Agronomic characteristics of elite soybean lines and the response to pod shattering

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Abstract. The soybean productivity can be increased simultaneously by improving the genetic potential and saving the yield losses due to pod shattering. The research aimed to evaluate the agronomic characters and seed yield of 14 soybean elite lines and two check varieties, and to identify their resistance to pod shattering. The experiment was conducted in two stages, i.e. field, and laboratory research. The field experiment was conducted in Mojokerto (East Java, Indonesia) from February to May 2019, and arranged in a randomized block design using 16 soybean genotypes with four replications. The pod shattering resistance was evaluated using the oven-dry method in the laboratory of ILETRI, Malang. The agronomic characters of days to flowering, days to maturity, 100 seed weight, and seed yield were significantly different among genotypes, meanwhile, the other agronomic characters (plant height, number of nodes, number of branches, number of empty pods, number of filled pods, seed weight per plant) were not significantly different. The range of seed yield of 14 elite lines was 2.76 – 3.14 t/ha, and the check varieties of Anjasmoro and Gema were 2.56 t/ha and 2.76 t/ha, respectively. All elite lines have a large seed size but medium maturity. The shattering evaluation obtained two resistant lines and twelve elite lines as very susceptible to pod shattering. Combination of the characters of high yield (based on the LSI value) and shattering resistance resulted in two elite lines with high yield and shatter-resistant, and eleven high yielding lines but susceptible to pod shattering. Those elite lines could be recommended for varietal development, but with the implication that the high yielded lines and susceptible to shattering need to be harvested immediately after maturity, thus were suggested to be developed in areas with no labor scarcity. On the contrary, it was possible to delay harvest for the resistant elite lines without causing significant yield losses.

1. Introduction

The problem in tropical areas for optimizing soybean production could be due to insect and pest infestation and also the yield losses due to pod shattering incidence. The percentage of yield losses due to pod shattering in soybean was estimated from 34 - 99% [1]. The number of losses was determined by plant external and internal factors. The internal factor was described as the shattering resistance level of a cultivar to pod shattering, meanwhile, the external factors could be due to environmental conditions and the availability of labor. The scarcity of labor at harvesting time has an effect on the delayed harvesting. The use of shattering susceptible cultivars will aggravate the shattered pods.

Pod shattering on soybean can be defined as the incidence of the opening soybean pod at plant maturity, especially on the ventral side of the pod which causes the seeds to release and fall to the ground. Based on those facts, then the most ideal way to minimize the yield losses is to provide soybean
shatter-resistant variety. This approach will have resulted in successful breeding because based on the various studies that have been conducted so far, pod shattering is an inherited trait [2,3] with the susceptibility was partially dominant over resistance [3,4,5].

The efforts to control yield loss due to pod shattering have been carried out in various soybean production centers, starting with the identification of shatter-resistant genotypes and recombining it with economic valuable characters such as high yield [6,7,8,9]. Pod shattering resistance is directly related to the pod characteristics, in terms of morphology, anatomy, and the chemical component of the pods [10,11]. Environmental factors, such as relative humidity and temperature also reported being associated with pod shattering resistance [12,13]. Integrating the determinants of soybean resistance to pod shattering with high yield as supporting agronomic characters would be the ideal choice to increase soybean yield productivity per unit area.

Several studies to identify the relationship between the agronomical plant characters with pod shattering resistance have been conducted. A non-significant relationship was reported between plant morphological traits, as well as the oil and protein content, with pod shattering resistance [8]. A study in Nigeria found that a late-maturing genotype has a greater shattering rate than an early maturing genotype [9]. The longer the maturing age of the plant, the older the pods will be and the pods will shatter more easily. Another study reported that a low number of pods per plant will decrease the shattering percentage, however, a large seed size will increase the pod shattering [14]. In fact, in terms of soybean seed yield, those two characters, namely the number of pods and the seed size, are the important characters needed to support high yield. A previous study showed that the number of pods and number of seeds per plant determines the soybean seed yield [15]. And there was also a pattern for a higher seed yield of a genotype with shorter growth periods that could be associated with better seed filling [16]. In maize, it was found that 1000 seed weight could be determinant in grain yield increase [17].

Based on those various studies, namely the performance of pod shattering resistance and seed yield, and the relationship among them, lead to the conception that it is necessary to develop soybean cultivars with high yield and shatter-resistance. Therefore, this study was designed to evaluate the agronomic characters and seed yield of soybean elite lines and to identify their resistance to pod shattering.

