THE RELATIONSHIP BETWEEN SEED COAT LIGNIN CONTENT AND SEED QUALITY OF SOYBEANS DURING STORAGE

HUBUNGAN ANTARA KANDUNGAN LIGNIN KULIT BENIH DENGAN MUTU BENIH SELAMA PENYIMPANAN

Marwanto, Marlin, dan Mulya Marlinda
Program Studi Agronomi, Fakultas Pertanian, Universitas Bengkulu

ABSTRACT

Lignin content of the soybean seed coat may have an effect on reducing seed deterioration during storage. A study was undertaken to evaluate genotypic differences in storage deterioration of soybeans and to relate seed coat lignin content with seed quality of soybeans during storage. Ten soybean genotypes with different seed coat lignin content were grown in research plots at Agriculture Faculty, Bengkulu University on November 2000. Seeds were hand harvested at R8 maturation and stored for 4 months at a room temperature and 75% RH. At the end of storage period each genotype was subjected to seed quality evaluation. There were substansial differences among the genotypes in the rate of seed deterioration during storage. Seed of B-3468 exhibited the highest storage potential. The seed coat lignin content was significantly and negatively correlated with membrane deterioration associated with a decline in soybean seed quality after storage.

Key words: soybean, lignin, seed coat, storability

ABSTRAK

Keberadaan lignin dalam kulit benih mungkin dapat mengurangi kemunduran mutu benih kedelai selama di penyimpanan. Tujuan penelitian ini adalah mengevaluasi keragaman daya simpan benih kedelai dan menghubungkan keberadaan lignin dalam kulit benih kedelai dengan daya simpannya. Sepuluh genotipe kedelai ditanam di petak percobaan Fakultas Pertanian Universitas Bengkulu pada bulan November 2000 dalam rancangan acak kelompok lengkap dengan tiga ulangan. Benih dipanen pada fase R8 dan disimpan selama 4 bulan di ruang penyimpanan bersuhu kamar dan berkelembaban 75%. Pada akhir periode simpan, setiap genotipe dievaluasi mutu benihnya. Setiap genotipe mempunyai daya simpan yang berbeda. Galur B-3468 mempunyai daya simpan terbaik. Kandungan lignin kulit benih memiliki hubungan yang bersifat nyata dan negatif dengan daya hantar listrik sebagai indikator mutu benih.

Kata kunci: kedelai, lignin, kulit benih, daya simpan

INTRODUCTION

A humid tropical climate characterized by high temperature and high relatif humidity is conducive to rapid deterioration of soybean [*Glycine max* (L.) Merrill] seed in storage. During storage its vigor, as well as viability, can deteriorate rapidly, making it impossible for farmers to use their own seed for planting in the next season due to its low quality. Chuntirapongsa (1992) further stated that storage at ambient storage and 75% RH for 4 month period were able to reveale soybean genotypic differences for resistance to seed deterioration during storage. Several studies on resistance to seed deterioration indicate that the seed coat plays an important role on resistance of seeds to deterioration (Dassou and Kueneman, 1984; Miranda *et al*., 1981; Nugraha, 1987) and its 'relative' impermeability to water absorption is the main factor involved (Kuo, 1989).

Lignin is a chemical compound found in the seed coat tissue since it is a constituent of cell walls. Its occurrence affects the rate of water absorption throughout the seed coat

(McDougal *et al*., 1996) and the impermeability of soybean seeds of certain genotypes as well (Tavares *et al*, 1987;). Its content in the soybean seed coat varies among genotypes (Alvares *et al*., 1997; Panobianco *et al*., 1999). Since lignin determines the rate of water absorption throughout the seed coat, its occurrence may have an effect on soybean seed deterioration during storage. However, few studies have considered the relationship between seed coat lignin content and resistant to soybean seed deterioration during storage.

The objectives of the study were (1) to evaluate genotypic differences in storage deterioration of soybeans, and (2) to relate seed coat lignin content with seed quality of soybeans during storage

METODOLOGY

Seeds of ten genotypes of soybeans consisting of 7 cultivars and 3 lines were used in these studies. The names of the lines and cultivars and their seed characteristics are given in Table 1. The seeds were produced specifically for the studies by growing them in research plots at Agriculture Faculty, Bengkulu University on November 2000 in a randomized complete block with three replications. Each genotype was planted in a single row 20m long with 1m between rows and 20cm between plants within a row.

