SOYBEAN SEED COAT CHARACTERISTICS AND ITS QUALITY LOSSES DURING INCUBATOR AGING AND STORAGE

KARAKTERISTIK KULIT BENIH KEDELAI DAN KEMUNDURAN MUTU NYA SELAMA DERAAN CUACA DAN PENYIMPANAN

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ABSTRACT

Deterioration of soybean seed quality has been associated with permeable seed coat. The objective of these studies were to (1) determine the role that seed coat characteristics play in soybean seed deterioration during incubator aging and storage, and (2) to relate resistance to incubator aging with resistance to storage. Eight small-seeded soybean genotypes with different characteristics of seed coats were grown in research plots at Agriculture Faculty, Bengkulu University on November 2000. For experiment I, pods were hand harvested at physiological maturity and subjected to incubator aging and non-aging as a control. For experiment II, seeds were hand harvested at R8 maturation and stored for 4 months at a room temperature and 75% RH in Agronomy Laboratory. After treated with aging of pod and ambient storage, each genotype from each replicate was subjected to seed quality evaluation. Incubator aging and ambient storage of soybean seeds lowered seed germination and increased electrolyte conductivity values. Most genotypes with slow imbibition rate of seed coat were resistant to incubator aging and ambient storage and had higher seed quality than those with rapid imbibition of seed coat. Among slow imbiber genotypes, seeds of Merapi and Kalitur were more resistant to aging treatment than B-3770 and B-3618. Seed coat permeability, which was inversely related to seed coat lignin content (r = - 0. 96 P<0.001) was correlated with seed germination (r = -0.77 P<0.05) and with electrolyte conductivity (r =0.75 P<0.05). A weak correlation was observed between seed coat permeability with AAG due to extreme seed deterioration of nearly all genotypes during accelerated aging of incubator aged seeds. The black-seeded genotypes were more resistant to aging than yellow-seeded genotypes. Seed coat lignin content was highly correlated with seed germination (r = -0.77 P<0.05) and with electrolyte conductivity (r =0.77 P<0.05). Positive correlations were observed between seed quality indicators following incubator aging and ambient storage. The coefficient correlation between seed germination following incubator aging and seed germination following 4 months of ambient storage was 0.85 (P<0.05) and between elctrical conductivity following incubator aging and electrical conductivity following 4 months of ambient storage was 0.73 (P<0.05). This indicates that most genotypes resistant to incubator aging were also resistant to seed deterioration in storage and vice versa.

Key words: soybean seed, incubator aging, ambient storage, seed coat permeability, seed viability, vigor

ABSTRAK

Kulit benih kedelai dianggap sebagai penentu mutu benihnya. Untuk mengetahui sampai sejauhmana peranannya, maka dua percobaan yang berbeda dilakukan dengan tujuan (1) menentukan peranan karakteristik kulit benih pada kemunduran mutu benih kedelai selama deraan cuaca dan penyimpanan, dan (2) menentukan kedekatan hubungan antara ketahanan benih kedelai terhadap deraan cuaca dan penyimpanan. Pada percobaan I, delapan genotipe benih kedelai yang diproduksi pada bulan November 2000 di petak percobaan Fakultas Pertanian didera dalam inkubator pada suhu 30^{0}C dan kelembaban 90% selama 10 hari, sedang pada percobaan II mereka disimpan di Laboratorium Agronomi selama empat bulan pada suhu kamar dan kelembaban 75%. Hasilnya menunjukkan bahwa genotipe kedelai berimbibisi lambat seperti Merapi dan Kalitur lebih tahan terhadap deraan cuaca dan penyimpanan daripada genotipe berimbibisi cepat seperti B-3770 dan B-3618. Permeabilitas sebagai penentu kemampuan kulit benih berimbibisi berhubungan erat dan terbalik (r = -0,77 P<0,05) dengan daya kecambah dan berhubungan positif (r = 0,75 P<0,005) dengan daya hantar listrik. Genotipe berkulit benih hitam lebih tahan terhadap deraan cuaca daripada genotipe berkulit benih kuning. Kandungan lignin kulit benih kedelai berhubungan erat dan positif (r = 0,77 P<0,05) dengan daya kecambah dan berhubungan negatif (r = -0,77 P<0,05) dengan daya hantar listrik. Antar variabel mutu benih yang memiliki hubungan erat setelah mengalami deraan cuaca maupun penyimpanan meliputi variabel daya kecambah dengan r =0,85 (P<0,05) dan variabel daya hantar listrik dengan r = 0,73 (P<0,05). Hal ini menunjukkan bahwa genotipe yang tahan terhadap deraan cuaca juga tahan terhadap penyimpanan.

