, Oliveira *et al*. (2014) observed that the permeability varies according to the genotype and developmental stage, whereas the pod lignin content had no influence on their permeability. However, there is still a need for exploratory studies to identify and understand the traits of soybean genotypes, and their interaction with water uptake.

Therefore, the objective of this study was to evaluate the water uptake of pods and seeds of soybean cultivars with different lignin contents after different soaking periods.

MATERIAL AND METHODS

The study was carried out at the research center Dr. Nilton Pereira da Costa, a technological nucleus of seeds and grains, of the Brazilian Agricultural Research Corporation, Embrapa Soybean, Londrina, PR, in the Laboratories of Physiology, Technology and Seed Chemistry (long. 23º11 'S; lat. 51º11' W; 620 m asl).

The experiment was arranged in a completely randomized, 7×10 factorial design, with four replications. The factors consisted of seven soybean cultivars (A, B, C, D, E, F and G) and 10 periods (1, 2, 3, 4, 5, 6, 7, 8, 24, 48 hours) of pod and seed soaking in water.

The seeds of the tested cultivars (Table 1) were produced in a greenhouse (model Van der Hoeven®), under partial temperature and relative humidity control. The environmental conditions in the greenhouse (air temperature and relative humidity) were monitored with a data logger HT-500 during the experiment (Figure 1).

Some hours before sowing, seeds of the evaluated cultivars were inoculated with the liquid inoculant BIAGRO NG®, containing *Bradyrhizobium japonicum* bacteria, strains SEMIA 5079 and 5080 ($5x10^9$ viable cells mL^{-1}), at a rate of 100 mL of the commercial product per 50 kg^{-1} seeds. The seeds were treated with the commercial fungicide Derosal Plus® (Carbendazin + Thiram) at 200 mL 100 kg^{-1} seeds, and after inoculation, sown in 9-L pots, containing soil classified as Ferralsols with a clayey texture, which was limed according to the crop requirements. Four seeds per pot were sown at a soil depth of 3 - 5 cm, and after seedling emergence, thinned to only two plants.

For each cultivar, four replications with 10 plants per cultivar were used, resulting in a total of 40 plants per genotype. The plants were irrigated daily with drip sprinklers. Cultural treatments (insecticide and fungicide applications) were carried out according to the requirement and recommendations for the crop.

Pods and seeds were harvested at the phenological growth stage R8 (at full maturation, when 95% of the pods had a mature color), as described by Ritchie *et al*. (1997). The samples were collected from the upper, middle and lower thirds of the plant and then sent to the laboratory for analysis, according to the following methodologies:

Moisture content of pods and seeds: the moisture content was determined by the oven method at 105 °C, with three replications per treatment, according to the methodology described in the Rules for Seed Analysis (Brasil, 2009). The results were expressed in percentage.

Pod and seed coat lignin content: determined in four replications of 100 seeds and 50 pods per treatment. The seeds, removed from the pods, were water-soaked for 12 hours to disassociate the seed coat from the cotyledon. Thereafter, the previously separated seed coats and pods were oven-dried at 105 °C for 24 hours. The dry matter was ground and sieved. Then, 0.3 g aliquots were weighed for the extraction of the cell wall proteins, and the resulting protein-free material was used to determine lignin by the acetyl bromide method (Moreira-Vilar *et al*., 2014). Results were expressed as percentages.

Water uptake rate in pods and seeds: the water uptake after 10 soaking periods (1, 2, 3, 4, 5, 6, 7, 8, 24 and 48 hours) by pods (with the seeds) and seeds was analyzed, in three replications with 25 individuals each, per treatment. Initially, the material of each replication was weighed (initial weight) and placed between two sheets of paper double-lined with pH – neutral filter (germitest paper), moistened with distilled water, and stored in plastic boxes. A water quantity of 2.5 times the paper weight was used (Brasil, 2009). These boxes were placed in a germinator at 25 ºC. At pre-determined times, the wet weight gain was determined by weighing. Each time, the pods and seeds were removed from the box, placed on paper to absorb external moisture, weighed and then placed back in the boxes and germinator.

After 48 hours, the pods and seeds were removed from the boxes and weighed (final weight). Based on the initial and final weight of each sample, weight gain was determined as water percentage taken up by the pods and seeds, during each soaking period, by the formula:

$$WG\,(\%) = \frac{(FW - IW)}{IW} * 100$$

Where: WG = percentage of water absorbed, in relation to the initial sample weight; IW = initial sample weight; FW = final sample weight (of each soaking period).

