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Physiological Quality of Conventional and RR Soybean Seeds Associated with Lignin Content

Cristiane Fortes Gris[1] and Edila Vilela de Resende Von Pinho[2]
[1]Federal Institute of Southern Mines
[2]Federal University of Lavras
Brazil

1. Introduction

The sale of genetically modified soybean seed resistant to the Roundup Ready (RR) herbicide has revolutionized the worldwide soybean market in recent years. According to data from the International Service for the Acquisition of Agri-Biotech Applications-ISAAA (2009), in 2009, for the first time, more than three-quarters (77%) of the 90 million hectares of soybeans grown globally were biotech; followed by cotton, with almost half (49%) of the 33 million hectares being biotech; by maize, with over a quarter (26%) of the 158 million hectares grown globally being biotech; and finally by canola, with 21% of the 31 million hectares being biotech. These numbers indicate not only increases in hectares, but also a strong and growing adherence of farmers around the world to this technology.

Considering the area planted to RR soybeans in the 2009/10 growing season throughout the world, from these 69.3 million hectares, a demand of approximately 4.2 million tons of RR soybean seeds may be estimated, which makes the international soybean seed market ever more expressive and competitive. In Brazil alone, up to November 2010, nearly 35% of the total soybean cultivars registered in the Ministry of Agriculture were RR genetically modified, this number having increased more than 443% in the last four growing seasons, a result of the increase in the number of breeding programs for obtaining RR cultivars.

It is known that the physiological quality of soybean seeds is controlled in large part by the genotype or cultivar, features of the plant, and more specifically those of the pod and the seed itself, determining a differential response of each cultivar and its levels of tolerance to seed deterioration, to adverse field conditions and even to mechanized harvesting. Among seed characteristics, the seed coat is one of the principal conditioning factors for germination vigor and longevity of seeds, with its characteristics being associated with susceptibility to mechanical damage, longevity and potential for seed deterioration, which may be influenced by the lignin content and the degree of seed coat permeability. Understanding of the structure and properties of the seed coat has contributed to explaining and altering seed behavior under certain environmental conditions.

In the case of soybeans, differences in the lignin content among seed coat have been observed by various authors (Tavares et al., 1987; Carbonell et al., 1992; Alvarez, 1994; Carbonell & Krzyzanowski, 1995; Panobianco, 1997; Menezes, 2008). In addition, a great deal of speculation has been generated in relation to the lignin content in the plant between RR genetically modified soybean cultivars and conventional cultivars (Coghlan, 1999; Gertz

Junior et al., 1999; Kuiper et al., 2001; Edmisten et al., 2006; Nodari & Destro, 2006), indicating overproduction of this substance of up to 20% more in RR cultivars. Such variation may occur not only in the vegetative parts of plants, but also in reproductive parts, such as pods and seeds.

The term lignin is used to designate a group of substances with similar chemical units indicated as polymers derived from "p-coumaryl", "conyferyl" e "sinapyl" alcohols (Lewis & Yamamoto, 1990). Impermeable to water, lignin is also very resistant to pressure and not very elastic and it is the most abundant plant polymer after cellulose, being found in greater quantity in the cell wall, around 60% to 90% (Egg-Mendonça, 2001), and its deposition occurs during the formation of the cell wall.

According to the authors, overproduction of lignin observed in the RR soybean plant in the US, and more recently in Brazil, is leading to deep stem fissures, with a significant number of plants in the field presenting bent or broken stems, and this effect possibly arises in the presence of water deficit and high temperatures.

Although the exact cause of the lignin behavior in this mechanism is still unknown, the hypothesis of overproduction of lignin in RR soybean plants is based on the fact of the precursors of the lignin molecule being formed in the same metabolic pathway, the pathway of shikimic acid, inhibited by the glyphosate herbicide. The inhibition of EPSPS enzymes by glyphosate present in this pathway leads to a deficiency in the production of amino acids and consequent death of the plants. That way, the sequence CP4 EPSPS, introduced in the genome of commercial soybean cultivars responsible for production of the protein CP4 enolpyruvylshikimate-3-phosphate synthase (EPSPS), an enzyme that participates in the biosynthesis of aromatic amino acids in plants and microorganisms, may be presenting the pleiotropic effect, thus modifying the lignin content in the plant.

Nevertheless, research in this area is still quite limited and the few results published do not compare conventional cultivars with their respective RR genetically modified versions, but refer to comparison between diverse genotypes and therefore do not isolate the effect of the inserted transgene. In this context, it is relevant to discuss the results of more recent research dealing with this issue in this chapter, principally looking at comparisons between conventional materials and their RR versions, which are essentially derivatives.

For that reason, in this chapter we will discuss results of research dealing with the physiological quality and the lignin content in RR and conventional soybean seeds submitted to different harvest times and spraying with glyphosate herbicide, produced in two different time periods and submitted to direct imbibition in water.

2. The lignification process and RR soybeans

The term lignin is used to designate a group of substances with similar chemical units. According to Panobianco (1997), the chemical structure of lignin is very complex and still not very well defined. Butler & Bailey (1973), cited by Silva (1981), refer to lignin as a polymer, 3-methoxy-phenyl-propanol and 3-5-dimethoxy-phenyl-propanol, bonded in varied proportions and in random sequence, leading to a great variety of products, which makes exact definition difficult. According to Esau (1976), lignin consists of an organic substance or mixture of organic substances with high carbon content, but different from carbohydrates, and which is found associated with cellulose on the walls of numerous cells.

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Yamamoto, 1990). Impermeable to water, lignin is also very resistant to pressure and not very elastic and it is the most abundant plant polymer after cellulose, being found in greater quantity in the cell wall, around 60% to 90% (Egg-Mendonça, 2001), and its deposition occurs during the formation of the cell wall.

The growth and development of the cell wall may be divided into two phases: growth of the primary wall, a phase in which the cell increases in size, and growth of the secondary wall, a phase in which deposition of lignin polymers occurs to the extent that the cell wall becomes progressively thicker as of the internal edge of the primary wall, in the direction of the center of the cell. The inclusion of lignin on the cell wall originates in the middle lamella, going in the direction of the interior of the secondary wall. According to Jung & Alen (1995), the effect of this lignin deposition pattern makes the middle lamella/primary cell wall region more intensely lignified.

This lignin deposition is important not only to lend rigidity and resistance to plant tissue, such as stem and leaves, but especially for the seed coat of soybean seeds, it has been correlated with resistance to mechanical damage (Alvarez, 1994; Panobianco, 1999), providing mechanical resistance to the tissue and protection against infestations by microorganisms to the cell wall (Rijo & Vasconcelos, 1983, cited by Tavares et al., 1987).

2.1 Lignification and the soybean seed coat

The seed coat is one of the main factors which determine germination capacity, vigor and longevity of seeds. It has a protective function during imbibition, avoiding cell rupture and loss of intracellular substances (Duke & Kakefuda, 1981), and also protects the embryonic axis (Carvalho & Nakagawa, 2000). It is derived from the integuments of the ovule where the primine gives rise to the testa and the secundine gives rise to the tegmen.

By means of a cross section of the testa of a soybean seed, three layers may be distinguished, the epidermis, the hypodermis and the inner parenchyma (Swanson et al., 1985). This last layer, composed of the spongy parenchyma, is present in the entire testa of the seed, except for the hilar region. It has from 6 to 8 cell layers, tangential to the surface of the testa, formed by thin walls and absent protoplasm, with the outermost part of this parenchyma being formed by large, elongated cells, while the innermost part by smaller and significantly branched cells (Esau, 1977).

