Identification and grouping of pod shattering resistance and agronomic characters performance from several soybean advanced lines

A. Krisnawati and M.M. Adie
Indonesian Legume and Tuber Crops Research Institute (ILETRI), Malang, East Java, Indonesia
Email: aydakrisnawati@pertanian.go.id

Abstract. The use of pod-shatter resistant variety is considered effective in reducing yield loss in soybeans. This study aimed to identify and grouping of pod shattering resistance and agronomic characters performance from several advanced soybean lines. The field experiment was conducted in Blitar (Indonesia) from January to April 2021, using 20 soybean advanced lines and two check cultivars. The laboratory study to identify the pod shattering resistance based on the oven-dry method was conducted in the Breeding Laboratory of ILETRI, Malang. The pod shattering resistance of 20 advanced lines was classified into five very resistant genotypes, three resistant genotypes, two moderate genotypes, nine susceptible genotypes, and a very susceptible genotype. The pod shattering resistance has a significant negative correlation with the pod width ($r = -0.472^*$). A wider pod resulted in a low percentage of shattering. The agronomic performance varied among all genotypes. Selection for pod shattering resistance and agronomic performance obtained two lines (G511H/Anjas//Anjas///Anjas-3-1 and Dega/Dega-2-105) as high yielding and very resistant to pod shattering. Those lines were suggested to be tested across diverse environments to determine their yield potential and stability.

1. Introduction
Yield losses in the food crops have become one of the major agricultural constraints in the world. Pod shattering is one of the factors that contribute to soybean yield losses. Several studies reported that the yield loss due to pod shattering in soybean ranged from 30-100% [1, 2]. Pod shattering has also become a serious threat in other crops, such as hairy vetch [3], rapeseed [4], and lentil [5]. An effort to reduce yield losses is to provide soybean varieties that are relatively resistant to pod shattering.

Losses in soybean yield due to pod shattering occur not only in the tropics but also in subtropical areas. As a result, several soybean-producing countries are implementing breeding strategies to save soybean yields due to pod shattering. In India, several soybean genotypes (Birssoya, Bragg, Dsb 21, DS 9712, DS 3101, KDS 775, KDS 904, MACS 450, and NRC 93) have been reported to be resistant to pod shattering [6]. The soybean genotype SJ-2 from Thailand was reported to be resistant to pod shattering based on a study conducted in Japan [7]. Several soybean genotypes were also reported in Nigeria, including TGX1955-10E, NG/AD/11/08/023, and NG/SA/07/100, which were good general combiners for resistance to pod shattering in soybean [8]. Obtaining pod-shatter-resistant genotypes not only prevents yield loss but also allows for a longer harvest period. As stated in the previous study
that pod-shatter resistant soybean varieties are also associated with longer harvest delays in the field [9].

External (non-genetic) and internal (genetic) factors are the determinants of soybean pod shattering resistance [10, 11]. Manipulation of internal factors to increase soybean resistance to pod shattering is easier in the era of climate change since it is environmentally friendly and results in a more durable resistance. Previous studies have shown that pod shattering resistance is genetically controlled and inherited [12-14]. A simultaneous selection for pod shattering resistance and agronomic characters of 591 F5 soybean populations has obtained 30 lines that were classified as highly resistant to pod shattering and the seed yield per plant was between 16.31 – 22.66 [15]. In Japan, it has identified the quantitative trait locus (QTL) controlling pod shattering in soybean (qPDH1), using the progeny of shattering-resistant cultivars derived from a Thai cultivar, SJ2 [7]. Those results were strengthened by a map-based cloning study that qPDH1 encodes a dirigent-like protein that regulates dehiscing force, the torsion of pod walls under low humidity [16].

The method for developing pod-shatter resistant varieties begins with recombination between parents, one of which is pod-shatter resistant, and then progresses to selection for pod-shatter resistance. Soybean lines that are already in the F7 phase or higher are classified as advanced generation lines. Selection can be carried out simultaneously in those populations based on the desired agronomic characters and combined with resistance to pod shattering. Selection of resistance to pod shattering can be done in the field [2, 17] or in the laboratory using the oven-dry method [14, 18]. The advantage of screening for pod shattering resistance using the oven-dry method is that it allows for the evaluation of large numbers of soybean genotypes in a shorter period of time, and the environment in the oven is more controlled and stable. As a result, the method is appropriate for selecting shatter resistance in segregated lines as well as advanced generation lines.

This study aimed to identify and grouping of pod shattering resistance and agronomic characters performance from several advanced soybean lines.

