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Selection of F6 soybean population for pod shattering resistance

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Abstract. *Krisnawati A, Soegianto A, Waluyo B, Kuswanto. 2019. Selection of F6 soybean population for pod shattering resistance. Biodiversitas 20: 3340-3346.* Pod shattering is one of the major soybean constraints in Indonesia. This research aimed to evaluate the resistance of soybean F6 population to pod shattering and characterize the selected lines for their agronomic performances. The materials used were 147 F6 lines derived from six crossing combinations. The check varieties consisted of Dega 1, Detap 1, and Anjasmoro. The experiment was arranged in a randomized block design with two replications. At R8 stage, thirty pods were randomly detached from five sample plants of each line to be used for evaluation of pod shattering resistance using an oven-dry method. The variability of pod shattering was showed after the treatment of 60°C oven temperature. The shattering resistance of 147 F6 lines was classified into 52 highly resistant, 49 resistant, two moderately resistant, ten susceptible, and 34 highly susceptible lines. The pod-shattering resistant lines could be used for further improvement in the breeding program. Anjasmoro variety was effective to be used as gene source for shattering resistant. Simultaneous selection based on yield (30% selection intensity) and shattering resistance selected six lines which have early maturity and large seed size, hence they could be further evaluated in several locations in the next breeding stage.

Keywords: Early maturity, *Glycine max,* large seed, high yield, simultaneous selection

INTRODUCTION

Pod shattering is one of the major constraints for soybean production in tropical regions of Indonesia, as well as in various soybean production centers in sub-tropical regions. Pod shattering is the opening of pod wall causing dispersal of seed as the crop reaches maturity (Bhor et al. 2014). The yield losses due to shattering may range from 34-99% (Tiwari and Bhatnagar 1991) depending on susceptibility of the variety, environmental factors (temperature and relative humidity), pod morphology and anatomy, and the duration of harvest delay after maturity (Zhang and Boahen 2010; Krisnawati and Adie 2017; Gaikwad and Bharud 2018; Zhang et al. 2018).

Several genetic studies to understand the genetic control of pod shattering in soybeans revealed different findings. Akpan (1988) reported that six to twelve genes were involved in the soybean pod shattering. On the other hand, Tukamuhabwa et al. (2002) suggested that pod-shattering was controlled by two genes and was influenced by non-allelic interactions resulting in classical dominant epistasis. Mohammed (2010) reported that the inheritance of pod shattering trait was under the influence of either duplicate recessive or dominant and recessive epistasis depending on the parental genotypes used. The identification of the markers for shattering resistance has been carried out by Funatsuki et al. (2008) which found a major quantitative trait locus (QTL), designated as qPDH1. Furthermore, Yamada et al. (2009) reported that shattering resistance behaves as a nearly recessive trait and molecular markers near qPDH1 could be used for marker-assisted selection for shattering resistance in soybean.

The most effective way to reduce the yield losses due to shattering is by using the shattering-resistant soybean variety. The successful development of shattering-resistant variety depends on the availability of source of resistance genes and a reliable selection method for shattering resistance (Romkaew and Umezaki 2006; Thakare et al. 2016). The selection method for shattering resistance can be performed in the field, and in the laboratory by using oven-dry method (Mohammed 2010; Bhor et al. 2014; Kuai et al. 2016). Tiwari and Bhatnagar (1991) reported that the use of oven-dry method was highly correlated with field shattering. Similarly, Agrawal et al. (2000) also obtained high correlation of shattering evaluation between field and laboratory conditions, and they suggested that laboratory method can be used as a tool for identifying pod shattering-resistant genotypes. The advantage of selection of soybean resistance using oven method are that it can provide a uniform and controlled environment, be able to select large quantities of population, and is faster (Krisnawati and Adie 2019).

Screening of soybean resistance to pod shattering has been carried out in several countries. In India, Tiwari and Bhatia (1995) obtained three genotypes resistant to pod shattering (Bragg, PK 416, and NRC), whereas Tukamuhabwa et al. (2002) in Nigeria obtained three very resistant genotypes (TGx 1448-2E, Duiker, and Nam 2). Research by Mohammed (2010) in Ghana obtained genotype TGX 1448-2E which categorized as resistant to shattering. Genotype SJ2 derived from Thailand was reported to be resistant to shattering, and had been used as gene source for genetic improvement of shattering resistant cultivars in Japan (Yamada et al. 2009). In Indonesia, Krisnawati and Adie (2017) examined several soybean genotypes and reported that Anjasmoro variety was resistant, whereas Grobogan and Argomulyo varieties were susceptible to shattering. Soybean genotypes that exhibited resistant to highly resistant to pod shattering could be used as source of resistant genes in the soybean breeding program for shattering resistance.

