

Full Length Research Paper

Lignification of the plant and related aspects of soaking seeds and soybean pod of RR and conventional cultivars

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There has been great speculation about differential responses of transgenic and conventional cultivars of soybean lignin content in its stem, pod and seeds. Each characteristic is associated with the soaking of seeds and pods. Increase of weight of intact soybean seeds and pods at different soaking periods and their relation to the lignin contents in RR and conventional soybean plants was determined. Samples of 20 pods and 50 seeds from the conventional cultivars, Jataí, Celeste, and Conquista and their respective transgenic RR versions Silvânia, Baliza, and Valiosa were utilized. The pods were immersed into water for 1, 3, 6, 9, 12, 24, and 48 h, and the seeds were immersed into water for 1, 2, 3, 4, 5, 6, 7, 9, 12, 24, and 48 h. The intact pods showed some resistance against water in the first few hours of soaking, but the seeds possess greater absorption at the onset and later stabilization. There were no differences between RR transgenic and conventional cultivars for lignin content in stem, leaf, pod, and seed coat and the IVH of intact pods was found. It was difficult to establish an association between the soaking rate and transgenic characteristic in the evaluated soybean cultivars.

Key words: Imbibition rate, lignin, water immersion.

INTRODUCTION

The genetically modified soybean (RR), resistant to the herbicide glyphosate, has been in Brazilian market for about 21 years ago, and their launch revolutionized the soybean market in Brazil and worldwide. In 2014/2015, the cultivation of these varieties reached 93.2% of the

planted area; about 29.10 million hectares were planted with this crop in Brazil. Transgenic intact soybean RR2 IPRO, which has stacked traits, in the second year of commercial adoption reached 5.2 million hectares planted, or 16.5% of the total soybean sown in 2014/2015

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(SNA, 2014).

Some writers have mentioned differential responses in lignin contents in RR and conventional cultivars due to excess lignification that occurs in RR transgenic cultivars (Coghlan, 1999; Kuiper et al., 2001; Edmisten et al., 2006; Nodari and Destro, 2006). However, research in this area is rather restricted, these claims being not based on comparative studies within the same genotype.

Lignin deposition gives strength and rigidity to plant tissues such as stems and leaves, especially soybean seed coat, which resists mechanical damage (Panobianco, 1997); it gives tissues strength and protection and also protects the cell wall from microorganisms' infestation (Rijo and Vasconcelos, 1983; Tavares et al., 1987). In this context, possible differential responses may become relevant to physiological quality of seeds, considering the relationship between the permeability of soybean seeds and the physiological quality.

Large characteristics of seed coats are associated with specific problems presented in the seeds, such as the susceptibility of mechanical damage, longevity and potential to deterioration that may be associated with their lignin content and levels of permeability of the seed coat. These aspects are considered for seed coat and can also be applied to pod and therefore be associated with the quality of seeds. Yaklich and Cregan (1981) have observed that the differences between soybean cultivars cannot be attributed just to environmental factors, but mainly to genetic differences between cultivars, such as soaking pods. Tully (1982) also mentioned that the incorporation of water impermeability in the pod would be most appropriate alternative to the impermeability seeds, verifying this characteristic variability between different soybean cultivars.

A good relationship between permeability of pods and quality of soybean seeds was reported by Pereira et al. (1985), who evaluated methods for the identification of genotypes with high quality seed. Costa et al. (2002), contrasting two soybean cultivars to water absorption in laboratory and field conditions, determined that the lower water absorption would be the probable cause of tolerance deterioration of seed of the cultivars. Braccini (1993) noted that the pod dunk test correlated negatively with premature aging tests, emergence in sand and emergency speed index, indicating that with increased permeability of the same the quality of seeds decreases. Therefore, the aim of this work is to determine the increase of weight of intact soybean seeds and pods at different soaking periods and their relation with the lignin contents in RR and conventional soybean plants.

MATERIALS AND METHODS

The experiment was conducted in Lavras, Minas Gerais, Brazil (21°14'S, 45°00'W, 918 m of altitude), an important region of coffee beans production. It was done in winter, in the agricultural year of 2007 (April - August). Soil in the experimental area is classified as

dystrophic red latosol. Climate in the region is type Cwa (wet moderate subtropical), according to the Köppen classification; it has mean annual temperature of 19.3°C and normal annual rainfall of 1530 mm (Dantas et al., 2007). The climatic data were collected in a meteorological station of the Instituto Nacional de Meteorologia (INMET) at the Universidade Federal de Lavras - UFLA (Figure 1).

