

The pod shattering resistance of soybean lines based on the shattering incidence and severity

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Abstract: The study is aimed at evaluating the pod shattering resistance of F₈ soybean lines based on the shattering incidence and shattering severity. The materials consist of fourteen F₈ soybean lines and two check cultivars. The pod shattering incidence was examined by using the oven-dry method, meanwhile, the shattering severity was evaluated based on the severity of the pod opening. The pod shattering resistance based on the shattering incidence resulted in five resistant lines (7–10% shattering), seven moderate lines (13–23% shattering), one susceptible line (53% shattering), and one very susceptible line (100% shattering). The pod shattering resistance based on the shattering severity showed that the pod opening on the ventral side differed between the lines and between the shattering degree, and it tends to form sigmoid curves with a different peak position for each shattering degree. The shattering severity of the resistant, moderate, and susceptible lines reached a peak at 60 °C, 50 °C, and 40 °C, respectively. A longer pod length indicated by the length of the dorsal ($r = 0.827^{**}$) and ventral ($r = 0.880^{**}$) sides of the pod, a higher total pod weight (0.827^{**}), and a larger seed size (0.794^{**}) will increase the degree of susceptibility to pod shattering. Those characteristics were considered to be the ones that should be used as the selection criteria in the breeding programme for pod shattering resistance in soybeans.

Keywords: *Glycine max*; oven-dry method; shatter-resistant; ventral side

Yield losses are a major problem in plants that may occur during planting, harvest, and post-harvest. In soybeans, pod shattering is one of the causes of yield losses. The amount of soybean yield losses was reported from negligible to significant (Tiwari & Bhatnagar 1991; Zhang & Boahen 2010; Bhor et al. 2014), which it can be determined by the duration of the harvest delay, environmental condition, and the susceptibility of the variety (Philbrook & Oplinger 1989; Tukamuhabwa et al. 2002; Zhang & Bellaloui 2012; Zhang et al. 2018). Other studies reported that pod shattering was associated with the variation in the anatomical, morphological, and physiological characteristics of the pod (Romkaew

et al. 2008; Gaikwad & Bharud 2018; Zhang et al. 2018). The pod moisture content is considered to have a strong relationship with the pod shattering in soybeans (Romkaew et al. 2007). Environmental relative humidity (RH) may also affect the pod shattering (Caviness 1965; Zhang & Singh 2020) by changing the pod moisture content (Metcalf et al. 1957). Romkaew et al. (2007) reported that low humidity during harvesting decreased the pod moisture content and resulted in higher shattering.

Pod shattering is the opening of the mature pod along the dorsal or ventral sutures and accompanied by the dispersal of the seeds after the plant reaches maturity (Bara et al. 2013). The pod opening was

driven not only by the weakening of the dorsal and ventral shattering zones, but also by the dehydration stress in the cells of the inner sclerenchyma layer (Zhang et al. 2018). Shattering (dehiscence) may be categorised as active or passive. Active dehiscence involves the production of stresses in the drying pods due to an in-built mechanism, which ultimately leads to dehiscence or shattering without any external disturbance. In contrast, passive dehiscence occurs solely from an external impact, and there are no built-in mechanisms to ensure dehiscence, such as the development of stresses in the fruit wall (Kadkol et al. 1989). A passive dehiscence mechanism was reported to be found in Brassica, meanwhile, active dehiscence was found in Fabaceae (Leguminosae) (Kadkol et al. 1986).

Soybean pods consist of two halves, produced from a single carpel enclosing the locule where the seeds are formed. The division of the two halves of the pod is followed by the development of clefts along the dorsal and ventral sutures of the parenchyma (Zhang et al. 2018). Soybean pod shattering has been reported to occur more frequently on the dorsal side than on the ventral side (Tsuchiya 1987; Tiwari & Bhatia 1995; Suzuki et al. 2009). In a soybean pod, the dorsal suture corresponds to the main vein of the carpel, whereas the ventral suture corresponds to the fusion of the two margins of the carpel (Carlson & Lersten 2004). The research on the common vetch by Dong et al. (2017) revealed the role of cell wall modifications and hydrolases genes in dissolving and disappearing the cell wall in the ventral suture of the pod, making the pod more susceptible to shattering.

The most promising effort to increase soybean resistance to pod shattering is through genetic improvement, i.e., through crossing between the parental lines, one of which carries a shattering resistance gene. For example, the soybean genotype from Thailand (SJ2) has been used as a source of resistant genes in Japan (Yamada et al. 2009). In Indonesia, the Anjasmoro variety is used as a source of the resistant gene to improve the shattering resistance (Krisnawati & Adie 2017a). Studies related to the molecular mechanism underlying the pod shattering resistance in soybeans have also been conducted. Funatsuki et al. (2014) found a gene, *Pdh1*, that promotes pod shattering by increasing the torsion of the dried pod walls. Meanwhile, Dong et al. (2014) identified a NAC gene, *SHAT1-5* (*Glyma16g02200*), which activates the secondary wall biosynthesis and promotes a significant thickening of the fibre cap cells in the soybean

pod wall. To date, several studies have successfully identified some loci underlying pod dehiscence in soybeans (Han et al. 2019; Hu et al. 2019).

In addition to the issue of pod shattering that commences in the soybean, the investigation on the pod shattering resistance based on the shattering severity, or the width of the opening pod, has never been performed. This study is important to reveal the difference in the pod shattering resistance pattern between the degrees of resistance based on the pod opening severity and the shattering incidence. Therefore, the objective of this study was to evaluate the pod shattering resistance of the F₈ soybean lines based on the shattering incidence and shattering severity.

MATERIAL AND METHODS

Plant material and field research. Sixteen soybean genotypes was used as the research material, consisting of fourteen promising lines (F₈ generation) and two check cultivars (Table 1). The check cultivars were the Dega 1 (high yielding, susceptible to shattering) and Detap 1 (high yielding, shatter-resistant) varieties. The F₈ lines were derived from the selection of crossing between the shatter-resistant variety (Anjasmoro) with several high yielding genotypes (G100H, Rajabasa, IAC100, Grobogan).