Seeds were harvested at harvest maturity (Fehr and Caviness, 1977) to establish a non-weathered category for the storage studies. As seed moisture content dropped below about 20%, the seeds were harvested by hand stripping of the pods. The pods were then dried with heated air (<35 ^{0}C) to reduce moisture content to 10-12% for threshing. The dried pods contained in jute bags were threshed by flailing and the seeds were separated from the pod walls and another plant parts by sieving. Sieving (round hole) was used to eliminate the small, immature and insect damaged seeds. Selected soybean soybean seed samples were then stored for a period of 4 months for storability study. Separate soybean seed samples were also drawn to evaluate seed coat lignin content and permeability.

The soybean seed storability of the 10 genotypes were compared under the storage conditions of about room temperature and 75% RH. Before storage, seed moisture content of each genotype was adjusted to about 10%. The seeds with similar initial physiological quality (>90%), as evaluated by the standard germination test were stored in a wooden humidity chamber of about 0.6 m3 capacity with a saturated sodium chloride solution in the bottom well to maintain 75% RH. The storage period was 4 month. Seeds of each genotype were contained in plastic mesh pouches and placed in the humidity chamber. The humidity chamber was positioned in a closed room.

At the end of storage period, seed quality of each genotype was evaluated by the following tests to determine storability of soybean seeds. The tests were seed moisture, eletrolyte conductivity, and standard germination and performed on seed from each genotype in each replication.

Seed moisture content was determined on seed fraction of the soybean sample immediately after 4 months period of storage. Samples of about 20 g in duplicate were placed in an oven at 105 ^{0}C for 24 hours to obtain dry weight and determine the amount of moisture lost. Seed moisture content was calculated on a wet weight basis and expressed in %.

To determine seed viability, fifty seeds were placed on mosit paper towels, which were rolled and placed inside plastic bags and kept at a room temperature. Germinated seeds were counted after 5 and 8 days. Dead seeds were removed after 5 days, while hard seeds after 8 days and counted with germinated seeds. The number of germinated seeds was expressed as a percentage of the total.

To determine seed electrical conductivity, for each genotype one set of two replicates of 25 seeds each were randomly drawn from seed fraction of the soybean sample. Seeds were assayed for electrical conductivity after being placed in a 200 mL - beaker glass with 100 mL of deionized water, at a room temperature for 24 hours. Conductivity values were determined with a Digital conductivity meter (Cole Palmer, Chicago-Illinois). The results were expressed in mmhos cm^{-2} g^{-1}.

The seed coat lignin content was determined using 1.0 g of seed coat tissue for each genotype by the sulphuric oxidation method (Van Soest and Wine, 1968).

To determine permeability of seed, for each genotype one set of two replicates of 10 g of seed were randomly drawn from seed fraction of the soybean sample. Initial seed moisture content of each genotype was adjusted to about 10%. Permeability of seed was determined following 2 hours of summersion in deionized water and expressed in g g^{-1} h^{-1}.

Analysis of variance of each variable was conducted as a complete random design.The means were separated by Duncan Multiple Range Test at the 0.05 level of probability. Regression analysis between percent lignin content of the seed coat of the tested genotypes and seed coat permeability, germination, and electrical conductivity was determined.

RESULTS AND DISCUSSION

Seed quality for the ten genotypes after 4 month storage at a room temperature of about 29 ^{0}C and 75% RH are shown in Table 2. Eventhough all genotypes deteriorated severely, a significant difference in the rate of deterioration of seeds of the ten genotypes under the rather adverse storage conditions as indicated by germination and electrolyte conductivity was still observed. One genotype, B-3468, consistently maintained higher seed viability as indicated by germination score than the others and might be classified as a good “storer”. While viability of seeds of B-3618, B-3770, Merapi, L-Batang, Meratus, L-Bewok, and Malabar had decreased to 40-55%, which might be classed as poor “storer”. Chuntirapongsa (1992) and Ferguson *et al.* (1990) reported the same results when working with different genotypes of soybeans. For breeding purposes, further studies are needed to determine the heritability of seed storability. If the studies indicate a high level of probability, a resistant genotype such as B-3468 can be exploited as sources of genes for better seed storability.

