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Genetic variability of soybean (*Glycine max* L. Merrill) genotypes for pod shattering resistance

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Abstract. Consumer demand for soybean (*Glycine max* L. Merrill) is not only high yielding variety, but also must be in accordance with industrial preferences, primarily for its shattering resistance. A total of 16 soybean lines were evaluated in Nganjuk, East Java, Indonesia from Feb to May 2018. The pod shattering evaluation was using oven-dry method. All tested lines have large seed size, except SAT-Ng-A4. Five lines have early days to maturity (< 80 d). The highest yielding line and large seeded size was SAT-Ng-AG-3 (4.06 t ha⁻¹), but it has medium maturity. Pod shattering evaluation which using a gradient system of temperature showed that oven temperature of 50 °C and 60 °C were able to differentiate the soybean resistance to pod shattering. Three very resistant lines and one resistant line were obtained after exposed to 60 °C. The soybean pods which allowed to dry at room temperature for 9 d and exposed to high temperature (80 °C) showed high variability on shattering resistance. Three resistant lines (SAT-Ng-A4, SAT-Ng-6-13, and SAT-Ng-5-5) were obtained from screening on both oven methods. Those three lines with high yield, large seed size, and medium maturity can be used as parental lines to improve soybean shattering resistance in the breeding program.

Keywords: Oven-dry method, maintain productivity, promising lines, tropical environment, yield losses

1. Introduction

Soybean (*Glycine max* L. Merrill) is the most important legume crop in Indonesia. The soybean domestic demand increases each year, but it still cannot be fulfilled by the domestic production. The constraints in increasing soybean national production is the losses due to pests' infestation and pod shattering. The most inexpensive and efficient effort to reduce yield loss due to pod shattering is by providing pod shattering resistant variety.

The soybean yield losses due to pod shattering vary among countries. IITA (in Khan et al.) [1] reported the yield losses ranged from 34 % to 100 %, whereas Tiwari & Bhatnagar (in [2]) reported the range of yield losses was 34 % to 99 %. The amount of yield losses caused by pod shattering were determined by environmental factors (rainfall, humidity, and temperature) and genetic factor [3–5]. The environmental factors caused pod shattering were consisted of dry weather, low humidity, high temperature, rapid temperature changes, and alternating wetting and drying [6].

Eliminating the environmental factor influencing the pod shattering is not a simple matter. The most ideal alternative is by providing soybean variety resistant to pod shattering. Krisnawati & Adie



[7] screened the resistance of thirty soybean genotypes to pod shattering, and successfully obtained thirteen resistant genotypes. It was also reported that pod wall thickness and pod length were play important roles in determining the soybean resistance to pod shattering.

Soybean resistance to pod shattering was reported to be controlled by genetic factor [8–10]. Each genotype has different resistance patterns. In canola (*Brassica napus* L.), the hemicellulose content was considered as an important factor in determining pod shattering resistance [11]. Also, pod shattering resistance also controlled by genetic factor [12]. The essential factors within soybean development for pod shattering resistance, beside determined by the availability of the gene source, is also the selection method which will be used in the screening on the pod shattering resistance. Krisnawati & Adie [6, 7] used oven-dry method to select soybean genotypes resistant to pod shattering. Furthermore, the using of oven dry method allows for simultaneous selection of large numbers of samples, faster, and provide a uniformity environment.

The provision of soybean variety for the tropics (Indonesia), is not solely for high yield and shatter resistant, but also must be combined with other agronomical attributes which in accordance with industrial preferences. This is due to soybeans are mostly used as raw material in industry of tempeh, hence the characters needed are early days to maturity and large-seeded size. The aims of the research were to evaluate the resistance of several soybean genotypes to pod shattering using oven-dry method, and to identify the performance of yield and yield components.

2. Materials and method

The research materials were 16 soybean genotypes, including the released varieties of Anjasmoro (pod shatter resistant) and Dega 1 (large seeded size and early maturity) as checks (Table 1). The field experiment was conducted in Nganjuk (East Java, Indonesia). The evaluation for shattering resistance using oven dry method was conducted in Plant Breeding laboratory of Indonesian Legume and Tuber Crops Research Institute.