The field experiment was conducted in Probolinggo, Indonesia (7°48'7.2"S, 113°9'32.4"E, 10 m a.s.l., E1 Oldeman climate type, rainfall of 2200 mm/year) during the dry season (July to October) 2019. The mean temperature and total rainfall during the growing season are presented in Figure 1.

The plants in the field trial were planted in a lowland after the rice planting without any soil tillage and arranged in a randomised block design with four replications. The plot size of each of the F₈ generation lines was 2 × 4.5 m, with a 40 × 15 cm planting distance, two seeds per hill. The plants were fertilised by 50 kg/ha urea, 100 kg/ha SP36, and 75 kg/ha KCl at the time of sowing. The pests and diseases were controlled by intensive monitoring.

Evaluation for pod shattering incidence. Three sample plants from each combination of the line and replication were randomly taken at the R₈ stage, i.e., when the plants reached full maturity or 95% of the pods had turned brown (full mature colour). The sample plants were then dried at room temperature for three days. The plants were leaned against a wall/fence in an upright position, in a place that was not

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Table 1. The pedigrees and characteristics of the tested soybean lines

Code	Genotype	Pedigree	Remark	F	Characteristics		
					DTF	DTM	SDZ
1	Anj/G100H-6	Anjasmoro × G100H	promising line	F ₈	34	84	14.38
2	Anj/G100H-14	Anjasmoro × G100H	promising line	F ₈	37	83	14.45
3	Anj/G100H-16	Anjasmoro × G100H	promising line	F ₈	34	82	15.09
4	Anj/G100H-21	Anjasmoro × G100H	promising line	F ₈	35	83	15.99
5	Anj/G100H-24	Anjasmoro × G100H	promising line	F ₈	34	82	15.15
6	Anj/G100H-28	Anjasmoro × G100H	promising line	F ₈	38	84	14.88
7	Anj/G100H-44	Anjasmoro × G100H	promising line	F ₈	36	84	16.43
8	Anj/IAC100-19	Anjasmoro × IAC100	promising line	F ₈	35	81	15.37
9	Anj/Rjbs-304	Anjasmoro × Rajabasa	promising line	F ₈	34	81	15.39
10	Anj/Rjbs-305	Anjasmoro × Rajabasa	promising line	F ₈	34	80	18.10
11	Anj/Rjbs-306	Anjasmoro × Rajabasa	promising line	F ₈	34	81	14.77
12	Anj/Rjbs-309	Anjasmoro × Rajabasa	promising line	F ₈	34	81	14.70
13	Anj/Rjbs-311	Anjasmoro × Rajabasa	promising line	F ₈	35	80	14.87
14	Grbg/Anj-2	Grobogan × Anjasmoro	promising line	F ₈	36	83	16.48
15	Dega 1	–	released variety	–	34	79	18.75
16	Detap 1	–	released variety	–	39	83	14.99

F – filial generation; DTF – days to flowering (day); DTM – days to maturity (day); SDZ – 100 seed weight (g)

exposed to direct sunlight. Thirty pods were randomly detached from each of the three sample plants, and placed in petri dishes (Ø = 15 cm).

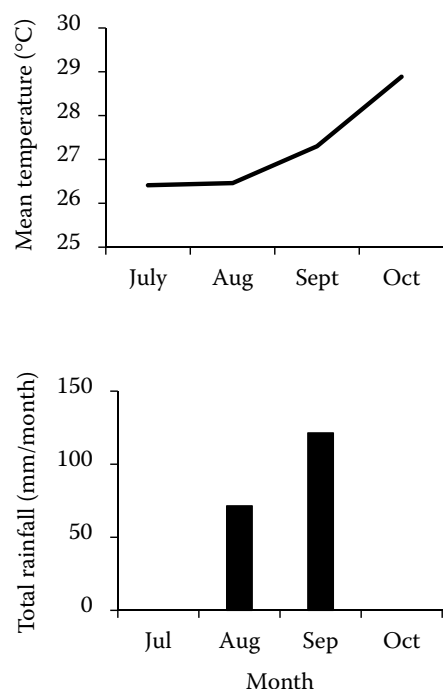


Figure 1. The mean temperature and rainfall during the growing season (July to October 2019)

The evaluation for the pod shattering incidence used the oven-dry method (Krisnawati & Adie 2017b) i.e., the sample pods were dried at 30 °C for three days, and then elevated to 40 °C (one day), 50 °C (one day), and 60 °C (one day). The pod shattering incidence was observed after being subjected to each oven temperature. The shattering incidence was calculated from the number of the shattered pods divided by the number of sample pods (thirty pods), in percentage. The degree of shattering resistance was determined after being subjected to 60 °C.

Evaluation for pod shattering severity. Three sample plants from each combination of the line and replication were randomly taken at the R₈ stage and then dried at room temperature for three days. Thirty pods were randomly detached from each of the three sample plants, and placed in petri dishes (Ø = 15 cm). The pod shattering based on the shattering severity was evaluated using the oven-dry method (Krisnawati & Adie 2017b), i.e., the sample pods were dried at 30 °C for three days, and then elevated to 40 °C (one day), 50 °C (one day), and 60 °C (one day). The shattering severity was observed after being subjected to an oven temperature of 30 °C (on the fourth day), 40 °C (on the fifth day), 50 °C (on the sixth day), and 60 °C (on the seventh day), respectively. The observation for the shattering

severity was based on the length of opening pod in the ventral side of the pod. The shattering severity was calculated by dividing the length of the opening pod on the ventral side by the total pod length on the ventral side, in percentage.

Observation. The classification of the resistance for the shattering incidence was based on the Asian Vegetable Research and Developmental Center (AVRDC 1979). The observation was also made for the pod characteristics, i.e., the number of seeds per pods, the pod length of the dorsal and ventral sides (Figure 2), the ratio of the length of the ventral and dorsal side, the total pod weight (g), and the 100 seed weight (g). The pod length of the dorsal side and the ventral side were measured using a sewing thread. Then, the thread was measured with a ruler to obtain their lengths (cm).

