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Evaluation of Mung Bean Genotypes Possessing Different Seed Coat Characteristics for Resistance to Field Weathering

Evaluasi Ketahanan Genotipe Kacang Hijau dengan Sifat Kulit Benih yang Berbeda terhadap Deraan Cuaca

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ABSTRACT

Seed coat characteristics may be involved in protecting mung bean seeds from field deterioration during delayed harvest. A field trial was conducted to evaluate mungbean genotypes for resistance to field weathering and to relate seed coat characteristics with tolerance to field weathering. Eleven mung bean genotypes of known seed weight, known lignin content in seed coat and known seed coat permeability were used in these studies. The seeds were planted in research plots at Agriculture Faculty, Bengkulu University on April 10, 2003 in a split-plot arrangement, with delayed harvest as a main plot and mung bean genotypes as a subplot with three replications.. Each genotype was subjected to field weathering by delaying harvest the pods for 0, 2 and 4 weeks past harvest maturity stage.and their seeds were subjected to accelerated aging and electrical conductivity test for seed quality evaluation. Delayed harvest caused a significant decrease in accelerated aging germination and a significant increase in seed leachate conductivity. Averaged over genotypes, accelerated aging germination were 95,3, 92,0 and 50,1% for the non-delayed, 2 week delayed, and 4 week delayed harvests, respectively, while seed leachate conductivity was 0,045, 0,143, and 0,264 mmhos cm^{-1} g^{-1} for the non-delayed, 2 week delayed, and 4 week delayed harvests, respectively. There were significant genotypic differences and a significant genotype x delayed harvest interaction for accelerated aging germination and seed leachate conductivity. Accelerated aging germination was significantly reduced by 4 week delay in harvest for all genotypes, but not by 2 week delay in harvest. Seed leachate conductivity was also significantly increased at both 2 and 4 week delay in harvest for all genotypes. Germinative responses of all genotypes to accelerated aging at 4 week delay in harvest ranked them very well in term of resistance to field weathering. Seeds of Bhakti, which had the highest percentage of hard seed exhibited the most resistant to field weathering. Some small-seeded genotypes were resistant to field weathering; others were susceptible. Regression analysis showed that accelerated aging germination was positively correlated with lignin content ($y = 23{,}62 + 624{,}75X$ and $r = 0{,}86$), while an inverse relationship was observed between conductivity of seed leachate and lignin content ($y = 0{,}39 - 2{,}86 X$ and $r = 0{,}96$) Overall, genotypic differences in resistance to field weathering was related to seed size, the degree of hardseededness and lignin content in seed coat.

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

INTRODUCTION

Producing and maintaining good quality of mung bean seed [*Vigna radiata* (L.) Wilczek] in the humid tropical regions for planting purposes is difficult mainly due to its susceptibility to field weathering damage. According to TeKrony *et al.* (1980) field weathering of seed is associated with unfavorable weather conditions during the ripening period and under this condition seed vigor as well as viability deteriorates rapidly mainly due to moisture, in the form of high humidity and precipitation, and temperature.

TeKrony *et al.* (1980) stated 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 in which the seeds were soaked 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).

Genotypic differences for resistance to field weathering have been reported by Marwanto (2003a, c; 2004c) and Dassou and Kueneman (1984) for soybean seeds., but not yet for mung bean seeds. Their tolerance have been associated with impermeable seed coat or hard seed character (Dassou and Kueneman, 1984; Miranda *et al.*, 1980). This approach can possibly be applied for mung bean seeds because they also belongs to a seed kind with impermeable seed coat character (Marwanto, 2007).

In addition to hard seed character, seed resistance to field weathering in soybeans was also associated with lignin content in seed coat (Marwanto, 2003c; 2004b) due to its impermeabilization effects (Tavares *et al.*, 1987). The same resitant mechanism may also be applicable for mung bean seeds since lignin content in their seed coat was also genotypically different (Marwanto, 2007). The objective of the study was (1) to evaluate mungbean genotypes for resistance to field weathering and (2) to relate seed coat characteristics with resistance to field weathering

METHODOLOGY

Eleven 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 delayed harvest as main plots 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.