Table 1. Description of 16 soybean genotypes

No	Genotype	Pedigree	Source ^a	Remark
1	SAT-Ng-8-5	G511 × Anjasmoro	ILETRI	Promising line
2	SAT-Ng-A4	G511 × Anjasmoro	ILETRI	Promising line
3	SAT-Ng-SBG-3	Anjasmoro × (Argomulyo × Lokal Jateng)	ILETRI	Promising line
4	SAT-Ng-1-1	G511 × Anjasmoro	ILETRI	Promising line
5	SAT-Ng-3-3	G511 × Anjasmoro	ILETRI	Promising line
6	SAT-Ng-6-13	G511 × Anjasmoro	ILETRI	Promising line
7	SAT-Ng-44-7	G511H × Argomulyo	ILETRI	Promising line
8	SAT-Ng-25	Mutiara × Argomulyo	ILETRI	Promising line
9	SAT-Ng-12	Anjasmoro × (Argomulyo × Lokal Jateng)	ILETRI	Promising line
10	SAT-Ng-GH-3	Grobogan × G100H	ILETRI	Promising line
11	SAT-Ng-5-5	G511 × Anjasmoro	ILETRI	Promising line
12	SAT-Ng-AG-3	Lokal Jateng × (Sinabung × Argomulyo)	ILETRI	Promising line
13	SAT-Ng-44-6	G511H × Argomulyo	ILETRI	Promising line
14	SAT-Ng-A-4	Argopuro × G100H	ILETRI	Promising line
15	Anjasmoro	-	ILETRI	Released variety
16	Dega 1	-	ILETRI	Released variety

^aILETRI = Indonesian Legume and Tuber Crops Research Institute

The field research was arranged in a randomized block design with 16 treatments and four replications. Each genotype was planted in 2.4 m × 4.5 m plot size, with 40 cm × 15 cm planting distance, two seeds per hill. Plants were fertilized with 250 kg Phonska and 100 kg SP-36 which applied entirely before planting.

When plants at R8 stage (full maturity), ten sample plants were randomly taken to be used for shattering evaluation using oven-dry method in the laboratory. The oven dry method consisted of two treatments of temperature, i.e. gradient temperature and high temperature. The gradient temperature method was followed a method by Krisnawati & Adie [7]. The high temperature method was done by allowed the sample plants to equilibrate for 3 d, 6 d, and 9 d at room temperature after which the sample pods were subjected to oven drying at 80 °C for 12 h.

The observed parameters of field experiment included days to maturity, 100 seed weight, and seed yield. Pod shattering percentage was calculated as = (number of shattered pods/number of pods) \times 100 %. The degree of pod shattering was classified as follows: very resistant (0 % shattering), resistant (1 % to 10 % shattering), moderately resistant (11 % to 25 % shattering), moderately susceptible (26 % to 50 %), and very susceptible (> 50 % shattering) [13].

3. Result and discussion

3.1. The analysis of variance

The analysis of variance showed that genotype was significant for all characters studied (table 2). There was difference between genotypes in the characters of days to maturity, seed size, and seed yield. Similarly, the use of gradient temperature starting from 40 °C showed significant pod shattering between genotypes. Furthermore, the use of different duration of the equilibration at room temperature also showed differences in shattering between genotypes.

Table 2. Analysis of variance for agronomic and pod shattering characteristics of 16 soybean genotypes

Character	Mean square	
	Replication	Genotype
Days to maturity (d)	0.250 ^{ns}	11.150 ^{**}
100 seed weight (g)	1.800 ^{ns}	11.955 [*]
Seed yield (t ha ⁻¹)	0.046 ^{ns}	0.269 [*]
Pod shattering at 40 °C	3.595 ^{ns}	8.843 ^{**}
Pod shattering at 50 °C	6.514 ^{ns}	39.340 ^{**}
Pod shattering at 60 °C	6.548 ^{ns}	56.080 ^{**}
Pod shattering by 3 d equilibration duration	6.616 ^{ns}	43.445 ^{**}
Pod shattering by 6 d equilibration duration	2.676 ^{ns}	45.860 ^{**}
Pod shattering by 9 d equilibration duration	0.778 ^{ns}	16.231 ^{**}

