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Genotypic Variation in Mung Beans Possessing Different Seed Coat Characteristics for Resistance to Incubator Weathering

Keragaman Resistensi Benih Kacang Hijau yang Berbeda Sifat Kulit Benihnya terhadap Incubator Weathering

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ABSTRACT

Under tropical conditions mung bean seed may lose vigor prior to harvest. A field trial was conducted to to determine mungbean genotypes with higher resistance to incubator weathering and to relate seed coat characteristics with resistance to incubator weathering. Nine mung bean genotypes of known seed weight, known lignin content in seed coat and known seed coat permeability were planted in research plots at Agriculture Faculty, Bengkulu University on April 10, 2003 in a split-plot arrangement, with incubator weathering period as a main plot and mung bean genotypes as a subplot with three replications.. At physiological maturity pods were harvested and kept in an incubator at 30^0C and 90% relative humidity for 0, 1 and 2 weeks. Their seeds were subjected to accelerated aging and electrical conductivity tests for seed quality evaluation. There were significant genotypic differences and a significant genotype x weathering period interaction for accelerated aging germination and seed leachate conductivity. A marked decline in accelerated aging germination and a significant increase in seed leachate conductivity for all genotypes was observed at 1 week weathering period and at this weathering period genotypic differences for resistnace to incubator weathering was revealed. Seeds of Bhakti, which had the highest percentage of hard seed exhibited the most resistant to incubator weathering. Some small-seeded genotypes were resistant to incubator weathering; others were susceptible. Regression analysis showed that accelerated aging germination was closely related (R^2 = 0,56*) to lignin content, while a inversely linear relationship (R^2 = 0,85**) was observed between conductivity of seed leachate and lignin content. Overall, there were genotypic differences in resistance to incubator weathering and this resistance was related to seed coat permeability, the degree of hardseededness, and lignin content in seed coat.

Keyword: mung bean, incubator weathering, lignin, seed coat permeability, hard seed

INTRODUCTION

High quality of mung bean [*Vigna radiata* (L.) Wilczek] seed is difficult to produce in the humid tropical regions due to its susceptibility to field weathering damage. Field weathering of seed is associated with unfavorable weather conditions during the ripening period and its occurrence was mainly due to moisture, in the form of high humidity and precipitation, and temperature (TeKrony *et al.,* 1980).

TeKrony et al. (1980) reported that seed viability was maintained at a relatively high level for 1 – 2 months following harvest maturity, but seed vigor began to decline within a few days after harvest maturity stage. In addition to a decrease in seed vigor, seed deterioration was also associated with the progressive loss of membrane integrity (Marwanto et al., 2003; Ching and Schoolcraft, 1968). Marwanto *et al.* (2003), working with soybeans and Ching and Schoolcraft (1968), working with crimson clover found that conductivity of seed leachate as a result of loss of membrane integrity was gretaer for aged seeds than for non-aged seeds.

In addition to physiological disruption by climatic factors, the cause of field weathering of seed is also associated with pathological infection such as Phomopsis infection (Dassou and Kueneman, 1984). However, the use of fungicides to control seed pathogens in the tropics including Phomopsis has met with a limited success (TeKrony *et al.*, 1980).

One of the most promising solutions to the problems of field weathering appears to be the development of cultivars that resist ‘weathering ‘ in the field. To provide genetic material for this purpose the identification of mung bean genotypes resistant to field weathering would be necessary. The common method used for this purpose in soybean is to delay harvest after plant maturity and then asses the seed quality (Dassou and Kueneman, 1984). When applied for mung beans; however, this method has several limitations. First, seed shatering was encountered when delayed harvest period was prolonged longer than 2 weeks. Second, seeds were subjected to different weathering period because pods did not mature at the same time. In an attempt to overcome these limitations, an incubator weathering or incubator aging method developed by Dassou and Kuenemann (1984) was employed. At this method pods at physiological maturity were harvested and kept at 30 ^{0}C and 90-95% relative humidity for 10 days. For mung bean seed, however, the exact period of incubator weathering effective to evaluate their resistance to field weathering is not well understood.

When subjected to delay in harvest, genotypic differences in mung beans for resistance to field weathering were observed (Marwanto, 2007b). The similar resistant differences was also reported by Marwanto (2003a; c) and Dassou and Kueneman (1984) in soybeans. Marwanto (2007b) further reported that the different resistance in mung beans was associated with impermeable seed coat or hard seed character. The similar resistant mechanism was also reported by Dassou and Kueneman (1984) and Miranda *et al.* (1980) in soybeans. In addition to hard seed character, lignin content in the seed coat, which differred among mung bean genotypes used in this study (Marwanto, 2007a) correlated with resistance to field weathering (Marwanto, 2007b). The similar resitant mechanism was also applicable for soybeans (Marwanto, 2003c; 2004). For mung bean seeds genotypic differences for resistance to incubator weathering and the role of seed coat characteristics on resistant to the weathering were not fully understood.