The data were analyzed for normality and homoscedasticity, by the Shapiro-Wilk and Hartley tests, respectively, which indicated that there was no need for transformation. Analysis of variance was performed and means between cultivars were compared by the Scott-Knott test at 5% probability. Regression analysis was performed for the soaking periods. Software SISVAR (Ferreira, 2011) was used for the analyses.

RESULTS AND DISCUSSION

The summary of analysis of variance for the interaction between cultivars and soaking periods, for the variables

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

Code	Cultivar	Type[1]	Habit[2]	Cycle	Group	Pubescence
A	BRS 1010 IPRO	I	Ind.	Early	6.1	Gray
B	BRS 284	C	Ind.	Early	6.3 a 7.1	Gray
C	NA 5909 RR	RR	Ind.	Early	5.9	Gray
D	BRSMG 752 S	C	Ind.	Semi Early	7.5	Brown
E	BRS Pintado	C	Det.	Medium	8.7	Gray
F	BRS Jiripoca	C	Det.	Medium	8.4	Gray
G	M 8210 IPRO	I	Det.	Early	8.2	Brown

[1] Soybean technology: I: intact; C: conventional and RR: Roundup Ready®.

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

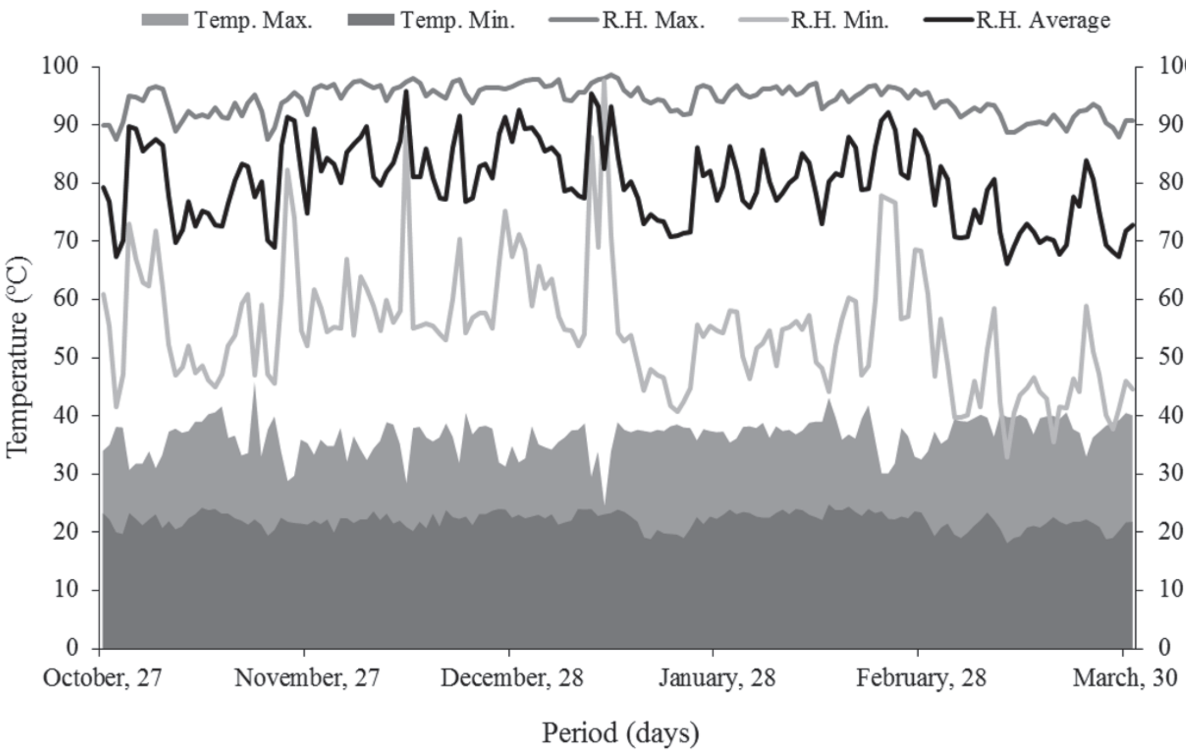

Figure 1: Maximum and minimum daily temperature (°C) and maximum, minimum and average daily relative humidity (R.H.) (%), for the growth period of soybean in a greenhouse.

