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Identification of Soybean Genotypes for Pod Shattering Resistance Associated with Agronomical and Morphological Characters

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Abstract

A yield loss caused by pod shattering is one of the obstacles to the improvement of soybean productivity in tropical areas. The aim of this study was to identify the resistance of soybean genotypes to pod shattering as affected by agronomical and morphological characters. The field study was conducted in Malang, Indonesia, using 150 soybean genotypes. Data were collected on agronomical traits, the percentage of pod shattering, and pod morphological traits. Identification for shattering resistance was done as per oven dry method. Percentage of pod shattering was ranged from 0 % up to 100 % shattering with a mean of 58.11 %. Pod shattering was found to be negatively correlated with a number of pod per plant, the thickness of the pod, and Y/Z (seed weight and pod weight ratio). The Identification obtained 66 very highly susceptible genotypes, 19 susceptible genotypes, 19 moderate genotypes, 38 resistant genotypes, and 8 very resistant genotypes. Two of eight very resistant genotypes (G511H/Anj//Anj///Anj////Anj-6-11 and G511H/Anj//Anj///Anj////Anj-5-4) have high yield, medium maturity day and large seed size. Those lines could be used as gene donor for soybean varietal improvement for shattering resistance, and recommended to propose as new improved soybean varieties resistant to pod shattering in Indonesia.

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INTRODUCTION

Soybean is the third most important crops after rice and maize in Indonesia. Pod shattering is one of major constraint associated with soybean production in the tropical ecology of Indonesia. It is due to soybean cultivation mostly planted in the second dry season (June / July until September / October), and characterized by hot and dry conditions, thereby pod shattering increases could lead to serious seed yield losses. In the USA, it was reported that seed shattering is considered as one of the major problems for soybean growers under the ESPS (early soybean production system) conditions (Zhang & Bellalouli, 2012).

Pod shattering refers to the opening of mature pods along the dorsal or ventral sutures of the soybean pod and dispersal of seed as the crop reaches maturity, as well as during harvesting (Bhor et al., 2014) resulting in seed loss. The yield loss due to pod shattering in soybean may range from 34 % to 100 % (Tefera et al., 2009; Khan et al., 2013) depend on the harvesting after maturity, environmental condition (Zhang & Boahen, 2010), chemical composition of the pod wall (Fitriana et al., 2009), plant growth regulator (Gulluoglu et al., 2006), anatomical structure of the pod, and genetic factor of the variety (Suzuki et al., 2009). Pod shattering is induced by low humidity, high temperature, and rapid temperature changes (Mohammed, 2010). Furthermore, it was also enhanced when dry-weather followed rains at harvesting (Liu et al., 2016; Kuai et al., 2016).

Pod shattering behavior of soybean variety was found to be associated with other agronomic, morphological, and physiological characteristics (Kang et al., 2009; Adeyeye et al., 2014). A report by Tiwari & Bhatia (1995) stated that that the thickness and length of the bundle cap on the dorsal side of the soybean pod and thickness of the pod were negatively and significantly correlated with the degree of pod shattering. Another study revealed that genotype with the small pod, less width and low volume/weight of seed was tolerant to pod shattering (Bara et al., 2013).

Shattering in soybean was the most important trait among the important characteristics of the soybean plant. Hence, the development of highly shattering-resistant cultivars in soybean is important to prevent significant seed loss. A study elucidated that resistance to pod shattering is one of great economic benefit to farmers in the hot tropics and areas where machines are used for harvesting regarding reduced yield losses and planning for time and labour (Zuo et al., 2014). A survey conducted in Benue state, Nigeria, revealed that resistance to pod shattering was a prerequisite for the adoption of any variety by the farming communities (Sanginga et al., 1999). A report by Funatsuki et al. (2008) stated that highly shattering-resistant cultivars had been preferably developed and cultivated in some regions where soybean cultivation has been carried out on a large scale with the use of combine harvesters.

A genetic variability is an important tool for the selection in the soybean varietal improvement program. Therefore, the breeding program for shattering resistance in soybean should be considered the affecting factors, the availability of gene source and suitable selection method. Further investigations are therefore needed in those aspects. Nowadays, the major emphasis of soybean variety improvement in Indonesia is focused on producing high-yielding cultivar, as well as for early maturity variety. Moreover, pod shattering becomes one of the problems in the improvement of potential soybean production. Hence, it is important to develop a new improved variety with pod shattering resistance to minimize yield losses. The objective of the research was to identify the resistance of soybean genotypes to pod shattering as affected by agronomical and morphological characters.

