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# Relationship between pod permeability and seed quality in soybean[1]

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ABSTRACT – The aim of this study was to evaluate the influence of pod wall permeability on the physiological quality of soybean seed. The cultivars studied were Sant'Ana, FT-2, FT-10, Bossier, Davis and the breeding line F 84-7-30, with a black seed coat. Pods were collected from plants at the R4, R5, R6, R7 and R8 development stages, which composed the treatments in regard to time of harvest. The parameters of permeability and the lignin content of the pods and the seeds within the pods were evaluated. The seeds were collected just after full maturity (R8), and the following tests were performed: germination, electrical conductivity, and tetrazolium, which determined seed viability and vigor. A randomized complete block design in a split-plot in time arrangement was used, with four replications per treatment. The soybean genotypes (six) composed the plots, and the split-plots consisted of the development stages (R4, R5, R6, R7 and R8). In seed evaluation, the same design was used, reducing the number of treatments to three in the split-plots (R6, R7 and R8). Pod permeability varied with the genotype and stage of development; this affected seed vigor, but not the viability of newly-harvested seeds. The pod lignin content did not show any influence on pod permeability.

Index terms: *Glycine max* (L.) Merrill., imbibition, lignin.

## Relação entre a permeabilidade da vagem e a qualidade da semente de soja

RESUMO - O objetivo do trabalho foi avaliar a influência da permeabilidade da parede da vagem na qualidade fisiológica das sementes de soja. As cultivares utilizadas foram Sant'Ana, FT-2, FT-10, Bossier, Davis e a linhagem F 84-7-30 com tegumento na cor preto. Coletaram-se vagens nos estádios de desenvolvimento R4, R5, R6, R7 e R8, sendo que os mesmos compuseram os tratamentos quanto à época de coleta. Avaliaram-se a permeabilidade e o conteúdo de lignina das vagens e das sementes no interior das mesmas. As sementes para os estudos foram obtidas logo após a maturidade plena (R8), sendo avaliadas a germinação, a condutividade elétrica e a viabilidade e o vigor pelo teste de tetrazólio. O delineamento experimental na avaliação das vagens foi em blocos ao acaso com parcelas subdivididas, sendo nas parcelas genótipos de soja (seis) e nas subparcelas estádios de desenvolvimento (R4, R5, R6, R7 e R8) com quatro blocos. Na avaliação das sementes utilizou-se o mesmo delineamento reduzindo o número de tratamentos para três nas subparcelas (R6, R7 e R8). A permeabilidade da vagem variou com o genótipo e com o estádio de desenvolvimento, e influenciou o vigor das sementes, entretanto, sem afetar a viabilidade das sementes recém-colhidas. O conteúdo de lignina nas vagens não teve influência sobre a permeabilidade das mesmas.

Termos para indexação: *Glycine max* (L.) Merrill., embebição, lignina.

## Introduction

Production of high quality soybean seeds in tropical and subtropical regions, especially at low latitudes, is hindered by diverse factors, among which is the occurrence of high temperatures, associated with high rainfall during seed maturation. These unfavorable environmental conditions lead to a fluctuation in seed moisture, resulting in reduction of germination and vigor. In addition, they favor the incidence of fungi associated with soybean seeds, such as *Phomopsis* spp., *Colletotrichum truncatum, Cercospora kikuchii* and *Fusarium* spp. (Pereira et al., 1985; Henning and França-Neto, 1980; França-Neto et al., 1994, 2007).

Among some of the properties that may contribute to improving the quality of soybean seeds, seed coat characteristics, semi-permeability of the pod walls, seed resistance to fungi, seed size, and the permeability of the cells of the component tissues of the seeds stand out (França-Neto et al., 1994).

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Thus, waxy pods, or those that through another reason have less permeability to moisture exchange between the environment and the seeds, may provide extra protection to the seeds against deterioration in the field (França-Neto and Krzyzanowski, 2000).

Tully (1982) proposed selection of soybean genotypes with pods impermeable to moisture. If these genotypes are found, and this characteristic exhibits a high degree of heritability, then it may be introduced in already existing cultivars.

Diverse researchers have observed genetic variability for the characteristic of pod wall permeability for soybean genotypes (Yaklich and Cregan, 1981; Tully, 1982; Dassou and Kueneman, 1984; Pereira et al., 1985). Among them, Pereira et al. (1985) observed a relationship between the lower permeability exhibited by the pod walls and the excellent physiological and health quality of the seeds since the low permeability of the pods provided greater protection to the seeds in the field during the final stages of maturation.

Tully (1982) developed a methodology for soybean genotype selection in regard to pod permeability, based on the principle of monitoring electron passage through the pod wall. Yaklich and Cregan (1981) measured the pod permeability of forty-six soybean cultivars through immersion of intact pods in water and subsequent evaluation of the moistening rate of the pods and the seeds; they observed significant differences in permeability among the different cultivars. Dassou and Kueneman (1984) used an incubation chamber to expose the seeds in pods to high temperature and moisture conditions, and the method allowed identification of soybean genotypes with high indices of tolerance to deterioration.

In relation to resistance to mechanical damage, this factor in the soybean seed is directly related to the lignin content present in the seed coat because this component is responsible for maintaining the integrity and structural cohesion of the plant fiber. Genotypes with more than 5% lignin in the seed coat are considered promising in relation to tolerance to mechanical damage (Alvarez et al., 1997).