The objective of the study was (1) to determine mungbean genotypes with higher resistance to incubator weathering and (2) to relate seed coat characteristics with resistance to incubator weathering

MATERIALS AND METHODS

Nine mung bean genotypes of known seed size and known seed coat characteristics (seed coat lignin content 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 in a split-plot arrangement, with period of incubator weathering as a main plot and mung bean genotypes as a subplot 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 3-4 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.

At physiological maturity stage pods from each genotype was harvested The pods were considered to reach physiological maturity stage when the first time their seeds reached maximum dry weight. The time of occurrence of physiological maturity was determined by harvesting 10 pods of each genotype at approximately daily intervals and measuring seed dry weight. The first harvest for seed dry weight determination was made at a few days before the physiological maturity stage was reached. Seed dry weight was measured gravimetrically after drying the seeds for 24 hours at 105 ^{0}C.

Pods from each genotype harvested at physiological maturity were subjected to incubator weathering. The weathering treatment were imposed by placing sufficient pods harvested at physiological maturity stage (maximum dry weight/ seed) on a wire mesh tray of 20X5X2.5cm. The tray was placed inside a plastic box of 30X10X5cm filled with 100 mL of NaCl solution to create a

90% relative humidity. A 10-mm gap was maintained between the salt solution surface and the seed tray. The box was covered with airtight lid and kept in oven at 30 ^{0}C for a period of 0, 1 and 2 weeks. After being imposed by a certain period of incubator weathering, pods were taken out and dried at 30 ^{0}C for 5 days and their seeds were subjected to quality evaluation.

To determine the effect of the weathering treatments on seed quality, mung bean seed of each genotype from each incubation period was subjected to accelerated aging test for seed vigor evaluation and electrical conductivity test for leachate conductivity evaluation.

Seed Quality Evaluation

In the accelerated aging test, 50 seeds from each treatment-replicate were subjected to a period of accelerated aging, 42 ^{0}C and near 100% RH, for 48 hours prior to standard germination test. They 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 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. 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. At the final count, the remaining normal seedlings and abnormal seedlings, dead seeds and hard seeds were counted. The percentages of germination (normal seedlings) and hard seeds were calculated separately and combined into a total viable seed percentage

In electrical conductivity test, a weighed sample of twenty five seeds were soaked in 40 ml distilled water for 12 hours at a room temperature. The electrical conductivity of seed leakage was determined with a Cole-Parmer conductivitimeter (Chicago, Illinois) and was expressed in mmhos cm^{-2} g^{-1}.

Seed Coat Characteristics Evaluation

The seed coat lignin content expressed as %ADL (Acid Delinted Lignin) 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 was 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}.

Data Analysis

Analysis of variance of each variable was 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 accelerated aging germination and conductivity of seed leachate following all weathering treatments with percent lignin content of the seed coat of the tested genotypes was also determined.

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 ab	0,029 d	4,74
Bhakti	0,070 a	0,008 e	4,34
Betet	0,054 bc	0,071 a	3,82
Kenari	0,010 fg	0,047 bc	5,61
Parkit	0,050 cd	0,039 c	5,07
Merak	0,070 a	0,043 c	6,64
IPB,M/97-13-60	0,042 d	0,056 b	6,27
VC-3012-B	0,016 ef	0,068 a	5,46
Vr,1686-3-8-B	0,007 g	0,042 c	4,00

Keterangan: *: Means separated within the same columns by Least Significant Difference, P = 0,05; **: Weight in grams of 100 seeds at 12% moisture; ***: Data taken from Marwanto (2007a)

Table 2. Effects of period of incubator weathering on the percentage of germinated seed (GS), hard seed (HS) and total viable seed (TVS) of mung bean