METHODS

The type of soil was Entisol Association and Inceptisol, the elevation was 335 m above sea level, and Oldeman climate type was C3. The research materials consist of 150 soybean genotypes, and the research was arranged in a randomized complete block design with two replications. Grobogan, Anjasmoro, and Argomulyo were used as check varieties. Each genotype was planted in 1.2 m × 4.5 m plot size with 40 cm × 15 cm planting distance, two plants per hill. Pests and diseases were controlled optimally. Drainage was applied to maintain optimum soil moisture. Fertilization with 250 kg ha^{-1} Phonska, 100 kg ha^{-1} SP 36, and 1 t ha^{-1} organic fertilizer at planting time. The data was collected started from days after planting to harvesting period (calculated if 95 % of the leaves have turned yellow), number of pods (taken from average of five randomly sample plants), 100 seed weight (g), and seed yield (randomly taken from the seed yield per plot and converted to t ha^{-1}).

Pod shattering identification was done as per oven dry method. The evaluation of pod shattering resistance sample was taken randomly, that is 25 fully matured pods of each plot. The samples were drying to the oven at 30 °C for three days, and the temperature was elevated 10 °C for the next four days, respectively. On the 7th day, the numbers of shattered pods were counted, and every genotype was classified into a different category based on the percentage of shattered pods.

Observations on the pod morphological traits consists of: length of pod (A), width of pod (B), length-width ratio (A/B), width-length ratio (B/A), thickness of pod (C), width at mid part of the pod (D), thickness of pod and width ratio (C/B). Observation sample was from 25 fully matured pods of each plot, consists of: seed weight from 25 pods (X), pod wall weight of 25 pods (Y), pod weight (weight of pod wall and seed) of 25 pods (Z), seed weight and pod weight ratio (Y/Z), and pod wall weight and pod weight ratio (X/Z).

Data were subjected to analysis of variance (ANOVA) and continued with DMRT at 5% significance level. Data on pod shattering was subjected to arcsine-square root transformation before statistical analysis. The data were also subjected to Pearson correlation analysis to determine the relationship between the pod shattering and agronomical as well as pod morphological traits.

RESULTS AND DISCUSSION

Analysis variance elucidated a significant variation in all agronomic and morphological pod characteristics, except for a number of pods per plant and pod width. Pod shattering also showed significant variability among genotypes (Table 1). The significant value revealed the existence of genotypic differences among the genotypes tested. The coefficient of variation (CV) ranged from 1.90% to 27.16%.

Mean, range, and standard deviation for observed traits are presented in Table 2. The performances of different soybean genotypes under field conditions was indicated by days to maturity, a number of pod per plant, 100 seed weight, seed yield. Days to maturity ranged from 76 d to 84 d with a mean of 79 d. Days to maturity is classified into late maturity (> 90 d), medium maturity (80 d to 90 d), and short maturity (< 80 d), thus all the observed genotypes including to early and medium maturity. Early maturing soybean provides many benefits, i.e. minimizing the yield loss due to drought stress, and increase the cropping intensity within a year (Krisnawati & Adie, 2008).

A number of pod per plant ranged from 27 to 66 with a mean of 42. The seed size, which reflected by 100 seed weight, consists of medium

Table 1. The scoring rate was as follows (Krisnawati & Adie, 2017)

Score	Description	Category
1	No pod shattering	Very Resistant
2	< 25% pod shattering	Resistant
3	25 – 50% pod shattering	Moderately Resistant
4	51 – 75% pod shattering	Highly Susceptible
5	> 75% pod shattering	Very Highly Susceptible

Table 2. Analysis of variance of 150 soybean genotypes.

Parameter	Mean Square		CV (%)
	Replication	Genotype	
Days to maturity (d)	41.813**	8.691**	1.90
Number of pod/plant	55.987ns	100.747ns	23.28
100 seed weight (g)	1.594ns	4.092**	5.85
Seed yield (t ha^{-1})	3.257**	0.439**	19.05
Length of pod (cm)	0.102ns	0.244**	7.19
Width of pod (cm)	0.108*	0.022ns	12.86
Width at mid part of pod (cm)	0.033**	0.005**	5.88
Thickness of pod (cm)	0.068ns	0.312**	7.29
Pod shattering (%)	0.604ns	22.833**	27.16
Weight of 25 seeds (g)	0.002ns	3.361**	10.73

CV = coefficient of variation, **= significant at 1 % probability level (p < 0.01), ns = not significant and large seeded. Seed yield varied from low yield (0.69 t ha^{-1}) to relatively high yield (2.96 t ha^{-1}) with a mean of 2.06 t ha^{-1}. In Indonesia, soybean seed size is divided into three categories: small (< 10 g per 100 seeds), medium (10 g to14 g per 100 seeds), and large size (> 14 g per 100 seeds) (Adie & Krisnawati, 2007). In this study, the average seed size was large seeded. Large seeded-size soybean is desirable trait in tempeh industry because it will produce tempeh with large volume (Krisdiana, 2005).