The study of lignin content in the pod may also be of great worth for seed technology because, hypothetically, pods with greater lignin content may exhibit less permeability to water, resulting in less seed deterioration in the field, especially in regions where there are inclement weather conditions during the maturation and pre-harvest stages. In addition, lignin may make the pod walls more resistant to insect and fungus attack, preserving seed quality. Romkaew et al. (2008) observed variability in lignin content in the pods among soybean genotypes, and that this is one of the factors to be considered in selection of material resistant to dehiscence of the pods.

Therefore, the study of the relationship of the permeability of the pod wall with soybean seed quality may contribute to genetic breeding of the crop, assisting in selection of materials with the ability to produce high quality seeds for low latitude tropical and subtropical regions. Thus, the aim of this study was to evaluate the effect of permeability of the pod wall on the physiological qualities of the soybean seed and verify the relationship between lignin content of the pod and its permeability.

## Materials and Methods

The present study was developed in the experimental fields and in the Seed Analysis Laboratories of Embrapa Soja in the city of Londrina, PR, Brazil. The cultivars were selected as a result of previous information available in the literature in regard to their physiological and plant health qualities. It was used the cultivar Sant'Ana, due to the low permeability of its pods (Pereira et al., 1985) and the good quality of its seeds (Gilioli et al., 1978); the cultivars FT-2, FT-10, Bossier and Davis, due to the distinct lignin content in the seed coat and its potential effect on physiological quality (Alvarez et al., 1997); and the breeding line F 84-7-30 of seeds with a black seed coat, due to the information available in regard to the high level of lignin in the seed coat and in regard to the physiological quality of its seeds (Krzyzanowski et al., 1999).

In the field, the experiment was set up in plots of 8 rows of 15 m, with 0.45 m spacing between rows and a population of 20 plants per meter for harvesting of pods and seeds from each cultivar. Seeds were sown on November 5, 2008 by the conventional method. The daily climate data of mean temperature and total rainfall during the harvest period were gathered from the meteorological station of Embrapa Soja and supplied by the Agrometeorological Laboratory of the same institution (Figure 1). Mean daily temperature oscillated from 20 to 28 ºC. The occurrence of rainfall was relatively small, being restricted to a few scattered days in the harvest period. The experimental area was irrigated when necessary.

The treatments for study of the pods were the periodical manual collections in the various stages of development, according to Ritchie et al. (1985), as follows: R4 (full pod), collected from February 1 to 9; R5 (beginning seed), collected from February 13 to 26; R6 (full seed), collected from March 6 to 20; R7 (beginning maturity), collected from March 18 to 25; and R8 (full maturity), collected from March 23 to April 6. In the study of seed quality, the pods were collected at R8 (full maturity) and threshed manually.

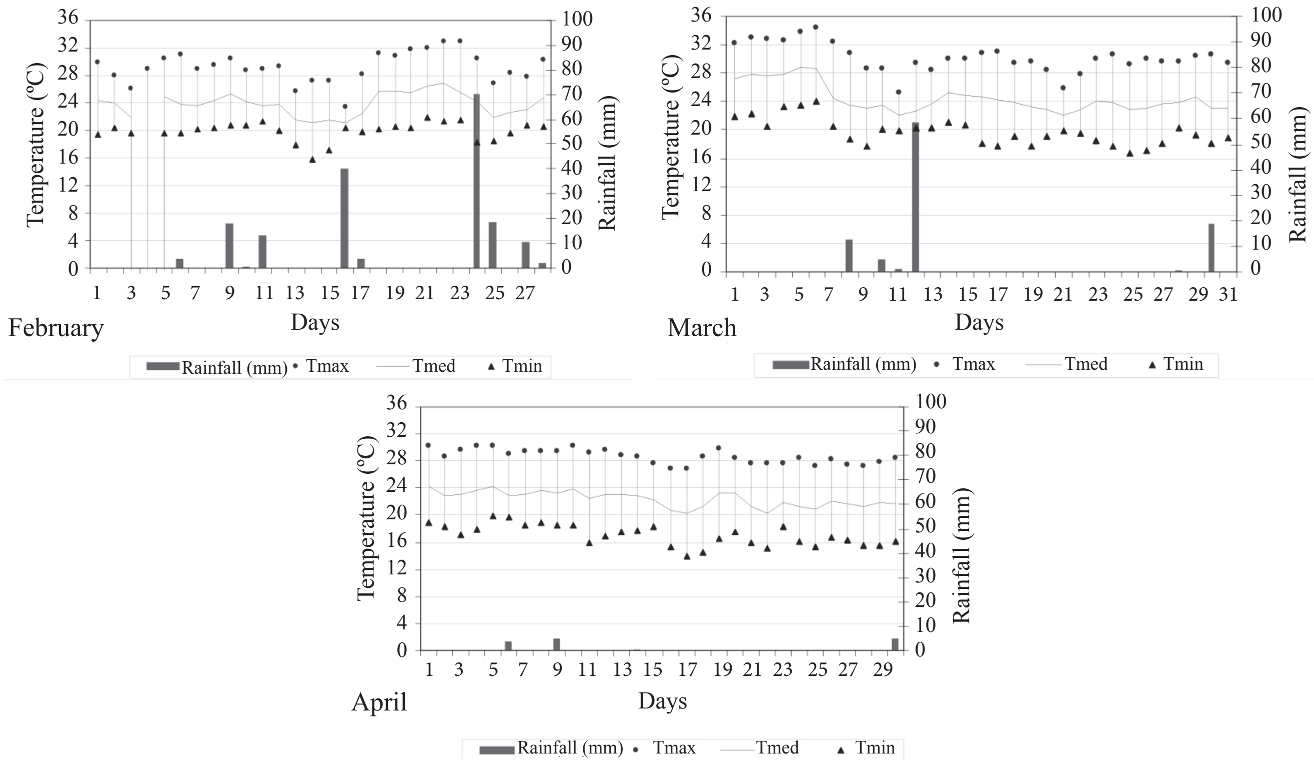


Figure 1. Rainfall and maximum and minimum temperatures on the Experimental Farm of Embrapa Soja, Londrina, PR, Brazil, in February, March and April 2009.