The intermediate layer of the testa, the hypodermis, is formed of cells in hourglass form, or pillar cells, or even osteosclereid cells. It consists of a uniform cell layer through the entire testa, except for the hilar region. The cell wall of its sclerenchyma cells is not uniform, with the presence of large intercellular spaces (Corner, 1951).

The epidermis, outside of the testa, remains uniseriate and gives rise to the palisade layer, characteristic of leguminosae seeds. This layer consists of macrosclereids (Malpighi cells) with wall of unequal thickness, having a cuticle present over their outermost wall. It cells are elongated and arranged perpendicular to the surface of the testa, with thick cell walls (Esau, 1976).

In soybean seeds, the thickness of the four testa layers altogether, including the cuticle, starting from the surface, may vary from 70 to 100 micrometers, there being variation among cultivars. Nevertheless, this characteristic is a constant with each cultivar and is controlled genetically (Caviness & Simpson, 1974). The presence or lack of pores and their quantity, shape and size on the surface of the testa is also controlled genetically. The pores seem to be related to water absorption, such that in hard seeds they are either absent or they exist in small quantity (Calero, 1981).

Morphological characteristics associated with the thickness and structure of the seed coat has also been related to the quality of soybean seeds. With the aid of a Scanning Electron Microscopy (SEM), it is possible to obtain a direct image of the atoms on the surface of a material, formed by secondary electrons and emitted from the surface of the irradiated specimen by the beam of primary electrons or by those scattered, which, in spite of generating poorer quality images, may indicate differences in the elementary composition of the sample. Designed basically for surface examination of samples, SEM allows the observation of internal surfaces if fractured and exposed, using principally secondary electrons (Alves, 2006).

Silva (2003), by means of scanning micrography of transversal sections of the testa of soybean seeds of the cultivars M-Soy 8400 and M-Soy 8411 observed three visible cell layers: palisade cell layers, an hourglass cell layer, and spongy parenchyma cells. The author evaluated the behavior of these cell layers that compose the testa of soybean seeds when they were exposed to five periods of accelerated aging (0, 24, 48, 72 and 96 hours) at 42º C and approximately 100% relative air humidity. For the cultivars evaluated, reduction in the thickness of the testa of the soybean seed was verified, which suggests collapse of the cells that compose such layers, which may be related to reduction of germination potential.

Menezes et al. (2009) evaluating the thickness and structure of the soybean seed coat (Figures 1 and 2) and the association of these characteristics with the physiological quality of the seeds, concluded that traits used for evaluation of physiological quality may be correlated with the lignin content of the seed coat. Nevertheless, according to the author, it was not possible to establish a relationship between the physiological quality of the soybean seeds and the anatomical aspects of the seed coat evaluated by SEM, emphasizing the need for refining the methodologies available for this purpose due to the difficulties of establishing the work area of common structures on the seeds, and of having observed that cell structures vary in different positions on the seed coat, which makes comparison of these structures among seeds of different genotypes difficult. In spite of that, in a general way, it was possible to observe that the lignin thickness on the palisade cell layers was greater when compared to the hourglass cell layers.
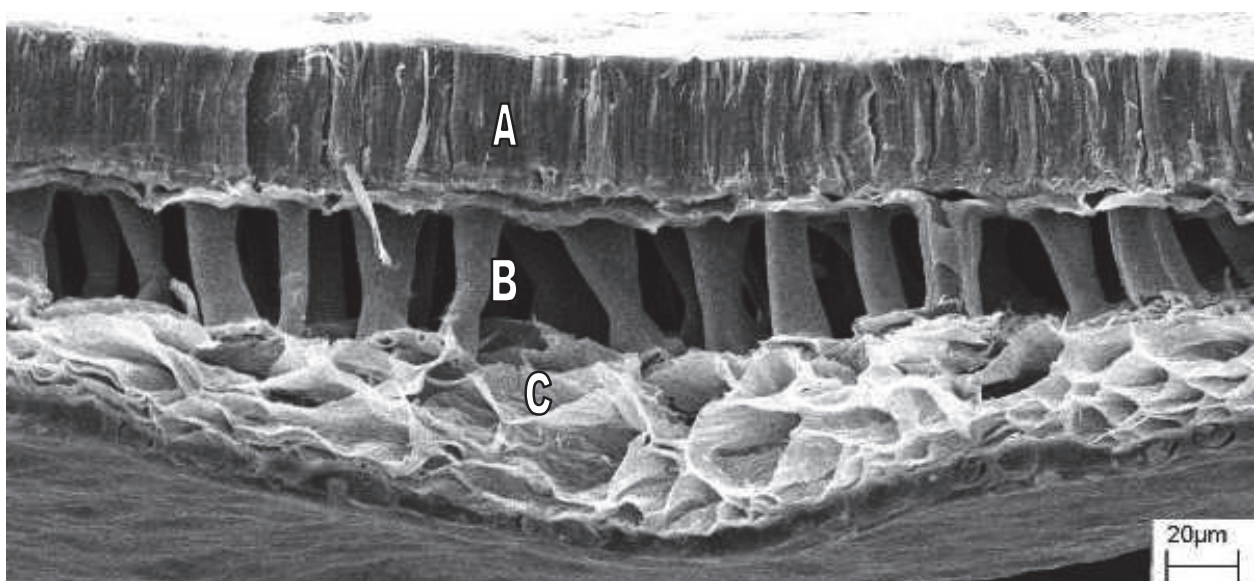

Fig. 1. Scanning micrography of the testa of the cultivar CD 201; A: palisade cell layer; B: hourglass cell layer and C: spongy parenchyma. Source: Menezes et al. (2009).

As is common in leguminosae, there is a particularly impermeable region on the walls of the upper part of the macrosclereids, which reflects light more intensely than the rest of the wall (Esau, 1965). What is called the conspicuous light line is visible in many wild soybean species, but is less prominent in cropped species (Alexandrova & Alexandrova, 1935, cited by Carlson & Lersten, 1987). This palisade layer drew the interest of researchers through the fact of its structure, and in certain hard seeds of leguminosae, being the cause of the high degree of impermeability of the seed coat, consequently affecting germination capacity (Esau, 1976).

Hard or impermeable seeds, according to Woodstock (1988), may be the result of compacted organization of cellulose microfibriles on the cell wall. This, for its part, may be impregnated with waterproof substances, such as lignin, waxes, suberins or tannin. They are abundantly composed of cellulose and hemicellulose polysaccharides, and of phenylpropanoid polymers such as lignin (McDougall et al., 1996).