2. Methods

2.1. Research materials
Sixteen genotypes which comprised of fourteen soybean elite lines and two check varieties were used in this study. Those elite lines were developed from various crossing combination, and through the selection procedures to obtain elite lines, namely F3 line. The check cultivars were Anjasmooro (medium maturity, large seed size, pod shatter-resistant), and Gema (early maturity, pod-shatter susceptible).

2.2. Field study
The field experiment to identify the agronomical and yield performances of 16 genotypes was carried out in Mojokerto (East Java, Indonesia) from February to May 2019. Soybeans were planted in lowland after rice planting with zero tillage system. The experimental design was randomized block with four replications. Each experimental unit consisted of 2 m × 4.5 m plot size with plant spacing of 40 cm × 15 cm, two plants/hill. Plants were fertilized with 250 kg/ha Phonska, 100 kg/ha SP 36, and organic fertilizer 1 t/ha which given entirely at planting to cover the planting hole. Mechanical weed control was performed at the plant age of two and four weeks after planting. The pests and diseases were intensively controlled starting from 10 days after planting. The data measured were days to maturity (day), days to flowering (day), plant height (cm), number of branches, number of filled pods, number of empty pods, 100 seed weight, seed weight per plant, and seed yield.
2.3. Laboratory study
The laboratory study was aimed to evaluate the pod shattering resistance of elite lines. The evaluation of shattering resistance was performed in the Laboratory of Breeding, Indonesian Legume and Tuber Crops Research Institute (ILETRI, Malang, East Java, Indonesia). The experiment was arranged in a randomized block design with 16 genotypes as treatment, and each treatment was replicated four times.

The pod samples for shattering evaluation were taken at R_{8} stage (when 95% of pods have attained maturity, leaves turn to yellow with brown pods) by randomly taken five plants from each combination of genotype and replication. Sample plants then were dried at room temperature for three days to equalize the moisture content of all pods. After that, thirty pods were randomly taken and placed in a petri dish (Ø = 15 cm), and subjected to oven drying. The oven-dry method was performed by using the oven temperature of 30°C for three days, and then elevated to 40°C (one day), 50°C (one day), and 60°C (one day) [6].

The percentage of pod shattering was observed after being subjected to each oven temperature treatment. The classification of the resistance based on the percentage of shattering after subjected to 60°C, following the Asian Vegetable Research and Developmental Center [18]: very resistant (0% shattering), resistant (1-10% shattering), moderate (11-25% shattering), susceptible (26 – 50% shattering), and very susceptible (>50% shattering).

2.4. Data analysis
Data collected were subjected to analysis of variance (ANOVA) using PROC GLM of SAS 9.1.3 [19] and heatmap analysis using RStudio Program ver. 1.3.959 [20]. The selection of genotypes based on yield was performed by using LSI (Least Significant Increase) [21].

3. Results and discussion

3.1. The analysis of variance
The analysis of variance for the ten traits measured showed that the genotype effects were highly significant (p > 0.01) for days to flowering, days to maturity, 100 seed weight, seed yield, and pod shattering (Table 1). It indicates the sufficient genetic variability present for those measured traits among the genotypes. Hence, it will provide a potential for improving these traits through a precise selection of desirable genotypes in soybean breeding program [9,22].

| Character                  | Replication  | Genotype  |
|---------------------------|--------------|-----------|
| Days to flowering (day)   | 9.4958<ns>   | 9.4958<*> |
| Days to maturity (day)    | 0.1822<ns>   | 28.0822<**> |
| Plant height (cm)         | 20.6393<ns>  | 74.5018<ns> |
| Number of branches        | 0.3370<ns>   | 0.2402<ns> |
| Number of filled pods     | 597.4166<**> | 92.1750<ns> |
| Number of empty pods      | 0.8406<ns>   | 0.1384<ns> |
| 100 seed weight (g)       | 0.6741<ns>   | 5.3407<**> |
| Seed weight per plant (g) | 102.9441<**> | 11.9972<ns> |
| Seed yield (t/ha)         | 0.0476<*>    | 0.0949<**> |
| Pod shattering (%)        | 5.2116<ns>   | 32.6544<**> |

<ns> = not significant, <*> = significant at 5 % probability level (p < 0.05), <**> = significant at 1 % probability level (p < 0.01).

