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Storability of Mung Bean Seeds Possessing Different Seed Coat Lignin Content under Simulated Adverse Conditions

Daya Simpan Benih Kacang Hijau Berkadar Lignin Beda di dalam Kulit Benihnya pada Kondisi Lingkungan Tercekam

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ABSTRACT

Lignin content in the seed coat may relate to genotypic differences among mung bean genotypes for resistance to adverse storage conditions. This study was undertaken to evaluate such presumption. Nine mung bean genotypes with different seed coat lignin content were grown in research plots at Agriculture Faculty, Bengkulu University on April 2003. Seeds were hand harvested at R8 maturation and stored for 8 months at a 30ºC and 75% relative humidity (RH). Samples of seeds from each genotype were taken at bimonthly interval and then subjected to seed quality evaluation. Treatments were arranged in a split plot design with storage period as main plots and genotypes subplots with three replications. There were genotypic differences among mung beans for resistance to adverse storage conditions as reflected by germination and accelerated aging germination (AAG) percentages. Germination and AAG percentages for all genotypes remained above 90% at the 0, 2, 4 and 6 month storage period. At the 8 month storage period, germination and AAG for three genotypes (Bhakti, Gelatik and Parkit) remained high (>80%) and they were classified as resistant to adverse storage conditions or a good storer. For the other genotypes germination and AAG declined to below 80% at the same storage period and they were classed as susceptible to adverse storage conditions or a poor storer. Good storer genotypes absorbed moisture at slower rate than poor storer genotypes. Seed moisture increased from near 11% at the 0 month storage period to only around 12% - 13% at the 8 month storage period. for good storer genotypes and from near 11% at the 0 month storage period to almost 14% - 15% at the 8 month storage period for poor storer genotypes. The rate of moisture absorption during storage was positively correlated with seed coat permeability (r = 0.93). Lignin content expressed as %ADL (acid delinted lignin) was correlated to germination percentage (r = 0.53) and with AAG percentage (r = 0.64). Overall, the involvement of lignin in resistant mechanism of mung bean seeds to adverse storage conditions was obviously through controlling seed coat permeability as shown by good correlation between lignin content and seed coat permeability (r = 0.69).

Keyword : mung bean, lignin, seed deterioration, seed viability, seed vigor, storability

INTRODUCTION

A humid tropical climate characterized by high temperature and high relatif humidity is very conducive to rapid deterioration of legume seeds such as mung bean [*Vigna radiata* (L.) Wilczek] seed in storage. During seed deterioration in storage, its vigor as well as viability, can deteriorate rapidly, making it impossible for the farmers to use their own seed for planting in the next season due to its low quality.

One of the most promising solutions to the problems of seed deterioration in storage appears to be the development of cultivars that resist adverse storage conditions. To provide genetic material for this purpose the identification of mung bean genotypes resistant to the conditions would be necessary. The common method used for this purpose in soybean is to store the seeds at high temperature and humidity for a certain period of time and then asses the seed quality regularly (Marwanto *et al.*, 2003; Chuntirapongsa, 1992).

The similar method has not been applied for mung bean.

Differences have been shown to exsist among genotypes in their resistance to adverse storage conditions in soybean (Marwanto *et al.*, 2003; Chuntirapongsa, 1992; Dassou and Kueneman, 1984). However, the same information is limitedly available for mung bean. In their selection among soybean lines for increased seed storability, they reported that seed size played important roles on resistance of seeds to deterioration in storage. They further suggested that soybean lines, adapted to adverse storage conditions, should be small-seeded, but not all small-seeded lines were resistant to adverse storage conditions. One possible explanation for the relationship between small seed size and good seed quality is the possitive correlation between seed size and seed coat permeability (Marwanto, 2007a, b;Dassou and Kueneman, 1984).