For each cultivar and in each treatment, the following factors were evaluated in the pods:

*a) Pods permeability:* evaluated by water absorption of the pods and seeds within them, which were immersed in water following the method proposed by Yaklich and Cregan (1981). Four replications of two subsamples of 20 newly-harvested pods were used, selecting those that did not exhibit visible damage. The pods were immersed in distilled water for 1, 6 and 24 h at 25 ºC and then placed on paper toweling to dry their surface for fifteen minutes. It was weighed the wet material and then the dry material (dried in an air-circulation laboratory oven at 100 ºC for 24 h). Only in the R6, R7 and R8 stages the pods and the seeds were separated after imbibition. Water absorption was determined by the difference between the initial water content of the pods and corresponding seeds, and after 1, 6 and 24 h of imbibition.

*b) Pod lignin content:* evaluated using four replications of 20 pods divided into two subsamples. After sterilization in NaClO (2%) for three minutes, the pods were rinsed in distilled water and placed for drying in a laboratory oven at 80 ºC for 24 h. The resulting dry material was ground and homogenized, and a 0.3 g sample was weighed for extraction of proteins. After that, from the material free of the proteins bound to the cell wall, the quantity of lignin was determined by the thioglycolic acid (TGA) method (Ferrarese et al., 2002).

In the analysis of pod lignin content and in evaluation of seed quality, after harvest, the pods and the seeds were packaged in paper bags and kept in dry cold storage (10 ºC and 50% relative humidity) up to the time of evaluation.

In the seeds from each cultivar, the following quality parameters were evaluated:

*a) Water content:* previously determined by the laboratory oven method at 105 °C ± 3 °C for 24 h (Brasil, 2009), using two replications of 20 seeds.

*b) Germination:* evaluated by the germination test, undertaken with four replications of 100 seeds, divided into two subsamples, as prescribed by the Rules for Testing Seeds (Brasil, 2009).

*c) Level of deterioration:* evaluated by the electrical conductivity test conducted with four replications of 200 seeds, divided into four subsamples, according to Vieira and Krzyzanowski (1999).

*d) Probable causes of reduction in seed vigor and the level of seed deterioration:* evaluated by the tetrazolium test, conducted with four replications of 100 seeds divided into two subsamples, according to the methodology of França-Neto et al. (1998). The seeds were classified to vigor and viability at levels from 1 to 8, and the types of damage were noted. Viability was represented by the sum of the seeds percentage belonging to classes 1 to 5; the vigor level, by classes 1 to 3; and loss of viability by classes 6 to 8. In this last category, the causes of loss of seeds physiological quality were characterized, i.e., through mechanical damage, deterioration from moisture, and damage brought about by stinkbugs.

A randomized block experimental design with split-plots was used in evaluation of the pods; the plots consisted of six genotypes (Sant´Ana, FT-2, FT-10, Bossier, Davis, and the breeding line F 84-7-30), and the split-plots consisted of the five stages of development (R4, R5, R6, R7 and R8), with four replications. In seed evaluation, the same design was used, varying only the number of treatments in the split-plots, which were R6, R7 and R8. All necessary tests were applied for use of analysis of variance to the distribution and independence of errors and homogeneity of variance of the errors of the treatments. Statistical analysis of the data for evaluations of pod permeability for each period of imbibition and lignin content of the pods and seeds was carried out in split-plots in time, with analysis of variance and comparison of mean values by the Scott-Knott test at 5% probability. Correlation was made of the mean values of pod and seed permeability, physiological quality of the seeds, and lignin content of the pods and seeds by the Student t test at 5% (Banzatto and Kronka, 2006).

## Results and Discussion

In the results of permeability of the pods and their seeds (Table 1), among the stages of development, it was observed greater water absorption in R8 and R7. In the initial stages (R4, R5 and R6), the pods and seeds absorbed little water, i.e., less than 5% of their initial weight, even after 24 h of imbibition, because the pods and seeds naturally displayed high moisture content (Table 2). Consequently, the statistical difference seen among the genotypes in these stages should not be considered a factor of importance to characterize them about pod permeability. Thus, these treatments were removed from this and from the other comparisons of this study.