Kata kunci: kedelai, deraan cuaca, penyimpanan, permeabilitas , viabilitas, vigor

INTRODUCTION

Humid, tropical environments are very conducive to seed deterioration and make the production of high quality soybean [*Glycine max* (L.) Merrill] seed in the field and maintenance of seed vigor during storage difficult. Deterioration of seed in the field prior to harvest is usually referred to as field weathering or as field deterioration or as preharvest weathering and in storage is called postharvest weathering. Several studies indicated that high temperature, humidity and precipitation play a critical role on pre and post harvest weathering. (Keigly and Mullen, 1986; TeKrony *et al*., 1980).

Genotypic differences in resistance to pre and postharvest weathering have been reported (Dassou and Kueneman, 1984; Marwanto, 2003b; Marwanto *et al*., 2003). Several studies on resistance to seed deterioration indicate that the seed coat characteristics plays an important role on resistance of seeds to deterioration (Dassou and Kueneman, 1984; Horlings *et al.*, 1991) 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). Since lignin determines the rate of water absorption throughout the seed coat, its occurrence may have an effect on soybean seed deterioration.

Dassou and Kueneman (1984) used different seed coat color in an incubator study to evaluate soybean genotypes for resistance to pre and post harvest weathering. They concluded that black-seeded genotypes were more resistant to weathering than yellow-seeded genotypes. In the same study, they also reported that seed coat permeability plays an important role on resistance to weathering

Most reports also emphasized that small-seeded genotypes were more resistant to pre and post harvest weathering than large-seeded genotypes (Dassou and Kueneman, 1984; Horlings *et al*., 1991). However, these studies were not able to determine if the resistance exhibited by small-seeded genotypes was as a result of their seed coat characteristic. In addition, few studies have been considered the role of soybean seed coat characteristic on pre and postharvest weathering resistance. The objective of this study was to (1) determine the role that seed coat characteristics play in soybean seed deterioration during incubator aging and storage, and (2) to relate resistance to incubator aging with resistance to storage.

METHODOLOGY

Experiment 1. Effect of Incubator Aging on Seed Quality of Soybeans

Eight small-seeded soybean genotypes with different characteristics of seed coats were used throghout this study. The first four of them stand for slow imbiber genotypes and the second four of them for rapid imbiber genotypes (Table 1). For seed production, three replicates of 10 row each of soybean seeds were grown on November 2000 at the Agriculture Faculty Research Plot in a split plot arrangement. Each genotype was planted in a single row 20m long with 1m between rows and 20cm between plants within a row. When plants reached physiological maturity stage (about 90% of the pods were yellow but before they had turned brown), 200 pods were hand harvested from each replicate and divided into two groups of 100 pods each. The pods from each group were then given the following treatment: (i) dried by hanging them in well-ventilated plastic bag for 2 to 3 weeks as a control. The pods then were hand-treshed. The moisture content of the seed after drying ranged from 11 to 13%. It was assumed that well-ventilated plastic bag provided a uniform drying environment which minimized seed deterioration during the dry-down period; (ii) subjected to 30 ^{0}C and 90% relative humidity for 10 days (incubator aging). After 10 days of incubator aging, the pods were removed from the incubator, force air dried to approximately 12% moisture content at 28 ^{0}C for 5 days and hand-threshed.. To determine the effect of the weathering treatments on seed quality, following treatments seeds were evaluated for viability by standard germination test, vigor by accelerated aging test, and leachate conductivity by electrolyte conductivity test.