In accordance with McDougall et al. (1996), the impermeability of the seed coat provided by lignin, exercises a significant effect on the speed and capacity of water absorption through it, thus interfering in the quantity of leached materials released to the outside during the imbibition phase of the seed germination process. Crocker (1948) already mentioned the need for better understanding of this mechanism since it was considered to be the best example of efficiency against water penetration and should therefore be better utilized by breeders in adjusting this characteristic to their needs. As general characteristics of soybean cultivars with a less permeable seed coat, one may cite better conservation potential, lower levels of infection by pathogens, greater vigor and viability, as well as resistance to reabsorption of moisture after maturation (Panobianco, 1999).
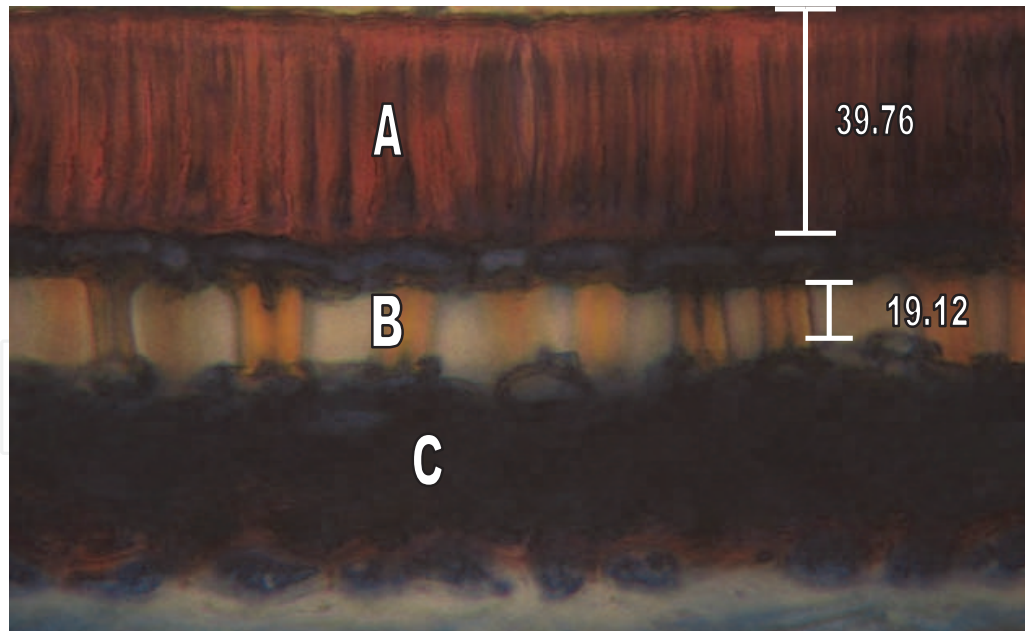

Fig. 2. Comparson of the thickness (m) of the palisade cell and hourglass cell layers obtained by SEM from the cultivar CD 206. A: palisade cell layer; B: hourglass cell layer and C: spongy parenchyma. Source: Menezes et al. (2009).

Tavares et al. (1987), studying structural characteristics of the seed coat of seeds of soybean lines, concluded that the total fiber content is not connected with impermeability; however,

in regard to the type of fiber, an accentuated increase in the lignin values was observed in the lines with impermeable seed coats (4.69% to 7.70%), differentiated from the values 1.80% to 3.18% found in lines with permeable seed coats. According to Brauns & Brauns (1960), cited by Tavares et al. (1987), the hydrophobic trait of lignin affects the hydrophilic bonds of the middle lamella and the removal of lignin interferes in the biological resistance of hydration in around 10.5% to 17% of the original tissue.

The occurrence of hard seeds in leguminosae has been attributed to both genetic and environmental factors (Donnelly, 1970). The percentage of hard seed exhibits considerable variability depending on the species or cultivar, the degree of maturity, the maturation conditions and the storage time. Thus, low air humidity during maturation results in a considerable increase in seed hardness (Baciu-Miclaus, 1970; Martins, 1989).

In soybeans, differences in the lignin content of the seed coat has been observed by various authors (Tavares et al., 1987; Carbonell et al., 1992; Alvarez, 1994; Carbonell & Krzyzanowski, 1995; Panobianco, 1999; Menezes et al., 2009; Gris et al., 2010;), and, in addition, differences have been reported in regard to the lignin content in the plant between genetically modified RR and conventional cultivars.

2.2 Lignin biosynthesis and RR soybeans

The advent of genetically modified soybeans, tolerant to the Roundup Ready© herbicide (RR), revolutionized the world soybean market. With the introduction of the CP4 EPSPS sequence in the genome of commercial soybean cultivars, which confers tolerance to the active ingredient glyphosate, the protein CP4 enolpyruvylshikimate-3-phosphate-synthase (EPSPS) is produced, an enzyme that participates in the biosynthesis of aromatic amino acids in plants and microorganisms. In the case of conventional cultivars, the inhibition of these enzymes by glyphosate, present in the shikimic acid pathway, leads to a deficiency in production of essential amino acids and consequent death of the plants, which does not occur in RR cultivars.

A great deal of speculation has been generated in relation to the lignin contents in the plant between genetically modified RR cultivars and conventional cultivars (Coghlan, 1999; Gertz Junior et al., 1999; Kuiper et al., 2001; Edmisten et al., 2006; Nodari & Destro, 2006).

In the late 1990s, some farmers in Georgia complained about the poor performance of their RR soybeans in years with a spring with drought and heat conditions. Scientists then carried out a comparative laboratory study of genetically modified and conventional soybeans (Gertz Junior et al. 1999). They found that the genetically modified plants were shorter, had a lower fresh weight, had less chlorophyll content, and, at high soil temperature of 40 °C to 50ºC, suffered from stem splitting. According to Coghlan (1999), the elevated levels of lignin deposited in the stem of soybean plants would be leading to this splitting due to the stiffening of the plants under high temperatures (45ºC), a problem also detected in genetically modified RR soybean crops in the USA, and which was to have led to considerable losses through falling of plants in hotter years (Nodari & Destro, 2006) as a consequence of overproduction of lignin in RR cultivars (Kuiper et al., 2001).

According to these authors, under stress conditions, losses in RR soybeans can arrive at 40% in comparison with conventional soybeans, brought about by greater production of lignin, up to 20% greater (Coghlan, 1999; Gertz et al., 1999). Nodari & Destro (2006), in a study undertaken in nine soybean crops in the state of Rio Grande do Sul (Brazil), observed that in the presence of drought and high temperatures, the RR soybean crops suffered more losses than conventional soybeans. The authors observed a large number of plants with deep stem

splitting and a significant quantity of these plants had bent or broken stems, around 50% to 70% of the plants, according to the authors, possibly due to overproduction of lignin in the RR material (Figure 3).
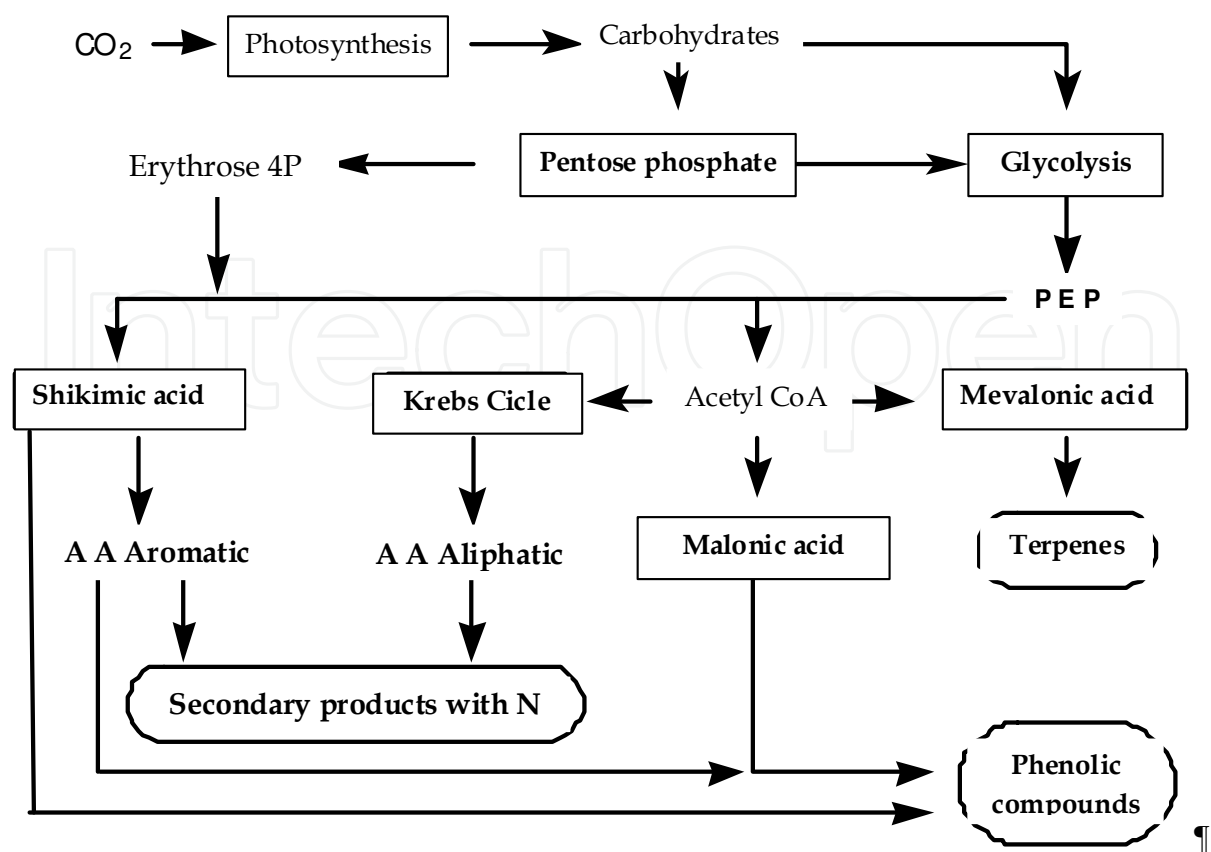