Table 1. Water absorption (%) by the pods collected in the R4, R5, R6, R7 and R8 stages of development , and by the seeds within the pods collected in the R6, R7 and R8 stages of six soybean genotypes for the imbibition periods of 1, 6 and 24 h.

| Periods | Genotype | Pods: water absorption (%) | | | | | Seeds: water absorption (%) | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | R4 | R5 | R6 | R7 | R8 | R6 | R7 | R8 | Mean Values |
| 1 h | Sant'Ana | 0.6 Ac[1] | 0.4 Ac | 0.0 Ac | 11.6 Ab | 34.4 Ba | 0.0 | 0.2 | 0.3 | 0.2 B |
| | Bossier | 0.0 Ac | 0.2 Ac | 0.1 Ac | 11.1 Ab | 29.7 Ca | 0.0 | 0.2 | 1.5 | 0.6 B |
| | Davis | 0.0 Ad | 0.5 Ad | 1.9 Ac | 7.1 Cb | 22.1 Ea | 1.1 | 2.5 | 1.9 | 1.8 A |
| | FT 2 | 0.1 Ab | 0.1 Ab | 0.7 Ab | 4.7 Db | 27.3 Da | 0.1 | 0.3 | 0.8 | 0.4 B |
| | FT 10 | 0.0 Ad | 0.0 Ad | 1.2 Ac | 9.7 Bb | 42.8 Aa | 0.1 | 0.0 | 1.3 | 0.5 B |
| | F 84-7-30 | 0.2 Ac | 0.8 Ab | 0.7 Ab | 1.7 Eb | 35.5 Ba | 0.5 | 0.4 | 1.0 | 0.6 B |
| | Mean Values | - | - | - | - | - | 0.3 b[1] | 0.6 b | 1.2 a | |
| CV% | | 9.6 | | | | | 167.1 | | | |
| 6 h | Sant'Ana | 0.8 Ac | 0.7 Bc | 1.0 Bc | 14.7 Bb | 49.2 Ca | 0.1 Bb | 0.2 Cb | 5.5 Ba | |
| | Bossier | 0.1 Ad | 2.3 Ad | 1.0 Bc | 13.0 Bb | 38.6 Da | 0.1 Bb | 0.3 Cb | 8.4 Aa | |
| | Davis | 0.6 Ad | 1.0 Bd | 1.9 Bc | 7.8 Db | 52.1 Ba | 0.5 Bb | 2.9 Ba | 3.6 Ca | |
| | FT 2 | 0.1 Ad | 0.1 Bd | 2.8 Ac | 23.3 Ab | 52.6 Ba | 0.3 Bb | 4.6 Aa | 5.3 Ba | |
| | FT 10 | 0.2 Ad | 0.0 Bd | 2.5 Ac | 13.3 Cb | 56.2 Aa | 0.6 Ab | 0.0 Cb | 7.5 Aa | |
| | F 84-7-30 | 0.6 Ac | 2.0 Ab | 1.7 Bb | 2.6 Eb | 38.5 Da | 1.9 Ab | 2.6 Bb | 7.9 Aa | |
| CV% | | 5.6 | | | | | 35.4 | | | |
| 24 h | Sant'Ana | 3.0 Ad | 1.8 Bd | 2.3 Ac | 19.2 Bb | 57.5 Aa | 0.4 Ac | 4.0 Bb | 25.6 Aa | |
| | Bossier | 1.2 Bd | 3.9 Ac | 1.5 Ad | 16.2 Cb | 57.3 Aa | 0.1 Ab | 1.4 Cb | 25.6 Aa | |
| | Davis | 2.2 Ac | 1.0 Bd | 3.0 Ac | 20.4 Bb | 55.8 Ba | 2.3 Ac | 4.6 Bb | 14.4 Ba | |
| | FT 2 | 0.5 Bd | 1.7 Bd | 3.8 Ac | 34.7 Ab | 58.5 Aa | 1.1 Ac | 5.4 Bb | 27.5 Aa | |
| | FT 10 | 0.4 Bd | 0.4 Bd | 2.4 Ac | 20.0 Bb | 53.9 Ca | 0.5 Ab | 0.6 Cb | 23.1 Aa | |
| | F 84-7-30 | 0.4 Bd | 1.6 Bc | 2.4 Ac | 12.9 Db | 54.6 Ca | 2.0 Ac | 10.9 Ab | 26.3 Aa | |
| CV% | | 6.3 | | | | | 14.7 | | | |

[1]Mean values followed by the same uppercase letter in the column and lowercase letter in the line do not differ among themselves by the Scott-Knott test at 5% probability.

Table 2. Moisture content (%) in the pods and corresponding seeds at the time of harvest of six soybean genotypes collected at different stages of development.

| Genotype | Initial pod moisture content (%) | | | | | Initial seed moisture content (%) | | |
|---|---|---|---|---|---|---|---|---|
| | R4 | R5 | R6 | R7 | R8 | R6 | R7 | R8 |
| Sant'Ana | 87.4 Ba[1] | 81.3 Ab | 75.3 Cc | 50.9 Cd | 9.0 Be | 64.1 Ba[1] | 48.4 Cb | 8.2 Ac |
| Bossier | 87.9 Ba | 79.5 Cb | 78.0 Ac | 50.3 Dd | 10.0 Ae | 66.6 Aa | 49.4 Cb | 7.4 Ac |
| Davis | 87.5 Ba | 80.4 Bb | 76.5 Bc | 55.4 Bd | 9.6 Ae | 63.3 Ba | 53.4 Ab | 7.2 Ac |
| FT 2 | 87.6 Ba | 80.4 Bb | 74.0 Dc | 31.2 Ed | 8.7 Be | 60.3 Da | 41.2 Db | 7.0 Ac |
| FT 10 | 88.7 Aa | 80.0 Bb | 77.5 Ac | 51.2 Cd | 8.4 Ae | 61.1 Da | 51.5 Bb | 7.3 Ac |
| F 84-7-30 | 88.1 Ba | 80.2 Bb | 75.2 Cc | 56.7 Ad | 9.3 Be | 62.0 Ca | 41.1 Db | 7.9 Ac |
| CV% | | | 0.64 | | | | 2.06 | |

[1]Mean values followed by the same uppercase letter in the column and lowercase letter in the line do not differ among themselves by the Scott-Knott test at 5% probability.