Experiment 2. Effect of Storage on Seed Quality of Soybeans

Eight small-seeded soybean genotypes used in the experiment I were also used in this storage study. For seed production, three replicates of 10 row each of soybean seeds were grown on November 2000 at the Agriculture Faculty Research Plot in a completely randomized block arrangement. Each genotype was planted in a single row 20m long with 1m between rows and 20cm between plants within a row. When each plant of soybean genotype reached harvest maturity (about 90% of the pods had turned dark brown) pods were harvested by hand stripping. Seed moisture content at this stage had dropped to about 20%. 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 seed samples were then stored for a period of four months in Agronomy Laboratory on April 2001 for storability study in a completely randomized design with three replications. Separate soybean seed samples were also drawn to evaluate seed coat characteristics such seed coat permeability and seed coat lignin content.

Before storage, seed moisture content of each genotype was adjusted to about 10%. Then, 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 m^3 capacity with a saturated sodium chloride solution in the bottom well to maintain 75% RH for four 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 standard germination and electrolyte conductivity and performed on seed from each genotype in each replication.

Procedures for Evaluating Viability, Vigor and Seed Coat Characteristics

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. Germination seeds were counted after 4 and 7 days. The number of germinated seeds were expressed as a percentage of the total.

In accelerated aging test, seeds were subjected to a period of accelerated aging, 42 ^{0}C and near 100% RH, for 48 hours prior to standard germination test. Fifty seeds from each replication were placed on a wire mesh tray of 20x5x2.5cm. The tray was placed inside a plastic box of 30x10x5cm 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 airtight lid and kept in oven at 42 ^{0}C for 48 hours. After aging, seeds were taken out of the aging box and subjected to standard germination test.

Table 1. Soybean genotypes with their seed coat characteristics used in this study.

Genotype	Seed Type^x	Seed Coat Color	100-seed Weight (g)	Lignin Content (% ADL)	Permeability (g g^{-1} hr^{-1})
B-3168	SS	Black	9.19	16.92	0.11
B-3770	SS	Black	8.57	16.83	0.08
Kalitur	SS	Black	8.44	23.12	0.09
Merapi	SR	Black	8.01	15.40	0.10
L. Batang	SR	Yellow	8.65	1.43	0.17
L. Brewok	SR	Yellow	7.78	0.95	0.18
Malabar	SR	Yellow	10.57	0.83	0.17
Meratus	SR	Yellow	10.59	1.36	0.19

*SS = Smal seed Slow Imbiber, SR = Small seed Rapid Imbiber

Table 2. Influence of pod aging and seed coat permeability on germination, accelerated aging germination and electrolyte conductivity.

Treatmen	Seed quality Indicators					
	Germination		Acc. aging germination		Electrolyte conductivity	
	Mean	Range^x	Mean	Range^x	Mean	Range^x
	--------------------%------------------------				-------mmho cm^{-2} g^{-1}----	
Slow imbiber, nonaged pod	78.93	74-98	68.53	56-94	0.32	0.20-0.37
Slow imbiber, incubator-aged pods	66.68	50-84	46.83	18-92	0.43	0.23-0.84
Rapid imbiber, nonaged pod	75.89	72-96	47.00	20-88	0.51	0.23-0.70
Rapid imbiber, incubator-aged pods	30.83	14-64	35.65	0.6-7.2	0.75	0.47-1.12
LSD (P<0.05) between means	8.74		10.05		0.10	

^{x} = Constitute the maximum and minimum value of the observation

To determine electrolyte conductivity, a weighed sample of twenty five seeds were soaked in 40 mL distilled water for 12 hours at a room temperature. The electrolyte conductivity of seed leakage was determined with a Cole-Parmer conductivitimeter (Chicago, Illinois) and was expressed in mmho 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 seed coat permeability, 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%. Seed coat permeability was determined following 2 hours of summersion in deionized water and expressed in g g^{-1} h^{-1}.