The experimental design was a randomized complete block design (RBD) with four replications. The experimental unit consisted of four rows with 6.0 m, and 0.50 m space from each one. In this case, the relevant areas are composed of two central rows of 0.50 m (useful area of 3.2 m²).

Three conventional cultivars and their RR transgenic versions were evaluated: BRS MG 46 'Conquista' versus BRS Valiosa RR, BRS MG 46 'Jataí' versus BRS Silvânia RR e BRS 'Celeste' versus BRS Baliza RR. When seeding, the seeds were treated with the commercial product Vitavax Thiram 200 SC® in the concentration of 250 ml⁻¹ 100 kg⁻¹ seed, and then inoculated with peat commercial product (minimum population of 1.200.000 cells/seed). The soybean seeding fertilization was performed according to the soil analysis and the second interpretations by Ribeiro et al. (1999). During thinning, there remained 16 plants/m linear density, and when required, cultural practices were performed according to recommendations for the crop. The average air temperature and precipitation in the region during the study period are given in Figure 1.

The lignin contents were determined in stem, leaf, vegetable and husk of soybean (Capeleti et al., 2005). The amounts of material to be analyzed, as well as electrical conductivity test (Vieira, 1994) and seed immersion test were done.

For seed immersion test, the seeds from the electrical conductivity test were used, subjected to submersion in water for 24 h at 25°C. Later, germination test was done. The number of normal and abnormal plants was evaluated for four days.

The plant tissues for lignin analysis were collected when they were between R7 and R8 stages (Fehr and Caviness, 1977) by selecting (Figure 1). There was daily change in air temperature and average rainfall from March to August 2007 (Meteorological Station of the Instituto Nacional de Meteorologia (INMET) at the Universidade Federal de Lavras - UFLA, MG, Brazil).

For soaking tests, pods with 2 or 3 seed each were selected, without apparent damage. They were collected with the scissors. The pods were threshed manually and retained in circular sieve of 5.55 and 6.35 mm. two replicates of 20 units per field block were used for soaking intact pods and 4 replicates of 50 units per field block to soak seeds. The samples were initially weighed and then immersed completely in 250 ml plastic cups containing demineralized water at 25 ± 2°C, for 1, 3, 6, 9, 12, 24 and 48 h for intact pods, using the methodology proposed by Boldt (1984), and 1, 2, 3, 4, 5, 6, 7, 9, 12, 24 and 48 h for seed according to Rocha et al. (1990).

To avoid the fluctuation of pods other plastic cups of the same volume were overlapped. After the soaking period, the glass is drained from the water and excess water from pods or seeds were eliminated by drying blotting paper. It was then weighed and soaked for a further period. With the initial and final weight of each sample, percentage of seed imbibition and intact pods was determined, for each soaking time, and the extracted intact seeds of pods, according to the equation:

$$E (\%) = [(PF - PI)] / PI \times 100$$

where E (%) = soaking percentage relative to the initial weight of the sample; PI = initial weight of the sample (for each period); PF = final weight of the sample after 48 h of immersion in water.

At the end of the soaking tests, the samples were subjected to 105°C for 24 h in an oven for final moisture determination; intact pods were determined separately in the water content of seeds and pods. Nakagawa et al. (2007) method was used to determine the