Observations on the physical traits consist of parts of the pod, i.e. the length of the pod, width of the pod, the width at mid part of the pod, the thickness of pod, and ratio of pod parts. A research by Bara et al. (2013) showed that those traits showed high genetic advance, which means that its phenotype reflects the genotype or assuming the absence of environmental effects. Hence, the selection for a specific genotype will be accurate within the limits imposed by the environmental effects. A further explanation by Rohman & Hussain (2003), a high genetic advance was associated with high value of heritability indicating additive gene effect in controlling the characters.

Percentage of pod shattering had a broad range; it was from no shattering up to 100% shattering. The mean shattering was 58.11%. The weight of seed from 25 samples of pods varied from 8.16 g to 15.92 g, with an average of 11.38 g. The weight of the pod wall also measured, and it ranged from 2.94 g to 7.24 g. Hence, the pod weight total (the weight of pod wall and seed) ranged from 11.09 g to 22.57 g (with a mean of 15.89 g). According to Bara et al. (2013), the thickness of pod as one of pod traits was more reliable for selection for improvement by simple selection procedure. This due to the trait was less influenced by the environment.

Identification for shattering resistance

Pod shattering as one of the major constraints in soybean could reduce the yield potential considerably. As a consequence, the management of pod shattering is great importance for achieving higher productivity. Furthermore, identification of resistant genotypes to pod shattering is one of the most important aspects of the management of pod shattering. However, the other countries (USA, Uganda, and Nigeria) have released soybean variety with pod shattering resistant, for example, 'Maksoy 1N' and 'Maksoy 2N' (Anonim, 2014a), Glenn (Anonim, 2014b), and TGX 1448-2E (Mohammed, 2010). In the present study, 150 soybean genotypes were evaluated for pod shattering resistance under laboratory condition. The pod shattering resistance

Table 3. Mean, range, and standard deviation for observed traits of 150 soybean genotypes.

Observation	Mean	Min	Max	SD
Days to maturity (d)	79	76	84	2.08
Number of pod per plant	42	27	66	7.10
100 seed weight (g)	15.80	13.18	22.13	1.43
Seed yield (t ha^{-1})	2.06	0.69	2.96	0.47
Length of pod (A) (cm)	4.36	3.55	5.32	0.34
Width of pod (B) (cm)	1.04	0.87	2.02	0.10
Width at mid part of pod (D) (cm)	0.91	0.75	1.05	0.05
Thickness of pod (C) (cm)	0.57	0.45	0.67	0.04
A/B	4.21	2.39	5.24	0.41
D/A	0.21	0.18	0.25	0.02
B/A	0.24	0.19	0.42	0.03
C/B	0.55	0.29	0.66	0.04
Pod shattering (%)	58.11	0.00	100.00	39.25
Weight of 25 seeds (X)	11.38	8.16	15.92	1.28
Weight of pod wall of 25 pods (Y)	4.51	2.94	7.24	0.77
Pod weight of 25 pods (Z)	15.89	11.09	22.57	1.92
X/Z	0.72	0.62	0.79	0.02
Y/Z	0.28	0.21	0.38	0.02

Min = minimal value, max = maximal value, SD = standard deviation was classified into five categories (Fig.1) with pod shattering percentage ranged from 0% to 100%. The genotypes resistance consists of very highly susceptible (66 genotypes or 44%), highly susceptible (19 genotypes or 12.67%), moderate (19 genotypes or 12.67%), resistant (38 genotypes or 25.33%), and very resistant (8 genotypes or 5.33%).

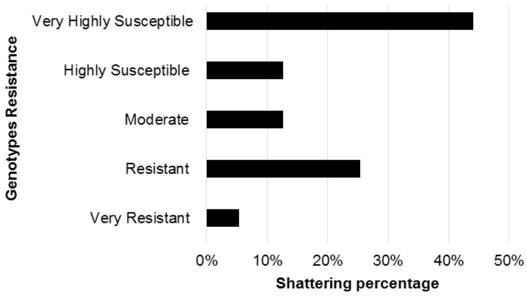

Figure 1. Pod shattering resistance category of 150 soybean genotypes.