3.2. Plant age character
Indonesia as a tropical area has mainly grown soybeans in a yearly cropping pattern of paddy-paddy-soybean. Thus, the plant age characteristic has become an important trait. The average of days to
flowering from the 16 genotypes was 32 days, and the number of days to maturity was 82 days (figures 1 and 2). In Indonesia, soybean maturity is classified as early maturity (< 80 days), medium maturity (80-90 days), and late maturity (> 90 days) [23]. Based on this classification, all the tested genotypes were classified as medium maturity.

3.3. Plant growth characters

In this study, the plant growth characters consisted of plant height, number of branches per plant, number of filled pods per plant, and the number of empty pods per plant (figure 3 to figure 6). The range of plant height was 55.38-70.13 cm (an average of 63.02 cm), the number of branches per plant ranged from 0.50-2.88 branches/plant (an average of 2.05), the number of filled pods ranged from 9.38-48.25 pods per plant (an average of 36.56), and the range of the number of empty pods per plant was 1.00-2.63 pods per plant (an average of 1.72).

Seed yield on soybean is a complex character, which is determined by various growth characters that interact with each other. Several growth characters were reported can contribute greatly to improve soybean yields in the future, such as higher biological yield, harvest index, number of pods per plant, lower number of branches per plant, plant height, and lodging score [24,25,26]. A study reported the important characters contributing to the soybean yield, namely plant height, number of clusters per plant, number of pods per plant, number of seeds per pod, and 100 seed weight [27]. Other studies confirmed the biological yield, harvest index, and the number of branches as important characters since they had the utmost correlation with seed yield [28]. Based on those studies above, it can be seen that the growth characters in soybean are important traits for improving yield productivity. Furthermore, seed yield in soybean are determined by various growth characters and differences in yield determinants were also depending on the genetic background of the soybean varieties used in the study.

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3.4. Seed characters

The seed characters were consisted of 100 seed weight, seed weight per plant, and seed yield (figure 7 to figure 9). The seed size (100 seed weight) and seed yield varied among soybean elite lines and the check cultivars, meanwhile, the seed weight per plant was relatively similar. The range of 100 seed weight was 12.86 – 17.39 g/100 seeds (an average of 14.63 g/100 seeds). The seed weight per plant ranged from 9.45 – 14.07 g (an average of 11.57 g per plant), meanwhile the seed yield ranged from 2.56 – 3.14 t/ha (an average of 2.92 t/ha).

In Indonesia, soybean classification for seed size in Indonesia could be divided into small (< 10 g/100 seeds), medium (10-14 g/100 seeds), and large size (> 14 g/100 seeds) [29]. In this study, most of genotypes were categorized as large seeded. Seed size character become an important trait in Indonesia, since the industries prefer the large seed size to be used as tempeh raw material [29,30]. A study reported that seed size was an important physical indicator of seed quality that affects the plant performance in the field. Furthermore, large seed was generally had better field performance than small seed [31]. However, other study showed that small seeded variety was less sensitive to deterioration in yield components under high temperature condition when compared to large seeded genotype [32].
Figure 9. Seed yield of 16 genotypes.
(a = not significantly different according to LSI test)

The role of seed weight per plant in soybean is to determine the seed yield. The importance of this character will be directly related to the plant population at harvest. The seed yield of the 16 soybean genotypes reflects the differences in genetic potential of each soybean genotype. Three elite lines were able to produce yield over 3.0 t/ha. The yields of check cultivars were lower than all the elite lines. A study in Brazilian macro-region obtained the range of seed yield of twelve soybean genotypes was between 2.00 - 2.53 t/ha [33], meanwhile, previous study obtained the range of seed yield from 2.90-4.09 t/ha with an average of 3.61 t/ha [34]. In Ethiopia, an evaluation for seed yield across locations obtained a range of yield from 1.55-2.90 t/ha [35]. The difference in seed yield could be attributed to the genetic potential of a genotype, environment, and crop management [36,37,38].

3.5. Pod shattering resistance
The evaluation of pod shattering resistance using the oven-dry method showed that all genotypes were remaining unshattered at 30°C and 40°C, respectively. The variation in the percentage of pod shattering was showed after subjected to 50°C and 60°C (figure 10). This observation was similar to earlier findings [39,40]. The average of pod shattering at 50°C was 40.27% and it increased to 68.95% at 60°C. The range of shattering at 50°C was 0-61%, meanwhile at 60°C was 2-100%.

The shattering resistance of fourteen elite lines after subjected to 60°C was divided into two groups, namely resistant (two lines) and very susceptible (twelve lines). A previous study showed a high variability was found at 60 °C, suggested that the temperature of 60 °C in the oven-drying method could be used to differentiate the shattering among the genotypes [40]. Screening for pod shattering resistance using the oven-dry method was reported to have many advantages, hence it is widely used to screen the resistance of soybean genotypes to pod shattering [3, 5, 9].