The pod characteristics were subjected to a boxplot analysis which consists of the minimum value, the first quartile (Q1), the median, the third quartile (Q3), the interquartile (IQ) range, and the maximum value. To observe the pattern of shattering severity of each line, the sample pods (30 pods) of each temperature (x -axis) were plotted against the shattering severity (%), respectively. To observe the shattering severity pattern on each shattering degree, the sample pods from all the temperatures (120 pods) of the susceptible lines, moderate lines, and resistant lines (x -axis) were plotted against the shattering severity (%), respectively. The relationship between the pod shattering with the pod characteristics was investigated using Pearson's correlation. The data were analysed by the statistical software Minitab (Ver. 14, 2004) for the descriptive statistics (boxplot) and Pearson's correlation coefficients.

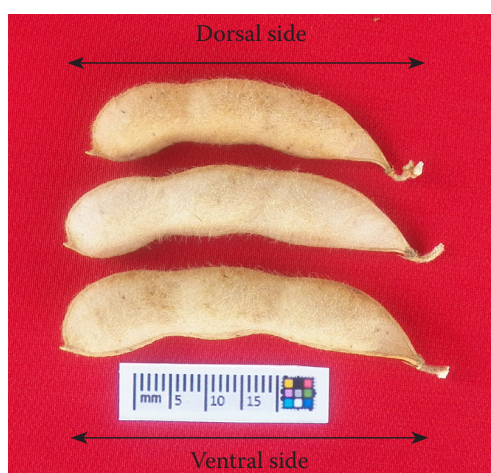


Figure 2. The ventral and dorsal sides of a mature soybean pod

RESULTS AND DISCUSSION

The performance of the pod characteristics.

Pod shattering in soybeans is related to the genetic constituent of the pod morphology. The characteristics of the pods varied between the F_8 soybean lines (Figure 3). Outliers were found in all the boxplots.

The length of the dorsal side ranged from 4.62 to 5.48 cm (average of 4.88 cm), with a median range of 4.62–5.48 cm. The range of the ventral length side was 4.00–4.81 cm (an average of 4.18 cm) with a median range of 3.96–4.84 cm. The length of the ventral side tended to show a similar pattern with the dorsal side. The soybean variety Dega 1 had the longest dorsal and ventral pod length, whereas Degtap 1 had the shortest dorsal and ventral size. The ratio of the length of the ventral to the dorsal side almost had a similar value, from 0.84 to 0.88 (an average of 0.85). The ratio between the V/D reflects the degree of the curvature of the soybean pod. The range of V/D was 0.85–1.36 with an average of 1.10. Most of the lines showed a similar degree of pod curvature. The average number of seeds per pod varied from 2.04 to 2.41 seeds per pod (an average of 2.30 seeds per pod).

Pod shattering incidence. Pod shattering has become an important problem in soybeans because it is related to the yield losses. In wild taxa, pod shattering (or pod dehiscence) is important for the propagation and adaptation of the offspring under various growth conditions. However, shattering is not a preferred feature in crop plants, because it makes harvesting difficult and often leads to significant production losses (Ogutcen et al. 2018). Soybean cultivars with shattering resistance and an economically beneficial agronomic characteristic are categorised as ideal varieties. Such varieties play an important role in saving the yield losses and the ability to secure the soybean production.

The pod shattering based on the shattering incidence showed a variation between lines at various degrees of oven temperatures (Table 2). At 30 °C, all the lines were resistant to the shattering. However, a gradual increase in the temperature provided a different response in the resistance for each line. The oven temperatures of 40 °C and 50 °C resulted in the degree of resistance from very resistant to very susceptible. At the end of the oven-drying treatment (60 °C), the degrees of resistance of the fourteen lines were classified into five resistant lines, seven moderate lines, one susceptible line, and one very

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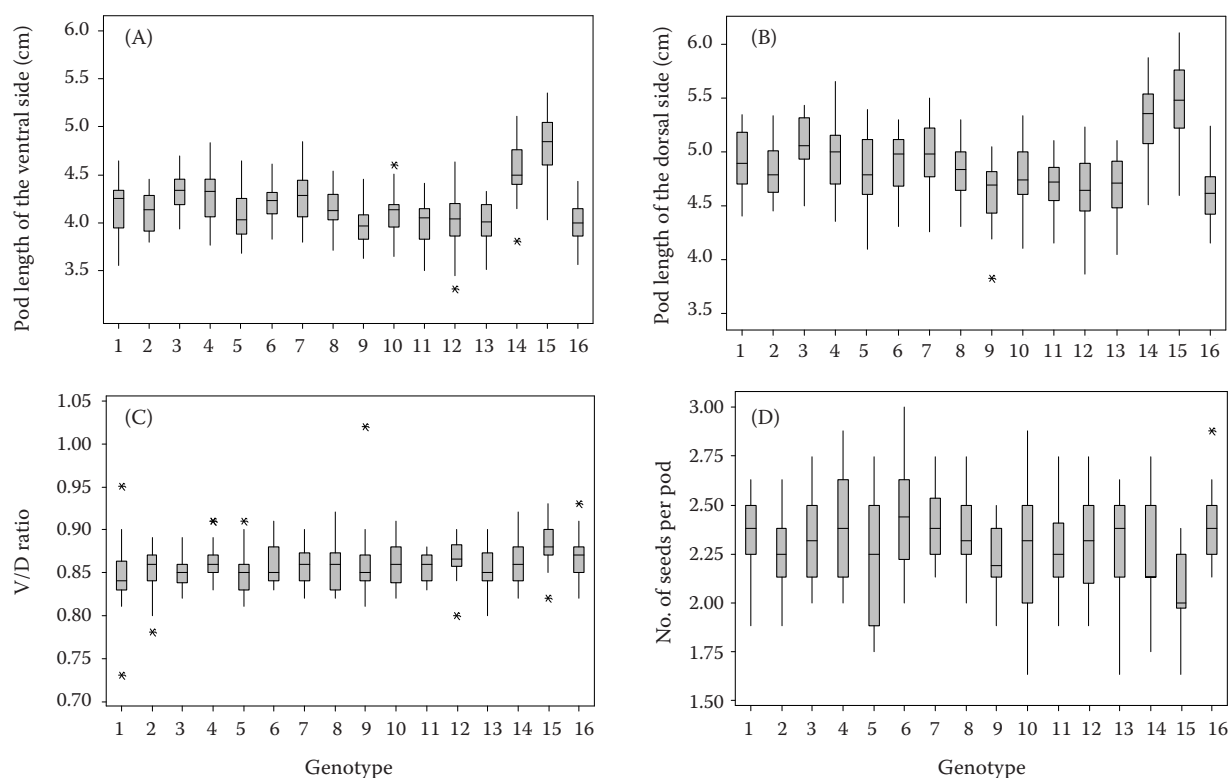