Fig. 3. Plants of the "Maradona" variety with broken stem (left), split (middle) and intact stem (right). Source: Nodari & Destro (2006).

The plants are responsible for the production of secondary metabolites that perform innumerable functions, among which the terpenes, the phenolic compounds and the alkaloids are considered as the most important. The secondary compounds are biosynthesized through three basic metabolic pathways, the acetate-mevalonate, the acetate-malonate and the acetate-shikimate (Érsek & Kiraly, 1986), also denominated simply as mevalonic acid pathway, malonic acid pathway and shikimic acid pathway, respectively (Taiz & Zeiger, 1998).

In superior plants, the shikimic acid pathway occurs in plastids, there also being evidence that it is present in the cytosol (Hrazdina & Jensen, 1992). This important metabolic pathway begins with phosphoenolpyruvate (PEP), derived from glycolysis, and the erythrose 4-P coming from the monophosphate pentose pathway and the Calvin cycle, resulting in the biosynthesis of the phenylalanine amino acids, tyrosine and tryptophan (Salisbury & Ross, 1992) (Figure 4).

According to Resende et al. (2003), the enzymes that participate in the initial and intermediary steps of the lignin biosynthesis pathway are common to the phenylpropanoid pathway (Figure 5). The metabolism of the phenylpropanoids includes a complex series of biochemical pathways that provide the plants with thousands of combinations. Many of these, according to Boatright et al. (2004), are intermediate in the synthesis of structural substances of the cells, such as lignin, if formed from shikimic acid, which forms the basic units of the cinnamic and p-coumaric acids (Simões & Spitzer, 2004).

Source: Adapted from Taiz & Zeiger (1998).

Fig. 4. Schematic representation of the shikimic, malonic and mevalonic acid pathways. ¶
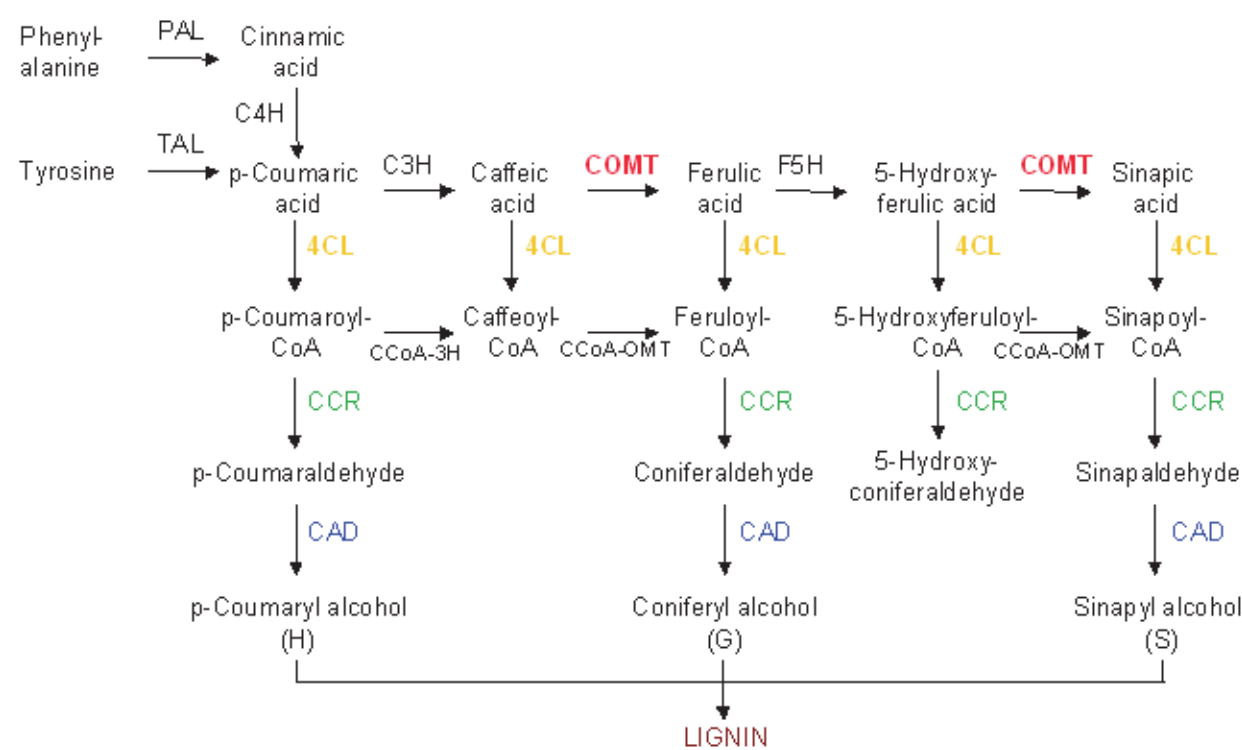

Fig. 5. Lignin biosynthesis pathway. Source: Baldoni (2010).

Lignin synthesis involves various enzymes and knowledge of them is important in studies in which the quality of soybean seeds and the lignin content is related (Baldoni, 2010). The complexity of the lignin biosynthesis pathways is attributed to various multifunctional enzymes, which also correspond to different gene families (Xu et al., 2009).

A considerable quantity of genes is attributed as participant in lignin synthesis, such as genes which regulate the activity of the enzymes phenylalanine ammonia-lyase (PAL), Cinnamate 4-Hydroxylase (C4H), 4-cumarate-CoA ligase (4CL), 4 Hydroxycinnamate 3-Hydroxylase (C3H), 5-Adenosyl-Methionine: Caffeate/5-Hydroxy (OMT), Ferulate-5-Hydroxylase (F5H), Hydroxycinnamoyl COA Reductase (CCR), cinnamyl alcohol dehydrogenase (CAD) (Boudet, 2000; Boudet, 2003; Darley et al., 2001).

Although the exact cause of lignin behavior under stress conditions in RR soybean cultivars is still unknown (Coghlan, 1999), possibly the alterations in the content of this biopolymer in the plant is due to the fact of the precursors of the lignin molecule being formed in the shikimic acid pathway, which is inhibited by the glysophate herbicide in conventional plants. The inhibition of EPSPS enzymes, present in this pathway by the glyphosate, lead to a deficiency in the production of amino acids and consequent death of the plants. That way, the CP4 EPSPS sequence introduced in the genome of the commercial soybean cultivars denominated RR, responsible for the production of the protein CP4 enolpyruvylshikimate-3-phosphate synthase (EPSPS), an enzyme that participates in the biosynthesis of aromatic amino acids in plants and microorganisms, may present the pleiotropic effect, thus modifying the lignin content in the plant.