Table 2: Summary of the analysis of variance for water uptake in pods (AP) and seeds (AS) of seven soybean cultivars (A, B, C, D, E, F and G), exposed to 10 water-soaking periods (1, 2, 3, 4, 5, 6, 7, 8, 24 and 48 hours). Isolated effect of cultivar on lignin content in pods (LP) and seed coats (LS)

F. V.	G. L.	Mean Squares			
		AP	AS	LP	LS
Cultivar (C)	6	26.54**	498.13**	10.96**	0.39**
Water-soaking (W)	9	1364.03**	14542.62**	-	-
C * W	54	12.12**	43.97**	-	-
Error	140	7.26	14.26	0.090	0.0290
Average	-	11.58	35.98	15.57	4.25
CV (%)	-	23.27	10.50	1.93	4.01

ns: not significant and **; *: significant at 1% and 5% probability, respectively, by the F test.

pod and seed water uptake, as well as the mean squares of each cultivar for the variables lignin contents of pods and seed coats are shown in Table 2.

For the variable pod water uptake, the interaction of cultivars by soaking periods indicated no significant differences between cultivars in the first hours of soaking (Table 4). This result may be associated with the timely harvest and the homogeneous initial water content of the evaluated treatments (see water content in Table 3). According to Cavariani *et al*. (2009) and Silva & Villela (2011), the speed of water penetration and tissue hydration is directly influenced by the initial water content. Thus highlighting the importance of standardizing the water content before installing tests related to tissue absorption, to find answers only for the evaluated assumptions, free of the influence of external agents.

Significant differences in pod water uptake were observed after 8 hours of soaking, when the water uptake of cultivar D was lowest (Table 4). After the 48-hours soaking period, the pods of cultivars D, E and F had taken up less water in the pods than the other genotypes.

For soybean pods, a higher percentage of water uptake can be detrimental to the seed quality, since the higher this value, the lower the tolerance of these genotypes to environmental variations, such as high temperatures associated with extremely high relative air humidity. The exposure of susceptible seeds to extreme conditions accelerates the deterioration process, causing irreversible damage to their quality, by reducing the germination potential and vigor drastically. According to França-Neto *et al*. (2000), pods with lower permeability represent an additional protection of the seeds against deterioration in the field under the changing moisture conditions of the environment.

Comparing the cultivar performance after different soaking periods, the pods of cultivar A absorbed higher amounts of water, while those of cultivars D, E and F less (Figure 2). This difference in water uptake between genotypes may be related to the tissue permeability of the pods. According to Oliveira *et al*. (2014), soybean genotypes differed in terms of pod water permeability.

According to our data, cultivars with a lower percentage of water uptake (Figure 2) had higher lignin contents in the pods (Table 5). Lignin is hydrophobic and plays an important role in water transport and mechanical resistance of crops, acting directly on tissue permeability and protection against environmental oscillations and possible changes in the water uptake speed (Zhao & Dixon, 2011).

Thus, these results indicate tolerance to weathering deterioration, since pods with a lower water uptake intensity reduce the exposure period of the seeds to unpredictable climate conditions. This reduction minimizes the deterioration process in the field, especially for short periods with high rainfall, i.e., when torrential rain occurs in the pre-harvest phase, as is often the case in the vast majority of soybean-producing regions in the country.

With regard to the seed water uptake, statistical differences were observed after 5 hours of soaking, and the cultivars with the lowest percentage for this variable were A, B and F (Table 4). At the end of the soaking period, the seed water uptake of cultivars E and G was higher than of the others.

Table 3: Characterization of water content in pods and seeds of soybean cultivars used in the experiment

Cultivar	Water content in pods (%)	Water content in seeds (%)
A*	13.0	13.7
B	13.5	14.3
C	14.0	12.3
D	13.2	13.9
E	13.7	12.0
F	12.7	12.2
G	13.0	12.7

*Cultivars: A = BRS 1010 IPRO; B = BRS 284; C = NA 5909 RR; D = BRSMG 752 S; E = BRS Pintado; F = BRS Jiripoca and G = M 8210 IPRO.

Table 4: Water uptake (%) in pods and seeds of soybean cultivars exposed to different soaking periods