Table 3. Influence of aging of pods of various genotypes with different seed coat characteristics on germination (Germ), accelerated aging germination (AAG) and electrolyte conductivity (EC).

Genotype	Seed quality indicators					
	Germn (%)		AAG (%)		EC (mmho cm^{-2} g^{-1})	
	Non-aged Pod	Incubator aged pod	Non-aged pod	Incubator aged pod	Non-aged Pod	Incubator aged pod
B-3168	78.7 a	54.0 b	62.7 a	52.0 a	0.35 b	0.57 a
B-3770	73.3 a	62.7 a	66.7 a	54.0 b	0.37 a	0.51 a
Kalitur	78.0 a	72.7 a	88.0 a	59.3 b	0.33 a	0.38 a
Merapi	80.7 a	77.73a	56.7 a	20.0 b	0.34 a	0.25 a
L. Batang	90.7 a	63.3 b	79.3 a	20.7 b	0.51 a	0.53 a
L. Brewok	64.7 a	62.0 a	49.3 a	43.3 a	0.53 b	0.87 a
Malabar	69.3 a	21.3 b	34.7 a	31.3 a	0.48 b	0.85 a
Meratus	78.7 a	19.3 b	24.7 a	4.7 a	0.50 b	0.74 a

x Means separation by t test (P = 0.05) of paired means within columns

Table 4. Correlations among seed coat lignin content, seed coat permeability, germination, accelerated aging germination, and electrolyte conductivity in soybean genotypes.

	Seed coat permeability	Germination	Accelerated aging germination	Electrolyte conductivity
Seed coat lignin content	-0.96**	0.77*	0.36 ns	-0.77*
Seed coat permability		-0.77*	-0.39 ns	0.75*
Germination			0.23 ns	-0.56*
Accelerated aging germination				-0.23 ns

*, ** = significant at P = 0.05 and 0.01, respectively ns = not significant

Statistical Analyses

Statistical analyses were conducted separately for incubator aging and storage studies. The data obtained from seed quality variables were analyzed using the analysis of variance. Means, when significantly different, were separated by *t* test of paired means and Duncan Multiple Range Test at the 0.05 level of probability. Correlation analyses was determined between seed coat characteristics and seed quality and also between resistance to incubator aging of soybean seeds with resistance to seed deterioration during storage.

RESULTS AND DISCUSSION

Experiment 1. Effect of Incubator Aging on Seed Quality Of Soybeans

The mean overall germination and accelerated aging germination values were higher for non-aged seeds, and electrolyte conductivity result was higher for incubator aged seeds (Table 2). This indicates that a simulated unfavorable condition contributed to soybean seed deterioration. Extreme seed deterioration of nearly all genotypes occurred during accelerated aging of incubator aged pods and masked their differences in resistance to incubator aging as shown by a wide difference between the maximum and minimum values of accelerated aging germination (Table 2). Therefore, only seed germination and electrolyte conductivity values were used as indicators for further seed quality evaluation following incubator aging and ambient storage and for further discussion the effects of incubator aging and ambient storage on soybean seed quality. The deleterious effects of unfavorable condition on reducing soybean seed quality were also reported by TeKrony *et al*. (1980) and Marwanto (2003b, 2004).

Table 5. Germination (Germ), and electrolyte conductivity (EC) for the 8 soybean genotypes after 4 months storage at ambient temperature of about 29 ^{0}C and 75% RH.