2. Materials and methods

2.1. Research material

The materials consisted of 20 soybean advanced lines and two check cultivars (Derap 1 and Detap 1). These lines are the result of a selection of several cross combinations, as shown in Table 1.

| No | Pedigree | Parental | Remark |
|----|----------|----------|--------|
|    |          | Female   | Male   |
| 1  | G511H/Anj///Anj///Anj-2-5 | G511H | Anjasmoro | Backcross |
| 2  | G511H/Anj///Anj///Anj-3-1 | G511H | Anjasmoro | Backcross |
| 3  | G511H/Arg///Arg///Arg-18-3 | G511H | Anjasmoro | Backcross |
| 4  | G511H/Anj///Anj///Anj-6-4 | G511H | Anjasmoro | Backcross |
| 5  | G511H/Anj///Anj///Anj-8-3 | G511H | Anjasmoro | Backcross |
| 6  | Dega/Degra-2-105 | Dega | Degra | Single cross |
| 7  | Grob/Degra-1-211 | Grobogan | Degra | Single cross |
| 8  | Degra/Grob-1-254 | Degra | Grobogan | Single cross |
| 9  | Anj/Degra-6-545 | Anjasmoro | Degra | Single cross |
| 10 | Dega/Mahameru-4-783 | Dega | Mahameru | Single cross |
| 11 | G100H/Anj-2-179 | G100H | Anjasmoro | Single cross |
| 12 | Grob/G100H-3-293 | Grobogan | G100H | Single cross |
| 13 | Grob/G100H-5-310 | Grobogan | G100H | Single cross |
| 14 | Grob/G100H-10-364 | Grobogan | G100H | Single cross |
| 15 | G100H/Anj-1-602 | G100H | Anjasmoro | Single cross |
2.2. Field research
The field study was conducted in Blitar (East Java, Indonesia) from January to April 2021. The experiment was arranged in a randomized block design with three replications. The study area was dryland. Before planting, the soil tillage was intensively performed. Each soybean advanced line was planted in a plot size of 2 m × 4.5 m with a plant spacing of 40 cm × 15 cm, two plants per hill. Plants were fertilized with 250 kg Phonska and 100 kg SP36, which were given entirely after planting. Weeds, pests, and diseases were intensively monitored. The observed agronomic data consisted of plant height, number of branches per plant, number of nodes per plant, number of pods per plant, 100 seed weight, and seed yield per plant.

2.3. Laboratory research
The evaluation for shattering resistance of soybean advanced lines using the oven-dry method [19] was conducted in the Laboratory of Plant Breeding, Indonesian Legume and Tuber Crops Research Institute (ILETRI) in Malang (East Java, Indonesia). When the plants were in the R8 phase (the leaves had begun to turn yellow), 10 plants were randomly chosen for each treatment and air-dried for three days with the plants standing. Thirty pods were randomly selected from three sample plants and placed in a petridish with a diameter of 15 cm. The petridish, which contained 30 soybean pods, was then placed in the oven. The oven temperature was set at 30°C for three days, then increased to 40°C for one day, then to 50°C for one day, and finally to 60°C for one day. Pod shattering percentage was observed after the oven-dry treatment of 60°C. The percentage of shattered pods was calculated as:

\[
\text{Pod shattering} = \frac{\text{number of shattered pods}}{30 \text{ pods}} \times 100 \%
\]

The grouping of shattering resistant follows the AVRDC [20]: very resistant (0%), resistant (1-10%), moderate (11-25%), susceptible (26 – 50%), and very susceptible (>50%). The observation was also made for the pod characteristics, i.e., pod length, pod width, and the ratio of the pod width and pod length.

2.4. Data analysis
The descriptive statistic was used for the agronomic characters and pod characteristics. The relationship between the pod shattering with the pod characteristics was investigated using Pearson’s correlation. The data were analyzed using the R statistical program [21] for the descriptive statistics and Pearson’s correlation coefficients.

3. Results and Discussion
3.1. Pod shattering resistance
Pod shattering is the opening of the soybean pod wall after the plant has matured, causing the seeds to detach from the pod wall and fall to the ground. The oven-dry method was used to assess the pod shattering resistance of 22 soybean genotypes. None of the tested genotypes had shattered pods after subjected to 30°C and to 40°C. The average of pod shattering was 9% after subjected to 50°C, and it increased to 21% after subjected to 60°C (Table 2). The use of the oven-dry method with a temperature of 60°C was able to screen the resistance of the soybean genotypes tested.