In R7, in the pod permeability parameter (Table 1), the line F 84-7-30 showed the least water absorption in the three periods. The cultivars Sant'Ana and Bossier had similar behavior with greater water absorption than the other cultivars in the period of 1h. The cultivar FT-2 showed little absorption at 1 h, but after 6 and 24 h of imbibition, it showed greater water absorption than the others. This may be explained by the fact of the FT-2 cultivar having been collected with the lowest initial moisture content (Table 2), which may be seen as a characteristic inherent to this cultivar since in the period of the pods collection in R7 (March 18 to 25) there was no rain (Figure 1) which could have altered the initial moisture content of the pods collected.

In relation to water absorption by the seeds within the pods in R7, the period of 24 h stood out as most efficient in exhibiting the differences among the genotypes, and the genotype that exhibited greatest absorption was the line F 84-7-30, followed by the cultivars FT-2, Davis and Sant´ana (Table 1).

In R8, in pod permeability, the cultivar FT-10 exhibited the greatest values of water absorption for 1 and 6 h, and FT-2 for 24 h, not differing statistically from Bossier and Sant´Ana.

In water absorption by the seeds within the pods, only 6 h of imbibition presents statistical difference among the genotypes. Bossier, FT-10, and the line F 84-7-30 had greater absorption than Davis, and the others were intermediate. At 24 h, Davis maintained the least absorption among the genotypes, which were not statistically different among themselves.

In general, it may be affirmed that in the R8 stage for the period of 24 h, there was imbibition stabilization of the pods and the seeds. Therefore, the period of imbibition of 6 h may be considered the treatment with most significant results to characterize the permeability of pods in R8 because it showed the greatest variation in imbibition among the genotypes. In this context, the cultivar FT-10 showed the greatest absorption of water by the pods and seeds. The cultivars Bossier and Davis exhibited the lowest imbibition of pods and seeds, respectively.

As described by Krul (1978) and by Yaklich and Cregan (1981), it was seen that the water passed through the pod wall and was absorbed by the seeds and, therefore, the water flux of soybean pods and seeds is selectively controlled by the pods. Nevertheless, imbibition of the pods followed a different pattern from that shown by the corresponding seeds, indicating that, in spite of the moisture content of the seeds being dependent on the quantity of moisture available in the pod, there was no direct relationship between water absorption by the pod wall and by the seeds within them; it is therefore a characteristic inherent to each genotype.

It may be affirmed that pod permeability was variable among the genotypes and also varied in the different stages of development, mainly due, in this case, to the moisture content at the time of harvest. The greatest variation among the genotypes was observed in the R7 stage, and the greatest absorption occurred in the R8 stage, when the pods exhibited the lowest water content at the time of harvest.

Similar to this study, Tully (1982), Dassou and Kueneman (1984), Pereira et al. (1985), and Yaklich and Cregan (1981) observed a difference among the genotypes for the pods studied. These differences were not totally foreseeable because according to the study of Pereira et al. (1985), the Sant'Ana cultivar exhibited the least permeability of the pod walls in R8, Bossier exhibited intermediate permeability, and Davis had one of the greatest values of water absorption by the seeds, indicating greater permeability of the pod walls. These results differ from those observed in this study; however, different methodologies were used to evaluate pod permeability since Pereira et al. (1985) evaluated the permeability of the pod walls through exposing the pods to an environment with high relative air humidity (95%).

In analysis of variance of the physiological quality of the seeds (Table 3), a significant statistical difference was seen among the genotypes. In the viability tests (G, TVS G, and TZ Viab), the genotypes Davis and FT-2 exhibited the lowest values, while in the vigor tests, the cultivar FT-10 had worse performance than the others. Thus, the electrical conductivity test identified the level of deterioration of the seed lots, and the highest quality seeds were those derived from the cultivars Davis and FT-2, followed by the line F 84-7-30 and Sant'Ana, and finally, by Bossier and FT-10, which had greater leaching than the others. Nevertheless, the values exhibited were at most 100 μS $cm^{-1}.g^{-1}$, indicating that all the lots exhibited viable seeds (Vieira et al., 1999).

Table 3. Physiological quality of the seeds of six soybean genotypes by the tests of electrical conductivity (EC) (μS $cm^{-1}.g^{-1}$), normal seedlings from the germination test (G) (%), total of viable seeds from the germination test (sum of normal seedlings and hard seeds) (TVS G) (%), Tetrazolium test for vigor (TZ Vigor) (%), viability (TZ Viab) (%), mechanical damage (MD) (%), deterioration from moisture (DetM) (%), and stink bug damage (SbD) (%), classes 1 - 8 and 6 - 8.