* = significant at 5 % probability level, ** = significant at 1 % probability level, ns = not significant

A high plants performance were showed in the field experiment. The range of seed yield was 3.15 t ha⁻¹ to 4.06 t ha⁻¹ with an average of 3.53 t ha⁻¹. The yield of check variety of Anjasmoro (3.71 t ha⁻¹) was higher than Dega 1 (3.24 t ha⁻¹) (table 3). Three soybean genotypes produced higher yield than Anjasmoro, namely SAT-Ng-AG-3 (4.06 t ha⁻¹), SAT-Ng-A-4 (3.93 t ha⁻¹), and SAT-Ng-44-7 (3.81 t ha⁻¹). The days to maturity vary from 78 d to 84 d with an average of 80 d. The days to maturity of Anjasmoro and Dega 1 were 79 d and 84 d, respectively. There were four genotypes with similar days to maturity with Dega 1.

The soybean seed size which was measured based on 100 seed weight, the range was from 13.55 g to 20.56 g per 100 seeds. The seed size of Dega 1 was largest than others, reached 20.56 g per 100 seeds. There was no tested genotypes with similar seed size with Dega 1. Moreover, the seed size of Anjasmoro was 15.12 g per 100 seeds, and there were six genotypes with seed size larger than Anjasmoro.

Observing the best three genotypes which produce yield over Anjasmoro, those genotypes have large seed size but medium maturity. The days of maturity of the highest yield genotype (SAT-Ng-AG-3) was 80 d.

Table 3. Phenotypic comparison of 16 soybean genotypes.

No	Genotype	Days to maturity (d)	100 seed weight (g)	Yield (t ha ⁻¹)
1	SAT-Ng-8-5	81	15.05	3.56
2	SAT-Ng-A4	81	13.55	3.56
3	SAT-Ng-SBG-3	79	17.63	3.37
4	SAT-Ng-1-1	80	14.68	3.59
5	SAT-Ng-3-3	81	15.40	3.24
6	SAT-Ng-6-13	82	14.78	3.40
7	SAT-Ng-44-7	81	14.61	3.81
8	SAT-Ng-25	79	16.52	3.59
9	SAT-Ng-12	78	16.54	3.21
10	SAT-Ng-GH-3	78	17.76	3.56
11	SAT-Ng-5-5	82	15.15	3.49
12	SAT-Ng-AG-3	80	15.58	4.06
13	SAT-Ng-44-6	81	17.68	3.15
14	SAT-Ng-A-4	82	15.09	3.93
15	Anjasmoro	84	15.12	3.71
16	Dega 1	79	20.56	3.24
Average		80	15.98	3.53

3.2. Pod shattering based on gradient temperature method

Evaluation of soybean resistance to pod shattering by using oven dry method with gradient temperature was presented in Table 4. The use of oven temperature of 30 °C for 3 d does not caused pod shattering. Such temperature was intended to equilibrate the moisture content of the pod. The variation in pod shattering vary from moderately resistant to very resistant when pods were exposed to oven temperature of 40 °C. However, this temperature was not shown the greater resistance of each genotype. If the temperature was increased to 50 °C, then the 16 tested genotypes have started to differentiate to very resistant (seven genotypes), moderately resistant (two genotypes), moderately susceptible (three genotypes), and very susceptible (four genotypes). At 50 °C, Anjasmoro was still categorized as resistant, but Dega 1 has already become very susceptible. At 60 °C, the soybean resistance scattered into four groups, i.e. very resistant (six genotypes), resistant (one genotype), moderately susceptible (one genotype), and very susceptible (eight genotypes). This results showed that the use of oven dry at 60 °C was considered as ideal temperature for selection on pod shattering resistance. Selection on those temperature will become a stringent selection, thus the selected genotypes will potentially have resistance to pod shattering in the field.

Table 4. Number of shattered pods using oven dry method.