Based on the grouping of pod shattering resistance, a total of six genotypes were classified as very resistant, four genotypes were resistant, two genotypes were moderate, nine genotypes were susceptible, and a genotype was very susceptible (Table 2). The advanced generation lines, which
were classified as very resistant, were descended from Anjasmoro as one of the parents, and were backcrossed 3-4 times. According to previous research, Anjasmoro was resistant to pod shattering [15].

Table 2. Identification and grouping of pod shattering resistance of 22 soybean genotypes

| No | Genotype                      | Pod shattering (%) | Resistance grouping at 60°C |
|----|--------------------------------|--------------------|-----------------------------|
|    |                                | 30°C   | 40°C | 50°C | 60°C |                                |
| 1  | G511H/Anj//Anj///Anj///Anj///Anj | 0      | 0    | 0    | 0    | Very resistant                |
| 2  | G511H/Anj//Anj///Anj-3-1        | 0      | 0    | 0    | 0    | Very resistant                |
| 3  | G511H/Arg///Arg///Arg///Arg-18-3| 0      | 0    | 0    | 0    | Very resistant                |
| 4  | G511H/Anj///Anj///Anj-6-4       | 0      | 0    | 3    | 7    | Resistant                     |
| 5  | G511H/Anj///Anj///Anj///Anj-8-3 | 0      | 0    | 0    | 0    | Very resistant                |
| 6  | Dega/Degra-2-105               | 0      | 0    | 0    | 0    | Very resistant                |
| 7  | Grob/Degra-1-211               | 0      | 0    | 31   | 86   | Very resistant                |
| 8  | Dega//Grob-1-254               | 0      | 0    | 27   | 40   | Susceptible                  |
| 9  | Anj//Degra-6-545               | 0      | 0    | 13   | 32   | Susceptible                  |
| 10 | Dega//Mahameru-4-783           | 0      | 0    | 17   | 50   | Susceptible                  |
| 11 | G100H/Anj-2-179                | 0      | 0    | 0    | 8    | Resistant                     |
| 12 | Grob/G100H-3-293               | 0      | 0    | 13   | 17   | Moderate                     |
| 13 | Grob/G100H-5-310               | 0      | 0    | 0    | 3    | Resistant                     |
| 14 | Grob/G100H-10-364              | 0      | 0    | 20   | 29   | Susceptible                  |
| 15 | G100H/Anj-1-602                | 0      | 0    | 2    | 29   | Susceptible                  |
| 16 | Grob/G100H-1-857               | 0      | 0    | 2    | 37   | Susceptible                  |
| 17 | Grob/G100H-3-881               | 0      | 0    | 22   | 33   | Susceptible                  |
| 18 | Dega//IAC100-7-1135            | 0      | 0    | 9    | 43   | Susceptible                  |
| 19 | Grob/G100H-2-1162              | 0      | 0    | 13   | 22   | Moderate                     |
| 20 | Grob/G100H-4-1195              | 0      | 0    | 19   | 30   | Susceptible                  |
| 21 | Derap 1                        | 0      | 0    | 0    | 0    | Very resistant                |
| 22 | Detap 1                        | 0      | 0    | 2    | 4    | Resistant                     |

**Average**: 0 0 9 21

3.2. Pod characteristics

The pod length, pod width, and the ratio of the pod width and pod length varied between the tested genotypes. The average pod length was 4.12 cm with a range between 3.62 – 4.44 cm. The range of pod width was between 0.91 – 1.14 cm with an average of 1.03 cm. the ratio of the pod width and pod length ranged from 0.23 – 0.27 cm with an average of 0.25 cm (Figure 1). Several advanced generation lines tested had pods that were longer than the average pod length. The longer pods do not appear to be followed by the wider pods. As a result, a wide range of pod characters could emerge in long pods, followed by wide pods. However, there are lines with relatively long pods but not overly wide pods.
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Figure 1. Pod length (a), pod width (b), and the ratio of the pod width and pod length (c) of 22 genotypes. Genotype code was according to Table 1.

The Pearson correlation between the pod shattering with pod characteristics was presented in Figure 2. The pod shattering resistance has a significant negative correlation with the pod width (r = -0.472*). A wider pod resulted in a low percentage of shattering. This result is in line with earlier finding [22], but it is not in agreement with other study [23]. This different result could be caused by the difference in the other pod characteristics which also affecting the shattering resistance. Several studies reported that other pod characteristics contributed to the pod shattering resistance, such as pod wall thickness, pod thickness, pod length, pod diameter, and pod weight [10, 19, 23, 24].