Hybridization and selection are the main methodologies in developing varieties. Selection for shattering resistance as well as for high yield in soybean F6 lines is an important stage in the varietal improvement program in Indonesia since the breeding activity for shattering resistance is still relatively new. Therefore, the aims of the study were to evaluate the resistance of soybean F6 population to pod shattering and characterize the selected lines for their agronomic performances.

MATERIALS AND METHODS

Study area

The research was conducted during the dry season (February to May 2018) at Jambegede Research Station (Malang, East Java, Indonesia) which located at the coordinate of 08°11'02" South Latitude and 112°33'32.9" East Longitude, 335 m above sea level, C3 (Oldeman) climate type, rainfall of 2300 mm/year, the range of temperature 23.5°C-32°C, 79% relative humidity, and soil type of Alfisol.

Plant materials

The plant materials used consisted of 147 lines of F6 segregating population and three checks varieties, namely Dega 1 (high yield, early maturity), Detap 1 (high yielding, shattering resistant, early maturity), and Anjasmoro (high yielding, shattering resistant, medium maturity). Those F6 segregating populations derived from six crosses combinations, which were developed during 2015 through hybridization. The gene source for shattering resistance was derived from cultivar of Anjasmoro, and was reciprocally crossed with three other soybean genotypes, i.e., G100H, Rajabasa, and Grobogan. The selection for high yield was made in segregating populations of five different generations (F2, F3, F4, F5, and F6) through pedigree method.

Field study

The experiment was arranged in a randomized block design with 150 genotypes as treatments, and each genotype was replicated twice. The research site was lowland (after rice planting) without soil tillage. Each genotype was planted in a plot size of 1.2×4.0 m^2 with plant spacing of 40 cm × 15 cm, two seeds per hill. Plants were fertilized with 50 kg ha^{-1} urea, 100 kg ha^{-1} SP36, and 75 kg ha^{-1} KCl which were applied at planting time. Weeding, pest, and disease were optimally monitoring.

Screening for shattering resistance

The selection method used for shattering resistance was an oven-dry method (Krisnawati and Adie 2017). When plants at R8 stage (leaves had started to turn yellow), three sample plants were randomly taken, and dried in the room temperature for three days in an upright position. A total of thirty pods from three sample plants of each genotype were randomly detached, and then placed in Petri dishes and kept in the oven. The oven temperature was set at 30°C for three days, and then elevated into 40°C (for one day), 50°C (for one day), 60°C (for one day). The degree of shattering resistance was classified according to AVRDC (1979) as follows: highly resistant (0% shattering), resistant (1-10% shattering), moderately resistant (11-25% shattering), moderately susceptible (26-50%), and highly susceptible (>50% shattering).

Observation

The observed parameters consisted of the shattered pods (after subjected to oven temperature of 30°C, 40°C, 50°C, 60°C, respectively), and agronomic characters (days to maturity, 100 seed weight, seed yield)

Selection for seed yield

Selection for seed yield, assuming the selection intensity of 30%, was calculated as proposed by Allard (1960):

$$X_s \ : \ \bar{x} + k \, . \, sf$$

Where:
X_s : selected line
$\bar{x}$: grand mean of all tested lines
k : selection intensity
sf : phenotypic standard deviation

Data analysis

Data were subjected to descriptive analysis which consisted of mean, minimum value, maximum value, and standard deviation. Agronomic data were subjected to analysis of variance (ANOVA).