Figure 3. The boxplots of the pod length of the ventral side (A), pod length of the dorsal side (B), the ratio of the ventral and dorsal side (C), and the number of seeds per pod (D)

The number of the genotype refers to Table 1; the extreme values (outliers) are marked with an asterisk on the boxplot

Table 2. The shattering incidence at various oven temperature levels

No.	F ₈ soybean lines	Pod shattering (%) at oven temperature			
		30 °C	40 °C	50 °C	60 °C
1	Anj/G100H-6	0	3	10	23
2	Anj/G100H-14	3	17	20	23
3	Anj/G100H-16	0	7	7	20
4	Anj/G100H-21	0	0	0	30
5	Anj/G100H-24	0	0	0	10
6	Anj/G100H-28	3	3	3	13
7	Anj/G100H-44	0	0	3	7
8	Anj/IAC100-19	0	3	3	20
9	Anj/Rjbs-304	0	0	0	7
10	Anj/Rjbs-305	0	3	3	10
11	Anj/Rjbs-306	0	7	7	10
12	Anj/Rjbs-309	0	3	3	20
13	Anj/ Rjbs-311	0	0	0	23
14	Grbg/Anj-2	0	23	43	53
15	Dega 1 (check cultivar)	0	80	100	100
16	Detap 1 (check cultivar)	0	0	3	10
Average		0	9	13	24

■ – very resistant; ■ – resistant; ■ – moderate; ■ – susceptible; ■ – very susceptible



Figure 4. The variation in the degree of shattering incidence of the F_8 soybean lines: very resistant (A), resistant (B), moderate (C), susceptible (D), very susceptible (E)

susceptible line. The variation in the degree of shattering incidence of the F_8 soybean lines is presented in Figure 4.

The shattering resistances of the check cultivars Detap 1 and Dega 1 were resistant and susceptible, respectively. In this study, the genetic improvement for the shattering resistance through hybridisation using a resistant parent (the Anjasmoro variety) resulted in five F_8 resistant lines that have comparable resistance with the check cultivar (Detap 1). Detap 1 was also derived from the progeny selection from the Anjasmoro variety as a resistant gene source. The effectiveness of the Anjasmoro variety as a parental line has been reported in the previous selection study (Krisnawati et al. 2019a).

According to Gan et al. (2008), screening breeding materials for resistance to shattering is difficult as pod shattering characteristics are also affected by factors other than genetics, such as the degree of pod maturity, the timing of the pod senescence, and the harvest method. The use of different methods to quantify the shattering resistance also affects the degree of resistance (Romkaew & Umezaki 2006). In this study, the use of the oven-drying method (Krisnawati & Adie 2017b) provides greater pressure

and potentially results in a high shatter-resistance genotype. There have been many studies in the use of the oven-drying method with varying temperatures to measure the degree of pod shattering in soybeans, such as oven-drying at 60 °C for 24 h (AVRDC 1979), oven-drying at 80 °C for 5 h (Tukamuhabwa et al. 2002), oven-drying at 60 °C for 12 h (Mohammed et al. 2014), and a hot air oven for 44 °C (6 hours in a day and ambient temperature at night) for 7 days (Bhor et al. 2014). In this study, the use of temperature gradients was intended to set the gradual drying of the pods and to show the critical shattering temperature which enables one to differentiate the pod shattering degree among the genotypes. Most of the lines shattered at 50 °C, and a high variability was found at 60 °C. It seems that the temperature of 60 °C in the oven-drying method could be used to differentiate the shattering among the genotypes.

The increase in the oven temperature causes different resistance changes between the lines. For example, four lines (Anj/G100H-21, Anj/G100H-24, Anj/Rjbs-304, and Anj/Rjbs-311) were shown to be very resistant after being subjected to an oven temperature 50 °C, but all of those four lines showed different resistance after being subjected to 60 °C.

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This shows that each line has a different buffering capacity, which can be caused by the genetic factor that is reflected by the differences in the anatomical, morphological and physiological characteristics of the pods (Zhang et al. 2018; Liu et al. 2019). Thus, pod shattering based on the severity of the opening pod was then investigated to obtain a further understanding of the difference in the resistance pattern at each oven temperature treatment and the degree of resistance, respectively.

Pod shattering severity. Pod shattering is the opening along the ventral or dorsal side of the pod, starting from the tip to the base of the pod. In this study, the observation of the soybean pod was focused on the ventral side, as it has been reported that the ventral suture is critical to the pod shattering in *Glycine max* (Agrawal et al. 2002; Christiansen et al. 2002; Dong & Wang 2015; Tu et al. 2019). The degree of resistance of a cultivar to pod shattering could be a combination of the severity and speed of opening the ventral side of the pod. The pod shattering severity was determined by the length of the pod opening on the ventral side. The value of the shattering severity was obtained by dividing the length of the pod opening on the ventral side by the total pod length on the ventral side, in percentage. An example of the pod opening at 100% severity is

presented in Figure 5. Figure 6 shows that each line has a different pattern of the pod opening (shattering severity) at each oven temperature treatment.