No	Genotype	Pod shattering (%)							
		30 °C	Criteria	40 °C	Criteria	50 °C	Criteria	60 °C	Criteria
1	SAT-Ng-8-5	0.00	VR	0.00	VR	0.00	VR	0.00	VR
2	SAT-Ng-A4	0.00	VR	0.00	VR	0.00	VR	0.00	VR
3	SAT-Ng-SB-3	0.00	VR	5.83	R	24.17	MR	45.00	MS
4	SAT-Ng-1-1	0.00	VR	0.00	VR	0.00	VR	0.00	VR
5	SAT-Ng-3-3	0.00	VR	0.00	VR	0.00	VR	0.00	VR
6	SAT-Ng-6-13	0.00	VR	0.00	VR	0.00	VR	0.00	VR
7	SAT-Ng-44-7	0.00	VR	30.00	MS	78.33	VS	100.00	VS
8	SAT-Ng-25	0.00	VR	10.00	R	30.83	MS	59.17	VS
9	SAT-Ng-12	0.00	VR	13.33	MR	66.67	VS	65.83	VS

Table 4 continue to the next page

Table 4. Continued

No	Genotype	Pod shattering (%)							
		30 °C	Criteria	40 °C	Criteria	50 °C	Criteria	60 °C	Criteria
10	SAT-Ng-GH-3	0.00	VR	3.33	R	38.33	MS	75.00	VS
11	SAT-Ng-5-5	0.00	VR	0.00	VR	0.00	VR	0.00	VR
12	SAT-Ng-AG-3	0.00	VR	16.67	MR	61.67	VS	96.67	VS
13	SAT-Ng-44-6	0.00	VR	10.00	R	47.50	MS	67.50	VS
14	SAT-Ng-A-4	0.00	VR	0.00	VR	12.50	MR	64.17	VS
15	Anjasmoro	0.00	VR	0.00	VR	0.00	VR	5.00	R
16	Dega 1	0.00	VR	15.00	MR	60.00	VS	70.00	VS
Average		0.00		6.51		26.25		40.52	

VR = very resistant, R = resistant, MR = moderately resistant, MS = moderately susceptible, VS = very susceptible

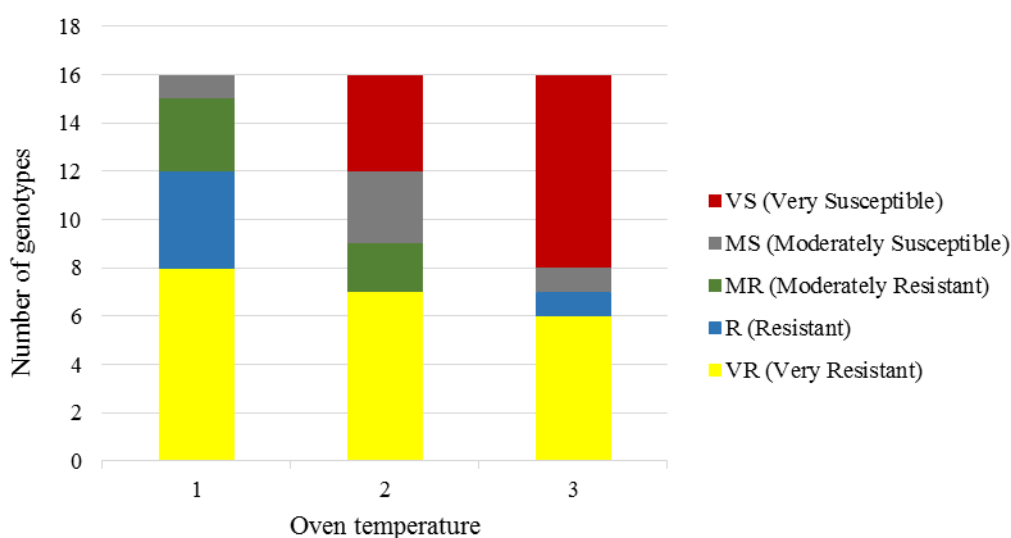


Figure 1. The degree of pod shattering of 16 soybean genotypes based on oven dry method (Oven temperature: 1 = 40 °C, 2 = 50 °C, 3 = 60 °C).

3.3. Pod shattering based on high temperature

In this study, the examination for soybean resistance to pod shattering by placing the soybean pods to equilibrate at room temperature for 3 d, 6 d, and 9 d, after which they were subjected to oven drying at 80 °C for 12 h.