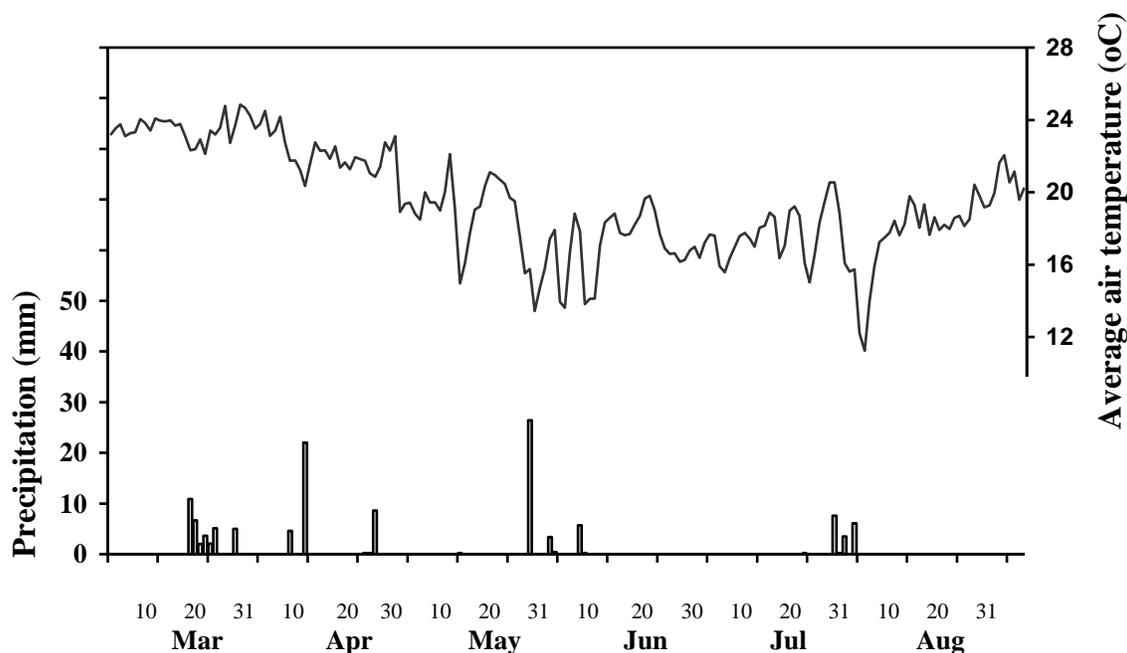


Figure 1. Daily change in air temperature and average rainfall from March to August 2007. Source: Meteorological Station of the Instituto Nacional de Meteorologia (INMET) at the Universidade Federal de Lavras - UFLA, MG, Brazil.

hydration rate index (IVH), based on the equation of germination speed index (GSI) of Edmond and Drapala (1958); the replacement of germination was given by the percentage of soaking.

Evaluation results were submitted to analysis of variance by F test (Storck et al., 2000) and when there was significance, the means were compared by Scheffé test (5% significance), through computer statistical software R (2008), for each soaking period, in contrast to conventional cultivars and their RR transgenic versions. For the variable hours of soaking, regression analysis was performed using quadratic model response plateau, which is the amount of time in which there is a stabilization value (% final soaking), given by:



RESULTS AND DISCUSSION

There was a significant effect for the interaction between cultivars and percentage of seed soaking (Table 1). For the cultivars, Conquista and Valiosae RR were observed different rates of seed soaking for 4, 5 and 6 h and for Jataí and Silvânia RR cultivars, it was 1 and 2 h. There was no statistical difference for the percentage of seed soaking for Celeste and Baliza RR cultivars.

After 4 h of immersion in water, it was observed that the seeds of the cultivar RR Valiosa absolved 55.24% of its weight in water; its value is statistically less than that 72.07% absolved by conventional Conquista.

Considering these three periods in which both cultivars showed differences in average, conventional seeds absolved 23.61% more water than RR seeds. This indicates greater restriction on water intake in these seeds.

According to Calero et al. (1981) and McDonald et al. (1988), probably the restriction on water ingress is due to the permeability of the seed coat, which according to these authors, acts as regulator of seed soaking. McDougall et al. (1996) emphasized that the seed coat impermeability awarded by the lignin, has a significant effect on the ability and speed of water absorption through this. However, it was not possible to establish relationship between increased permeability of the seed coat and lower lignin content. So, in this work, the cultivars showed no differences for this trait (Table 4).

According to McDonald et al. (1988), soybean seeds absorb approximately 80% water for the first 3 h of soaking, seeds coats having a relevant role in this process. In Table 1, it was observed that within 3 h, only Conquista and Valiosa cultivars RR showed absorptions smaller than 70% water by weight, and the cultivars, Jataí and Silvânia RR absorbed more than 90%.

It is noteworthy that among the materials evaluated, the seeds of Jataí and Silvânia RR had the lowest percentages at final soaking. However, they have absorbed during the first 2 h, 73.58 and 90.68% water weight, respectively. This indicates increased permeability of the seed coat for these crops compared to

Table 1. Averages of contrasts obtained for soaking percentage of conventional soybean varieties of seeds and their transgenic versions RR. South of Minas, Brazil, 2007.