Figure 10. Pod shattering percentage after subjected different temperatures.
3.6. Simultaneous selection

The seed yield and the pod shattering resistance of fourteen elite lines and two check cultivars were presented in table 2. Based on the LSI value by using the seed yield of the best check cultivar, Gema, the LSI value was 2.86 t/ha. Thus, of the 14 elite lines, 13 of them had higher seed yield than the LSI value, and of these 13 lines, four of them had yielded over 3 t/ha (Figure 9).

The classification for pod shattering resistance showed that twelve elite lines were very susceptible, meanwhile, only two elite lines were categorized as resistant to pod shattering (PL-MJ19-13 and PL-MJ19-14). Combination of the characters of high yield (based on the LSI value and shattering resistance resulted in two elite lines with high yield and shatter-resistant, and eleven high yielding lines but susceptible to pod shattering.

The agronomic data of all elite lines and the check cultivar was presented in Figure 11. The high yield and shatter-resistant elite lines (PL-MJ19-13 and PL-MJ19-14) showed a good performance of plant growth and seed characters. The simultaneous selection based on the high yield and pod shattering resistance was to optimize the yield productivity of elite soybean lines. High yielding elite lines but shatter susceptible were recommended to be developed in areas with no labor scarcity, thus the harvesting can be done immediately after plant maturity. On the contrary, the high yielding and shatter-resistant elite lines could be delayed for harvesting without causing significant yield losses.

Table 2. Seed yield and pod shattering of 14 elite lines and two check cultivars.

| No | Genotype     | Seed yield (t/ha) | Pod shattering (%) | Remarks                      |
|----|--------------|-------------------|--------------------|------------------------------|
| 1  | PL-MJ19-01   | 2.87              | 72                 | High yield, shatter-susceptible |
| 2  | PL-MJ19-02   | 2.76              | 78                 | High yield, shatter-susceptible |
| 3  | PL-MJ19-03   | 2.89              | 85                 | High yield, shatter-susceptible |
| 4  | PL-MJ19-04   | 3.14              | 90                 | High yield, shatter-susceptible |
| 5  | PL-MJ19-05   | 2.97              | 86                 | High yield, shatter-susceptible |
| 6  | PL-MJ19-06   | 2.95              | 69                 | High yield, shatter-susceptible |
| 7  | PL-MJ19-07   | 2.92              | 87                 | High yield, shatter-susceptible |
| 8  | PL-MJ19-08   | 2.98              | 82                 | High yield, shatter-susceptible |
| 9  | PL-MJ19-09   | 2.88              | 85                 | High yield, shatter-susceptible |
| 10 | PL-MJ19-10   | 3.12              | 88                 | High yield, shatter-susceptible |
| 11 | PL-MJ19-11   | 2.95              | 81                 | High yield, shatter-susceptible |
| 12 | PL-MJ19-12   | 3.09              | 85                 | High yield, shatter-susceptible |
| 13 | PL-MJ19-13   | 3.01              | 7                  | High yield, shatter-resistant |
| 14 | PL-MJ19-14   | 2.97              | 8                  | High yield, shatter-resistant |
| 15 | Anjasmoro    | 2.56              | 2                  | Shatter-resistant cultivar    |
| 16 | Gema         | 2.67              | 100                | Very susceptible cultivar     |

Mean  | 2.92    | 68.95              |
LSI (Gema) | 2.86    |

LSI = Least Significance Increase
Figure 11. Heat map analysis of the agronomic data and pod shattering resistance of 14 elite lines and two check cultivars, where the dark and light boxes indicate high values and low values, respectively. DTF = days to flowering, DTM = days to maturity, PHT = plant height, NOB = number of branches, NFP = number of filled pods, NEP = number of empty pods, SWG = 100 seed weight, ISW = seed weight per plant, YLD = seed yield.

4. Conclusion
The increase in soybean productivity has the potential to be carried out by providing soybean cultivars with high yield and pod-shatter resistant. Eleven elite lines with high yield, but very susceptible to pod shattering. Two elite lines showed high yield and shatter-resistant. Those elite lines could be recommended for varietal development with different implementations in the field. The high yielded lines but susceptible to shattering were suggested to be developed in areas with no labor scarcity where it can be immediately harvested after plant maturity, meanwhile, the resistant elite lines could be delayed without causing significant yield losses.

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