The placing of soybean pods at room temperature for 3 d and then subjected to oven drying at 80 °C for 12 h did not showed high variability in pod shattering resistance. The use of 6 d of equilibration duration showed the variability in shattering resistance, i.e. the resistance of 16 genotypes were grouped into resistant (three genotypes), moderately resistant (three genotypes), moderately susceptible (one genotype), and very susceptible (nine genotypes). Furthermore, the equilibration duration for 9 d caused most of all genotypes become very susceptible to pod shattering. Anjasmoro variety was categorized as resistant when placed at three days of room temperature. However, it become susceptible when the equilibration durations at room temperature were 6 and 9 d, respectively.

Based on the research results on the equilibration duration at room temperature (Table 5), then during the screening for pod shattering using high temperature, the placing of soybean pods at room

temperature for 6 d was considered as ideal pre-treatment before they were subjected to oven drying at 80 °C for 12 h.

Table 5. Number of shattered pods using high temperature method.

No	Genotype	Pod shattering (%) at 80 °C for 12 h					
		3 d*	Criteria	6 d*	Criteria	9 d*	Criteria
1	SAT-Ng-8-5	0.83	R	12.50	MR	78.33	VS
2	SAT-Ng-A4	0.00	R	0.83	R	20.83	MR
3	SAT-Ng-SBG-3	85.00	VS	94.17	VS	95.00	VS
4	SAT-Ng-1-1	0.00	VR	16.67	MR	65.00	VS
5	SAT-Ng-3-3	0.00	VR	19.17	MR	68.33	VS
6	SAT-Ng-6-13	0.00	VR	0.83	R	15.83	MR
7	SAT-Ng-44-7	75.00	VS	96.67	VS	100.00	VS
8	SAT-Ng-25	59.17	VS	75.00	VS	83.33	VS
9	SAT-Ng-12	53.33	VS	87.50	VS	95.00	VS
10	SAT-Ng-GH-3	25.00	MR	84.17	VS	95.83	VS
11	SAT-Ng-5-5	0.83	R	3.33	R	57.50	VS
12	SAT-Ng-AG-3	72.50	VS	76.67	VS	100.00	VS
13	SAT-Ng-44-6	50.83	VS	73.33	VS	91.67	VS
14	SAT-Ng-A-4	56.67	VS	85.83	VS	88.33	VS
15	Anjasgoro	5.83	R	29.17	MS	50.83	VS
16	Dega 1	71.67	VS	87.50	VS	99.17	VS
Average		34.79		52.71		75.31	

* = equilibration duration

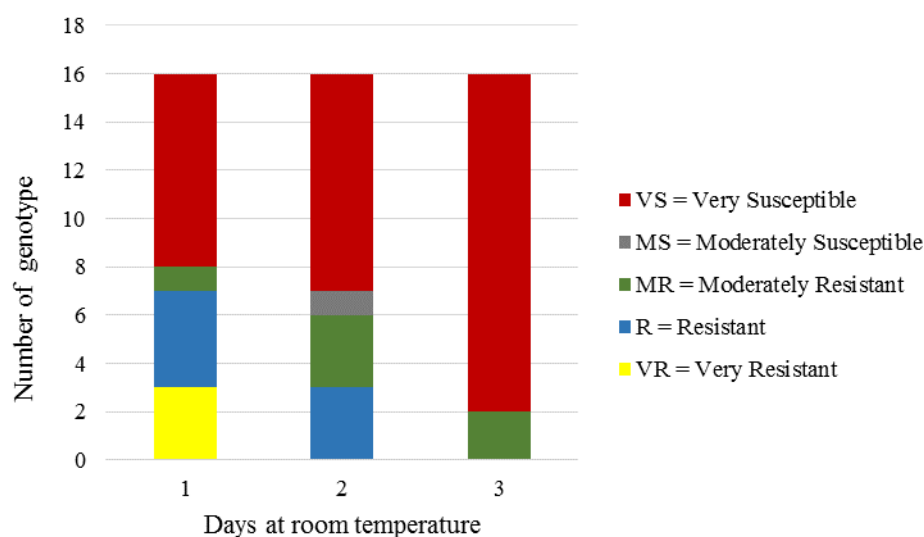


Figure 2. The degree of pod shattering of 16 soybean genotypes based on high temperature method (Days at room temperature: 1 = 3 d, 2 = 6 d, 3 = 9 d).