Time (h)	Averages of contrasts					
	Conquista vs. Valiosa RR		Celeste vs. Baliza RR		Jataí vs. Silvânia RR	
1	20.81 ^a	13.85 ^a	23.12 ^a	35.21 ^a	45.22 ^b	63.68 ^a
2	37.92 ^a	27.77 ^a	48.03 ^a	58.45 ^a	73.58 ^b	90.68 ^a
3	53.96 ^a	40.11 ^a	72.43 ^a	76.91 ^a	96.34 ^a	106.91 ^a
4	72.07 ^a	55.24 ^b	97.45 ^a	93.59 ^a	110.41 ^a	115.94 ^a
5	87.49 ^a	70.91 ^b	113.62 ^a	107.13 ^a	117.77 ^a	120.44 ^a
6	99.25 ^a	83.23 ^b	123.67 ^a	117.62 ^a	123.72 ^a	124.11 ^a
7	108.50 ^a	94.16 ^a	129.90 ^a	124.67 ^a	126.70 ^a	126.15 ^a
9	120.08 ^a	107.56 ^a	135.07 ^a	131.60 ^a	128.73 ^a	127.83 ^a
12	127.95 ^a	118.56 ^a	137.86 ^a	135.65 ^a	129.70 ^a	128.21 ^a
24	131.75 ^a	122.67 ^a	137.39 ^a	135.95 ^a	128.17 ^a	125.94 ^a
48	136.55 ^a	136.73 ^a	140.18 ^a	135.51 ^a	127.85 ^a	125.29 ^a

*Means followed by the same letter in the line, between the contrasts, do not differ by Scheffé test at 5% probability.

Table 2. Averages values for the variables in which the contrasts between conventional soybean cultivars and their transgenic versions RR showed significance. South of Minas, Brazil, 2007.

Variable	Averages	
	Jataí	Silvânia RR
Water soaking (%)	59.00 ^a	36.00 ^b
Electrical conductivity ($\mu\text{S}/\text{cm}/\text{g}$ of seeds)	76.54 ^b	100.25 ^a

*Means followed by the same letter in the line, between the contrasts, do not differ by Scheffé test at 5% probability.

others; faster stabilization occurred at 5.81 and 6.67 h, respectively, getting for these times some statistical differences.

Soaking differences occurred for both varieties with 1 and 2 h of soaking. Silvânia RR cultivar was more permeable to water with about 90% of after 2 h Rodrigues et al. (2006) studied preview hydration of soybean seeds, and found sharp increase in the water content within the first 3 h; there was 6 h of relative stabilization.

These results are against those obtained for physiological quality of seeds of the two cultivars (Table 2). Once, the cultivar Silvânia RR had smaller number of normal germinated seedlings after being immersed in water, and greater value electrical conductivity, possibly because of higher membrane permeability and damages caused by the rapid entry of water.

Figure 1 shows the regressions for hours within each cultivar, indicating the average stabilization values (X_0) and the percentage of soaking (P) at X_0 time to seed. It can be inferred there are different periods of stabilization of soaking seeds of cultivars, Jataí/Silvânia RR, Celeste/Baliza RR e Conquista/Valiosa RR, which showed an average of 6.24, 9.10 and 13.53 h for

stabilization, respectively. However, the final imbibition values were not significantly different, and the average concentration was between 126.71 and 136.12% absorbed water (Figure 2).

According to Labouriau (1983), soaking speed is affected when environmental conditions vary, but the maximum amount of water absorbed at this stage does not change. This is because this maximum is a property of hydrophilic colloids seed, conditioned by maturity and/or the storage. In soybeans, proteins are primarily responsible for the soaking phenomenon, due to their hydrophilic nature (Rocha et al., 1990). However, the composition and protein in the seeds produced have not been determined in this work.

Costa et al. (2002) observed variations in water absorption speed among soybean cultivars, until the eighth hour absorption when the authors observed a quadratic response to this variable. These results contrary are to this work. The percentage of seed soaking is framed in quadratic model; indicating that the amount of absorbed water became lower, stabilizing at the last hour.