Genotype	Incubator weathering period (weeks)								
	0			1			2		
	GS	HS	TVS	GS	HS	TVS	GS	HS	TVS
	----------- % ---------								
Gelatik	85,0	10,0	95,0 a A	64,0	10,0	74,0 b AB	29,3	4,0	33,3 c BC
Bhakti	82,7	10,0	92,8 a A	74,7	6,0	80,8 b A	54,0	6,7	60,8 c A
Betet	92,0	1,3	93,3 a A	62,0	2,0	64,0 b B	30,0	0,3	30,3 c C
Kenari	89,4	2,3	91,8 a A	46,7	1,3	48,0 b C	11,0	0,3	11,3 c D
Parkit	90,0	5,3	95,3 a A	76,7	2,0	78,8 b A	70,0	0,7	70,7 b A
Merak	83,0	7,0	90,0 a A	60,0	4,7	64,8 b B	64,0	0,7	64,7 b A
IPB.M/97-13-60	91,3	6,0	97,3 a A	40,0	4,0	44,0 b C	36,0	0,7	36,7 b BC
VC-3012-B	90,0	5,0	95,0 a A	40,0	6,0	46,0 b C	38,7	2,0	40,7 b B
Vr.1686-3-8-B	90,0	6,0	96,0 a A	48,0	5,3	53,3 b C	27,3	4,0	31,3 c BC

Keterangan: *Numbers within the same column followed the same capital letter differed significantly at ? = 0,05; **: Numbers within the same row followed the same small letter differed significantly at ? = 0,05

Table 3. Effects of period of incubator weathering on leachate conductivity of mung bean seeds

Genotype	Incubator weathering period (weeks)		
	0	1	2
	------- Leachate conductivity (mmhos cm $^{-2}$ g^{-1}) -------		
Gelatik	0,051 a A	0,189 b D	0,214 b D
Bhakti	0,049 a A	0,180 b D	0,276 c BCD
Betet	0,043 a A	0,190 b D	0,231 b CD
Kenari	0,039 a A	0,343 b AB	0,718 c A
Parkit	0,039 a A	0,258 b CD	0,343 c B
Merak	0,041 a A	0,226 b CD	0,220 b D
IPB,M/97-13-60	0,045 a A	0,289 b BC	0,333 b BC
VC-3012-B	0,050 a A	0,380 b A	0,660 c A
Vr,1686-3-8-B	0,052 a A	0,342 b AB	0,796 c A

Keterangan: *Numbers within the same column followed the same capital letter differed significantly at ? = 0,05; **: Numbers within the same row followed the same small letter differed significantly at ? = 0,05

RESULTS AND DISCUSSION

No rainfall occurred at physiological maturity when seeds of all genotypes were scheduled for harvest. Rainfall more than 5 mm only occurred a few days before the stage was attained (data not shown). This good environmental condition prior to physiological maturity contributed to a good seed quality for all genotypes. Simulated unfavorable conditions during incubator weathering mainly high humidity and high temperature contributed to seed deterioration of mung bean as indicated by a decrease in accelerated aging germination (Table 2) and an increase in seed leachate conductivity (Table 3). Averaged over genotypes, accelerated aging germination as an indicator of seed vigor were 94,0, 61,5 and 42,2% for the non-weathered, 1 week and 2 week weathered seeds, respectively. Average over genotypes, seed leachate conductivity was 0,045, 0,266, and 0,421 mmhos cm^{-2} g^{-1} for the non-weathered, 1 week and 2 week weathered seeds, respectively. These results indicated that deterioration in mung bean seeds as a result of incubator weathering was manifested by a decrease in germinative response after accelerated aging and an increase in conductivity of seed leachate. Other researchers working with different seeds have also reported the decline in seed quality associated with incubator weatheing. These included Marwanto (2007 b) in mung beans and Marwanto (2004) and Dassou and Kueneman (1984) in soybeans.

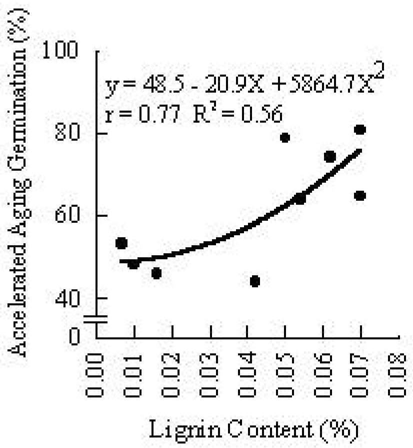

Figure 1. Relationship between seed coat lignin content and accelerated aging germination for nine mung bean genotypes

The analysis of variance showed that there was a significant genotype X weathering period interaction for accelerated aging germination and seed leachate conductivity. The significant genotype X weathering period interaction for both seed quality indicators was due to genotypic differences in the rate of decrease in seed vigor (Table 2) and in membrane damage (Table 3). As shown in Table 2 and 3, a marked decline in accelerated aging germination and a significant increase in seed leachate conductivity for all genotypes was observed at 1 week weathering period and at this weathering period genotypic differences for resistnace to incubator weathering was revealed.. Therefore, this weathering period could be used by breeders to determine differences in genotype's potential to resist seed deterioration during field weathering. Meanwhile, the 2 week weathering period caused extreme seed deterioration for all genotypes and masked their differences in resistance. The accelerated aging germination had dropped to below 80% for all genotypes and even to below 50%.for almost all genotypes.