The very resistant genotypes in this research showed no shattered pods. This finding is in line with research by Tukamuhabwa et al. (2002) which found three soybean genotypes (TGx 1448-2E, Duiker and Nam 2) demonstrated a high level of shattering resistance by showing no loss over the harvesting period. Thus, the use of resistant varieties was recommended as good sources of resistance in breeding for shattering resistance, and the use of susceptible varieties should be avoided since they start shattering on commencement of maturity resulting high yield loss. Another study by Khan et al. (2013) found that pod shattering percentage ranged from 8.7% (Himsoy-1560) to 93.3% (Punjab-1), and there is no variety resistant to pod shattering. Screening for shattering resistance by Bara et al. (2013) showed that shattering percentage ranged from 0.6730% (JSM 170) to 67.05% (JSM 131) with a mean of 19.11%. He also found that the rate of seed shattering accelerated after 7 days, and enhanced with the age of the matured pod. With the age of the plant, pod shattering is also influenced by the high temperature at the time of maturity. Screening for pod shattering resistance using oven method by Krisnawati et al. (2015), obtained the various degree of shattering (0% to 80%). Another study by Krisnawati & Adie (2016) found a number of shattered pods in the laboratory (oven method) ranged from 7 to 26 pods (22.2% to 87.2%). Enhancement in shattering resistance may promote productivity, harvesting of uniformly ripe seeds, efficiency of seed recovery and improved oil extraction. Moreover, it also promote the adjustment in harvesting and threshing time; reduction in cost of production, problem of volunteer plants (Morgan et al., 1998), and longevity of seed (Bara et al., 2013).

(A) (B)

Figure 2. Identification for pod shattering resistance using oven method; (A) Susceptible genotype; (B) Resistant genotype

Table 3 showed the effect of different agronomic parameters and morphological characteristics of soybean pod on pod shattering. Pod shattering behavior of soybean variety was reported to be associated with other agronomic characteristics. Adeyeye et al. (2014) observed many variabilities existed regarding vegetative growth, seed yield and shattering ability among the varieties tested. It revealed the existence of genotypic differences among varieties. In this research, the 100 seed weight, width of the pod, and width at mid part of the pod had no significant effect on a number of shattered pods per plant, which indicated that these parameters would not be useful as an index for pod shattering selection. Research by Tsuciya (1987) also found that 100 seed weight had no significant effect on pod shattering, whereas Bara et al. (2013) reported a significant and positive association of shattering percentage with pod width and width at mid part of the pod.

Furthermore, pod shattering was found to be negatively correlated with number of pod per plant, thickness of the pod, and Y/Z ratio. It implied that the increasing in the number of pod per plant, the thicker of the thickness of the pod, and the higher of Y/Z ratio will have a lower pod shattering, respectively. Meanwhile, the length of the pod and X/Z ratio were found to be significantly correlated to pod shattering. It means that the longer length of the pod, the pod shattering is also enhanced; as well as an increase of pod wall weight will result in a higher pod shattering percentage. This finding is in agreement with Adeyeye et al. (2014) and Bhatia & Tiwari (1994), which recommended the large seed (bigger diameter) and pod thickness as reliable index and indicator in selecting for shattering in soybean breeding program. Similarly, the newest study by Krisnawati & Adie (2017) revealed that pod length is one of the essential factors associated with pod shattering resistance, as well as pod wall thickness. However, this is not in agreement with the earlier report by Morgan et al. (1998) which stated that genotype with the small pod (with less width and weight of periphery region) and low volume/weight of seed has a low shattering percentage. The knowledge of correlation existing between characters is of great use in breeding programmes to easily identify those characters that may use as selection indices (Adeyeye et al., 2014).

In this study, the thicker pod and the higher of Y/Z ratio (larger seed size) will result to a lower pod shattering. Various studies of pod anatomy in detail have been conducted, and certain anatomical structures of the soybean pod have been recognized as important for resistance to shattering. Examination of the dehiscence zone of soybean pod and the expression analysis of the soybean endo polygalacturonase transcript revealed that the endo polygalacturonase was primarily found in dehiscence-related tissue and was presumably involved in the breakdown of the middle lamella before dehiscence (Christiansen et al., 2002). A study by Dong et al. (2014) revealed that the excessively lignified fiber cap cells (FCC) with the abscission layer unchanged in the soybean pod ventral suture as the key cellular feature of the shattering-resistant trait. Meanwhile, Funatsuki et al., (2014) revealed important aspects of pod shattering, namely, the dehiscing force and the associated regulatory gene.