Table 1. Descriptive data for pod-shattering of 147 F6 soybean lines and three check varieties

Descriptive analysis	Pod shattering (%) at oven temperature (°C)			
	30	40	50	60
Minimum value	0	0	0	0
Maximal value	0	23	47	100
Standard deviation	0	3.13	11.40	36.38
Mean	0	1	6	24

Table 2. Classification for pod shattering resistance of 147 F6 soybean lines and three check varieties

Genotypes	Number of F6 lines on each level of shattering resistance					Total
	HR	R	M	S	HS	
F6 lines:						
Anjasmoro/G100H	11 (44%)	8 (32%)	1 (4%)	3 (12%)	2 (8%)	25
G100H/ Anjasmoro	6 (40%)	2 (13%)	0 (0%)	0 (0%)	7 (47%)	15
Anjasmoro/IAC 100	4 (57%)	3 (43%)	0 (0%)	0 (0%)	0 (0%)	7
Anjasmoro/Rajabasa	30 (31%)	36 (37%)	1 (1%)	6 (6%)	24 (25%)	97
Rajabasa/Anjasmoro	0 (0%)	0 (0%)	0 (0%)	1 (50%)	1 (50%)	2
Grobogan/Anjasmoro	1 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1
Total	52 (35%)	49 (33%)	2 (1%)	10 (7%)	34 (23%)	147
Checks:						
Dega 1	0	0	0	0	1	1
Detap 1	1	0	0	0	0	1
Anjasmoro	1	0	0	0	0	1

Note: HR: highly resistant, R: resistant, M: moderately resistant, S: susceptible, HS: highly susceptible

RESULTS AND DISCUSSION

Pod shattering resistance

Descriptive data of 147 F6 soybean lines and three check varieties showed that no shattered pods at oven temperature of 30 °C. At 40 °C, the pod shattering ranged from 0-23% with an average of 1%. The range of shattering level was between 0-47% (an average of 6%) at 50°C. The average of shattering increased to 24%, with range of 0-100% at 60°C (Table 1, Figure 1). The oven temperature between 30^0 to 40°C was more intended to reduce pod water content. Based on the range of shattering, the use of temperature of 60°C in the oven-dry method was considered as the most effective temperature for screening pod shattering resistance.

Pod shattering at 60°C varied between cross combinations (Table 2). A total of 147 F6 tested lines, 52 lines were categorized as highly resistant to pod shattering, 49 lines were resistant, two lines were moderately resistant, ten lines were susceptible, and the rest (34 lines) were highly susceptible to shattering (Table 2, Figure 2).

The distribution of shattering varied for each cross combination (Table 2). The F6 soybean lines derived from cross combination of Anjasmoro and IAC100 (seven lines), four lines (57%) showed highly resistant to shattering. The cross combination between Anjasmoro and G100H which consisted of 25 lines, 11 lines (44%) were highly resistant to shattering. The F6 soybean lines derived from cross combination between G100H and Anjasmoro showed six highly resistant lines (40%) of 15 tested lines, whereas the cross combination between Anjasmoro and Rajabasa showed 30 highly resistant lines (31%) of 97 tested lines. The check variety Dega 1 was highly susceptible, whereas the Detap 1 and Anjasmoro were highly resistant to shattering.

Performance of agronomic characters

The analysis of variance for three primary agronomical characters (days to maturity, 100 seed weight, seed yield) of 147 F6 soybean lines and three check varieties showed a significant difference between lines for those three characters. The coefficient of variation ranged from 3.19-16.51% (Table 3). The days to maturity ranged from early (77 days) to medium maturity (86 days), the seed size (100 seed weight) ranged from small size (9.27 g per 100 seeds) to large seed size (17.92 g per 100 seeds), and the seed yield ranged from 0.81-2.29 $t.ha^{-1}$ (an average of 1.54 $t.ha^{-1}$). The check varieties of Dega 1 and Detap 1 have early maturity, whereas Anjasmoro variety has medium maturity. The seed size of Dega 1 and Detap 1 were large, meanwhile, Anjasmoro has medium seed size. The seed yield of those three check varieties ranged from 1.34-1.65 $t.ha^{-1}$.

The distribution of days to maturity of 147 F6 lines consists of early maturity (40 lines) and medium maturity (107 lines) (Figure 3). The seed size was divided into small seed (three lines), medium (105 lines), and large-seeded (39 lines) (Figure 4). Selection for seed yield using the formula given by Allard (1960) with the intensity of selection 30% (k = 1.16). Based on the Allard's formula, then the selection limit for yield was 1.96 $t.ha^{-1}$. By those selection limits, it was selected six F6 soybean lines with seed yield over 1.96 $t.ha^{-1}$ (Figure 5). The yield of Dega 1, Detap 1, and Anjasmoro were 1.14, 1.65, and 1.34 $t.ha^{-1}$, respectively.