Chuntirapongsa (1992) and Dassou and Kueneman (1984) also reported that some soybean lines with a hard seed coat were resistant to adverse storage conditions, but not all genotypes with resistance to deterioration in storage were hard-seeded.In other studies Marwanto (2007a, 2003b), Panobianco *et al.* (1999) and Tavares *et al.* (1987) reported that seed coat impermeability of soybean was chemically influenced by its lignin content. McDougal *et al.* (1996) also reported that its occurrence in seed coat as a constituent of cell walls affects the rate of water absorption throughout the seed coat. Since lignin determines the rate of water absorption throughout the seed coat, its occurrence had an effect on reducing deterioration of soybean seed during storage (Marwanto *et al.*, 2003). However, the same relationship between seed coat lignin content and resistant to deterioration during storage in mung bean seed is not well understood.

The objectives of the study were (1) to evaluate genotypic differences in resistance to adverse storage conditions, and (2) to relate seed coat lignin content with resistance to adverse storage conditions.

MATERIALS AND METHODS

Procedures for Seed Production

Nine mung bean genotypes of known seed size and seed coat permeability were used in these studies (Table 1) The seeds were planted in research plots at Agriculture Faculty, Bengkulu University on April 10, 2003 with three replications. Each genotype was planted in a plot consisting of a single raised bed, 65 cm wide and 4 meters long. Two rows were planted per bed. Row spacing was 35 cm between rows within beds and 65 cm between beds. Seeds were planted in hills 20 cm apart with 2-3 seeds per hill. For optimum growth N, P, and K fertilizer at a rate of 100, 80 and 75 kg ha^{-1} was applied prior to planting.

Table 1. Selected mung bean genotypes used in this study with their lignin content expressed as % ADL (acid delinted lignin), seed coat permeability (P) and seed weight.***.

Genotipe	Lignin Content (%ADL)	P* (g g^{-1} hr^{-1})	100-Seed Weight ** (g)
Gelatik	0.062	0.029	4.74
Bhakti	0.070	0.008	4.34
Betet	0.054	0.071	3.82
Kenari	0.010	0.047	5.61
Parkit	0.050	0.039	5.07
Merak	0.070	0.043	6.64
IPB.M/97-13-60	0.042	0.056	6.27
VC-3012-B	0.016	0.068	5.46
Vr.1686-3-8-B	0.007	0.042	4.00

Keterangan : *: Permeability of seed determined following two hours submersion in dionized water; **: Weight in grams of 100 seeds at 11% moisture; ***: Data taken from Marwanto (2007a)

Table 2. Total viable seed percentages for nine selected mung beans at bimontly intervals during 8 month storage at 30 °C and 75% RH.

Genotype	Storage Period (months)				
	0	2	4	6	8
	---------- Germination (%) ----------				
Gelatik	97.3 a	98.7 a	95.3 a	96.3 a	83.0 b
	A	A	A	A	AB
Bhakti	92.7 a	94.0 a	98.7 a	95.3 a	88.3 a
	A	A	A	A	A
Betet	96.0 a	98.0 a	98.0 a	97.3 a	78.0 b
	A	A	A	A	ABC
Kenari	96.7 a	96.7 a	98.7 a	97.3 a	77.7 b
	A	A	A	A	ABC
Parkit	96.0 a	98.7 a	93.3 a	96.0 a	80.0 b
	A	A	A	A	ABC
Merak	100.0 a	100.0 a	99.3 a	99.7 a	74.7 b
	A	A	A	A	BC
IPB.M/97-13-60	98.0 a	95.3 a	93.3 a	95.7 a	70.0 b
	A	A	A	A	C
VC-3012-B	98.7 a	96.7 a	100.0 a	98.3 a	78.7 b
	A	A	A	A	ABC
Vr.1686-3-8-B	98.0 a	98.7 a	98.0 a	98.3 a	70.0 b
	A	A	A	A	C

Keterangan : *: Numbers within the same column followed the same capital letter differed significantly at $\alpha = 0.05$; **: Numbers within the same row followed the same small letter differed significantly at $\alpha = 0.05$

Table 3. Accelerated aging responses in terms of total viable seed percentages for nine selected mung beans at bimontly intervals during 8 month storage at 30 °C and 75% RH.

