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Technical note

Identification of soybean seed coat damage

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Abstract

The objective of this research was to evaluate the tests for detection of mechanical damages in soybeans seeds. Thus, it was compared the use of sodium hypochlorite solution (2.0% and 5.25%), water and tetrazolium solution (0.075%). The study was conducted in soybean seeds, from the cultivars Embrapa 48 and FTS Águia with, respectively, 8.08% and 6.06% of lignin on seed coat, which were harvested mechanically and manually. The seed quality was evaluated by water content, germination and vigor (tetrazolium and electrical conductivity tests). The mechanical damages in soybean seeds, of immediate effect, can be determined by the use of the 2.0% sodium hypochlorite solution.

Key words: *Glycine max* (L.) Merrill, seed integrity, harvest.

Identificação de danos mecânicos em sementes de soja

Resumo

O objetivo dessa pesquisa foi avaliar a eficiência da utilização de testes para determinar os danos mecânicos em sementes de soja. Assim, foi comparada a utilização das soluções 2,0% e 5,25% de hipoclorito de sódio, da água e da solução 0,075% de tetrazólio. A pesquisa foi conduzida com sementes de soja, cultivares Embrapa 48 e FTS Águia que têm, respectivamente, 8,08% e 6,06% de lignina no seed coato, e que foram colhidas manualmente e à máquina. Paralelamente as sementes foram avaliadas pelo teor de água, germinação e pelo vigor (testes de tetrazólio e de condutividade elétrica). Os danos mecânicos de efeitos imediatos nas sementes de soja podem ser determinados pela utilização da solução 2,0% de hipoclorito de sódio.

Palavras-chave: *Glycine max* (L.) Merrill, integridade da semente, colheita.

Identificación de daños mecánicos en semillas de soja

Resumen

El objetivo de esta investigación fue evaluar la eficacia de la utilización de test para determinar el daño mecánico en las semillas de soja. Así, se comparó el uso de la solución de 2,0% y 5,25% de hipoclorito de sodio, agua y solución de tetrazolio 0,075%. La investigación se llevó a cabo con semillas de soja, cultivar Embrapa 48 y FTS Águila que tienen, respectivamente, 8,08% y 6,06% de lignina en seed coato y que fueron cosechadas a mano y máquina. En paralelo las semillas fueran evaluadas por el teor de agua, germinación y vigor (tests de tetrazolio y conductividad eléctrica). Se puede determinar los daños mecánicos de efectos inmediatos de las semillas de soja mediante el uso de solución de 2,0% de hipoclorito de sodio.

Palabras clave: *Glycine max* (L.) Merrill, la integridad de las semillas, cosecha.

Introduction

The mechanical harvest and the processing consist in one of the main sources of mechanical damages in soybean seeds (MARCONDES et al., 2005; OBANDO FLOR et al., 2004). According with FRANÇA NETO and HENNING (1984) and PAIVA et al. (2000) during the harvest phase, the seed gets particularly susceptible to mechanical damage, of immediate or later effect. Since that the vital parts of the embryonic axis are situated under not thick seed coat, which practically does not provide protection. The same can occur for the seeds when they are at the line of processing, mainly in function of the continuous moving and of the susceptible falls in the seeds mass, factors connected to this operation (OLIVEIRA et al., 1999).

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Some tests are indicated to assess the mechanical damage in soybean seeds and are used in programs of quality control during the harvest, transport and processing phases. The tetrazolium test described by FRANÇA NETO et al. (1998), is widely used in the quality control by seed producer companies, because it evaluates besides the mechanical damages, the vigor, viability, damages by moisture and by stinkbugs.

Another simple and easy test is the sodium hypochlorite, described by COPELAND and McDONALD (1995) and recommended for soybean seeds by KRZYZANOWSKI et al. (2004). In this test, the seeds are immerse by a period of ten minutes in a solution of sodium hypochlorite at the concentration of 5.25%, which enables to determine the percentage of damaged seeds. This test can also be done using water.

Considering that the assessment of physical damages is essential for the preservation of soybean seeds quality and that there are no studies related to the improvement of available tests, this research was conducted with the objective of comparing the efficiency of the methods provided to determine the mechanical damages in soybean seeds, and verify the possibility of use of the sodium hypochlorite 2.0% solution, aiming to reduce the concentration of this product.