Previously, based on the shattering incidence, five resistant genotypes (four lines and a check cultivar) were identified with a pod shattering percentage that ranged from 7–10% (Table 2). The pod opening pattern on the ventral side showed that most of those shattered pods had 100% opening pod at 60 °C, and only several lines had a shattering severity under 30%. This result was contradictory to that obtained in the susceptible lines. Most of the susceptible lines had a 100% pod opening on the ventral side which started at 40 °C, hence the 100% shattering severity for all the rest of the shattered pods were achieved at 60 °C. The moderately resistant lines showed a gradual shattering severity. At 50 °C, the pod opening on the ventral side of shattered pods was about 30% and then increase to 100% at 60 °C.

The patterns of pod shattering severity on the ventral side of the resistant lines, moderate, and susceptible are presented in Figure 7. The resistant lines showed a 100% pod opening of the shattered pods started mostly at 60 °C. The 100% shattering severity was low and it was indicated by the sigmoid curve with a relatively broad peak at the right end. The moderately resistant lines showed the shattering



Figure 5. The pod opening at 100% shattering severity

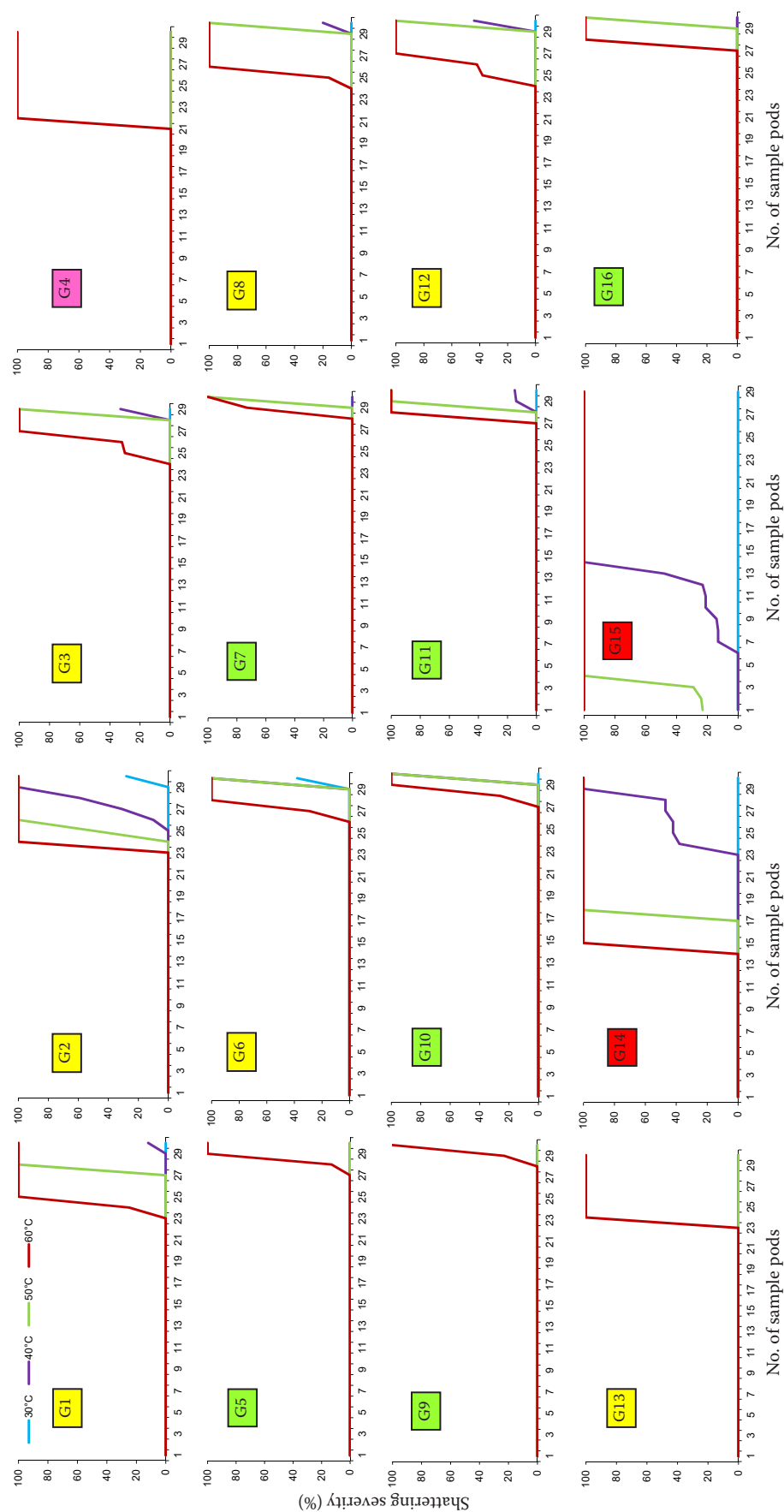


Figure 6. The shattering severity of the F_8 soybean lines and check cultivars

The letter G (stands for genotype) followed by the number (1–16) represents the genotype code, refer to Table 1; the background colour of the genotype code represents the shattering degree (green – resistant, yellow – moderate, pink – susceptible, red – very susceptible); the number of sample pods of a genotype was 30 pods