The superior storability of B-3468 line was probably attributed to its slower rate of imbibition as reflected by its low permeability (Table 1) and relatively low seed moisture content (Table 2). Table 1 and 2 showed that B-3468 had the smallest seed coat permeability and relatively low moisture content. While genotypes with poor storability such as Meratus, Malabar, and L-Batang tended to have high seed coat permeability and seed moisture content. The proposed reasons were in agreement with Kuo (1989), who reported that soybean seed with low seed coat permeability tended to imbibe water at slower rate than others and this ‘delayed imbiber’ might resist absorption of water (moisture) during storage and then protect seed from deterioration. He further stated that the respiraton rate of seed was accelerated with increased seed moisture content and this respiration interferes with the seed quality of rapid-imbibed seeds to greater extent than slow-imbibed seeds.

Eventhough seed viability after storage was influenced by seed coat permeability and lignin occurrence in the seed coat determines its permeability, a significant relationship between seed coat lignin content and seed germination was not found. Regression analysis showed that a non-significant positive relationship (R^2 = 0.193,P=0.204) was observed between seed coat lignin content and seed germination (Fig. 1). This suggests that lignin content of the soybean seed coat weakly involved in reducing the rate of seed deterioration in storage. A possible explanation for the lack of significant correlation between these two variables was due to extreme seed deterioration of nearly all genotypes making their seed germination dropped drastically to low and unwidely spread values (Table 2). Consequently, this masked their real difference for resistance to storage deterioration.

A decline in seed quality in this study was also followed by an increase in membrane deterioration as reflected by electrolyte conductivity. This result was in agreement with other reports (Chuntirapongsa, 1992)

Table 1. Selected soybean genotypes, with their seed coat characteristics used in this study.

Genotype	Seed characteristics			
	Seed Coat Color	100-Seed Weight (g)	Lignin Content (%ADL) [x]	Permeability ($g\ g^{-1}\ h^{-1}$) [x]
B-3468	Black	10.12	17.285 b	0.082 d
B-3618	Black	9.19	16.920 b	0.086 cd
B-3770	Black	8.57	16.830 b	0.104 bcd
Merapi	Black	8.01	15.305 b	0.107 bcd
Kalitur	Black	8.57	23.120 a	0.086 cd
Lokon	Yellow	10.34	1.255 c	0.094 cd
L.Batang	Yellow	8.65	1.425 c	0.165 ab
Meratus	Yellow	10.59	1.355 c	0.165 ab
L.Brewok	Yellow	7.78	0.950 c	0.179 ab
Malabar	Yellow	10.57	0.830 c	0.185 a

[x] Means separated within columns by Duncan's multiple range test, P=0.05

Table 2. Seed moisture content (SMC), germination (Germ), and electrical conductivity (EC) for the 10 selected soybean genotypes after 4 months storage at ambient temperature of about 29 ^{0}C and 75% RH

Soybean genotypes	SMC(%)[x]	Germ (%)[x]	EC (mmhos $cm^{-2}\ g^{-1}$)[x]
B-3468	11.24 bcd	72.66 a	0.24 e
B-3618	11.53 bcd	51.33 b	0.25 de
B-3770	11.09 d	51.00 b	0.31 c
Merapi	11.57 bcd	53.00 b	0.30 cd
Kalitur	11.19 cd	48.33 b	0.29 cd
Lokon	11.47 bcd	46.67 b	0.38 b
L.Batang	12.88 a	50.33 b	0.27 cde
Meratus	11.53 bcd	40.00 c	0.37 be
L.Brewok	11.63bc	55.00 b	0.35 b
Malabar	11.70 ab	46.67 b	0.43 a

[x] Means separated within columns by Duncan's multiple range test, P=0.05

The lowest electrolyte conductivity was possessed by B-3468, indicating that it sufferred the least physiological disruption of membranes during storage. The least membrane disruption of B-3468 was well understood since it had the least seed coat permeability. When related to seed coat lignin content, there was a significantly negative relationship (R^2=0.496, P=0.023) between seed coat lignin content and seed electrolyte conductivity (Fig. 2), thus establishing the great influence of seed coat lignin content on membrane deterioration associated with a decline in seed quality after storage. The similar result was also reported by Panobianco *et.al.* (1999).