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 columns by Least Significant Difference, P = 0,05; **: Weight in grams of 100 seeds at 12% moisture

Table 2. Temperature (temp.), rainfall and relative humidity (RH) collected from Pulau Bai Weather Station, Bengkulu from May 20 to July 2 2007.

Date	Weather Condition			Date	Weather Conditions		
	Temp. (°C)	Rainfall (mm)	RH (%)		Temp. (°C)	Rainfall (mm)	RH (%)
May 20	27,2	0	81	June 11	27,0	0	81
May 21	26,4	0	83	June 12	27,1	0	84
May 22	26,3	trace	90	June 13**	27,0	0	88
May 23	27,1	0	90	June 14	26,0	5	84
May 24	26,9	0	89	June 15	26,8	0	89
May 25*	27,3	0	86	June 16	26,4	2	94
May 26	27,1	0	86	June 17	23,1	8	82
May 27	26,2	0	83	June 18**	25,8	0	82
May 28	26,1	0	84	June 19	31,8	13	82
May 29	26,4	0	85	June 20	26,9	9	76
May 30*	26,9	0	90	June 21	26,8	8	80
May 31	26,7	0	90	June 22	25,9	25	80
June 1	27,3	0	83	June 23	25,5	27	81
June 2	26,8	0	85	June 24	26,2	0	85
June 3	26,8	0	84	June 25	27,0	0	82
June 4	26,6	3	88	June 26	27,2	0	87
June 5	25,4	0	91	June 27***	25,9	0	81
June 6	26,4	8	86	June 28	27,0	0	86
June 7	25,7	8	87	June 29	26,8	0	82
June 8	26,4	8	87	June 30	26,5	0	82
June 9	26,5	0	83	Juli 1	27,0	0	85
June 10	26,5	0	83	Juli 2***	28,0	0	84

Keterangan : ^{x} : trace means rainfall less than 1 mm; ^{y} : 0 means no raifall; *: first harvest dates; **: second harvest dates; ***: third harvest dates

To evaluate its resistance to field weathering each genotype was subjected to field weathering by delaying harvest the pods for 0, 2 and 4 weeks past harvest maturity stage. After being imposed by a certain period of delayed harvest, pods were hand-harvested and their seeds were subjected to quality evaluation. The pods were considered to reach harvest maturity stage when more than 90% of them was dark brown or black in color and the first time their seeds dried to less than 14% moisture content. The time of occurrence of harvest maturity was determined by harvesting 10 pods of each genotype at approximately daily intervals and measuring seed moisture content. The first harvest for seed moisture determination was made at a few days before the harvest maturity stage was reached. Seed moisture (wet weight basis) was measured gravimetrically after drying the seeds for 24 hours at 105 °C.

At the end of each delayed harvest treatment, sufficient pods from each genotype were hand harvested to obtain 75 g of dry seed of high quality. The pods were then dried with heated air (<35 °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. To determine the effect of the weathering treatments on seed quality, mung bean seed of each genotype from each harvest was subjected to accelerated aging test for seed vigor evaluation and electrical conductivity test for conductivity of seed leachate evaluation.

Table 3. Effects of period of field weathering on the percentage of germinated seed (GS), hard seed (HS) and total viable seed (TVS) of mung bean.