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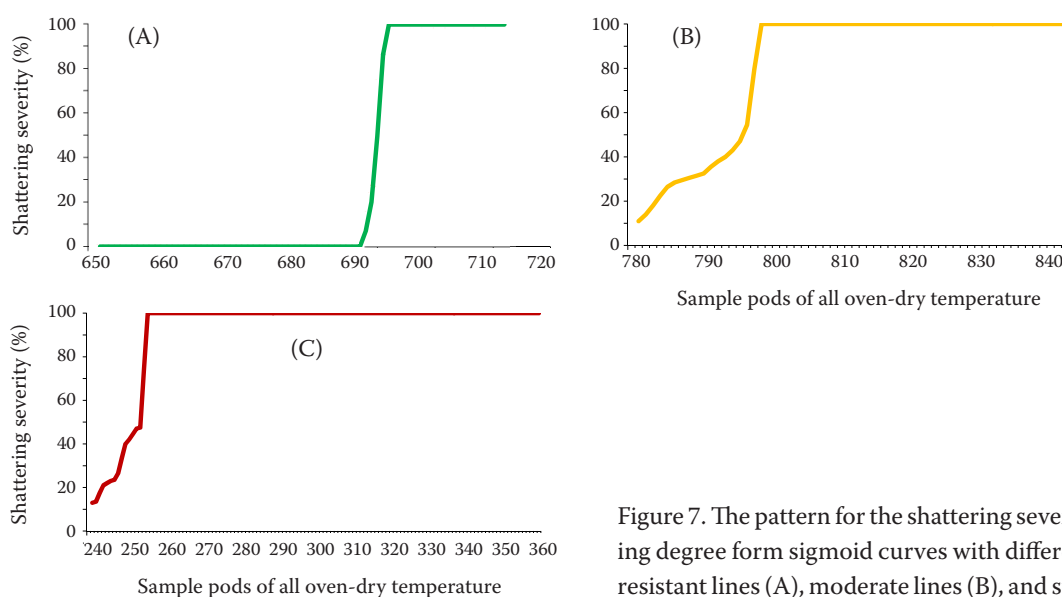


Figure 7. The pattern for the shattering severity of each shattering degree form sigmoid curves with different peak positions: resistant lines (A), moderate lines (B), and susceptible lines (C)

severity tended to form a sigmoid curve, in which the pod opening started mostly at 50 °C and then reached 100% at 60 °C. The percentage of shattering severity of the susceptible lines at 100% was high. It formed a sigmoid curve with the highest peak starting at the left end, in which the pod opening of the shattered pods started mostly at 40 °C. The pattern of shattering indicates that the tissues are under tension. Either natural or mechanical conditions stimulate the rapid separation of the tissue at a specific point (Spence et al. 1996).

So far, no studies have investigated the severity of the pod opening in soybeans. Since the pod shattering in soybeans takes place first at the ventral suture (Dong et al. 2014), the study of this part is important so that the pattern of opening the pods can be used as a basis for identifying the resistance of each soybean genotype to the pod shattering. Also, it will provide

fundamental information to further study and fully understand the shattering mechanism and the factors involved and to formulate strategies for managing or solving the frontline issue of pod shattering.

The relationship between the pod characteristics with shattering. The relationship between the pod characteristics with the pod shattering showed a similar pattern (Table 3) in the oven temperature of 50 °C and 60 °C. The pod shattering at 50 °C had a strong positive correlation with the length of the dorsal side ($r = 0.811^{**}$), the length of the ventral side ($r = 0.862^{**}$), the V/D ratio ($r = 0.863^{**}$), the total pod weight (0.842^{**}), and the 100-seed weight (0.805^{**}), but it had a strong negative correlation with the number of seeds per pod ($r = -0.710^{**}$). The pod shattering at 60 °C with those pod characteristics also showed a similar correlation value, i.e., the length of the dorsal side ($r = 0.827^{**}$), the

Table 3. The correlation between the pod shattering with several pod characteristics

Characters	PS ₆₀	PLD	PLV	V/D	S/P	TW	B100
PS ₅₀		0.811 ^{**}	0.862 ^{**}	0.863 ^{**}	-0.710 ^{**}	0.842 ^{**}	0.805 ^{**}
PLD	0.827 ^{**}		0.985 ^{**}	0.984 ^{**}	-0.334	0.690 ^{**}	0.526 [*]
PLV	0.880 ^{**}	0.985 ^{**}		1.000 ^{**}	-0.372	0.778 ^{**}	0.629 ^{**}
V/D	0.862 ^{**}	0.984 ^{**}	1.000 ^{**}		-0.376	0.782 ^{**}	0.634 ^{**}
S/P	-0.612 [*]	-0.334	-0.372	-0.376		-0.613 ^{**}	-0.648 ^{**}
TW	0.827 ^{**}	0.690 ^{**}	0.778 ^{**}	0.782 ^{**}	-0.613 [*]		0.884 ^{**}
B100	0.794 ^{**}	0.526 [*]	0.629 ^{**}	0.634 ^{**}	-0.648 ^{**}	0.884 ^{**}	

PS₅₀ – pod shattering at 50 °C; PS₆₀ – pod shattering at 60 °C; PLD – pod length of the dorsal side; PLV – pod length of the ventral side; V/D – ratio of the length of ventral and dorsal side; S/P – number of seeds per pod; TW – total pod weight; B100 – 100 seed weight; ^{*}significant at 5% probability level ($P < 0.05$); ^{**}significant at 1% probability level ($P < 0.01$)

length of the ventral side ($r = 0.880^{**}$), the V/D ratio ($r = 0.862^{**}$), the total pod weight (0.827^{**}), the 100-seed weight (0.794^{**}), and the number of seeds per pod ($r = -0.612^{*}$). A previous study by Kataliko (2019) also found a negative correlation between the pod shattering and the number of seeds per pod ($r = -0.13^{*}$).

The result of this study showed that the longer the pod length, which was indicated by the length of the dorsal and ventral sides of the pods, will increase the degree of the pod shattering in the soybean pods. The longer dorsal and ventral sides of the pods indicate the longer part of the pod suture, hence, it will facilitate the pod opening more. A longer pod may be assumed to result in a thinner pod wall. The previous study revealed that the pod length characteristic may play a role as a determinant factor in the pod shattering resistance in soybeans (Krisnawati et al. 2019b). Small pods are also presumed to be composed of thin pod walls (Suzuki et al. 2009).