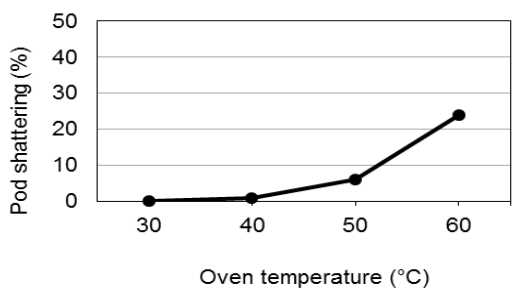

Figure 1. The average of pod-shattering from 147 F6 soybean lines and three check varieties at different oven temperatures

Simultaneous selection

The simultaneous selection of 147 F6 soybean lines and three check varieties was focused on the pod shattering resistance and high yield. A total of six lines were selected based on their high yield (based on the Allard's formula, with the selection intensity 30% or k = 1.16) and shattering resistance. The degree of resistance of those six lines ranged from resistant to highly resistant. The selected lines and their agronomic characters (maturity and seed size) were presented in Table 5. Of six selected lines, three lines (Anjasmoro/G100H-6, Anjasmoro/G100H-28, and Anjasmoro/G100H-44) have early maturity and large seed size, two lines (Anjasmoro/Rajabasa-27 and Anjasmoro/ Rajabasa-168) have medium maturity and medium seed size, and the rest (Anjasmoro/G100H-32) has an early maturity but medium seed size.

Discussion

The increase of labor scarcity in the food crop sector and followed by the uncertainty of the season due to climate change can delay harvesting in soybeans, leading to seed yield loss caused by pod shattering. In tropical area, such as Indonesia, pod shattering has become a major abiotic problem, since soybeans cultivations are mostly conducted during the second dry season (soybean planting period of June/July to September/October) which characterized by high temperature and low humidity (Sudaryono et al. 2016; Adie and Krisnawati 2016).

In this study, the F6 soybean lines derived from crosses made in 2005 using Anjasmoro variety as source of resistant parent for shattering resistance. The use of Anjasmoro as gene source for shattering resistance has a great potential to increase soybean resistance to pod shattering as seen that 52% and 49% of 147 F6 lines were highly resistant and resistant, respectively. Resistant cultivars can be developed by introducing resistance genes into susceptible cultivars through hybridization. Genetic improvement, by introducing resistance genes was reported more effective, less costly, not subject to environmental conditions, and easier for growers to implement (Thakare et al. 2016). In Japan, genetic improvement of shattering resistant cultivars used genotype SJ2 derived from Thailand which was reported to be resistant to shattering (Yamada et al. 2009).

Screening for shattering resistance showed that the degree of resistance of F6 soybean lines ranged from highly resistant to highly susceptible. Similar results also obtained in the previous study on the F5 population. Furthermore, a total of 104 populations (16.40%) were selected out of 591 F5 lines and were classified as highly resistant to pod shattering (Krisnawati et al. 2019). Other studies were also obtained the range of shattering from highly resistant to highly susceptible (Romkaew and Umezaki 2006; Adie and Krisnawati 2016). However, other studies (Adeyeye et al. 2014; Antwi-Boasiako 2017; Barate et al. 2018) did not find genotypes with zero shatterings or classified as highly resistant genotypes. The pod-shattering resistant lines identified in the present study could be used for further breeding strategies and improvement of soybean plant. Bhor et al. (2014) stated that genotypic characteristics played a major role in the overall expression of pod shattering, suggesting that these differences could be attributed to difference in genetic information of soybean genotypes and environmental condition during plant's growth and development (Tukamuhabwa et al. 2002; Richard 2018). Zhang and Bellaloui (2012) reported that different weather patterns, especially temperature and rainfall in a year could be essential factors affecting seed shattering patterns. Furthermore, the anatomical and morphological of the pod also reported determining the shattering resistant (Bara et al. 2013; Krisnawati and Adie 2017; Zhang et al. 2018).