In contrast, Toledo (2008) observed linear response of soybean cultivars as the capacity of water absorption by

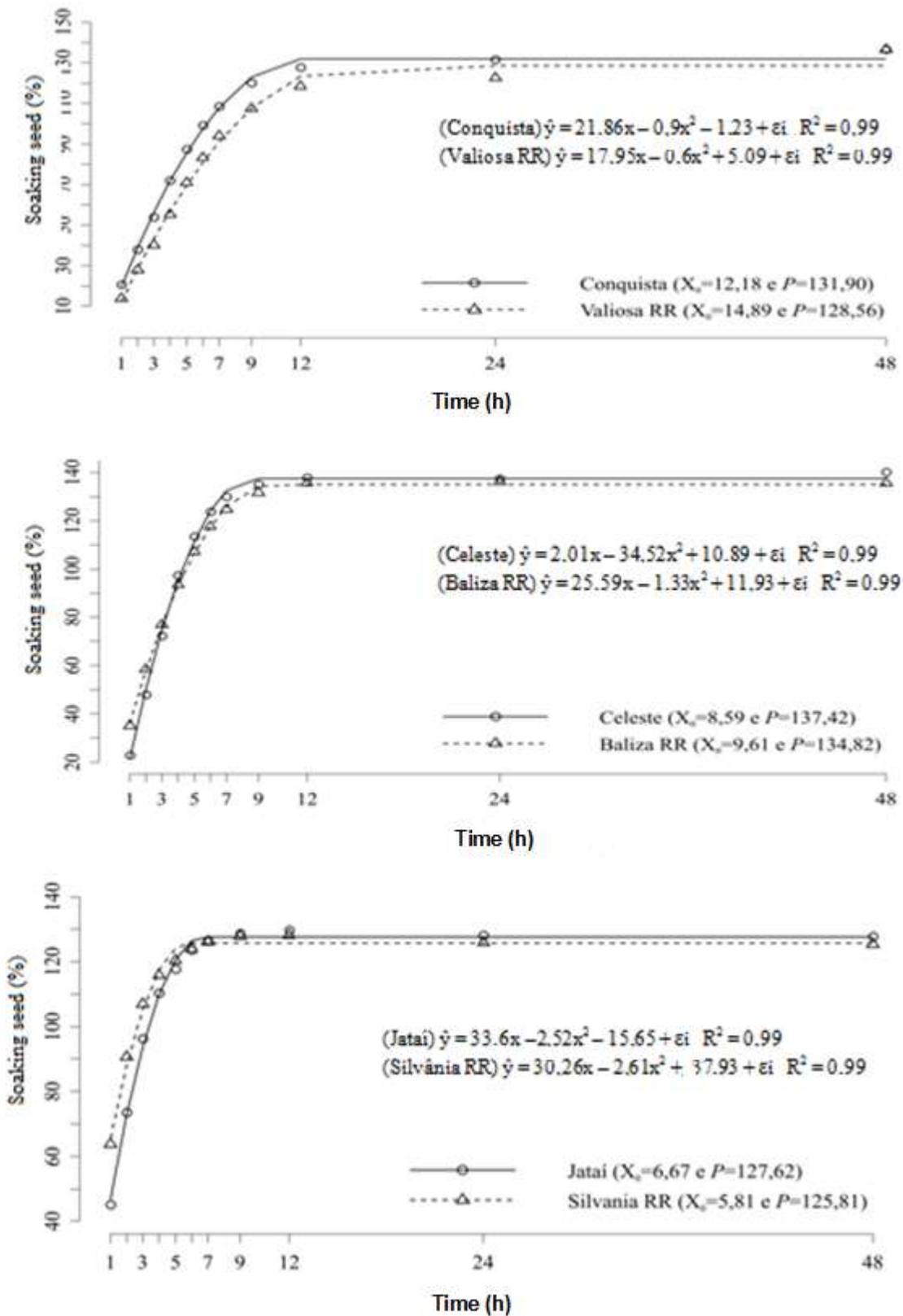


Figure 2. Averages values (symbols) and estimated (lines), stabilization of average time (X₀) and average weight value in time X₀ (P) obtained in the regression analysis for the percentage of seed soaking the conventional soybean cultivars and their transgenic versions RR. South of Minas, Brazil, 2007.