The characteristic of the length of the dorsal side with the ventral side has a strong relationship, and thus, the effect can also be found in the V/D ratio. Furthermore, a higher total pod weight and 100-seed weight tend to increase the pod shattering. It can be assumed that a higher total pod weight will result in a larger pod size (length and/or width), as well as the 100-seed weight, which shows the seed size. An interesting fact was shown by the characteristic of the number of seeds per pod, where a fewer number of seeds per pod will increase the resistance to the pod shattering. As a result, the smaller number of seeds in the pod may reduce the pressure on the pod wall produced by the seeds. A previous study reported that the pod shattering resistance positively correlated to the pod wall weight (Morgan 1998).

Pod characteristics have been studied in detail, and some anatomical and morphological structures of the soybean pod have been recognised as being important for the shatter resistance (Tsuciya 1987; Tiwari & Bhatia 1995; Christiansen et al. 2002; Bara et al. 2013). In this study, the longer pod length, which is specifically shown by the length of the dorsal and ventral side of the pod, the total pod weight and the seed size were more significant in determining the soybean resistance to the pod shattering. This finding suggests the use of those characteristics as the selection criteria to the pod shattering resistance in a soybean breeding programme. Another study by Tiwari and Bhatia (1995) reported that the thickness and length of the bundle cap on the dorsal side of the

pod and the thickness of the pod are negatively and significantly correlated to the degree of pod shattering. Meanwhile, according to Dong et al. (2014), the excessively lignified fibre cap cells (FCCs) with an abscission layer unchanged in the pod ventral suture are the main cellular characteristic of the shattering-resistant trait. However, further study related to other anatomical and morphological factors that may be involved is needed. Since the pod shattering can also be affected by climatic and environmental factors (Tukamuhabwa et al. 2002), hence, the pod shattering resistance over the years need to be considered in the further research.

CONCLUSION

The screening for pod shattering based on the shattering incidence resulted in five resistant lines, seven moderate lines, one susceptible line, and one very susceptible line. The pod shattering resistance based on the shattering severity showed that the pod opening on the ventral side differs between the lines for each level of resistance. The resistant lines showed that the pod opening started from 60 °C, and it forms a curve with a peak at the right end. The moderately resistant lines tend to form a sigmoid curve which the pod opening mostly started at 50 °C. The shattering severity pattern of the susceptible lines formed a curve with the highest peak starting at the left end, in which the pod opening of the shattered pods mostly started at 40 °C. A longer pod length, indicated by the length of the dorsal and ventral side of the pod, a higher total pod weight, and a larger seed size will increase the degree of the pod shattering susceptibility. Thus, those characteristics should potentially be used as the selection criteria to the pod shattering resistance in a soybean breeding programme.

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REFERENCES

- Agrawal A.P., Basarkar P.W., Salimath P.M., Patil S.A. (2002): Role of cell wall-degrading enzymes in pod shattering process of soybean *Glycine max* (L) Merrill. *Current Science*, 82: 58–61.
- AVRDC (1979): Soybean Report. Shanhsua, Asian Vegetable Research and Development Centre.