In spite of all those studies suggesting the pleiotropic effect of the transgene under high stress conditions in laboratory tests in the USA, some authors suggest that it might not be detected until specific environmental conditions are observed, which usually does not occur in field conditions. In this sense, the quantification of lignin in the plant, and consequently in pods and the seed coat of soybeans, become necessary in field conditions, principally with a view toward comparisons between conventional materials and their RR versions, which are essentially derivatives, since the previous reports refer to diverse genotypes, thus not isolating the effect of the inserted transgene. It is worth highlighting that scientific studies that truly prove the pleiotropic effect of the RR transgene under any characteristics are rare in the literature, with most of them being based only on observations and not on scientific results.

Therefore, we will further discuss some results of research obtained in Brazil in which the relation lignin versus RR and conventional soybean cultivars under diverse aspects was evaluated, emphasizing contents of this polymer in the plant, pod and seed coat.

3. Conventional and RR genetically modified soybeans: Some results in Brazil

3.1 Physiological quality and lignin content in the seed coats submitted to different harvest times

The viability period of the soybean seed is extremely variable, depending both on genetic characteristics and environmental effects during the phases of development, harvest, processing and storage. Once unfavorable conditions occur in some of these phases, physiological damages may result in losses to seed quality, with the intensity of these damages varying with the genetic factors intrinsic to each cultivar. Various researchers have emphasized the possibility of use of the seed with seed coat with a certain degree of

impermeability to water as an alternative for avoiding loss of quality in the field (Gilioli & França Neto, 1982; Peske & Pereira, 1983; Hartwig & Potts, 1987), with delay in harvest and determination of the lignin content in the seed coat being methodologies suggested for genetic breeding programs for evaluation of the quality of soybean seeds (França Neto & Krzyzanowski, 2003).

Within this context, the work presented below (Gris et al., 2010) was conducted with the purpose of evaluating the physiological quality and lignin content in the seed coat of the conventional and RR soybean seeds collected at three different times in Lavras (MG), Brazil. Thus, the seeds of ten cultivars collected at stages R7, R8 and 20 days of harvest delay (R8+20) were submitted to tests for evaluation of physiological quality and lignin content. Harvest stages were determined according to Fehr & Caviness (1977).

We observed differences in the physiological quality of seeds among the different harvest times for the cultivars BRS 134, BRS 247 RR, Conquista, Jataí and Silvânia RR, with reduction in viability with harvest delay (R8 + 20). In a similar way, when submitted to accelerated aging, the seeds of the cultivars BRS 245 RR, BRS 134, BRS Jataí and Silvânia RR also underwent a reduction in vigor with harvest delay (Table 1). Braccini et al. (2003), studying the response of 15 genotypes of soybeans to harvest delay, also observed a significant reduction in germination percentage and vigor of seeds when they were submitted to harvest 30 days after the R8 stage of development.

Cultivars	Germination			Accelerated Aging			Electrical Conductivity		
	R7	R8	R8 + 20	R7	R8	R8 + 20	R7	R8	R8 + 20
Celeste	94.75a	96.50a	95.50a	94.75a	97.50a	91.50a	77.01a	82.42a	94.76a
Baliza RR	94.25a	93.00a	91.00a	91.50a	88.50a	84.00a	83.47b	90.86b	118.01a
BRS 133	91.25a	93.00a	88.00a	91.25a	96.50a	87.50a	93.66b	82.61b	107.15a
BRS 245 RR	91.75a	96.50a	90.50a	97.75a	99.50a	87.50b	94.79a	99.11a	97.37a
BRS 134	91.75a	90.50a	79.00b	94.00a	95.00a	75.50b	86.81a	87.02a	97.65a
BRS 247 RR	96.50a	98.50a	87.50b	94.00a	96.00a	87.00a	76.38a	85.18b	102.44b
Conquista	85.75a	90.00a	78.00b	89.75a	88.50a	84.00a	93.87b	85.23b	118.25a
Valiosa RR	89.75a	83.00a	84.50a	87.50a	92.00a	87.50a	98.15b	90.01b	112.56a
Jataí	91.50a	89.00a	76.50b	93.25a	87.00a	64.00b	83.42b	88.43b	152.70a
Silvânia RR	93.00a	91.50a	82.00b	92.50a	92.00a	71.00b	92.92b	89.61b	143.74a

Means followed by the same letter in the line for each determination do not differ among themselves by the Scott-Knott test at the 5% significance level.

Table 1. Means of the germination and accelerated aging test (% of normal seedlings) from seeds of soybean cultivars and their respective RR genetically modified forms, 2007/08 harvest. UFLA, Lavras, MG, Brazil.

We observed that the greatest decreases in seed vigor by the accelerated aging test (Table 1), when the harvest delay and the mean of stages R7 and R8 are contrasted, occurred for the

cultivars Jataí and Silvânia RR, which presented, on average, losses in vigor of 40.82% and 29.93% respectively, indicating that not always cultivars that have high seed quality when collected near physiological maturity have greater tolerance to deterioration with delay of harvest. And, moreover, the greatest values of electrical conductivity were observed for the majority of seeds of the cultivars collected 20 days after the R8 stage, with exception of the cultivar BRS 247 RR, in which reduction in seed vigor was observed as of the R8 stage, and of the cultivars Celeste, BRS 245 RR and BRS 134 that did not undergo any alterations with the time of harvest.

As degradation of the cellular membranes is constituted hypothetically in the first event of the deterioration process (Delouche & Baskin, 1973), tests that evaluate membrane integrity, such as the electrical conductivity test, would theoretically be the most sensitive for estimating seed vigor, which is in agreement with the results obtained in this study, in which said test stood out in detecting differences of viability between the harvest times in seven of the ten cultivars evaluated. We emphasize that the electrical conductivity values observed in this study were situated from 77.01 μS cm^{-1} g^{-1} to 98.15 μS cm^{-1} g^{-1} for the R7 harvest time, 82.42 $\mu S.cm^{-1}.g^{-1}$ to 99.11 $\mu S.cm^{-1}.g^{-1}$ for the R8 harvest time and 94.76 $\mu S.cm^{-1}.g^{-1}$ and 152.70 $\mu S.cm^{-1}.g^{-1}$ for the 20 days after R8, values which demonstrate the growing trend of leachates released by the seeds with delay in harvest.

When we analyze the percentage of mechanical damage in seeds (Table 2), we observe the greatest values with delay of harvest for the cultivars Conquista (12.5%), Jataí (16.0%) and Silvânia RR (15.0%), which was not observed for the other cultivars studied. In addition, we also observed that by the germination test of seeds submitted to the water immersion test, three of the ten cultivars evaluated were differentiated in regard to the percentage of normal seedlings, however, with distinct responses. The lowest germination values when collected in R8 were observed in seeds of the cultivar BRS 245 RR; in those of the cultivar BRS 247 RR there was a reduction in germination when collected in R8 and R8 + 20; and finally in those of the cultivar Silvânia RR the lowest germinative power was verified when collected in R7 and R8. Various authors emphasize that soybean cultivars and lines behave differently in regard to degree of tolerance to delay of harvest (Lin & Severo, 1982; Rocha, 1982; Boldt, 1984), indicating that this trait may influence maintenance of the physiological quality of the seeds.

For the lignin content in the soybean seed coat, we can observe greater lignin content in the seed coat of seeds collected in the R7 and R8 + 20 stages, as well as for the cultivar Silvânia RR, when contrasted with its conventional version Jataí (Table 3).

When we observe the data of percentage of deformed abnormal seedlings, characterized by root curling, typical of damage by rapid imbibition, we observe a smaller number of abnormal seedlings due to the greater number of dead seeds with harvest delay. Giurizatto et al. (2003) affirm that the deteriorated seeds imbibe more rapidly and are therefore more prone to greater damage through imbibition, which is in agreement with the results obtained in this study.