Genotype	Storage Period (months)				
	0	2	4	6	8
	-------- Accelerated Aging Germination (%) --------				
Gelatik	96.7 a	94.7 a b	92.7 a b	94.7 a b	84.3 b
	A	A	A	A	A
Bhakti	98.7 a	94.0 ab	91.3 ab	88.0 ab	86.7 b
	A	A	A	A	A
Betet	95.3 a	91.3 a	95.3 a	94.0 a	61.7 b
	A	A	A	A	CD
Kenari	97.3 a	93.3 a	96.7 a	95.7 a	51.7 b
	A	A	A	A	D
Parkit	95.3 a	92.7 ab	93.3 ab	93.7 ab	83.0 b
	A	A	A	A	A
Merak	97.3 a	96.7 a	96.7 a	96.7 a	77.7 b
	A	A	A	A	AB
IPB.M/97-13-60	95.3 a	96.0 a	90.0 a	90.0 a	67.0 b
	A	A	A	A	BC
VC-3012-B	93.3 a	98.0 a	92.0 a	96.3 a	67.7 b
	A	A	A	A	BC
Vr.1686-3-8-B	97.3 a	93.3 a	95.3 a	95.3 a	63.7 b
	A	A	A	A	C

Keterangan : *: Numbers within the same column followed the same capital letter differed significantly at $\alpha = 0.05$; **: Numbers within the same row followed the same small letter differed significantly at $\alpha = 0.05$

Seeds were harvested by hand stripping of the pods at harvest maturity when 90% of mung bean pods turned dark brown and their seed moisture content had dropped about 20% to establish a non-weathered category. The pods were then dried with heated air (<35 °C) to reduce

moisture content to about 12% threshed in jute bags by flailing. Then, 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 mung bean seed samples from each genotype were further evaluated for its initial physiological quality by the standard germination test and then their seed moisture content were adjusted to about 11% Only seed samples with high initial quality (>90%) were used for storage study. Separate seed samples were also drawn to evaluate seed coat lignin content.

Procedures for Evaluating Resistance to Adverse Storage Conditions

Resistance to adverse storage conditions for the nine mung bean genotypes of known seed coat lignin content were evaluated by subjecting their seeds at about 30 °C and 75% relative humidity (RH) for periods of 0 (control), 2, 4, 6 and 8 months in a controlled storage chamber. The storage chamber was made from plexiglass about 0.3 m^3 capacity. A saturated sodium chloride solution was added in the bottom well of the chamber to maintain the 75% RH condition in the chamber. A shelf was placed inside the chamber to support the seeds and a 15-cm gap was maintained between the solution surface and the shelf.. The chamber was then divided into 5 small boxes with a partition and each box made up of one of the storage period treatment. Seeds of 100 g from each genotype and each storage period were contained in plastic mesh pouches and placed in each box of the storage chamber. The chamber was then covered with an airtight lid and positioned in a room with temperature controlled at about 30°C. At the end of each storage period samples of seeds from each genotype were taken and then subjected to seed quality evaluation in term of seed viability and vigor by standard germination and accelerated aging tests for seed viability and vigor evaluation, respectively. While seed moisture test was made only at the beginning of 0 month storage period and at the end of 8 month storage period. All these tests were performed on seed from each genotype in each replication.

Seed Quality Evaluation and Lignin Determination

Seed moisture content was determined on seed fraction of the mung bean seed sample. For each seed moisture evaluation, samples of about 20 g in duplicate were placed in an oven at 105°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 %.