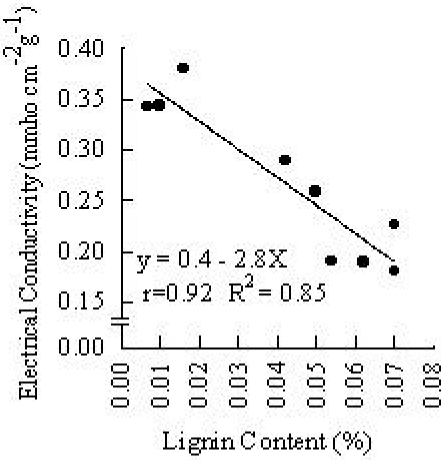

Figure 2. Relationship between seed coat lignin content and electrical conductivity for nine mung bean genotypes

Among the genotypes subjected to 1 week weathering period, Bhakti was identified as being the most resistant to weathering as indicated by the highest score of accelerated aging germination (>80%) (Table 2) and the lowest value of conductivity of seed leachate (Table 3).

The superior resistance of Bhakti to incubator weathering was apparently related to its expression of hardseededness, which tends to be higher than the others except for VC-3012-B. However, hardseededness was not the only mechanism of resistance in this study since VC-3012-B, which had the same percentage of hard seed as Bhakti had poor accelerted aging germination value. This result was supported by Dassou and Kueneman (1984), who reperted that soybean with good seed quality had hard seeds, but not all hard-seeded genotypes had a good seed quality.

Seed size appeared to play a role in resistance to incubator weathering among the genotypes tested in this study. Bhakti with the highest seed vigor had relatively small seeds, but other small-seeded genotypes such as Betet and Vr.1686-3-8-B had a poor seed vigor. This result emphasized that genotypes with good quality had relatively small seeds, but not all small-seeded genotypes had good seed quality. The similar result was also reported by Dassou and Kueneman (1984) for soybeans.

The superior resistance of small-seeded genotype such as Bhakti to incubator weathering was obviously related to seed coat permeability. This genotype, which was the most resistant to incubator weathering had the lowest seed coat permeability. While the other small-seeded genotype such as Betet, which was susceptible to incubator weathering had the highest seed coat permeability. This result indicates that resistance to incubator weathering for mung beans was related more to seed coat permeability than seed size. The similar result was also reported by Dassou and Kueneman (1984) for soybean. According to Kuo (1989) seeds with low seed coat permeability might gain their protection against incubator weathering from a seed coat which tends to imbibe water at slower rate than those with high seed coat permeability.

Lignin content in the seed coat expressed as %ADL also involved in protecting mung bean seeds from incubator weathering. Regression analysis showed that there was a quadratic relationship (R^2 = 0,56*) between accelerated aging germination to lignin content (Fig. 1), thus establishing positive influence of lignin content on maintaining seed vigor during incubator weathering. Meanwhile, an inversely linear relationship (R^2 = 0,85**) was observed between conductivity of seed leachate to lignin content (Fig. 2); the lower the lignin content in the seed coat, the higher the values of the electrical conductivity. The similar results were also reported by Marwanto (2007b) in mung bean and Marwanto (2003b, c) and Marwanto *et al.* (2003) in soybean. The positive effects of lignin on protecting mung bean seeds against incubator weathering was understood since lignin in the seed coat has impermeabilization characteristics and exerts an important effect on the capacity and velocity of absorption of water throughout the seed coat (McDougall *et al.*, 1997). The proposed reasons were in agreement with Kuo (1989), who reported that soybean seeds with low seed coat permeability tended to imbibe water at slower rate. The results obtained from this study showed that mung bean seeds with high lignin content tended to have low permeability (Table 1). Thus, the involvement of lignin in resistant mechanism of mung bean seeds to incubator weathering was through controlling seed coat permeability.

CONCLUSIONS

Mung bean genotypes differred significantly in their ability to maintain seed qaulity during incubator weathering. Some genotypes with good seed quality had relatively small seeds, but not all small-seeded genotypes had a good seed quality. Genotypic differences in resistance to incubator weathering was related more to seed coat permeability than seed size. Some genotypes with good seed quality had hard seeds, but not all hard-seeded genotypes had a good seed quality. Genotypic differences in resistance to incubator weathering were also related to seed coat lignin content, which was a characteristic that varied among mung bean genotypes. The accelerated aging responses as a measure of seed vigor appeared to be more sensitive indicators of deterioration in mung bean seeds during incubator weathering. than conductivity of seed leachate.

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