Genotype	Seed Quality Indicators	
	Germ (%)^x	EC (mmho cm^{-2} g^{-1})^x
B-3618	51.33 a	0.25 de
B-3770	51.00 a	0.31 c
Kalitur	48.33 ab	0.29 cd
Merapi	53.00 a	0.30 cd
L. Batang	50.33 a	0.27 cde
L. Brewok	55.00 a	0.35 b
Malabar	46.67 bc	0.43 a
Meratus	40.00 c	0.37 be

^{x} Means separation within colums by Duncan's Multiple Range Test, P = 0.05

Seed coat permeability affected all seed quality indicators. Incubator aged seed of type SR had the lowest seed quality, while non-aged seed of type SS had higher seed quality than all treatments. This indicates that not all small-seeded genotypes were resistant to incubator aging and the mechanism of resistance was more affected by seed coat permeability rather than small seed size. This has also been reported by other investigators (Horlings *et al*., 1991; Marwanto, 2003c).

As stated earlier, genotypes with slow imbibition seed coat type were more resistant to aging treatment than those with rapid imbibition seed coat type, as shown by higher germination and lower electrolyte conductivity values. Table 3 showed the genotypes more tolerant to incubator aging. Among slow imbiber genotypes, seeds of Merapi and Kalitur were more resistant to aging treatment than B-3770 and B-3618 as shown by the two seed quality indicators. Their germination and electrolyte conductivity values for aged seeds had no significant difference with those for non-aged seeds. The two genotypes with better seed quality were black-seeded genotypes.

Among rapid imbibition genotypes, seed of L-Batang was identified as being more resistant to aging treatment followed by L-Brewok as shown by seed germination and electrolyte conductivity scores. However, these yellow-seeded genotypes did not exhibit greater resistant to incubator aging than black-seeded genotypes as shown by mean seed germination and electrolyte conductivity values (Table 2). The mean seed germination and electrolyte conductivity for four yellow-seeded genotypes were 41.5% and 0.75 mmho cm^{-2} g^{-1}, respectively, compared to 66.7% and 0.43 mmho cm^{-2} g^{-1}, respectively following incubator aging for four black-seeded genotypes. Based on the two seed quality indicators, the black-seeded genotypes were superior to the yellow-seeded genotypes in resistance to incubator aging. This is consistent with result reported by Dassou and Kueneman (1984) and Marwanto (2003c).

Several studies have suggested that seed coat permeability can provide protection against seed deterioration (Dassou and Kueneman, 1984; Horlings *et al*., 1991). In this study, a significant correlation was obtained between seed coat permeability and resistant to incubator aging (Table 4). Significant correlation coefficients were found between seed coat permeability with germination (r = -0.77) and with electrical conductivity (r = 0.75), but a non significant correlation coefficient was observed between seed coat permeability with accelerated aging germination (r = - 0.39). These suggest that seed coat permeability was involved in reducing seed germinability and seed membrane integrity during incubator aging, but not involved in reducing accelerated aging germination.A weak correlation between seed coat permeability and accelerated aging germination was due to extreme seed deterioration of nearly all genotypes during accelerated aging of incubator aged seeds. The extreme seed deterioration of nearly all genotypes masked their difference in resistance to incubator aging as shown by wide difference between the maximum and minimum values of accelerated aging germination values (Table 2).

Seed coat lignin content also appeared to play a significant role in reducing seed deterioration. It was positively correlated with seed germinability (r = 0.77 P<0.05) and with accelerated aging germination (r = 0.36 P>0.05), but negatively correlated with electrolyte conductivity (r = - 0.77 P<0.05). The possitive effect of seed coat lignin content on reducing seed deterioration during incubator aging was related to its impermeability effect on soybean seed coat (Marwanto, 2003 a, c; Panobianco *et al*, 1999). A significant negative correlation (r = - 0.96 P<0.01) was found between seed coat lignin content and seed coat permeability. This indicates that lignin occurrence in the seed coat exerts an important effect on the capacity of absorbtion of water throughout seed coat.