Standard germination test (SGT), which was used to evaluate seed viability, was made on three replications of 50 seeds. The test was determined on mung bean seed sample taken after 0, 2, 4, 6 and 8 month period of storage.The fifty seeds were placed on mosit paper towels, which were rolled and placed inside plastic bags and kept at a room temperature. Germinated seeds (normal seedlings) were counted after 4 and 7 days. Dead seeds were removed after 4 days, while hard seeds after 7 days. The percentage of all germinated seeds and hard seeds were counted separately and then combined into total viable seeds percentage.

Accelerated aging test, which was used to evaluate seed vigor, was made on three replications of 50 seeds. The test was determined on mung bean seed sample taken after 0, 2, 4, 6 and 8 month period of storage. The fifty seeds from each treatment-replicate were subjected to a period of accelerated aging, 42 °C and near 100% RH, for 48 hours prior to standard germination test. The seeds were placed on a wire mesh tray of 20X5X2.5cm. The tray was placed inside a plastic box of 30X10X5cm and the box was filled with 100ml of water. A 10-mm gap was maintained between the water surface and the seed tray. The box was covered with an airtight lid and kept in oven at 42 °C for 48 hours. After aging, seeds were taken out of the aging box and subjected to standard germination test. In standard germination test, fifty seeds from each replication were place on moist paper towels which were rolled and placed inside plastic bags and kept at a room temperature. Germinated seeds (normal seedlings) were counted after 4 and 7 days. Dead seeds were removed after 4 days, while hard seeds

after 7 days. The percentage of all germinated seeds and hard seeds were counted separately and then combined into total viable seeds percentage.

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

Data Analysis

Genotypes and storage period which included in this study as treatments were arranged in a split-plot design with period of storage as main plot and mung bean genotypes as subplots with three replications. Analysis of variance of each variable was also conducted as a split plot design (Steel and Torrie, 1980). The means were separated by Duncan Multiple Range Test at the 0.05 level of probability. Regression analysis between viability and vigor with percent lignin content of the seed coat of the tested genotypes was also determined.

RESULTS AND DISCUSSION

Climatological data (data not shown) showed that almost no rainfall occurred several days before and after all genotypes reached harvest maturity stage although at this period daily average temperatures and relative humidity were above 26°C and 85%, respectively. Less rainfall during this seed maturation was probably responsible for almost maximum level of seed vigor of all genotypes when their seeds were harvested.

Simulated unfavorable conditions during storage mainly high humidity and high temperature contributed to seed deterioration of mung bean. Less seed deterioration occurred until 6 month storage period, while severe seed deterioration only occurred after 8 month storage period as indicated by a decrease in germination (Table 2) and accelerated aging germination (Table 3). Averaged over genotypes, germination as indicator of seed viability were 97.0, 97.4, 97.2, 97.1 and 77.8% for 0, 2, 4, 6 and 8 month storage, respectively. Average over genotypes, accelerated aging germination as an indicator of seed were 96.3, 94.4, 93.7, 93.8 and 71.5% for 0, 2, 4, 6 and 8 month storage, respectively. These results indicated that deterioration in mung bean seeds as a result of adverse storage conditions was manifested by a decrease in germination capacity and germinative response after accelerated aging.

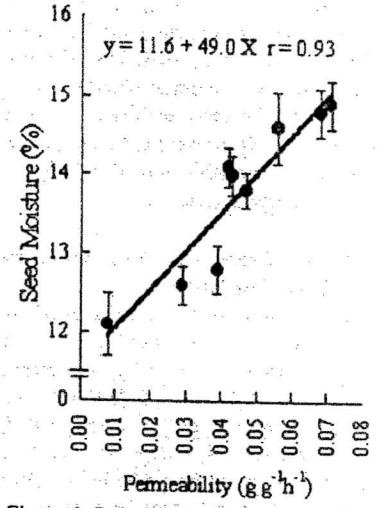

Figure 1. Relationship between seed coat permeability and seed moisture for nine mung bean genotypes. Vertical bars = s.e