Figure 2. Pearson correlation between pod shattering with pod characteristics. The limit of significant correlation is ≥ ± 0.50. PSH = pod shattering, PLG = pod length, PWD = pod width, RTO = the ratio of the pod width and pod length.

3.3. Agronomic performance

The agronomic character of soybean is one that is related to one another, especially when it is associated with economic value characters, such as seed yield. The average plant height of the 22 genotypes tested was 67.77 cm, with a range of 54.33 - 85 cm. The number of branches per plant varied between the soybean genotypes tested, ranging from 0.89 to 5.22, with an average of 2.91 branches/plant. The number of nodes per plant ranged from 7.11 to 26.89, with an average of 16.12 nodes/plant (Figure 3).

Yield characters such as seed size and seed/plant weight showed significant variation among the soybean genotypes tested. The weight of 100 seeds ranged from 13.27 g/100 seeds to 21.74 g/100 seeds, with an average weight of 16.12 g/100 seeds. The seed yield ranged from 8.04 to 17.74 g/plant, with an average of 13.57 g/plant (Figure 3). Most of the advanced lines were classified as having large
seed sizes. Seed yield per plant also revealed 10 advanced generation lines with seed weights (13.77 – 17.74 g/plant) greater than the average seed yield of all tested genotypes.

Figure 3. Agronomic performance of 22 soybean genotypes: (a) plant height, (b) number of branches per plant, (c) number of nodes per plant, (d) 100 seed weight, and (e) seed weight per plant. Genotype code was according to Table 1.

3.4. Selection of advanced lines
In this study, five very resistant and three resistant lines to pod shattering of 20 soybean advanced lines were successfully obtained (Table 3). Of these eight genotypes, four of them (G511H/Anj///Anj-3-1, G511H/Anj///Anj-6-4, Dega/Degra-2-105, and Grob/ G100H-5-310) were yielded over the mean yield of 22 genotypes. In addition to being very resistant to pod shattering, the G511H/Anj///Anj-3-1 also produced a higher yield (17.09 g/plant) and have a large seed size (18.81 g/100 seeds). The Dega/Degra-2-105 as a very resistant line, it produced the second-best yield
and also has a larger seed size (20.08 g/100 seeds). Among the three resistant lines, Grob/G100H-5-310 had the best yield (14.42 g/plant) and have a comparable seed size with Dega/Degra-2-105. However, the high yielding and very resistant genotypes (G511H/Anj///Anj///Anj-3-1 and Dega/Degra-2-105) were suggested to be re-tested in a more diverse soybean growing environment to determine their yield potential and stability.

Table 3. Selected soybean advanced lines that are very resistant and resistant to pod shattering

| No. | Genotype                     | Pod shattering at 60°C (%) and the degree of resistance | 100 seed weight (g) | Seed weigh/plant (g) |
|-----|------------------------------|--------------------------------------------------------|---------------------|----------------------|
| 1   | G511H/Anj///Anj///Anj-2-5     | 0 (VR)                                                 | 17.10               | 10.02                |
| 2   | G511H/Anj///Anj-3-1          | 0 (VR)                                                 | 18.81               | 17.09                |
| 3   | G511H/Arg///Arg///Arg-18-3   | 0 (VR)                                                 | 19.21               | 11.08                |
| 4   | G511H/Anj///Anj-6-4          | 7 (R)                                                  | 19.53               | 13.77                |
| 5   | G511H/Anj///Anj///Anj-8-3    | 0 (VR)                                                 | 18.12               | 10.60                |
| 6   | Dega/Degra-2-105            | 0 (VR)                                                 | 20.08               | 14.73                |
| 7   | G100H/Anj-2-179             | 8 (R)                                                  | 21.74               | 8.04                 |
| 8   | Grob/G100H-5-310            | 3 (R)                                                  | 20.33               | 14.42                |

Check cultivars:

- Derap 1 0 (VR) 13.27 10.52
- Detap 1 4 (R) 17.04 14.31

Average of 22 genotypes 21 18.60 13.57

4. Conclusion
The identification for pod shattering resistance using the oven-dry method had successfully screened the 22 soybean advanced lines into six very resistant genotypes, four resistant genotypes, two moderate genotypes, nine susceptible genotypes, and a very susceptible genotype. Two soybean advanced lines, G511H/Anj///Anj///Anj-3-1 and Dega/Degra-2-105 produced high yield and resistant to pod shattering. Those lines were suggested to be tested across diverse environments to determine their yield potential and stability.

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