| Genotype | EC | G | TVS G | TZ Vigor | TZ Viab | MD (1-8) | MD (6-8) | DetM (1-8) | DetM (6-8) | SbD (1-8) | SbD (6-8) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | μS $cm^{-1}.g^{-1}$ | (%) | (%) | (%) | (%) | (%) | (%) | (%) | (%) | (%) | (%) |
| Sant'Ana | 74.2 C[1] | 94.3 A | 97.3 A | 68.5 A | 93.0 A | 0 B | 0 | 73.5 A | 1.9 B | 16.5 B | 3.3 A |
| Bossier | 88.5 D | 97.3 A | 97.3 A | 70.8 A | 94.5 A | 0 B | 0 | 58.0 B | 1.3 B | 19.8 B | 4.0 A |
| Davis | 55.3 A | 86.7 C | 93.8 B | 65.3 A | 87.0 B | 0 B | 0 | 71.0 A | 7.7 A | 16.0 B | 3.3 A |
| FT-2 | 61.4 B | 91.8 B | 94.3 B | 74.5 A | 96.0 A | 0 B | 0 | 53.8 B | 0.3 C | 20.0 B | 2.5 A |
| FT-10 | 100.8 E | 96.0 A | 96.0 A | 54.5 C | 92.0 A | 0 B | 0 | 51.3 B | 1.8 B | 36.8 A | 6.3 A |
| F 84-7-30 | 72.5 C | 96.8 A | 97.3 A | 62.0 B | 94.3 A | 9.5 A | 0 | 21.0 C | 0.3 C | 34.3 A | 5.0 A |
| CV% | 4.9 | 1.8 | 1.6 | 7.4 | 3.0 | 170.3 | - | 13.2 | 35.7 | 29.2 | 74.1 |

[1]Mean values followed by the same letter in the column do not differ among themselves by the Scott-Knott test at 5% probability.

There was significant correlation between pod and seed permeability and seed quality (Table 4). The electrical conductivity test showed positive and significant correlation with water absorption by the pod in R8 in the period of 1 h, and by the corresponding seeds for 6 h. The germination test also showed this correlation, indicating that the permeability of the pods does not affect seed viability.

Among the correlations, it was also observed that water absorption by the pod in R8 for 1 h reflected the damages by stink bug seen in the seeds by the tetrazolium test, which resulted in a decline especially in seed vigor. Krul (1978) also observed that the differences in pod moisture content may also have an effect on seed deterioration.

The significant negative correlation between deterioration from moisture, characterized in the tetrazolium test, and germination and viability by the tetrazolium test indicates that this was the main cause in the decline in germination potential of the seeds. The significant positive correlation between stink bug damage, for its part, and electrical conductivity and vigor determined by the tetrazolium test indicate that this was the main cause in the decline in seed vigor.

In the Davis cultivar, the presence of hard seeds in the germination and tetrazolium tests (Table 3) justifies the lower permeability of the pods of this cultivar, observed by lower water absorption of the corresponding seeds of the pods in R8, after the periods of 6 and 24 h of immersion (Table 1).

For all these reasons, it may be affirmed that the presence of hard seeds in the seed lots is an interesting characteristic for, as Potts et al. (1978) affirmed, genotypes with high frequency of hard seeds proved to be resistant to deterioration in the field. Nevertheless, selecting lines that exhibit hard seeds is not a practical solution since these seeds soak up moisture slowly and germinate irregularly, unless they are scarified. Yaklich and Cregan (1981) also observed lower permeability of the pods through lower water content of the corresponding seeds in the cultivars with a tendency toward hard seeds.

Pereira et al. (1985), in their study with fifteen genotypes of soybean, observed that the cultivar Sant'Ana exhibited seeds with the best germination and vigor indices, and the lowest fungal infection rates. The authors attributed the excellent physiological and health qualities of the seeds of this cultivar to the lower permeability exhibited by their pod walls. These results were not observed in this study, though the Sant'Ana cultivar showed good quality seeds; the high percentage of seeds with moisture damage decrease seed quality and indicated that the pods were not totally effective in protecting the seeds against deterioration in the field.

In analysis of lignin content in the pods and in the coat of the seeds within the pod, there was statistically significant interaction between the genotypes and the stages of development (Table 5), therefore indicating that this is a characteristic that varies with the genotype, in agreement with the studies of Baldoni et al. (2013), Romkaew et al. (2008), and Alvarez et al. (1997).

Table 4. Simple correlation between permeability of the pods and corresponding seeds, for different periods of imbibition, and physiological quality of the seeds [tests of electrical conductivity (EC), germination (G), total of viable seeds from the germination test (sum of normal seedlings and hard seeds) (TVS G), test of tetrazolium for vigor (TZ Vigor), viability (TZ Viab.), deterioration from moisture (DetM), and stink bug damage (SbD), classes 1 to 8 and 6 to 8] of six soybean genotypes collected at different stages of development.