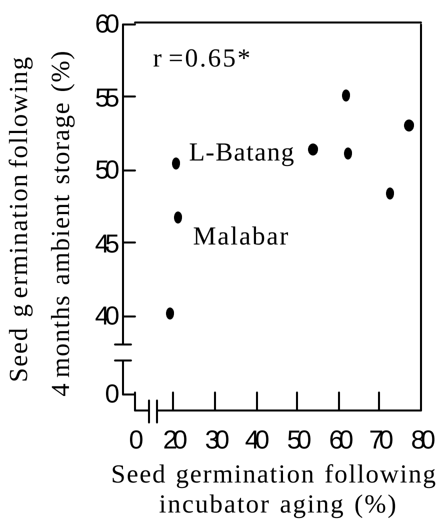

Fig. 1. Relationship between seed germination following incubator aging and seed germination following 4 monthsstorage.

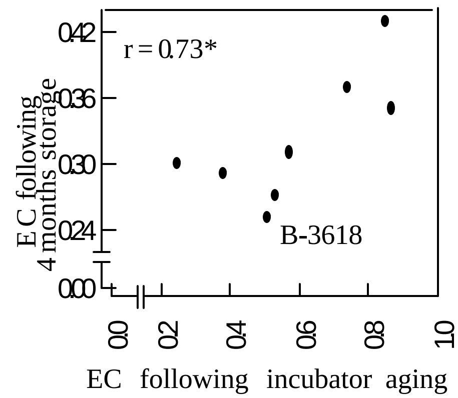

Fig 2. Relationship between EC following incubator aging and EC following 4 months storage.

Experiment II. Effect of Storage on Seed Quality of Soybean

Seed quality for the eight genotypes after 4 month storage at a room temperature of about 29^{0}C and 75% RH are shown in Table 5. Eventhough all genotypes deteriorated severely, a significant difference in the rate of deterioration of seeds of the eight genotypes under the rather adverse storage conditions as indicated by germination and electrolyte conductivity was still observed. Genotypes such as Merapi, B-3618 and B-3770 for black-seeded genotypes and L-Batang and L-Bewok for yellow-seeded genotypes 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 Malabar and Meratus with high permeable seed coat and Kalitur with low seed coat permeability had decreased to below 50%, which might be classed as poor “storer”. Similar results was also reported by Chuntirapongsa (1992) when working with different genotypes of soybeans.

Fig. 1 and 2 show that positive correlations were observed between seed quality indicators after incubator aging and after ambient storage. The coefficient correlation between seed germination following incubator aging and seed germination following 4 months of ambient storage was 0.85 ($P<0.05$) (Fig. 1) and between elctrolyte conductivity (EC) following incubator aging and electrolyte conductivity following 4 months of ambient storage was 0.73 ($P<0.05$) (Fig 2.) This indicates that most genotypes resistant to incubator aging were also resistant to deterioration in storage and vice versa. The similar result was also reported by Dassou and Kueneman (1984).

CONCLUSIONS

Incubator aging of soybean pods and 4 months ambient storage resulted in a lowering of seed quality as indicated by decreasing seed germination and increasing electrolyte conductivity scores. Among genotypes included in these studies, there were significant genotypes differences in resistance to incubator aging and seed deterioration in storage. Black-seeded genotypes were more resistant to incubator aging than yellow-seeded genotypes. Seed coat permeability, which was inversely related to seed coat lignin content, played a significant role in decreasing seed quality during incubator aging. Genotypes with resistance to incubator aging were slow imbibiton seeded types. Among slow imbiber genotypes, seeds of Merapi and Kalitur were more resistant to aging treatment than B-3770 and B-3618. Seed coat lignin also greatly influenced resistance to the aging. Most genotypes resistant to incubator aging were also resistant to seed deterioration in storage and vice versa.

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