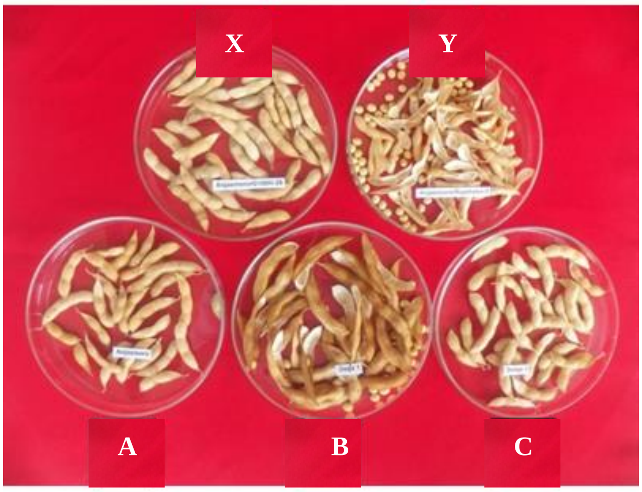

Figure 2. Pod shattering of highly resistant (X) and highly susceptible F6 lines (Y) and check varieties (A=Anjasmoro, B=Dega 1, C=Detap 1)

Table 3. Analysis of variance for agronomic characters of 147 F6 soybean lines and three check varieties

Characters	Mean square		CV (%)
	Replication	Genotype	
Days to maturity (days)	0.0833^{ns}	8.1664**	3.19
100 seed weight (g)	18.5256**	5.1241**	7.21
Seed yield (t.ha^{-1})	3.1827**	0.1330**	16.51

Note: ** = significant at 1 % probability level ($p < 0.01$), ns = not significant, CV = coefficient of variation

Table 4. Descriptive data for agronomic characters of 147 F6 soybean lines and three check varieties

Descriptive analysis	Days to maturity (days)	100 seed weight (g)	Seed yield (t.ha^{-1})
Minimum value	77	9.27	0.81
Maximal value	86	17.92	2.29
Standard deviation	2.92	1.62	0.26
Mean	82	12.97	1.54
Checks			
Dega 1	77	17.92	1.14
Detap 1	78	14.72	1.65
Anjasmoro	82	13.41	1.34
Selection intensity (30%)			1.96

Table 5. Simultaneous selection of F6 soybean lines for pod shattering resistance and high yield

F6 soybean lines	Pod shattering %	Pod shattering Criteria	Seed yield ($t.ha^{-1}$)	Days to maturity (hr)	100 seed weight (g)	Description
Anjasmoro/G100H-6	0	HR	2.15	77	15.26	Early maturity, large seed size
Anjasmoro/G100H-28	0	HR	2.01	78	14.98	Early maturity, large seed size
Anjasmoro/G100H-32	3	R	2.29	78	13.93	Early maturity, medium seed size
Anjasmoro/G100H-44	2	R	2.10	78	15.76	Early maturity, large seed size
Anjasmoro/Rajabasa-27	2	R	2.04	80	12.99	Medium maturity, medium seed size
Anjasmoro/Rajabasa-168	2	R	1.97	84	11.36	Medium maturity, medium seed size
Check:						
Dega 1	57	HS	1.14	77	17.92	
Detap 1	0	HR	1.65	78	14.72	
Anjasmoro	0	HR	1.34	82	13.41	
Mean	24		1.54	79	12.97	
Selection intensity (30%) for yield ($t.ha^{-1}$)			1.96			

Note: HR: highly resistant, R: resistant, M: moderately resistant, S: susceptible, HS: highly susceptible. Classification of maturity: early (< 80 days), medium (80-90 days), late (> 90 days), classification of seed size: small (< 10 g per 100 seeds), medium (10-14 g per 100 seeds), large (>14 g per 100 seeds)

In Indonesia, soybean is cultivated in the upland during the rainy season (soybean planting period of November/ December to February/March), or in the wetland during the dry season (soybean planting period of February/March to June/July and planting period of June/July to September/ October. Soybeans are widely cultivated during the second dry season within planting period of June/July to September/October, and this condition will cause a higher possibility of the occurrence of pod shattering. Furthermore, soybean cultivation is mainly following a yearly planting pattern of paddy-paddy-soybean. Based on those facts, the strategy to develop soybean improved varieties is not only aimed for shattering resistance but must be combined with other important characters, such as early maturity and large seed size. Simultaneous selection based on shattering resistance coupled with yield (30% selection intensity based on Allard's formula) on the 147 F6 soybean lines obtained six selected lines. The degree of resistance of all lines was resistant to highly resistant, and the yield was over 1.96 $t.ha^{-1}$Selection based on yield and shattering on soybean also have been done by Richard (2018) which obtained five high yield lines and were resistant to pod shattering. High yielding variety is still become main purpose in the soybean varietal improvement program in Indonesia. The combination of yield with shatter-resistant characters will play important role in minimizing the yield losses. In India, it was reported that the critical phase of pod shattering occurs when the harvest was delayed up to 10 days, and by using resistant varieties (JS 335, JSM 170, and MAUS 61-2) were able to minimize the yield losses (Bara et al. 2013).