Table 3. Averages of contrasts obtained for soaking percentage of intact legumes of conventional soybean cultivars and their transgenic versions RR. South of Minas, Brazil, 2007.

Time (h)	Means of contrasts					
	Conquista vs. Valiosa RR		Celeste vs. Baliza RR		Jataí vs. Silvânia RR	
1	7.67 ^a	7.64 ^a	10.30 ^a	7.12 ^a	4.46 ^a	4.23 ^a
3	13.12 ^a	11.89 ^a	18.71 ^a	14.03 ^a	9.21 ^a	7.34 ^a
6	19.48 ^a	16.21 ^a	25.98 ^a	22.17 ^a	10.49 ^a	9.54 ^a
9	26.78 ^a	21.31 ^a	34.48 ^a	29.64 ^a	13.63 ^a	13.34 ^a
12	33.60 ^a	24.83 ^a	42.64 ^a	37.80 ^a	16.74 ^a	17.09 ^a
24	51.75 ^a	39.20 ^b	61.02 ^a	55.92 ^a	28.07 ^a	29.27 ^a
48	70.74 ^a	56.79 ^b	80.81 ^a	74.43 ^a	43.49 ^a	43.78 ^a

*Means followed by the same letter in the line, between the contrasts, do not differ by Scheffé test at 5% probability.

the seeds as a function of time, indicating gradual increases in the amount of water absorbed within 8 h of imbibition. Santos et al. (2007) observed that seeds of cultivars Embrapa 48 and BRS 133, evaluated for 24 h of hydration, also showed linear adjustment of the data. However, it is noteworthy that Santos et al. (2007) and Toledo (2008) used the methodology of the humid atmosphere and moistened paper method, respectively, which provided slower seed absorption process.

Souza et al. (2004), evaluating the physiological quality of bean seeds by water absorption in different storage periods, observed genetic variability for both percentage of water absorption as for the germination and emergence speed. The difference was accentuated with the storage time of seed. These authors observed that the water absorption decreased during storage, as the emergence speed and germination rate increased; indicating that probably for bean seeds, water absorption does not seem to affect the seed vigor.

About the cumulative percentage of water gain by legumes (Table 3), it was observed that the cultivar Valiosa RR showed lower soaking rate than its conventional version, possibly indicating greater restriction on the water ingress through the walls of RR legumes. These results were similar with the seed soaking test (Table 1), where legumes of the cultivar Conquista had 32% more water absorption than the cultivar, Valiosa RR.

Regarding the evolution of the process of water absorption by intact pods (Table 3), there were observed differentiations in periods of 24 to 48 h between Conquista and Valiosa RR cultivars; there were no other differences between RR and conventional materials. It is noteworthy that when the X_0 stabilization time is not among the periods when there was a statistical difference, do not consider different the X_0 and P values between RR and conventional materials.

Contrasting Figures 2 and 3, in the process of seed soaking there are fast initial absorption of water, with subsequent stabilization, of at most 13.54 h; while in the intact pods, there was resistance to water ingress in the

first hours of soaking, taking stabilization time of the same, with the exception of Celeste and Baliza RR cultivars that exceeded 48 h. This shows that the period of performing the test was not sufficient for the stabilization of soaking pods.

Among the cultivars evaluated, as well as for soaking seeds, Jataí and Silvânia RR had lower percentage (52.25 and 42.26%), respectively, as well as smaller values of soaking in all periods when compared to other cultivars. As for seeds, it was not possible to establish an association between the soaking rate of pods and the transgenic characteristic of soybean cultivars evaluated. Table 4 shows the significant effect of the hydration rate index (IVH) on the seeds of the cultivars, Conquista and Valiosa RR; with no differences between RR and conventional cultivars for the other variables.

There was a higher rate of hydration of seeds to cultivate transgenic Valiosa RR (5.23 g/h) when compared to conventional Conquista (3.84 g/h). However, the results obtained are contrary to the soaking percentages for these cultivars, wherein the cultivar Conquista, in some periods, exceeded Valiosa cultivar. These results of IVH are justified since the weight increments are multiplied by soaking period. To cultivate Valiosa, there were additions of soaking in the last periods, 24 and 48 h, than those observed for the cultivar Conquista, which resulted in higher IVH for this cultivar.