Genotype	Field Weathering Period (weeks)								
	0			2			4		
	GS	HS	TVS	GS	HS	TVS	GS	HS	TVS
					---------- % ---------				
Gelatik	94,0	4,0	98,0 a* A**	68,0	14,6	82,6 b C	37,4	14,6	52,0 c DE
Bhakti	86,0	6,6	92,6 ab A	42,7	52,6	95,3 a A	36,0	48,0	84,0 b A
Betet	82,0	11,3	93,3 a A	60,0	36,0	96,0 a A	26,0	26,0	52,0 b DE
Kenari	79,3	12,3	91,6 a A	63,3	24,0	87,3 a BC	22,0	22,0	44,0 b E
Parkit	86,6	8,6	95,2 a A	83,3	8,3	91,6 a ABC	24,0	30,0	54,0 b CD
Merak	84,6	5,4	90,0 a A	62,0	36,0	98,0 a A	36,0	28,0	64,0 b B
IPB.M/97-13-60	91,3	6,0	97,3 a A	72,7	7,3	90,0 a AB	40,0	14,0	54,0 b CD
VC-3012-B	100,0	0,0	100,0 a A	63,3	30,7	94,0 a AB	8,6	22,0	30,6 b F
Vr.1686-3-8-B	92,6	4,0	96,0 a A	70,3	23,0	93,3 a AB	10,0	10,0	20,0 b G

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 $\alpha = 0,05$

Seed Quality Evaluation

In the accelerated aging test, 50 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. 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 100 mL 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 °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 leachate was determined with a Cole-Parmer conductivitimeter (Chicago, Illinois) and was expressed in mmhos cm^{-1} 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.

RESULTS AND DISCUSSION

Harvest schedule presented in Table 2 showed that genotypes of Gelatik, Bhakti, Betet, Kenari and Parkit reached harvest maturity stage at the same time on May 25, 2003, 5 days earlier than those of Merak, IPB.M/97-13-60, VC-3012-B and Vr.1686-3-8-B. Loss of seed due to shaterring problem occurred for all genotypes when pods were scheduled for the third harvest, but not for the first and second harvest. Less rainfall and high temperature a few days before the third harvest period were probably responsible for shaterring problem to occur.

Climatological data presented in Table 2 also showed that almost no rainfall occurred several days before 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

No rainfall occurred at harvest maturity, two and four weeks after harvest maturity stage when seeds of all genotypes were scheduled for harvest. Rainfall more than 5 mm only occurred from June 6 to June 8 and from June 19 to June 23, a few days before the second and the third harvest was scheduled. Dry condition in the field during seed maturation and delayed harvest was obvious due to dry season that occurred during this experiment.

Table 4. Effects of period of field weathering on leachate conductivity of mung bean seeds.

Genotype	Field Weathering Period (weeks)		
	0	2	4
	------- Leachate Conductivity (mmhos cm $^{-2}$ g^{-1}) -------		
Gelatik	0,051 a* A**	0,141 b C	0,214 c D
Bhakti	0,049 a A	0,097 b F	0,181 c E
Betet	0,043 a A	0,194 b A	0,220 b CD
Kenari	0,039 a A	0,177 b A	0,343 c B
Parkit	0,039 a A	0,125 b D	0,220 c CD
Merak	0,041 a A	0,154 b BC	0,189 c E
IPB.M/97-13-60	0,045 a A	0,186 b A	0,289 c C
VC-3012-B	0,050 a A	0,107 b EF	0,380 c A
Vr.1686-3-8-B	0,052 a A	0,104 b EF	0,342 c B

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 $\alpha = 0,05$

Delayed harvest significantly reduced seed vigor as indicated by reducing accelerated aging germination (Table 3) and and increasing seed leachate conductivity (Table 4). Averaged over genotypes, seed vigor as measured by accelerated aging germination were 95,3; 92,0 and 50,1% for the non-delayed, 2 week delayed, and 4 week delayed harvests, respectively. Average over genotypes, seed leachate conductivity was 0,045; 0,143, and 0,264 mmhos cm^{-1} g^{-1} for the non-delayed, 2 week delayed, and 4 week delayed harvests, respectively. These results indicate that deterioration in mung bean seeds as a result of field weathering was reflected 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 delayed harvest. These included Sukarman (1992) in mung bean, Marwanto (2004c) and TeKrony *et al.* (1980) in soybeans, and Marwanto (2004a) in cowpea.