In addition to yield, the consumers' preferences for soybean varieties include large seed size and early maturity. The days to maturity of 147 F6 lines consist of early maturity and medium maturity, whereas the seed size consists of small, medium, and large-seeded. Three selected lines (Anjasmoro/G100H-6, Anjasmoro/G100H-28, and Anjasmoro/G100H-44) have early maturity and large seed size. Soybean maturity day is different among countries. In Indonesia, soybean maturity is classified into late maturity

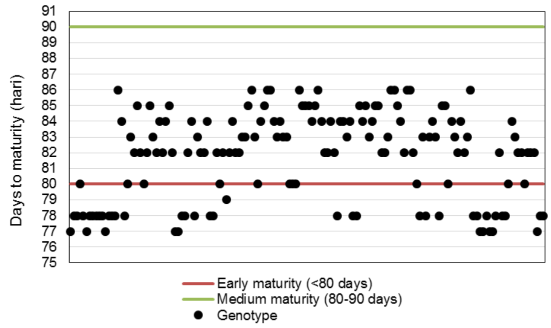

Figure 3. Distribution of days to maturity of 147 F6 soybean lines and three check varieties

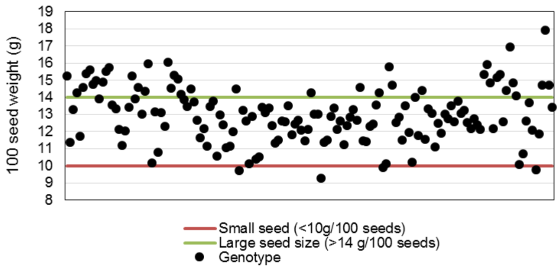

Figure 4. Distribution of seed size of 147 F6 soybean lines and three check varieties

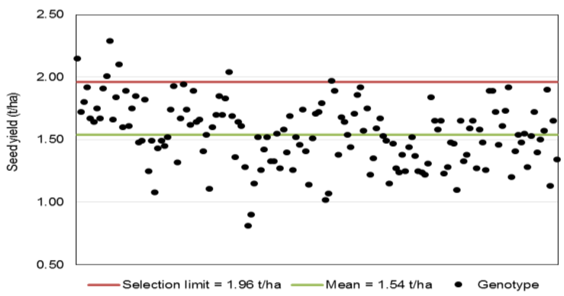

Figure 5. Distribution of seed yield of 147 F6 soybean lines and three check varieties

(> 90 days), medium maturity (80-90 days), and early maturity (<80 days). The soybean variety development in di Indonesia in the last five years has been aiming at developing soybean early maturing varieties (Adie and Krisnawati 2018).

Early maturing variety is essential to character because it can reduce the potential for harvest delays, more tolerant of drought and pests through the escape mechanism, and increasing the cropping index (Adie and Krisnawati 2017). In Indonesia, soybeans are mostly cultivated following the yearly planting pattern of paddy-paddy-soybean, hence an early maturing soybean is considered suitable for those conditions. In term of seed size, it is important character which contributes to the yield productivity and also become the determinant factor of tempeh quality (Kuswantoro et al. 2014). Large-seeded soybeans are considered more suitable for raw material of tempeh because produce a brighter colored and larger volume of tempeh (Krisdiana 2005).

In conclusion, the increase of shattering resistant on soybean could use the gene source of resistant derived from Anjasmoro variety. Screening for shattering resistance of 147 crosses combinations by using Anjasmoro as parental resulted in 52 highly resistant lines and 49 resistant lines which further can be utilized for improvement in the breeding program. The simultaneous selection was able to obtain three lines with high yield and shatter-resistant, and also showing early maturity and large seed size, respectively. Those lines could be further evaluated in several locations in the next breeding stage.

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