The RR and conventional cultivars did not differ in the percentage of final soaking of seeds from the soaking test of pods which, absorbed on average 70.69, 86.22 and 72.15% water by weight; this is contrasting with Conquista/Valiosa RR, Celeste/Baliza and Jataí RR/RR Silvânia, which were observed in the final water content of 41, 46 and 41%, respectively (Table 4). It is noteworthy that this final water content obtained at the end of the soaked seed test (64% for cultivars Conquista and Valiosa RR and 61% for other cultivars) demonstrate the important role of pod in the quality of soybean seeds, acting as a regulator of water absorption.

This regulatory function of pods can be better viewed for Jataí and Silvânia RR cultivars, when observing the

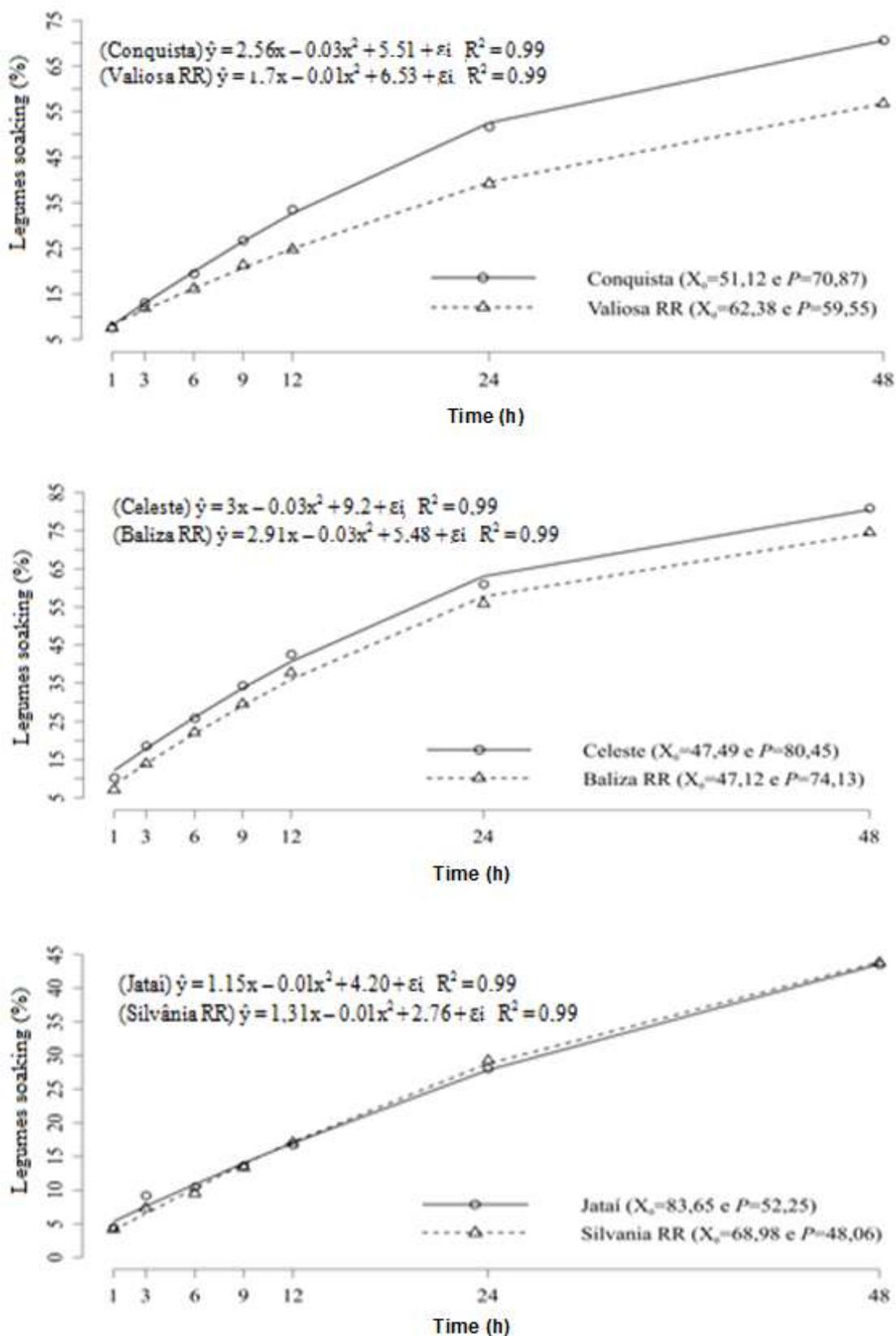


Figure 3. Averages values (symbols) and estimated (lines), stabilization of average time (X_0) and average weight value in time X_0 (P) obtained in the regression analysis for soaking percentage of intact pods, conventional soybean cultivars and their versions transgenic RR. South of Minas, Brazil, 2007.