Many researchers had agreed that the decline in soybean seed quality during delayed harvest was due to high temperature and humidity (Dassou and Kueneman, 1984; TeKrony *et al.*, 1980). In this study the decline in mung bean seed quality as reflected by a decrease in accelerated aging germination and an increase in seed leachate conductivity during delayed harvest was also due to high temperature and humidity. The mean air temperature and humidity during this period was above 26 ^{0}C and 85%, respectively (Table 2).

The analysis of variance revealed significant genotypic differences and a significant genotype X delayed harvest interaction for accelerated aging germination and seed leachate conductivity. The significant genotype X delayed harvest interaction for both accelerated aging germination and seed leachate conductivity was due to genotypic differences in the rate of decrease in accelerated aging germination (Table 3) and in membrane damage (Table 4) as harvest date was delayed. As shown in Table 3 and 4, accelerated aging germination was significantly reduced by 4 week delay in harvest for all genotypes but not by 2 week delay in harvest. Seed leachate conductivity was also significantly increased at both 2 and 4 week delay in harvest for all genotypes.

It has been known that delayed harvest has been successfully used to evaluate genotipic resistance to field weathering in soybean (TeKrony *et al.*, 1980) and in cowpea (Marwanto, 2004a). In this study this method was also applicable to evaluate seed tolerance to field weathering in mung beans. From the three different period of delayed harvest in this study, the non delayed harvest did not cause a marked decline in seed vigor for all genotypes. Their accelerated aging germination remained above 90%. for all genoytpes. There was also a little change in seed vigor at the 2 week delayed harvest. The only significant decline in seed vigor for almost all genotypes occurred at the 4 week delayed harvest. In other words the 4 week delayed harvest caused seed deterioration of almost all genotypes and revealed genotypic differences for resistnace to field weathering. Therefore, this method could be used by breeders to determine differences in genotype's potential to resist seed deterioration during field weathering.

Among mung bean genotypes included in this study, seeds of Bhakti, which had the highest percentage of hard seed exhibited the most resistant to field weathering as indicated by the highest score of accelerated aging germination (Table 3) and the lowest value of conductivity of seed leachate (Table 4). The superior resistance of this genotype to field weathering was apparently related to its expression of hardseededness, which tends to be higher than the others. The similar result was also reported by Chuntirapongsa (1992) and Dassou and Kueneman (1984) in soybeans.

Seed size appeared to play a role in resistance to field 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 superior resistance of small-seeded genotypes such as Bhakti to field weathering was obviously related to seed coat permeability. This genotype, which was the most resistant to field weathering had the lowest seed coat permeability. While the other small-seeded genotype such as Betet, which was susceptible to field weathering had the highest seed coat permeability. This result indicates that resistance to field 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 soybeans. According to Kuo (1989) seeds with low seed coat permeability might gain their protection against field 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 field weathering. Regression analysis produced a significantly high correlation between resistance of mung bean seeds to field weathering as reflected by accelerated aging germination and conductivity of seed leachate values with the lignin content in the seed coat. Accelerated aging germination was positively correlated with lignin content ($y = 23{,}62+624{,}75X$ and $r = 0{,}86$), thus establishing positive influence of lignin content on maintaining seed vigor during field weathering. Meanwhile, an inverse relationship was observed between conductivity of seed leachate and lignin content ($y = 0{,}39–2{,}86\ X$ and $r = 0{,}96$); 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 (2003b, c) and Marwanto et al. (2003) in soybean. The positive effects of lignin on protecting mung bean seeds from field 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 field weathering was through controlling seed coat permeability.

CONCLUSIONS

Among the genotypes included in this study, there were genotypic differences in resistance to field weathering and this resistance was related to the degree of hardseededness, seed size and lignin content in seed coat. The accelerated aging responses and conductivity of seed leachate as a measure of seed vigor appeared to be sensitive indicators of deterioration in mung bean seeds during field weathering.

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