Material and Methods

The research was conducted in the laboratory of Seed Analyzes of the Department of Vegetal Production, in the University of São Paulo, Luiz de Queiroz Superior School of Agriculture (USP/ESALQ), in Piracicaba, São Paulo.

It were used two cultivars of soybean, FTS Águia and Emprapa 48, which were evaluated, regarding the contents of lignin of the seed coat, by the method indicated by ALVAREZ et al. (1997). This procedure allowed to classify the seeds of the cultivar FTS Águia with 8.08% of lignin, as the most resistant to mechanical damage; and for the seeds of the cultivar Embrapa 48, with 6.96% of lignin, as the most sensitive to mechanical damage.

For the experiment in the field were installed six plots for each cultivar, three were plots destined to mechanical harvest and the other three to manual harvest. The preparation of soil, fertilization, irrigation and phytosanitary control and of invasive plants were done according the recommendation for the soybean crops indicated by the EMBRAPA (2006).

For the mechanical harvest was employed the harvester for experimental area, brand Hege®, Model 140, with the rotation of the cylinder regulated in 510 rpm and speed of machine of approximately 4 kilometers hour^{-1} (EMBRAPA, 2006). In the manual harvest phase, the plants were cut and then stored in raffia sacks for the transport and packaging, until the moment of the manual trail, for the obtainment of seeds

Soon after, was determined the degree of seeds moisture (BRASIL, 2009), then the seeds were dried at 29 °C, until they achieve 11.0% of water. Next, the seeds were packed in paper bags and stored at 14° C and 45% of relative humidity of the air until the moment of evaluation.

For the physical and physiological assessments of the seeds from each cultivar were used two or four repetitions, depending on the test, for each field plot, according to the described:

1) Determination of the moisture degree: done by the method of greenhouse at 105±3 °C, for 24 hours, using two sub samples of 5.0g of seeds for each repetition coming of the field (BRASIL, 2009). The results were expressed in percentage for each of water, humid basis.

2) Germination Test: 12 repetitions of 50 seeds were distributed in paper, brand Germitest, wet with quantity of water equivalent to 2.5 times the mass of the dry substrate and kept in a germinator at 25° C. The assessments were performed at five and eight days after sowing (BRASIL, 2009). The results were expressed in percentage of normal plants

3) Electric conductivity: it were assessed 12 repetitions of 50 seeds per treatment; weight with precision of 0.01g and placed to soak in plastic cups (capacity of 200 mL) having 75 mL of deionized water, during 24 hours, at 25 °C (VIEIRA and KRZYZANOWSKI, 1999). Next, was determined the electrical conductivity in a conductivimeter of the brand Digimed®, CD-21, being the results expressed by the average µS cm^{-1} g^{-1} of seed.

4) Tetrazolium test (TZ): 12 repetitions of 50 seeds were hydrated in paper, brand Germitest, wet with quantity of water equivalent to 2.5 time its weight, during 16 hours, at 25 °C. In the next step, the seeds were placed in plastic cups and totally submersed in the solution 0.075% of chloride of 2,3,5-triphenyl chloride of tetrazolium. The seeds were placed in a greenhouse, in the dark, at a temperature of 40 °C, per 150 minutes. Past this period, the seeds were washed in running water and analyzed one by one,

registering the number of seeds potentially germinable (viability), as those included in the class of seeds of 1 to 5; as potentially vigorous (vigor), those included in the class of seeds of 1 to 3; and as non viable (caused by mechanical damage), those included in the class of seeds of 6 to 8 (FRANÇA NETO et al., 1998). The results were expressed in average percentage of viable seeds, vigorous seeds and dead seeds due to the mechanical damages, for each treatment.

5) Test of sodium hypochlorite: for this were used 12 repetitions of 50 seeds immerse in a solution of 5.25% of sodium hypochlorite (KRZYZANOWSKI et al. 2004), and 2.0% of sodium hypochlorite, during 10 minutes. Following, the seeds were retrieved of the solution and each repetition was distributed on sheets of paper for the counting of the number of swollen seeds (damaged). The results were expressed in average percentage of swollen seeds.

6) Evaluation of the mechanical damage with water: we used 12 repetitions of 50 seeds, placed in a petri dish and covered with water during five minutes. Then, the seeds were distributed over sheets of paper for the counting of the number of swollen seeds (damaged). The results were expressed in average percentage of swollen seeds.