According to Alpert & Oliver (2002) the cellular membranes have two main states, one more fluid or "crystalline liquid" and another less fluid or "gel", remaining, when organized, in the crystalline phase. In a dry seed, the membranes are found in the gel phase and therefore do not constitute an efficient barrier to contain the release of solutes. When the seeds are exposed to rapid imbibitions, the water penetrates before the membrane can be reverted to the crystalline liquid phase, with damage occurring to the cells; thus, the transition between these two phases in the configuration of the membrane constitutes the fundamental cause of possible injuries during imbibition of seeds, which makes the study of the role of lignin in the seed coat even more important.

Cultivars	Mechanical Damage			Germination after Immersion Normal seedlings			Germination after Immersion Abnormal curled seedlings		
	R7	R8	R8 + 20	R7	R8	R8 + 20	R7	R8	R8 + 20
Celeste	3.50a	2.50a	3.00a	62.50a	70.50a	62.00a	20.00a	14.50a	11.00a
Baliza RR	3.00a	3.00a	6.00a	50.00a	46.50a	44.50a	17.50a	24.00a	12.00a
BRS 133	3.00a	1.00a	2.00a	55.00a	49.50a	43.50a	14.50b	26.00a	17.00b
BRS 245 RR	2.50a	2.50a	5.00a	46.00a	22.50b	43.50a	19.00a	17.00b	32.00b
BRS 134	1.50a	1.50a	1.00a	51.00a	47.50a	36.00a	26.00a	26.50a	23.00a
BRS 247 RR	1.50a	1.00a	3.50a	63.00a	50.50b	41.00b	15.50b	32.00a	23.00b
Conquista	6.00b	4.50b	12.50a	38.00a	33.00a	35.00a	10.50a	6.00a	1.00a
Valiosa RR	5.50a	4.50a	5.50a	35.50a	25.50a	36.00a	9.00a	4.50a	3.50a
Jataí	2.50b	3.50b	16.00a	20.50a	29.50a	26.00a	32.00a	41.50a	2.50b
Silvânia RR	4.50b	5.00b	15.00a	21.50b	28.50b	40.50a	30.00a	26.00a	1.50b

Means followed by the same letter in the line for each determination do not differ among themselves by the Scott-Knott test at the 5% significance level.

Table 2. Means obtained for mechanical damage (%) and germination after water immersion (% of normal seedlings and abnormal curled) of soybean cultivar seeds and their genetically modified RR forms, 2007/08 harvest. UFLA, Lavras, MG, Brazil.

These differences observed for the lignin content among the harvest times are not biologically explainable, having possibly been detected due to the low coefficient of variation (CV) obtained for this variable. When we analyze the sole significant contrast, for its part, the genetically modified cultivar Silvânia RR presented greater lignin content in the seed coat than its respective conventional cultivar Jataí. Nevertheless, as an isolated fact, among the five RR combinations versus the conventional versions tested, in our view it does not justify a greater inference regarding pleiotropy of the RR transgene.

Lignin Content				
Harvest Stages			Cultivars	
R7	R8	R8 + 20	Jataí	Silvânia RR
0.2685a	0.2385b	0.2615a	0.3008b	0.4167a

Means followed by the same letter in the column do not differ among themselves by the Scott-Knott test at the 5% significance level.

Table 3. Means with a significant difference obtained for lignin content in the soybean seed coat (%), 2007/08 harvest, UFLA, Lavras, MG, Brazil.

In general, we can conclude that in spite of there being behavioral differences in regard to tolerance to harvest delay among the different cultivars evaluated, we did not observe consistent results in regard to a comparison of the RR versus conventional cultivar, not indicating, for the conditions of this test, any sign of pleiotropy.

3.2 Physiological quality and lignin content in the plants submitted to spraying with glyphosate

Glyphosate (N-phosphonomethyl glycine) is one of the most used herbicides in weed control throughout the world, making up nearly 12% of global herbicide sales and presenting more than 150 commercial brands (Kruse et al., 2000). The emergence of RR genetically modified soybeans increased the use of this molecule in soybeans crops in a considerable way and, along with this, also the environmental concern due to exclusive and indiscriminate use of this herbicide.

According to Sanino et al. (1999), although pesticides (especially glyphosate) may have a beneficial effect on agricultural productivity, the potential risk of these chemical compounds in the environment must be considered, which makes greater studies regarding the behavior of glyphosate under tropical conditions relevant. Within this context we aimed to evaluate the physiological quality of genetically modified RR soybean seeds and the lignin contents of plants submitted to spraying with glyphosate herbicide (Gris, 2009).

In Tables 4 and 5 we present the mean results for the variables analyzed when the soybean plants were submitted to spraying with glyphosate herbicide and water (greenhouse test) and spraying with glyphosate herbicide or manual weeding (field test) respectively.

Cultivars	Germination		Accelerated Aging		Seed Coat Lignin	
	Water	Herbicide	Water	Herbicide	Water	Herbicide
Valiosa RR	91.0a	89.50a	98.75a	97.50a	0.33a	0.26a
BRS 245 RR	94.25a	93.75a	98.50a	96.75a	0.21a	0.22a

Means followed by the same letter in the line for each determination do not differ among themselves by the Scott-Knott test at the 5% significance level.

Table 4. Means of germination and Accelerated aging (% of normal seedlings) and lignin content in the seed coat (%) of genetically modified RR soybean seeds submitted to spraying with water and glyphosate herbicide, 2007/08 harvest, Lavras, MG, Brazil, greenhouse test.

We observe that application of the glyphosate herbicide did not alter the physiological quality of the soybean seeds nor the lignin contents in the seed coat and in the plant for the two tests evaluated. These results are not in agreement with those obtained by Sanino et al. (1999), who studying the effect of application of glyphosate herbicide in soybeans observed, in a general way, reduction in the physiological quality of the RR seeds, as well as considerable reduction in activity of the enzyme α-amylase in terms of time. It is worth emphasizing that such a study was carried out comparing only 2 soybean cultivars, one conventional and one genetically modified RR variety, and that the two did not represent the same genotype, since they originated from different parentages.

In this study (Gris, 2009) we obtained a significant response only for the interaction cultivar versus treatments, when the values of electrical conductivity of the seeds produced in the field test were evaluated (Table 5), in which we observed that seeds of the cultivars Baliza RR and BRS 247 RR had their values reduced and increased respectively when the same spraying was performed. Such a differential response may possibly be explained by the different capacity of the genes inserted in the RR cultivars in expressing tolerance to the glyphosate herbicide, which according to Lacerda & Matallo (2008) may or may not occur in a homogeneous manner among cultivars and even within the same cultivar, as well as other factors inherent to the genetics of each cultivar.