<https://doi.org/10.17221/20/2020-CJGPB>

- Bara N., Khare D., Srivastava A.N. (2013): Studies on the factors affecting pod shattering in soybean. *Indian Journal of Genetics and Plant Breeding*, 73: 270–277.
- Bhor T.J., Chimote V.P., Deshmukh M.P. (2014): Inheritance of pod shattering in soybean [*Glycine max* (L.) Merrill]. *Electric Journal of Plant Breeding*, 5: 671–676.
- Carlson J.B., Lersten N.R. (2004): Reproductive morphology. In: Boema H.R., Specht J.E. (eds.): *Soybeans: Improvement, Production, and Uses*. Agronomy Monograph No. 16. Madison, ASA, CSSA, and SSSA.
- Caviness C.E. (1965): Effects of relative humidity on pod dehiscence in soybeans. *Crop Science*, 5: 511–513.
- Christiansen L.C., Degan F.D., Ulvskov P., Borkhardt B. (2002): Examination of the dehiscence zone in soybean pods and isolation of a dehiscence-related endopolygalacturonase gene. *Plant, Cell and Environment*, 25: 479–490.
- Dong Y., Wang Y. (2015): Seed shattering: from models to crops. *Frontiers in Plant Science*, 6: 476.
- Dong Y., Yang X., Liu J., Wang B., Liu B., Wang Y. (2014): Pod shattering resistance associated with domestication is mediated by a NAC gene in soybean. *Nature Communications*, 5: 3352.
- Dong R., Dong D., Luo D., Zhou Q., Chai X., Zhang J., Xie W., Liu W., Dong Y., Wang Y., Liu Z. (2017): Transcriptome analyses reveal candidate pod shattering-associated genes involved in the pod ventral sutures of common vetch (*Vicia sativa* L.). *Frontiers in Plant Science*, 8: 649.
- Funatsuki H., Suzuki M., Hirose A., Inaba H., Yamada T., Hajika M., Komatsu K., Katayama T., Sayama T., Ishimoto M., Fujino K. (2014): Molecular basis of a shattering resistance boosting global dissemination of soybean. *Proceedings of the National Academy of Sciences of the USA*, 111: 17797–17802.
- Gaikwad A.P., Bharud R.W. (2018): Effect of harvesting stages and biochemical factors on pod shattering in soybean, *Glycine max* (L.) Merrill. *International Journal of Current Microbiology and Applied Sciences*, 7: 1015–1026.
- Gan Y., Malhi S.S., Brandt S.A., McDonald C.L. (2008): Assessment of seed shattering resistance and yield loss in five oilseed crops. *Canadian Journal of Plant Science*, 88: 267–270.
- Han J., Han D., Guo Y., Yan H., Wei Z., Tian Y., Qiu L. (2019): QTL mapping pod dehiscence resistance in soybean (*Glycine max* L. Merr.) using specific-locus amplified fragment sequencing. *Theoretical and Applied Genetics*, 132: 2253–2272.
- Hu D., Kan G., Hu W., Li Y., Hao D., Li X., Yang H., Yang Z., He X., Huang F., Yu D. (2019): Identification of loci and candidate genes responsible for pod dehiscence in soybean via genome-wide association analysis across multiple environments. *Frontiers in Plant Science*, 10: 811.
- Kadkol G.P., Beilharz V.C., Halloran G.M., MacMillan R.H. (1986): Anatomical basis of shatter-resistance in the oil-seed Brassicas. *Australian Journal of Botany*, 34: 595–601.
- Kadkol G.P., Halloran G.M., MacMillan R.H., Caviness C.E. (1989): Shatter resistance in crop plants. *Critical Reviews in Plant Sciences*, 8: 169–188.
- Kataliko R.K., Kimani P.M., Muthomi J.W., Wanderi W.S., Olubayo F.M., Nzuve F.M. (2019): Resistance and correlation of pod shattering and selected agronomic traits in soybeans. *Journal of Plant Studies*, 8: 39–48.
- Krisnawati A., Adie M.M. (2017a): Variability on morphological characters associated with pod shattering resistance in soybean. *Biodiversitas*, 18: 73–77.
- Krisnawati A., Adie M.M. (2017b): Identification of soybean genotypes for pod shattering resistance associated with agronomical and morphological characters. *Biosaintifika*, 9: 193–200.
- Krisnawati A., Soegianto A., Waluyo B., Kuswanto (2019a): Selection of F₆ soybean population for pod shattering resistance. *Biodiversitas*, 20: 3340–3346.
- Krisnawati A., Adie M.M., Soegianto A., Waluyo B., Kuswanto (2019b): Pod shattering resistance and agronomic traits in F₅ segregating populations of soybean. *SABRAO Journal of Breeding and Genetics*, 51: 266–280.
- Liu X., Tu B., Zhang Q., Herbert S.J. (2019): Physiological and molecular aspects of pod shattering resistance in crops. *Czech Journal of Genetics and Plant Breeding*, 55: 87–92.
- Metcalfe D.S., Johnson I.J., Shaw R.H. (1957): The relation between pod dehiscence, relative humidity and moisture equilibrium in birdsfoot trefoil, *Lotus corniculatus*. *Agronomy Journal*, 49: 130–134.
- Mohammed H., Akromah R., Abudulai M., Mashark S.A., Issah A. (2014): Genetic analysis of resistance to pod shattering in soybean. *Journal of Crop Improvement*, 28: 17–26.
- Morgan C.L., Bruce D.M., Child R.D., Ladbroke Z.L., Arthur A.E. (1998): Genetic variation for silique shatter resistance among lines of oilseed rape developed from synthetic *B. napus*. *Field Crops Research*, 58: 153–165.
- Ogutcen E., Pandey A., Khan M.K., Marques E., Penmet-sa R.V., Kahraman A., von Wettberg E.J.B. (2018): Pod shattering: a homologous series of variation underlying domestication and an avenue for crop improvement. *Agronomy*, 8: 137.
- Philbrook B., Oplinger E.S. (1989): Soybean field losses as influenced by harvest delays. *Agronomy Journal*, 81: 251–258.
- Romkaew J., Umezaki T. (2006): Pod dehiscence in soybean: assessing methods and varietal difference. *Plant Production Science*, 9: 373–382.

- Romkaew J., Umezaki T., Suzuki K., Nagaya Y. (2007): Pod dehiscence in relation to pod position and moisture content in soybean. *Plant Production Science*, 10: 292–296.
- Romkaew J., Nagaya Y., Goto M., Suzuki K., Umezaki T. (2008): Pod dehiscence in relation to chemical components of pod shell in soybean. *Plant Production Science*, 11: 278–282.
- Spence J., Vercher Y., Gates P., Harris N. (1996): Pod shatter in *Arabidopsis thaliana*, *Brassica napus* and *B. juncea*. *Journal of Microscopy*, 181: 195–203.
- Suzuki M., Fujino K., Funatsuki H.A. (2009): Major soybean QTL, qPDH1, controls pod dehiscence without marked morphological change. *Plant Production Science*, 12: 217–223.
- Tiwari S.P., Bhatnagar P.S. (1991): Pod shattering as related to other agronomic attributes in soybean. *Tropical Agriculture*, 68: 102–103.
- Tiwari S.P., Bhatia V.S. (1995): Characterization of pod anatomy associated with resistance to pod-shattering in soybeans. *Annals of Botany*, 72: 483–85.
- Tsuciya T. (1987): Physiological and genetic analysis of pod shattering in soybeans. *Japan Agricultural Research Quarterly*, 21: 166–175.
- Tu B., Liu C., Wang X., Li Y., Zhang Q., Liu X., Herbert S.J. (2019): Greater anatomical differences of pod ventral suture in shatter-susceptible and shatter resistant soybean cultivars. *Crop Science*, 59: 2784–2793.
- Tukamuhabwa P., Dashiell K.E., Rubaihayo P., Nabasirye M. (2002): Determination of field yield loss and effect of environment on pod shattering in soybean. *African Crop Science Journal*, 10: 203–209.
- Yamada T., Funatsuki H., Hagihara S., Fujita S., Tanaka Y., Tsuji H., Hajika M. (2009): A major QTL, qPDH1, is commonly involved in shattering resistance of soybean cultivars. *Breeding Science*, 59: 435–440.
- Zhang J., Singh A.K. (2020): Genetic control and geo-climate adaptation of pod dehiscence provide novel insights into soybean domestication. *Genes Genomes Genetics*, 10: 545–554.
- Zhang L., Boahen S. (2010): Evaluation of critical shattering time of early-maturity soybeans under early soybean production system. *Agriculture and Biology Journal of North America*, 1: 440–447.
- Zhang L., Bellalloui N. (2012): Effects of planting dates on shattering patterns under early soybean production system. *American Journal of Plant Sciences*, 3: 119–124.
- Zhang Q., Tu B., Liu C., Liu X. (2018): Pod anatomy, morphology and dehiscing forces in pod dehiscence of soybean (*Glycine max* (L.) Merrill). *Flora*, 248: 48–53.

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