The agronomic characters of genotypes with very resistant category were presented in Table 4. The eight selected genotypes have maturity from 78 days to 83 days, 100 seed weight ranged from 14.06 g per 100 seeds to 15.84 g per 100 seeds, and the seed yield ranged from 1.31 t ha^{-1} to 2.60 t ha^{-1}.

All the check varieties have short maturing day and large seed size. The popular variety of Anjasmoro was categorized as resistant to pod shattering. Meanwhile the varieties of Grobogan and Argomulyo categorized as very high susceptible, respectively.

The incorporation of high yielding and pod shattering resistant is one of pursued goal in Indonesian soybean breeding programmes. In this study, the genotype with the shortest maturing day was G511H/Anjasmoro//Anjasmoro-5-6, but it produced a low yield (1.70 t ha^{-1}). There were two high yielding genotypes (G511H/Anj//Anj///Anj////Anj-6-11 and G511H/Anj// Anj///Anj////Anj-5-4), higher than the check cultivars used. Both of lines have characteristics of medium maturity day, large seed size, and produced a yield of 2.52 t ha^{-1} and 2.60 t ha^{-1}, respectively. Since pod shattering is a qualitative heritable trait (Yamada et al., 2009; Mohammed, 2010; Sujata et al., 2012), thus these lines could be used as donor for shattering resistance, or could be proceed to the next selection step of breeding to be released as new soybean varieties with high yielding and pod shattering resistance, considering that those desirable characteristics are important for tropical area of Indonesia.

Table 4. Correlation analysis among agronomical, morphological characters and shattering percentage of 150 soybean genotypes.

Characteristic	1	2	3	4	5	6	7	8	9
(1) Pod shattering	1								
(2) Number of pod per plant	-0.171*	1							
(3) 100 seed weight	0.092ns	-0.210**	1						
(4) Length of pod (A)	0.440**	0.354**	-0.246**	1					
(5) Width of pod (B)	-0.139ns	0.124ns	-0.093ns	0.193*	1				
(6) Width at mid part of pod (D)	0.027ns	0.408**	-0.156ns	0.411**	0.277**	1			
(7) Thickness of pod (C)	-0.441*	0.291**	-0.069ns	-0.110ns	0.326**	0.274**	1		
(8) Y/Z ratio	-0.3375**	-0.1566ns	0.0792ns	-0.3942**	0.057ns	-0.1580**	0.1585ns	1	
(9) X/Z ratio	0.3374**	-0.156ns	0.079ns	-0.394**	-0.057ns	-0.158**	0.158ns	-1.000**	1

Y/Z = seed weight and pod weight ratio, X/Z = pod wall weight and pod weight ratio, **= significant at 1 % probability level ($p < 0.01$), * = significant at 5 % probability level ($p < 0.05$), ns = not significant.

Table 5. Agronomic characters of pod shattering resistance genotypes.

Genotypes	Days to maturity (d)	100 seeds weight (g)	Yield (t ha^{-1})	Category
G 511 H/Anjasmoro-1-7	81	14.60	1.98	VR
G 511 H/Anjasmoro//Anjasmoro-5-2	81	14.96	1.61	VR
G 511 H/Argom//Argom-2-1	81	14.73	2.15	VR
G 511 H/Anjasmoro//Anjasmoro-5-6	78	14.20	1.70	VR
G 511 H/Anj//Anj///Anj////Anj-6-11	82	15.84	2.52	VR
G 511 H/Anj//Anj///Anj////Anj-6-12	84	14.06	1.32	VR
G 511 H/Anjasmoro//Anjasmoro-5	81	15.69	2.03	VR
G 511 H/Anj//Anj///Anj////Anj-5-4	83	14.80	2.60	VR
Check varieties:				
Anjasmoro	79	14.65	2.37	R
Grobogan	77	18.71	1.46	VHS
Argomulyo	77	15.49	2.41	VHS

VR = very resistant, R = resistant, VHS = very highly susceptible

CONCLUSION

Pod shattering elucidated significant variability among genotypes. The genotypes resistance consists of very highly susceptible (66 genotypes), highly susceptible (19 genotypes), moderate (19 genotypes), resistant (38 genotypes), and very resistant (8 genotypes). The thicker of the thickness of the pod and the higher of Y/Z ratio (larger seed size) will result to a lower pod shattering. Two very resistant genotypes (G511H/Anj//Anj///Anj////Anj-6-11 and G511H/Anj// Anj///Anj////Anj-5-4) have high yield, medium maturity day and large seed size. Those lines could be proposed as new improved soybean varieties in Indonesia.

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