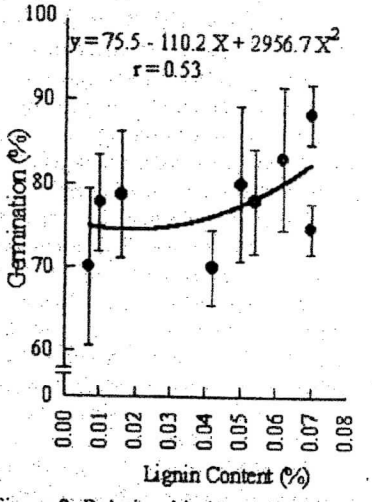

Figure 2. Relationship between seed coat lignin content and germination for nine mung bean genotypes. Vertical bars = s.e

The analysis of variance showed that there was a significant genotype X seed storage period interaction for germination and accelerated aging germination. This interaction for both seed quality indicators was due to genotypic differences in the rate of decrease in seed viability (Table 2) and seed vigor (Table 3). Other researchers working with different seeds have also reported the decline in seed quality associated with adverse storage conditions. These included Marwanto (2004), Chuntirapongsa (1992) and Dassou and Kueneman (1984) in soybeans.

As shown in Table 2 and 3, a marked decline in germination and accelerated aging germination all genotypes was observed only at 8 month storage period and at this storage period genotypic differences for resistnace to adverse storage conditions was revealed. Therefore, this storage period could be used by breeders to determine differences in genotype's potential to resist seed deterioration during storage for mung bean. Meanwhile, storage period at 0, 2, 4 and 6 month did not cause a marked decline in seed viability and vigor for all genotypes. At these storage periods their germination and accelerated aging germination remained above 90% for all genoytpes.

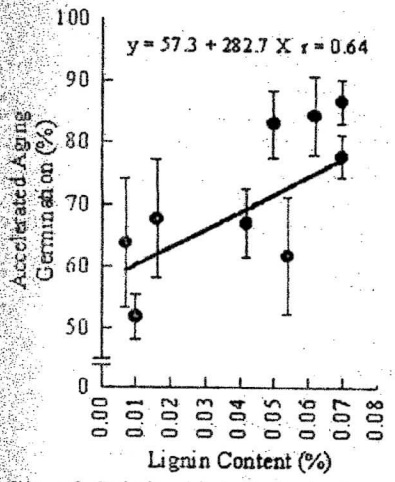

Figure 3. Relationship between seed coat lignin content and accelerated aging germination for nine mung bean genotypes. Vertical bars = s.e

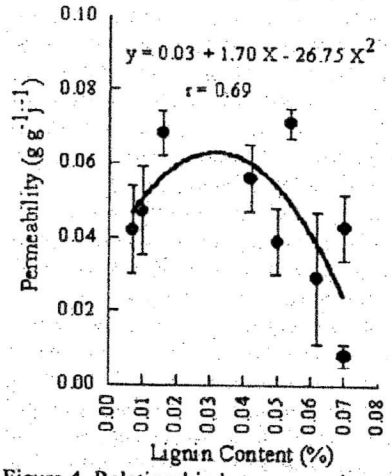

Figure 4. Relationship between seed coat lignin content and permeability of seed coat for nine mung bean genotypes. Vertical bars = s.e

Among the genotypes subjected to 8 month storage period three genotypes, Bhakti, Gelatik and Parkit, consistently maintained high seed viability and seed vigor (>80%) and might be classified as a good "storer". Among these three genotypes, Bhakti was identified as being the most resistant to adverse storage conditions as indicated by the highest score of germination (Table 2) and accelerated aging germination (Table 3) followed by Gelatik and Parkit respectively. Similar superior resistance was also exhibited by seed of Bhakti when it was exposed to unfavorable conditions during natural aging (Marwanto, 2007b) and incubator aging (Marwanto, 2008a). Meanwhile, viability and vigor of seeds of Betet, Kenari, Merak, IPB.M/97-13-60, VC-3012-B and Vr.1686-3-8-B had decreased to below 80%, which might be classed as a poor "storer". For breeding purposes, however, 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 Bhakti can be exploited as sources of genes for better seed storability.