Cultivars	Germination		Accelerated Aging		Mechanical Damage		ESI	
	Weeding	Herbicide	Weeding	Herbicide	Weeding	Herbicide	Weeding	Herbicide
Baliza RR	93.50a	96.50a	88.00a	90.50a	0.75a	0.75a	7.07a	7.14a
BRS 245 RR	89.00a	93.50a	97.00a	95.50a	1.00a	0.00a	7.24a	7.12a
BRS 247 RR	96.50a	97.75a	94.50a	91.50a	0.75a	1.75a	7.19a	7.07a
Silvânia RR	93.00a	93.00a	87.00a	88.00a	3.00a	2.00a	7.44a	7.00a
Valiosa RR	86.00a	89.00a	84.50a	83.50a	2.00a	2.25a	7.42a	7.39a

Cultivars	Electrical Conduct.		Seed Coat Lignin		Pod Lignin		Stem Lignin	
	Weeding	Herbicide	Weeding	Herbicide	Weeding	Herbicide	Weeding	Herbicide
Baliza RR	61.0a	48.0b	0.24a	0.23a	8.66a	8.09a	12.71a	13.61a
BRS 245 RR	69.0a	70.0a	0.19a	0.19a	8.41a	7.81a	12.20a	13.07a
BRS 247 RR	52.0b	70.0a	0.20a	0.20a	9.26a	8.57a	13.43a	12.91a
Silvânia RR	69.0a	69.0a	0.29a	0.30a	9.61a	9.03a	20.23a	18.50a
Valiosa RR	46.0a	40.0a	0.27a	0.30a	7.87a	7.70a	14.62a	13.35a

Means followed by the same letter in the line for each determination do not differ among themselves by the Scott-Knott test at the 5% significance level.

Table 5. Means of germination and Accelerated aging (% of normal seedlings), Mechanical damage (%), Emergence speed index – ESI (days), Electrical conductivity ($\mu S.cm^{-1}.g^{-1}$), Lignin content in the seed coat, pod and stem (%) of genetically modified RR soybean cultivars submitted to manual weeding and spraying with glyphosate herbicide, 2007/08 harvest, Lavras, MG, Brazil, field test.

It is worth emphasizing that since degradation of the cellular membranes is constituted hypothetically in the first event of the deterioration process (Delouche & Baskin, 1973), tests such as electrical conductivity that evaluate membrane integrity are theoretically most sensitive for estimating seed vigor, which possibly, allied with the affirmations of Lacerda & Matallo (2008), would explain the alterations only in the conductivity values.

The absence of a significant response for treatments with weeding and spraying with the glyphosate herbicide indicate that in a general way they did not influence the physiological quality of the seeds, nor the lignin content in the soybean plants. According to Cole & Cerdeira (1982) the blocking of the shikimate pathway due to the action of the glyphosate leads to the accumulation of shikimic acid with many physiological and ecological implications, which, according to Duke & Hoagland (1985) and Becerril et al. (1989), may result in synthesis of indol acetic acid of other plant hormones, chlorophyll synthesis, phytoalexin and lignin synthesis and protein synthesis, and affect photosynthesis, respiration, transpiration, permeability of membranes and other factors.

In addition, other studies have shown that applications of glyphosate in crops interfere in nutrient absorption, increase pests and diseases, reducing crop vigor and yield (Antoniou et al., 2010). According to compilation of data made by these authors, glyphosate reduces nutrient absorption by plants, immobilizing trace elements such as iron and manganese in the soil, as well as avoiding their transport from the roots to the above ground part

(Strautman, 2007). As a result, RR soybean plants treated with glyphosate have lower levels of manganese and other nutrients and reduction in growth of budding and roots (Zobiole et al., 2010). It is worth emphasizing that the seeds produced in the two tests described in this secondary heading are being tested in regard to variation in chemical composition, data which should soon be published.

Both in the field test and in the greenhouse test, it was not possible to relate physiological quality of the seeds and lignin content in their seed coat. We observed significant differences only among the cultivars evaluated, which presented different responses when submitted to the different vigor tests, as well as lignin content, which was already expected, in terms of the great genetic variability among them.

We conclude from these tests that there is a differential response for the electrical conductivity values of the seeds when the plants of different soybean cultivars are submitted to spraying with the glyphosate herbicide; nevertheless, we did not observe a difference in the lignin contents in the stem, in the pod and in the seed coat of the soybean seeds in the cultivars evaluated when submitted to spraying with the glyphosate herbicide.

3.3 Agronomic characteristics and quality of soybean seeds produced at different times

It is known that different planting times, influenced by different environmental conditions, may be determining factors for the development of seed deterioration tolerance mechanisms and therefore for the quality of soybean seeds. Considered as a seed deterioration tolerance mechanism, the impermeability of the seed coat, characterized principally by seeds with greater lignin content, hinders water penetration in the seed coat. In a similar way to alterations in the germination process and in manifestation of vigor, in terms of the climate in the seed production phase, environmental conditions may also in some way affect the metabolism and chemical constitution of the seeds.

As we have already seen in this chapter, according to some authors, overproduction of lignin in RR soybean plants may be associated with the presence of water deficit and high temperatures during cropping, indicating that the environmental conditions found in the field during crop development may affect lignin production in the plant in an expressive way.

With this objective, we compared agronomic traits of the plant, physiological quality and seed health and lignin content in the seed coat of RR and conventional seeds produced in different time periods, summer and winter (Gris, 2009), with the determinations: plant height, height of insertion of the first pod and number of pods per plant, weight of 1000 seeds (Brasil, 1992), lignin content in the seed coat (Capeleti et al., 2005), incidence of mechanical damage (Marcos Filho et al., 1987), germination and dry matter of normal seedling from germination (Brasil, 1992), emergence speed index and germination speed index (Edmond & Drapala, 1958), final stand in the seed bed (counting at 24 days after seeding), accelerated aging at 42°C for 72h (Marcos Filho, 1999), electrical conductivity (Vieira, 1994), water immersion test of seeds and seed health, evaluating the infestation percentage (Machado, 2000) and intensity of the inoculums. The data of inoculum density were weighted by the McKinney formula (1923):

$$II(\%) = \frac{\Sigma(F \quad n) \quad 100}{(N \quad M)}$$

In which: II = inoculum intensity, F = number of seeds with a determined score, n = score observed, N = total number of seeds evaluated and M = maximum score of the scale.

In Table 6, we present a summary of the mean results for the variables in which the contrasts (RR cultivar versus conventional cultivar) presented a significant difference, for both harvests, in which among all the characteristics evaluated, significant results for the contrasts evaluated were few.

For the electrical conductivity test, we observed a greater value for the conventional cultivar Jataí (76.54 μS.cm^{-1}.g^{-1}) when compared to the cultivar Silvânia RR (100.25 μS.cm^{-1}.g^{-1}). According to Vieira & Krzyzanowski (1999) for lots of high vigor soybean seeds, the standard conductivity values should be situated at most up to 70-80 μS.cm^{-1}.g^{-1}, however with a strong trend to present medium vigor. Nevertheless, in spite of the high value of electrical conductivity observed in seeds of the cultivar Silvânia RR, we did not observe differences between the two cultivars in the germination and vigor tests, which, according to José et al. (2004), may indicate that there are cultivars with greater efficiency in membrane reorganization, not resulting in damages, strictly speaking.

Variables	Means – Summer 2006/07 harvest				
Plant height (m)	Jataí	1.56 a	vs	Silvânia RR	1.41 b
Number Pods/plant	Jataí	110.00 a	vs	Silvânia RR	57.50 b
Germination (%)	BRS 133	95.50 a	vs	BRS 245 RR	87.25 b
Weight of 1000 seeds (g)	BRS 134	155.50 a	vs	BRS 247 RR	142.70 b
Emergence Speed Index	BRS 134	7.16 b	vs	BRS 247 RR	7.55 a
Lignin Seed Coat (%)	Celeste	0.20 b	vs	Baliza RR	0.26 a
Variables	Means – Winter 2007 harvest				
Electrical conductivity (μS.cm^{-1}.g^{-1})	Jataí	76.54 b	vs	Silvânia RR	100.25 a

Capital letters followed by the same letter in the line do not differ among themselves by the Scheffe Test, at the 5% significance level.

Table 6. Mean values for some variables in which the contrasts between the conventional soybean cultivar and its genetically modified RR version presented significance, summer and winter harvest, Lavras, MG, Brazil.