3.4. Selection of soybean genotypes

The ideal soybean varieties which will be developed in the Indonesian tropical area are not only those with high production, but also must have other characters, i.e. resistant to pod shattering and in accordance with consumers' preferences. Three high yielding genotypes (SAT-Ng-44-7, SAT-Ng-AG-

3, and SAT-Ng-A-4) were susceptible to pod shattering. Based on the evaluation for shattering resistance by using both of gradient and high oven temperature, there were three consistently resistant genotypes, i.e. SAT-Ng-A4, SAT-Ng-6-13, and SAT-Ng-5-5, which have yielded 3.56 t ha^{-1} , 3.40 t ha^{-1} and 3.49 t ha^{-1} , respectively. Those genotypes have medium maturity, but only SAT-Ng-6-13 and SAT-Ng-5-5 which have large seed size (Table 6).

The evaluation for pod shattering resistance using high temperature gives heavier selection pressures compared to those of gradient temperature method. Genotypes that were classified as very resistant based on gradient temperature method become resistant when evaluated using high temperature method. Antwi-Boasiako [14] using oven temperature of 80°C and obtained six moderate genotypes to pod shattering. The gradient temperature of oven dry method have been used to assess the resistance of 150 soybean genotypes, and has obtained eight very resistant genotypes [6]. The using of oven dry method in the evaluation of pod shattering resistance is considered as practical method and allowed to screen samples in large quantities. A screening method using desiccator was able to classify 25 soybean genotypes derived from Japan and Thailand [2]. In this study, the combination characters of a genotype with shatter resistant, large seed size, and early maturity was not obtained. In future, those three combination characters combined with high yield are needed to strengthen the soybean productivity in Indonesia.

Table 6. Relationship between agronomic characteristics with pod shattering.

No	Genotypes	Agronomic characters			Pod shattering (%)			
		Days to maturity (d)	100 seed weight (g)	Yield (t ha^{-1})	Gradient temperature	Criteria	High temperature	Criteria
1	SAT-Ng-8-5	80.75	15.05	3.56	0.00	VR	12.50	MR
2	SAT-Ng-A4	81.25	13.55	3.56	0.00	VR	0.83	R
3	SAT-Ng-SBG-3	78.50	17.63	3.37	45.00	MS	94.17	VS
4	SAT-Ng-1-1	80.25	14.68	3.59	0.00	VR	16.67	MR
5	SAT-Ng-3-3	80.50	15.40	3.24	0.00	VR	19.17	MR
6	SAT-Ng-6-13	82.25	14.78	3.40	0.00	VR	0.83	R
7	SAT-Ng-44-7	80.50	14.61	3.81	100.00	VS	96.67	VS
8	SAT-Ng-25	78.75	16.52	3.59	59.17	VS	75.00	VS
9	SAT-Ng-12	78.00	16.54	3.21	65.83	VS	87.50	VS
10	SAT-Ng-GH-3	78.25	17.76	3.56	75.00	VS	84.17	VS
11	SAT-Ng-5-5	82.00	15.15	3.49	0.00	VR	3.33	R
12	SAT-Ng-AG-3	80.00	15.58	4.06	96.67	VS	76.67	VS
13	SAT-Ng-44-6	81.25	17.68	3.15	67.50	VS	73.33	VS
14	SAT-Ng-A-4	82.25	15.09	3.93	64.17	VS	85.83	VS
15	Anjasromo	83.75	15.12	3.71	5.00	R	29.17	MS
16	Dega 1	78.75	20.56	3.24	70.00	VS	87.50	VS
Average		80.43	15.98	3.53	40.52		52.71	

VR = very resistant, R = resistant, MR = moderately resistant, MS = moderately susceptible, VS = very susceptible

4. Conclusion

Selection on the soybean resistance to pod shattering in the tropical area of Indonesia could be done by using oven dry method with gradient temperature or high temperature by 9 d of equilibration duration at room temperature. Soybean genotypes SAT-Ng-A4 and SAT-Ng-5-5 could be developed in the tropics, such as Indonesia, or could be used as genes source for the development of soybean resistance to pod shattering.

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