The data obtained in the germination tests, of tetrazolium (viability and vigor) and electric conductivity was submitted to the variance analysis, considering the completely randomized design. The results of evaluation of mechanical damage by tests of sodium hypochlorite, concentrations of 2.0% and 5.25%, with water and by tetrazolium were analyzed in factorial scheme (2 manners of harvest X 4 methods for the determination of mechanical damages). The data was not transformed for the analysis of variance, the software used the ASSISTAT, version 7.5 beta (SILVA, 2008). The averages were compared by the Tukey test (5%).

Results and Discussion

At the moment of harvest, the seeds of the cultivar Embrapa 48, manually harvested had 18.0% of water and the mechanically harvested had 20.0%, and for the cultivar FTS Águia the seeds manually harvested had 17.0% of water and the mechanically harvested had 18.0%. The degree of humidity of the seeds from both cultivars in the study presented similar values, which indicated that there was no interference of this parameter in the results of analysis.

By the values of germination (Table 1) for seeds from both cultivars harvested mechanically, there was a significant reduction in the quantity of normal seedlings. With regard to the vigor, (Table 1), was significant the reduction of quality of the seeds from the cultivar Embrapa 48, harvested mechanically, in function of the results obtained from the tests of tetrazolium and electric conductivity, now for the seeds from the FTS Águia the reduction of quality was only verified by the tests of tetrazolium. These results are probably associated to the greater tolerance to mechanical damages of seeds from the cultivar FTS Águia, due to the greater content of lignin in the seed coat of seed, which confers the seeds at the moment of the mechanical harvest or in the benefiting, lesser sensibility to physical injuries (CABALLERO AGUERO, 1994).

Table 1. Germination tests, viability (TZ), vigor (TZ) and of electric conductivity in soybean seeds, of the Emprapa 48 and FTS Águia cultivars, manually or mechanical harvested.

Harvest	Germination	Viability (TZ)	Vigor (TZ)	Electric Conductivity
Embrapa 48	----------%----------			---- µS cm^{-1} g^{-1} ----
Manually	76 a	98 a	91 a	101.67 b
Mechanically	50 b	85 b	74 b	124,.7 a
CV (%)	9.8	1.3	4.0	4.7
FTS Águia	----------%----------			---- µS cm^{-1} g^{-1} ----
Manually	92 a	97 a	91 a	86.38 a
Mechanically	83 b	93 a	76 a	97.53 a
CV (%)	8.1	3.3	5.0	7.8

Averages followed by the same letter in the column do not differ between themselves by Tukey test $p<0.05$.

The results of the electric conductivity test (Table 1) for the seeds of the cultivar FTS Águia, which has more lignin in the seed coat, did not present significant differences in function of the methods of harvest. PANOBIANCO et al. (1999) verified that in seed coat which has more lignin, there is lesser exchange of solutes between the seed and the external environment, thus, there is interference in the quantity of leachates released. For the seeds of the cultivar Embrapa 48, the quantity of leachates released by the seeds, was influenced by the type of harvest, since there was a greater liberation of exudates by the seeds mechanically harvest, which interfered with the integrity of the cell membranes of these seeds. Therefore, membranes poorly structured and damaged cells are, generally, associated with the deterioration process of the seed and, therefore, to the seed vigor (HESLEHURST, 1988).

In this research the soybean seeds of the cultivar Embrapa 48 were considered lesser resistant to the mechanical damages in relation to the cultivar FTS Águia, this classification was based on the 5% content of lignin related by FRANÇA NETO et al. (2007).

The results of the determinations of mechanical damages of the seeds from the cultivar Embrapa 48 and FTS Águia (Table 2) assessed by the sodium hypochlorite solutions, concentrations of 2.0% and 5.25%, by water and tetrazolium salt, indicated that the seeds harvested mechanically had more mechanical damages. According to ANDRADE et. al. (1999), the mechanical harvest is one of the most important sources of physical damages to the seeds, specially at the moment of the mechanical trail, i. e. in the moment in which considerable forces are applied in the seed, in order to separate than from the structure which contains it.

The seeds of the cultivar Embrapa 48 present more mechanical damage than the seeds of the cultivar FTS Águia, independently of the test used to assess physical damage (Table 2), confirming the relation between the lignin content of the seed coat and the resistance to mechanical damage, as stated FRANÇA NETO et al. (1998) and SANTOS et al. (2007).