| Correlation | | EC | G | TVS G | TZ Viab. | TZ Vigor | DetM (1-8) | DetM (6-8) | SbD (1-8) | SbD (6-8) |
|---|---|---|---|---|---|---|---|---|---|---|
| Physiological Quality of the Seeds | | | | | | | | | | |
| Germination | | 0.78* | | | | | | | | |
| TVS G | | 0.59 | 0.87* | | | | | | | |
| TZ Viab. | | 0.26 | 0.68 | 0.46 | | | | | | |
| TZ Vigor | | -0.54 | -0.22 | -0.16 | 0.39 | | | | | |
| DetM (1-8) | | -0.18 | -0.53 | -0.33 | -0.45 | 0.33 | | | | |
| DetM (6-8) | | -0.43 | -0.81* | -0.59 | -0.96* | -0.13 | 0.57 | | | |
| SbD (1-8) | | 0.60 | 0.57 | 0.31 | 0.20 | -0.78* | -0.77 | -0.44 | | |
| SbD (6-8) | | 0.79* | 0.58 | 0.41 | -0.04 | -0.90* | -0.51 | -0.21 | 0.90* | |
| Permeability | | | | | | | | | | |
| Pod R7 | 1 h | 0.49 | 0.14 | 0.26 | -0.16 | 0.01 | 0.74* | 0.16 | -0.35 | 0.02 |
| | 6 h | -0.01 | -0.10 | -0.31 | 0.44 | 0.54 | 0.44 | -0.29 | -0.36 | -0.47 |
| | 24 h | -0.38 | -0.45 | -0.69 | 0.24 | 0.50 | 0.29 | -0.08 | -0.33 | -0.57 |
| Pod R8 | 1 h | 0.81* | 0.74* | 0.60 | 0.31 | -0.69 | -0.40 | -0.55 | 0.79* | 0.82* |
| | 6 h | -0.06 | -0.53 | -0.66 | -0.38 | -0.23 | 0.49 | 0.35 | -0.04 | -0.04 |
| | 24 h | -0.42 | -0.19 | -0.12 | 0.41 | 0.96* | 0.48 | -0.16 | -0.80* | -0.88* |
| Seed R7 | 1 h | -0.68 | -0.87* | -0.67 | -0.85* | 0.02 | 0.34 | 0.93* | -0.43 | -0.34 |
| | 6 h | -0.80* | -0.57 | -0.68 | -0.06 | 0.43 | -0.23 | 0.08 | -0.19 | -0.54 |
| | 24 h | -0.55 | -0.04 | 0.07 | 0.17 | 0.09 | -0.64 | -0.18 | 0.17 | -0.14 |
| Seed R8 | 1 h | 0.00 | -0.33 | -0.43 | -0.60 | -0.26 | 0.03 | 0.62 | 0.04 | 0.21 |
| | 6 h | 0.79* | 0.95* | 0.77 | 0.58 | -0.29 | -0.62 | -0.71 | 0.64 | 0.66 |
| | 24 h | 0.33 | 0.75* | 0.58 | 0.98* | 0.29 | -0.44 | -0.98* | 0.26 | 0.03 |

*significant by the T test at 5%.

Table 5. Lignin (%) in the pods and in the seed coat of seeds of six soybean genotypes collected at different stages of development.

| Genotype | Lignin Pods (%) | | | | | Lignin Seeds (%) | | |
|---|---|---|---|---|---|---|---|---|
| | R4 | R5 | R6 | R7 | R8 | R6 | R7 | R8 |
| Sant'Ana | 5.1 Bc[1] | 5.2 Bc | 8.9 Bb | 10.6 Ba | 10.4 Ba | 2.9 Ab[1] | 2.9 Ab | 3.8 Fa |
| Bossier | 7.0 Ad | 5.2 Be | 9.2 Bc | 12.2 Aa | 10.4 Bb | 2.5 Bc | 3.0 Ab | 4.9 Da |
| Davis | 5.4 Bc | 5.8 Ac | 8.8 Bb | 9.8 Ba | 10.0 Ba | 2.4 Bc | 3.0 Ab | 4.5 Ea |
| FT-2 | 5.3 Bd | 6.4 Ac | 9.2 Bb | 11.5 Aa | 9.9 Bb | 2.8 Ab | 3.1 Ab | 6.0 Ba |
| FT-10 | 7.2 Ac | 6.1 Ad | 9.8 Bb | 11.3 Aa | 11.7 Aa | 2.7 Ac | 3.2 Ab | 5.3 Ca |
| F 84-7-30 | 5.4 Bb | 5.4 Bb | 11.1 Aa | 10.3 Ba | 10.3 Ba | 2.9 Ab | 3.3 Ab | 10.0 Aa |
| CV% | | | 7.0 | | | | 6.7 | |

[1]Mean values followed by the same uppercase letter in the column and lowercase letter in the line do not differ among themselves by the Scott-Knott test at 5% probability.

The percentage of lignin in the pods increased with their development up to R7, and then stabilized or declined in R8. The exception was the line F 84-7-30, with seeds with a black seed coat, which reached maximum lignin concentration in R6. In seeds, the lignin content in the seed coat also increased with maturity, reaching a maximum in R8, for all the genotypes studied. These results show that there was lignin transport from the pod to the seed during maturation.

Among the data of maximum lignin accumulation, it canbe observed that Bossier exhibited the greatest lignin content in the pod and Davis the least, while the other genotypes were intermediate. In the seeds, it was observed that the line F 84-7-30 showed the greatest percentage of lignin in the seed coat, followed by the cultivars FT-2, FT-10, Bossier, Davis and Sant'Ana. Alvarez et al. (1997) observed similar results among the cultivars they studied, with the greatest lignin content in the seed coat for the seeds of the cultivar FT-2, followed by FT-10, Bossier and Davis.

Correlation analysis was carried out between the lignin content in the pod and in the seed coat of the corresponding seeds at the different stages and the parameters studied (Table 6). Among the statistically significant correlations, those that exhibited the most relevant results, which may contribute to research, stand out. There was significant positive correlation of the lignin content in the pod in R6 with the seed in R7 and R8. Therefore, the characteristic of the genotype in relation to the lignin content in the seed coat would already be defined in R6. In agreement with this assertion, Baldoni et al. (2013) also observed expression of the genes C4H and PAL, precursors of lignin, in soybean seeds in the R5 and R6 stages.