Cultivar	Soaking periods (hours)									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**24**	**48**
Pods										
A*	2.0a	2.4a	3.8a	5.0a	8.7a	12.0a	15.1a	17.8a	26.9a	34.3a
B	1.9a	2.6a	4.0a	4.4a	7.4a	10.7a	12.3a	16.2a	21.1b	28.6b
C	1.5a	1.7a	2.4a	5.3a	8.8a	11.5a	15.2a	17.3a	21.6b	30.7b
D	2.3a	2.7a	3.6a	4.7a	6.7a	10.1a	13.7a	13.3b	19.2b	22.8c
E	3.9a	4.7a	7.0a	8.8a	9.8a	11.4a	13.3a	15.3a	19.5b	20.7c
F	3.5a	4.8a	6.8a	8.5a	10.0a	11.3a	13.4a	15.5a	19.6b	21.7c
G	4.2a	4.7a	7.1a	7.9a	9.7a	11.4a	13.9a	16.4a	21.8b	26.5b
CV (%)	23.27									
Seeds										
A	4.7a	10.0a	19.9a	21.1a	24.0b	30.0b	36.8a	39.7b	71.1b	82.7c
B	5.4a	9.2a	12.6a	18.0a	20.7b	22.8c	29.1b	31.4c	61.0c	73.4d
C	6.3a	11.6a	20.5a	25.5a	30.3a	41.0a	43.5a	49.9a	71.5b	81.7c
D	8.2a	11.6a	16.7a	19.8a	27.0a	38.5a	40.7a	45.9a	79.2a	93.4b
E	11.2a	15.1a	18.3a	21.4a	29.0a	34.8a	40.2a	45.7a	80.2a	99.0a
F	11.4a	15.3a	14.6a	19.3a	22.6b	28.7b	32.0b	35.3c	71.3b	89.6b
G	10.3a	14.7a	16.7a	22.9a	28.0a	34.2a	40.3a	46.3a	81.4a	99.7a
CV (%)	10.50									

Means followed by the same letter in a column do not differ from each other by the Scott-Knott test, at 5% probability. *Cultivars: A = BRS 1010 IPRO; B = BRS 284; C = NA 5909 RR; D = BRSMG 752 S; E = BRS Pintado; F = BRS Jiripoca and G = M 8210 IPRO.

Regarding the tested soaking periods, cultivars D, E, F and G absorbed the biggest amount of water (Figure 3). A higher water uptake by the seeds, aside from increasing weathering damage, can also impair the normal development of seedlings in the germination phase. This occurs due to an inadequate reorganization of the membranes during imbibition, causing irreversible damage to the embryo. In a study with soybean cultivars with contrasting seed coat colors, Bahry *et al*. (2017) found that black seeds take up water more slowly, allowing a reorganization of their membrane systems and thus a reduction of the stress caused by an accelerated during water uptake for the germination process.

In a comparison of the different increase rates in water uptake with the lignin contents of the seed coat, it was not possible to associate the results of each period and throughout the soaking periods. This result demonstrates that the seeds of the tested cultivars, with seed coat lignin contents between 3 and 5% (Table 5), similarly to most cultivars currently sold on the market, do not differ in terms of water uptake. For Bahry *et al*. (2015), seed water uptake is more closely related with the genotype-environment interaction than with the relationship between seed coat and lignin concentration. However, it is important to highlight the relationship of lignin with some attributes that make up seed quality, as mentioned by Krzyzanowski & França-Neto (2021).

In view of the above, more contrasting lignin contents in the seed coat would be necessary to detect possible differences in water uptake. However, this trait has been overlooked in breeding programs over the years, due to the focus on grain yields. Consequently, it is now necessary to identify the other characteristics related to tissue water uptake, in contribution to the selection for promising lines for high-quality seed production in breeding programs. This fact was confirmed by Cavariani *et al*. (2009), who stated that the imbibition speed of soybean seeds was not only influenced by the lignin content of the seed coat, but also by the thickness of the palisade and spongy parenchyma and hypodermis of the tissues.

Table 5: Lignin content in pods and seed coats of soybean cultivars

Cultivar	LP (%)	LS (%)
A*	13.46 D	4.27 B
B	14.10 C	4.20 B
C	15.34 B	3.60 C
D	16.13 A	4.58 A
E	16.19 A	4.47 A
F	18.56 A	4.26 B
G	15.18 B	4.35 B
CV (%)	1.93	4.01

Means followed by the same letter in a column do not differ from each other by the Scott-Knott test, at 5% probability. LP: Lignin content in the pod; LS: Lignin content in seed coat. *Cultivars: A = BRS 1010 IPRO; B = BRS 284; C = NA 5909 RR; D = BRSMG 752 S; E = BRS Pintado; F = BRS Jiripoca and G = M 8210 IPRO.