The superior resistance to adverse storage conditions for Bhakti, Gelatik and Parkit was attributed to its slower rate of moisture reabsorption during storage as reflected by its relatively low seed moisture content. Their seed

moisture content at 8 month storage period were 12.1% for Bhakti, 12.6% for Gelatik and 12.8% for Parkit. While the other genotypes had a seed moisture of 13.8% for Kenari, 14.0% for Merak, 14.1% for Vr.1686-3-8-B, 14.6% for IPB.M/97-13-60, 14.8% for VC-3012-B and 14.5% for Betet, respectively. The slower rate of moisture reabsorption during storage exhibited by these three genotypes was probably a result of lower seed coat permeability as shown by a highly positive correlation (r = 0.93) between seed moisture and seed coat permeability (Figure 1). These three genotypes with less permeable seed coat experienced less exposure to moisture reabsorption during storage and tended to absorb moisture at a slower rate than the other genotypes with more permeable seed coat and this would protect them from deterioration due to adverse conditions during storage. As a result of this decresead response, genotypes with less permeable seed coat could be expected to have lower levels of moisture at any storage period and higher level of seed viability and vigor than those with more permeable seed coat. The proposed reasons were in agreement with Marwanto (2003a, c) and Kuo (1989) who worked with soybeans. They reported that soybean seeds with low seed coat permeability tended to imbibe moisture at slower rate than others and this 'delayed imbiber' might resist absorption of moisture during storage and then protect seed from deterioration. Kuo (1989) 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.

The superior resistance to adverse storage conditions for the three genotypes, Bhakti, Gelatik and Parkit was probably related to lignin content in their seed coat as well. Regression analysis showed that lignin content in the seed coat was correlated (r = 0.53) with germination (Figure 2). A positive correlation (r = 0.64) was also found between accelerated aging germination and lignin content in the seed coat (Fig. 3). These two figures showed that genotypes with both high seed viability and vigor and were classed as a good storer tended to have high lignin content in the seed coat, while genotypes with both low seed viability and vigor and were classed as a poor storer tended to have low lignin content in the seed coat. These positive correlations suggest that lignin content in the seed coat may be involved in reducing the rate of seed deterioration due adverse storage conditions, thus establishing positive influence of lignin content on maintaining seed viability and vigor during storage. Researchers working with other species also found a positive correlation between resistance to adverse conditions and lignin content in the seed coat These included Marwanto (2003a, c), Marwanto et al. (2003) and Chuntirapongsa (1992) in soybeans and Marwanto (2008a , 2007b) in mung bean.

The positive effects of lignin on protecting mung bean seeds against adverse storage conditions was understood since lignin in the seed coat has impermeabilization characteristics and exerts an important effect on the capacity and velocity of absorption of moisture throughout the seed coat (McDougall *et al.*, 1996). The result of this experiment showed that lignin content in the seed coat was correlated (r = 0.69.) with seed coat permeability (Figure 4). This figure showed that genotypes with high lignin content in the seed coat tended to have low seed coat permeability, while genotypes with low lignin content tended to have both high seed coat permeability. This result was in agreement with Marwanto (2003a, c) and McDougall *et al.* (1996) who worked with soybeans. Thus, the involvement of lignin in resistant mechanism of mung bean seeds to adverse storage conditions was through controlling seed coat permeability.

CONCLUSIONS

Among genotypes included in this study, there were genotypic differences in resistance to adverse storage conditions. The different resistance to adverse storage conditions was related to lignin content in the seed coat, which tended to be higher in the genotypes with less permeable seed coat.

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