Table 6. Simple correlation between lignin content in the pods and in the seed coat of seeds and the permeability of the pods and corresponding seeds at different periods of imbibition, and the physiological quality of the seeds (tests of electrical conductivity, germination, tetrazolium for vigor and viability, deterioration from moisture, and stink bug damage, classes 1 to 8 and 6 to 8), of six soybean genotypes collected at different stages of development.

| Lignin Content | | Pod | | | | | Seeds | | |
|---|---|---|---|---|---|---|---|---|---|
| | | R4 | R5 | R6 | R7 | R8 | R6 | R7 | R8 |
| Lignin Content Seed | R6 | -0.34 | -0.02 | 0.52 | -0.08 | 0.09 | | 0.43 | 0.55 |
| | R7 | 0.22 | 0.37 | 0.85* | -0.02 | 0.33 | | | 0.83* |
| | R8 | -0.16 | 0.00 | 0.94* | -0.17 | -0.12 | | | |
| Pod Permeability R7 | 1 h | 0.47 | -0.34 | -0.75* | 0.36 | 0.41 | -0.39 | -0.70 | -0.85* |
| | 6 h | 0.00 | 0.51 | -0.82* | 0.58 | -0.04 | 0.03 | -0.34 | -0.52 |
| | 24 h | -0.29 | 0.79* | -0.50 | 0.24 | -0.27 | 0.02 | -0.12 | -0.28 |
| Pod Permeability R8 | 1 h | 0.49 | -0.08 | 0.37 | 0.19 | 0.85* | 0.58 | 0.48 | 0.23 |
| | 6 h | -0.02 | 0.72 | -0.41 | -0.17 | 0.33 | -0.10 | -0.10 | -0.52 |
| | 24 h | -0.40 | 0.03 | 0.72 | 0.38 | -0.63 | -0.06 | -0.67 | 0.42 |
| Seed Permeability R7 | 1 h | -0.37 | 0.11 | 0.50 | -0.68 | -0.45 | -0.62 | -0.24 | -0.17 |
| | 6 h | -0.63 | 0.58 | 0.22 | -0.25 | -0.69 | 0.07 | 0.24 | 0.37 |
| | 24 h | 0.69 | -0.11 | 0.72 | -0.53 | -0.55 | 0.55 | 0.43 | 0.81* |
| Seed Permeability R8 | 1 h | 0.44 | 0.15 | 0.24 | -0.05 | 0.05 | -0.82* | 0.13 | 0.04 |
| | 6 h | 0.66 | -0.35 | 0.51 | 0.59 | 0.49 | 0.29 | 0.48 | 0.44 |
| | 24 h | 0.03 | -0.10 | -0.01 | 0.60 | 0.05 | 0.72 | 0.23 | 0.34 |
| Physiological Quality of the seeds | | | | | | | | | |
| Electrical Conductivity | | 0.88* | -0.17 | -0.01 | 0.56 | 0.88* | 0.09 | 0.26 | -0.06 |
| Germination | | 0.52 | -0.41 | 0.47 | 0.57 | 0.49 | 0.48 | 0.36 | 0.36 |
| Tetrazolium Viability | | 0.05 | 0.00 | -0.05 | 0.68 | -0.04 | 0.61 | 0.25 | 0.36 |
| Tetrazolium Vigor | | -0.46 | -0.06 | -0.54 | 0.31 | -0.79* | -0.11 | -0.56 | -0.23 |
| Det. from Moisture (1-8) | | -0.07 | -0.04 | -0.96* | -0.02 | -0.09 | -0.55 | -0.90* | -0.97* |
| Det. from Moisture (6-8) | | -0.15 | 0.04 | -0.53 | -0.60 | -0.18 | -0.73 | -0.40 | -0.45 |
| Stink bug Damage (1-8) | | 0.45 | 0.18 | 0.78* | 0.05 | 0.68 | 0.46 | 0.90* | 0.64 |
| Stink bug Damage (6-8) | | 0.69 | -0.05 | 0.56 | 0.06 | 0.85* | 0.18 | 0.66 | 0.35 |

*significant by the T test at 5%.

In correlation of lignin content with permeability of the pods and seeds, negative correlations are considered relevant because they indicate that the greatest lignin content resulted in low permeability.

Thus, significant negative correlation was observed between permeability of the pod in R7 for 1 and 6 h of imbibition and the lignin content of the pods in R6, and between imbibition of seeds in R8 for 1h and the percentage of lignin in the seed in R6. Nevertheless, there were more positive correlations than negative. Therefore, it can not be concluded that the results indicated a clear relationship between pod and seed permeability and lignin content. In a similar manner, Romkaew et al. (2008) observed the relationship between lignin content and pod dehiscence, but did not observe a relationship between moisture retention in the cultivars resistant and susceptible to pod dehiscence.

In the study of correlation between the quantity of lignin and the physiological quality of the seeds, there was significant negative correlation between lignin content in the pod in R6 and in the seed in R7 and R8 for deterioration from moisture, evaluated in the tetrazolium test. This result indicates that greater lignin content in the pod and in the seeds may contribute to a decrease in deterioration from moisture. Nevertheless, a significant positive correlation was observed between lignin content in the seed in R7 and in the pod in R6 and R8 and stink bug damage, indicating that the lignin content in the pod did not make the pod walls more resistant to insect attack, disagreeing with the suppositions of Alvarez et al. (1997).

Thus, it may be inferred that the lignin content confers resistance to the pods and seeds, but does not have an effect on their permeability, i.e., it does not contribute to the oscillation in the moisture content of the seeds within the pods.

## Conclusions

The permeability of the pod varies with the genotype and with the stage of development, and it has an effect on seed vigor, though it does not affect the viability of the newly-harvested seeds.

The lignin content in the pods does not have an effect on the permeability of the pods.

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