Panobianco (1997) upon reporting variation in electrical conductivity of soybean seeds and the lignin content in their seed coat affirms that the genotype may alter the electrical conductivity for seeds with the same standard of physiological quality. Nevertheless, we did not observe significant differences between the cultivars Jataí and Silvânia RR in regard to lignin content in the seed coat, indicating that, in this case, it may not have been responsible for the variation in electrical conductivity observed. In the same way, it was not possible to relate the difference in the lignin contents in the seed coat, observed between the cultivars Celeste (0.20%) and Baliza RR (0.26%), and the results of physiological quality of the two, produced in the summer harvest, since they differed only for this characteristic. It is worth highlighting that in spite of the differences found for these two cultivars, it was not possible through the incidence of mechanical damage to detect any differences between the cultivars studied.

Upon observing the contrasts established between the RR and conventional cultivars, we can infer that the cultivars Jataí and Silvânia RR presented the greatest number of significant differences among the variable studied (Table 6), not only in relation to the physiological quality of the seeds, but also in regard to agronomic traits, such as plant height and number of pods per plant.

When we analyze the mean values of plant height and number of pods per plant, we verify once more that the conventional cultivar Jataí showed superiority to the cultivar Silvânia RR, such that for number of pods/plant, these values were up to 91.3% greater. Nevertheless, it is worth emphasizing that for these two cultivars in field conditions, we observed the greatest variations in regard to the phenological cycle, with greater uniformity in maturation and a shorter cycle, around 10 days, of the conventional cultivar Jataí in relation to the genetically modified RR cultivar. It is fitting to highlight that in spite of the RR cultivars tested in this study being essentially derivatives of the respective conventional cultivars, by means of backcrossings, the genotype of the recurrent genitor is not always recovered, due to number fewer recurrence cycles which may consequently result in variations between both materials. Nevertheless, for these cultivars, there is no information on the number of backcrossing cycles used.

When we evaluate the physiological quality of the seeds by means of the germination test in the summer harvest and of the germination speed index (IGV) in the winter harvest, we do not observe a relationship between the significant results for these variables, with the contrasts BRS 133 versus BRS 245 RR and Conquista versus Valiosa RR being differentiated respectively. For both results, the conventional cultivars showed superiority to the genetically modified RR cultivars, with the conventional cultivar BRS 133, with 95% of normal seedlings, overcoming the cultivar BRS 245 RR, with 87%, by approximately 9.5%, when they were produced in the summer harvest. Nevertheless, by the results in reference to the Emergence Speed Index, we observe a lower value for the genetically modified cultivar BRS 247 RR (7.55 days) in comparison with the conventional cultivar BRS 134 (7.16), which once more shows the inconsistency of data that justify a pleiotropic effect of the RR gene on lignin production.

It is worth emphasizing that in spite of the results found in this study, with exception of the variables Emergence Speed Index and lignin in the seed coat, the RR cultivars stood out in relation to the conventional cultivars; most of the significant contrasts, were seen to be isolated, in only one of the harvests or one of the tests in the midst of various comparisons among physiological quality of the seeds, therefore not indicating substantial differences of quality between the RR and conventional materials.

According to Menezes (2008) the physiological quality of soybean seeds is influenced by the maternal or extra-chromosome effect, just as is the cytoplasmatic inheritance, with the physical characteristics of the seed coat, of maternal origin, not being sole determinants of the physiological quality of the seeds. According to this author, the study of genetic control for seed quality indicates the effect of the general and specific combination capacity, which suggests the presence of additive and non-additive gene effects for physiological quality of soybean seeds. Therefore, the quality of seeds may not be attributed only to their seed coat and consequently to their lignin contents, but also to genes present in the nucleus.

When we analyze the results obtained in the seed health test (Figure 6), we observe that the cultivars BRS 133, BRS 245 RR, BRS 134 and BRS 247 RR presented the lowest percentages of infection and infection indexes (severity), when produced in the summer, indicating that the environmental conditions during the seed maturation period were responsible for seed health quality. In these cultivars a shorter phenological cycle and semi-early maturity was observed, which provided for the maturation period outside of the rainy period.

According to Delouche (1975), the alternating of dry and wet days during the maturation phase until harvest, which occurs with greater facility in the summer, can increase the incidence of diseases in a differentiated way at the end of the cycle of the seeds produced. Within this context, the seed becomes not only an easy target for the action of microorganisms, which considerably reduce its viability, but they also come to be efficient

vehicles for dissemination of pathogens (Machado, 2000). This situation may be visualized principally for the cultivars Jataí and Silvânia RR, which remained for a greater period in the field, and presented the greatest percentages of infection, 39% and 38% (Figure 6A), and also the greatest indexes of infection by the pathogen Phomopsis, 35% and 26% (Figure 6B), respectively. It is worth emphasizing that when produced in winter conditions, under a controlled irrigation system, without rains in the seed maturation period, the presence of pathogens was not observed for any seeds.

A - Seed health

B - Severity in seeds

Fig. 6. Average values for infection percentage (A) and infection indexes - severity (B) in the seed health test of conventional soybeans and the genetically modified RR versions, summer harvest.

In relation to the RR versus conventional contrasts, we observe that in spite of the cultivars tested in this study having their origin in the same genotype by successive backcrossings, when observed in the field, we verified that some presented perceptible cycle variations, maintaining the cultivars Conquista and Celeste for more days in the field in relation to the cultivars Valiosa RR and Baliza RR, respectively; enough so that the first, subjected to rains and high temperatures, presented slightly greater values in the seed health and severity test. In this case, we cannot attribute the differences of RR versus conventional contrast, observed in Figure 6A and 6B, to the effect of the RR transgene, but rather to environmental conditions associated with difference of cycle.

In view of the above, in spite of some authors suggesting the pleiotropic effect of the transgene CP4 EPSPS on lignin overproduction in the plant, it was not possible for us to identify the pleiotropic effect in the cultivars studied in this and in the other studies described here, which indicates that the alterations of lignin content in the plant, observed by those authors under normal climatic conditions, are not due to the fact of the lignin molecule precursors being formed in the shikimic acid pathway.   Thus, the sequence CP4 EPSPS, introduced in the genome of commercial soybean cultivars, responsible for the production of the protein CP4 enolpyruvylshikimate-3-phosphate-synthase (EPSPS), an enzyme that participates in the biosynthesis of aromatic amino acids in plants and microorganisms, seems not to be associated with lignin contents in the plant and in the soybean seed coat, and it seems that there are no substantial differences in regard to the agronomic traits and physiological quality of seeds between conventional and genetically modified RR cultivars.

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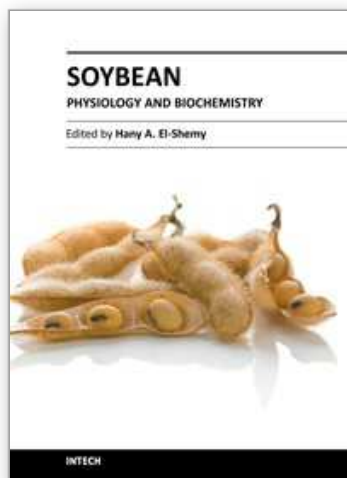Crocker, W. (1948). *Growth of plants.* New York: Reinohold. 459p.

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Soybean Physiology and Biochemistry

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Worldwide, soybean seed proteins represent a major source of amino acids for human and animal nutrition. Soybean seeds are an important and economical source of protein in the diet of many developed and developing countries. Soy is a complete protein and soyfoods are rich in vitamins and minerals.Soybean protein provides all the essential amino acids in the amounts needed for human health. Recent research suggests that soy may also lower risk of prostate, colon and breast cancers as well as osteoporosis and other bone health problems and alleviate hot flashes associated with menopause. This volume is expected to be useful for student, researchers and public who are interested in soybean.

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