The negative relationship between these two variables was understood since lignin has im-permeabilisation effects, thus affecting the way and the amount of leakage of electrolyte from the seeds to imbibing solution (Tavares *et al.*, 1987).

The effect of lignin occurrence on seed coat permeability as reported by Tavares et al. (1987) was also observed in this study. Regression analysis showed that seed coat lignin content was significantly and negatively correlated (R^2 = 0.615, P = 0.07) with seed coat permeability (Fig. 3). This indicates that an increase in seed coat lignin content was inversely matched with a decline in seed coat permeability.

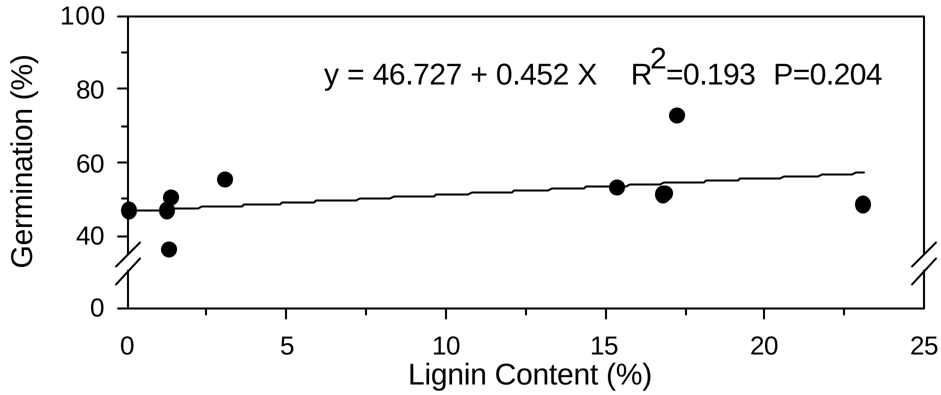

Figure 1. Relationship between seed coat lignin content and seed germination for ten soybean cultivars.

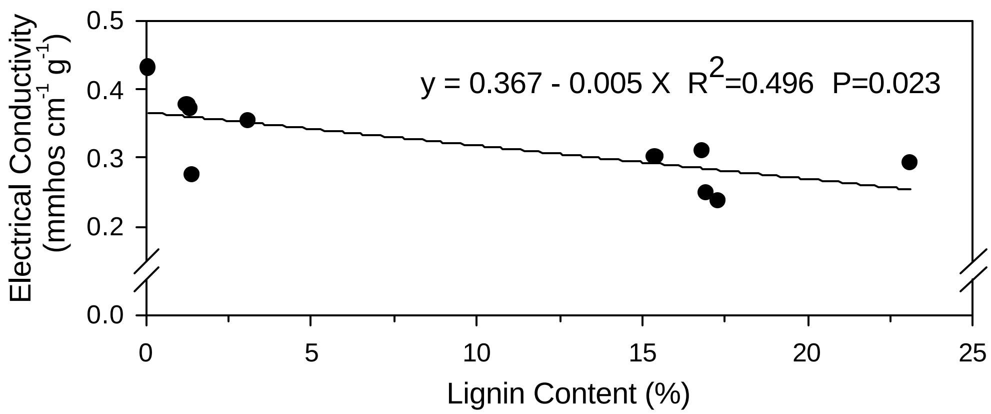

Figure 2. Relationship between seed coat lignin content and electrollyte conductivity for ten soybean cultivars.