Table 4. Averages of contrasts obtained for the variables studied (I to IX) of conventional soybean cultivars and their transgenic versions RR. South of Minas, 2007.

Variable	Averages of contrasts					
	Conquista vs. Valiosa RR		Celeste vs. Baliza RR		Jataí vs. Silvânia RR	
I	3.84b	5.23 ^a	2.64 ^a	2.04 ^a	1.30 ^a	0.87 ^a
II	11.62 ^a	10.50 ^a	10.38 ^a	10.10 ^a	6.98 ^a	7.57 ^a
III	64.47 ^a	76.92 ^a	89.45 ^a	82.99 ^a	66.08 ^a	78.22 ^a
IV	0.26 ^a	0.25 ^a	0.21 ^a	0.21 ^a	0.39 ^a	0.39 ^a
V	7.62 ^a	7.64 ^a	7.92 ^a	7.77 ^a	7.85 ^a	7.51 ^a
VI	12.45a	12.43 ^a	12.09 ^a	12.71 ^a	11.53 ^a	12.66 ^a
VII	6.62 ^a	7.19 ^a	5.88 ^a	5.33 ^a	6.34 ^a	6.20 ^a
VIII*	39.07	43.27	45.19	47.01	39.68	43.60
IX*	64.11	63.24	62.95	58.35	61.66	60.95

(I) IVH of seeds (g/h), (II) IVH of intact pods (g/h), (III) Final percentage of seed soaking in soak test of intact pods (%), (IV) Lignin in seed coat (%), (V) Pod lignin (%), (VI) Lignin in stem (%), (VII) Lignin in leaf (%), (VIII) Final moisture of the seeds after pods soaking test (%), (IX) Final moisture of the seeds after seed soaking test (%). Means followed by the same letter in the line, between the contrasts, do not differ by Scheffé test at 5% probability. *No data analyzed statistically.

results of physiological quality (Table 2) and the data obtained from the soaking of seeds and intact pods. When the seeds were submitted for vigor tests characterized by immersion in water as seed soaking, germination after immersion in water and electrical conductivity, there were the highest percentages of soaking in the early hours of the test, in addition to the smaller number of normal seedlings after immersion and the highest electrical conductivity values.

However, when subjected to other vigor tests, which did not use direct immersion in water, the seeds of these cultivars showed high vigor. This allows one to infer that the seed coat of Jataí and Silvânia RR cultivars, despite having the highest values of lignin, is not the mainly responsible for maintaining the physiological quality of seeds, and possibly the pod is an important ally of the seed, in maintaining the physiological quality. It acts as a regulator of water absorption, especially during the final maturation process. These results are different from the work of Braccini (1993), which suggests that increased permeability of pods is correlated with the reduction of the quality of soybean seeds; which according to Pereira et al. (1985), it should be explored further improving the processes of high quality seeds. The results observed for the lignin content in stem, leaf and pods were similar among all materials tested (Table 4).

As for the lignin content in the seed coat, the greater values were observed for the Jataí and Silvânia RR cultivars, when compared with the other cultivars. However, from the results, it is inferred that the seed water absorption does not appear to be associated only with the lignin, since these cultivars had the highest percentages of soaking in the early hours of the test. According to Calero et al. (1981), soybean cultivars with slow absorption of water can have elongated pores and teguments with waxy material embedded in the

epidermis, one fact that makes the water imbibition process slower. However, these characteristics were not evaluated in this work.

Conclusion

There were no differences in the lignin content of the stem, leaf, pod and seed coat between the transgenic and conventional soybean cultivars. It was not possible to establish an association between the soaking rate in seeds and pods, and evaluate the transgenic characteristic of the soybean cultivars. The pod soaking correlates with the physiological quality of soybean seeds.

Conflict of interests

The authors have not declared any conflict interests.

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