As for the methods used for the quantification of mechanical damages in the soybean seeds, Table 2, the sodium hypochlorite enabled to characterize the seeds harvested by machine, for both cultivars, as those with the highest percentages of mechanical injuries. KRZYZANOWSKI et al. (2004), had already considered the efficiency of the use of the solution at 5.25% of sodium hypochlorite for the determination of ruptures in the seed coat, in a quick manner, being possible to be performed successive times during the stages of control of quality of the seeds, even in the field.

On the other hand, the results of this study, Table 2, indicated that the solution of sodium hypochlorite at 2.0%, can be used in the replacement to the concentration at 5.25%, due to the statistical similarities between the results of the cultivar Embrapa 48, with the use of hypochlorite at 5.25% and water, even considering the statistical superiority of the test with tetrazolium. For the seeds of the cultivar FTS Águia, the results obtained with the solution at 2.0% of sodium hypochlorite indicated similar efficiency to the one with the tetrazolium test, for the determination of damage in the seed coat, and superior to the one determined by the 5.25% sodium hypochlorite solution and with the use of water. This

Table 2. Mechanical damage in soybean seeds, of the Embrapa 48 and FTS Águia cultivars, harvested manually and mechanically, by the tests of sodium hypochlorite (concentrations of 2.0% and 5.25%), with water and the tetrazolium solution (seeds classes from 6 to 8).

Harvest	Sodium Hypochlorite		Mechanical damage	Tetrazolium
	[2.0%]	[5.25%]	with water	(6 a 8)
Embrapa 48	---------- % of seeds with mechanical damages ----------			
Manually	3 bA	3 bA	2 bB	3 bA
Mechanically	6 aB	6 aB	6 aB	12 aA
CV (%)	11.7			
FTS Águia	---------- % of seeds with mechanical damages ----------			
Manually	1 bA	1 bA	1 bA	1 bA
Mechanically	4 aA	2 aC	3 aB	4 aA
CV (%)	24.5			

Averages followed by the same letter in the column do not differ between themselves by Tukey test p<0.05.

results favors the indication of the use of commercial sodium hypochlorite, which generally, is sold with 2.0% of concentration.

Considering the seeds of the cultivar which has more lignin in the seed coat (cultivar FTS Águia) and the mechanical harvest, factor which favors the physical damage in the soybean seeds, is possible to state that the use of the 2.0% sodium hypochlorite solution has similar efficiency to the tetrazolium solution to assess physical damages of immediate effect in soybean seeds.

The use of the tetrazolium test to assess the mechanical damages in soybean seeds enables the diagnosis of possible causes of physiological quality reduction of these seeds, as the mechanical damages of immediate and latent effects (FRANÇA NETO et al., 1998). Even though the sodium hypochlorite tests at 2.0% and at 5.25% and with water determine only the damage of immediate effect, they are tests which can be used in field, especially during harvest, enabling the possibility to keep control of the seed quality.

By the analysis of the results of the seeds from the cultivar Embrapa 48 was possible to verify that the tetrazolium test was to most efficient for the determination of the mechanical damages of seeds mechanically harvested. Besides, the intensity of damages determined in these seeds passes to limit suggested by KRZYZANOWSKI et al. (2004), more than 10%, which characterized the damage as excessive of these seeds.

When the analysis was carried out on seeds more resistant to mechanical damage (FTS Águia), the quantity of physical damages determined by the tetrazolium test, Table 2, was similar to the test with 2.0% of sodium hypochlorite. Such fact becomes relevant in the choice of the alternative method (sodium hypochlorite 2.0%), to quantify the mechanical damage of immediate effect in soybean seeds.

As states PAIVA et al. (2000), the mechanical damages determined by the sodium hypochlorite test are classified as of immediate effect. Because, the damages of latent effect injure the internal tissues of the seed, and are of hard detect by these methods. Fact this, that justifies the superior quantity of mechanical damage of seeds of the cultivar Embrapa 48 (lesser resistant to mechanical damage) when analyzed by the tetrazolium test (Table 2).

However, is essential to highlight that in function of the quick obtaining of results (less than fifteen minutes) and ease of execution, the sodium hypochlorite test with solution of 2.0% has similar or superior efficiency to the test with 5.25% with advantages regarding concentration reductions of the used product, of cost and risk to the user and to the environment.

Conclusions

The mechanical damage in soybean seeds, of immediate effect can be determined by the use of the solution at 2.0% of sodium hypochlorite.

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