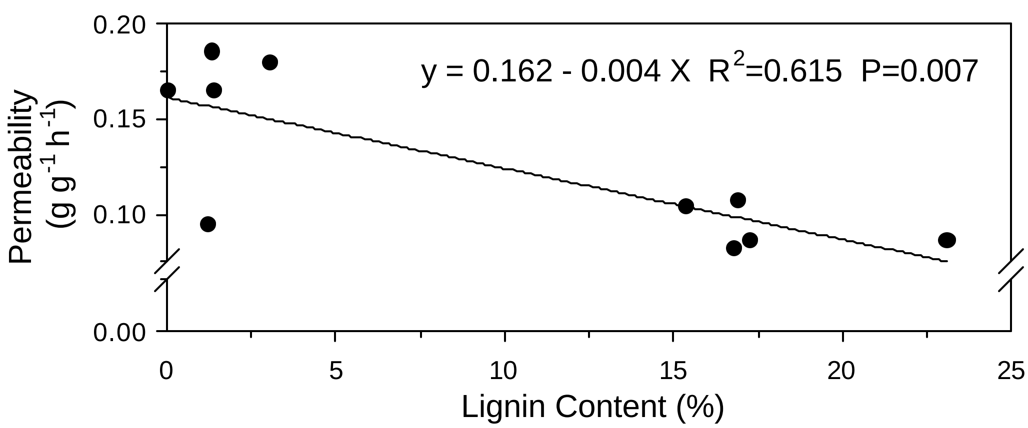

Figure 3. Relationship between seed coat lignin content and seed coat permeability for ten soybean cultivars.

CONCLUSIONS

The results indicate that soybean genotypes differred significantly in their ability to maintain seed quality during storage. Seed of B-3468 exhibited the highest storage potential. The seed coat lignin content was significantly and negatively related to membrane deterioration associated with a decline in soybean seed quality after storage, but not well related to the decline in seed viability after storage.

REFERENCES

Alvarez, P.J.C., F.C. Krzyzanowski, J.MG. Mandarino, and J.B. Franca Neto. 1997. Relationship between soybean seed coat lignin content and resistance to mechanical damage. Seed Sci Tech. 25:209-214.

Chuntirapongsa, S. 1992. Effects of seed coat color on storability of soybean seeds. Dissertation (Ph.D.). Miss. State Univ., Miss. State, MS.

Dassou, S. and E.A.Kueneman. 1984. Screening methodology for resistance to field weathering of soybean seed. Crop Sci. 24:774-779.

Fehr, W.R. and C.E. Caviness. 1977. Stages of soybean development. Ames. Iowa State University/Cooperative Extention Service. Special Report No. 80, 11p.

Ferguson, J.M., D.M. TeKrony, and D.B. Egli. 1990. Changes in early soybean seed and axes deterioration: I. Seed quality and mitochondrial respiration. Crop Sci. 30: 175-179.

Kuo, W.H.J. 1989. Delayed-permeability of soybean seed: Characteristic and screening methodology. Seed Sci. Tech. 13:322-325.

McDougall, G.J. I.M. Morrison, D. Stewart and J.R. Hillman. 1996. Plant cell walls as dietary fiber: range, structure, processing and function. J. Sci. Food and Agric. 70:133-150.

Miranda, F.M., C.H. Andrews, and K.W. Roy. 1980. Seed coat impermeability in soybean associated with resistance to seedborne infection and weathering. Agron. Abstr. 1980:111.

Nugraha, U. 1987. Association of seed coat color and permeability in soybeans with resistance to weathering stress and adverse storage conditions. Dissertation (Ph.D.). Miss. State Univ., Miss. State, MS.

Panobianco, D., R.D. Vieira, F.C. Krzyzanow ski, and J.B. Franca Neto. 1999. Electrical conductivity of soybean seeds and correlation with seed coat lignin content. Seed Sci. & Tech. 27: 945-949.

Tavares, D.Q., M.A. C. Miranda, C.Y. Umino and G.M. Dias. 1987. Structural characteristics of seed coat of permeable and impermeable of seeds of soybeans breeding line. Revista braseleira de Botanica. 10: 147-153.

Van Soest, P.J. and R.H. Wine. 1968. Determination of lignin and cellulose in acid detergent fiber with permanganate.J.Assoc.Off. Agric. Chem. 51:780-785