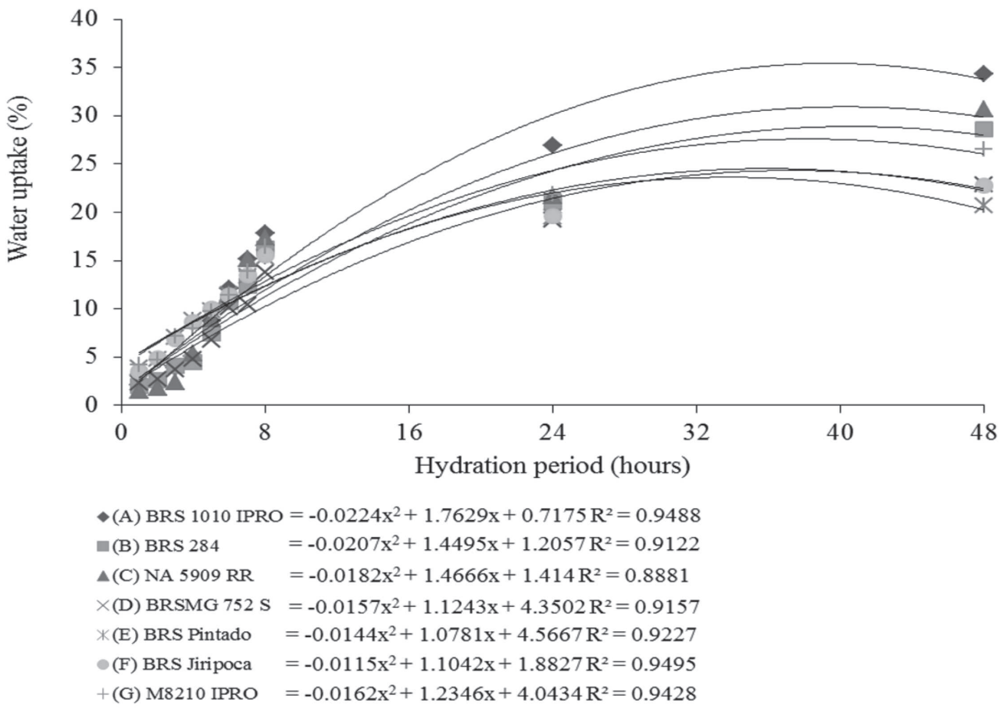

Figure 2: Water uptake curves of pods of soybean cultivars, exposed to different soaking periods.

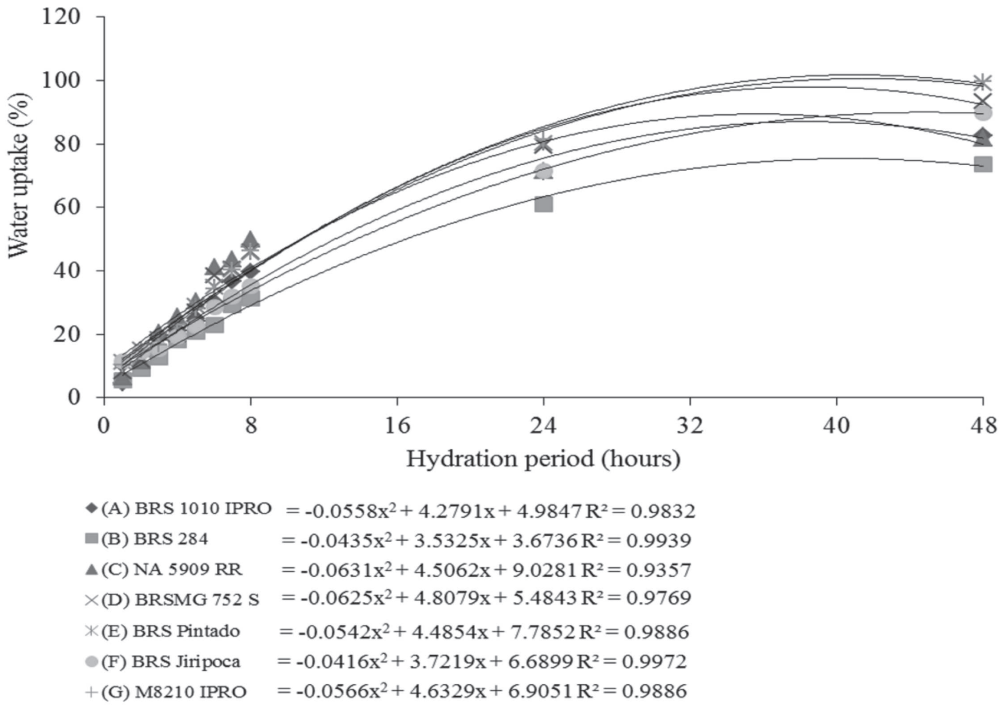

Figure 3: Water uptake curves of seeds of soybean cultivars, exposed to different soaking periods.

CONCLUSIONS

Soybean cultivars with high lignin contents in the pods have a lower rate of water uptake.

The lignin content of the seed coats, within the evaluated limits, does not interfere with the water uptake by the seeds.

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