BIODIVERSITAS
Volume 18, Number 1, January 2017
Pages: 73-77

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d180111

Variability on morphological characters associated with pod shattering resistance in soybean

AYDA KRISNAWATI♥, M. MUCHLISH ADIE
Indonesia Legumes and Tuber Crop Research Institute. Jl. Raya Kendalpayak Km 8, PO Box 66 Malang 65101, East Java, Indonesia. Tel.: +62-341-801468, 801075, Fax.: +62-341-801496, ♥email: my_ayda@yahoo.com

Manuscript received: 29 February 2016. Revision accepted: 16 November 2016.

Abstract. *Krisnawati A, Adie MM. 2016. Variability on morphological characters associated with pod shattering resistance in soybean. Biodiversitas 17: 73-77.* Pod shattering is one of the major constraint associated with soybean production during dry season in Indonesia. The objectives of the study were to investigate varietal difference of pod shattering and to identify the morphological pod characters related to pod shattering. The field study was carried out in Blitar (East Java, Indonesia) during the dry season 2015. Morphological traits of pod were studied for their association with pod shattering trait in 30 soybean genotypes. The results showed significant differences between genotypes for all characters studied. The degree of shattering varied among genotypes with shattering percentage ranging from 2.5% (G511H/Argom//Argom-2-1) to 100% (Grobogan) with mean of 30%.However, among the 30 genotypes studied, 13 genotypes were relatively resistant, 11 genotypes moderate, 1 genotype susceptible, and 5 genotypes were very highly susceptible. Further path coefficient analysis indicated direct effects of the pod wall thickness and pod length on shattering percentage while other causal effects were small. These characters (pod wall thickness and pod length) may play role as determinant factors in pod shattering resistance. Therefore, soybean resistance to pod shattering could be enhanced by increasing thickness of the pod wall.

Keywords: *Glycine max*, pod characteristics, pod shattering, resistance

INTRODUCTION

In Indonesia, soybean is mostly cultivated during the second dry season, from June/July until September/ October. The typical characteristics of dry season are high temperature and low humidity, which adversely affect soybean growth and development. One of the major constraints to soybean productivity in this growing season is pod shattering.

Pod shattering is the opening of mature pod along the dorsal or ventral sutures and followed by seed dispersal when the crop reaches maturity and during harvesting (Bara et al. 2013). The pod shattering may result from external factors such as contact with other pods, racemes, or harvesting machinery, which separate the vascular connections across the dehiscence zone of pod wall (Peterson et al. 1996). The significant yield losses due to pod shattering have been reported (IITA 1986; Tiwari and Bhatnagar 1991; Tefera et al. 2009; Krisnawati et al. 2015; Krisnawati and Adie 2016), ranging from 34% to 100% in severe cases. The degree of shattering depends upon the time of harvesting, environmental condition, anatomical structure of the pod, chemical composition of the pod wall, application of plant growth regulators, and genotype (Tiwari and Bhatia 1995; Srivastava et al. 1998; Gulluoglu et al. 2006; Zhang and Boahen 2010).

Environmental factors, such as drought stress during pod maturation has also been found to result in a weak pod structure, leading pod to shatter. Delayed harvesting also increased the degree of shattering (Philbrook and Oplinger 1989; Tukamuhabwa et al. 2002). Other environmental factors that could cause pod shattering include low humidity, high temperature, rapid temperature changes, and alternating wetting and drying (Tsuchiya 1987; Agrawal et al. 2002). Tiwari and Bhatnagar (1989) reported that rains followed by dry weather at harvest will enhance pod shattering. However, among those factors, the genotypic characteristics play a major role in the overall expression of pod shattering (Bhor et al. 2014).

Earlier studies have revealed that pod shattering is a qualitative heritable trait (Caviness 1969) with multiple genes governing the trait (Carpenter and Fehr 1986; Tukamuhabwa et al. 2000; Sujata et al. 2012). Mohammed (2010) reported that inheritance of resistance to pod shattering was under the influence of either duplicate recessive or dominant and recessive epistasis depending on the parental genotypes used in the cross. A studies of pod anatomy (Esau 1977), showed that certain anatomical and morphological structures of the soybean pod have been recognized as determinant factors within pod shattering resistance (Tsuciya 1987; Tiwari and Bhatia 1995; Bara et al. 2013; Adeyeye et al. 2014).

Varietal differences in pod shattering resistance among soybean genotypes have been evaluated in various countries, and resulted in several soybean genotypes which resistant and/or tolerant to pod shattering, such as ‘Bragg’, ‘PK 416’, and ‘NRC 3’ (Tiwari and Bhatia 1995), ‘JS-1515’, ‘JS-1608’ and ‘JS-1625’ (Upadhyaya and Paradkar 1991), ‘TGX 1448-2E’ (Mohammed 2010), ‘Maksoy 1N’ and ‘Maksoy 2N’ (AGRA 2014), ‘Glenn’ (VCIA 2014), ‘JS 335’, ‘JSM 170’, and ‘MAUS 61-2’ (Agrawal et al. 2003; Bara et al. 2013). Those genotypes could be potentially utilized as source of genes for pod shattering resistance in breeding programs.

Since pod shattering resistance was reported to be associated with morphological, and physiological characteristics of the pods, the knowledge on morphological and physiological characteristics will provide useful information for selection of suitable parents, as well as segregating populations for developing soybean shattering resistant.

The objectives of the research were to classify the varietal difference of pod shattering and to identify the morphological pod characters related to pod shattering.

MATERIALS AND METHODS

The field study was carried out in Blitar (East Java, Indonesia) during the dry season 2015 (June to September 2015). The type of soil was Alluvial, elevation of 355 m above sea level, and C3 of Oldeman climate type (5-6 wet month consecutively). The genetic material consists of 30 soybean genotypes. The experimental design was arranged in a randomized block design with four replicates. Each line was planted on 2.4 m × 4.5 m plot size, 40 cm × 15 cm plant distance, two plants per hill. Fertilizer of 50 kg ha^{-1} Urea, 100 kg ha^{-1} SP36 and 75 kg ha^{-1} KCl were applied before sowing time. Pests and diseases were controlled optimally, and drainage was applied to maintain optimum soil moisture. Pod was harvested when 95% of the leaf turned yellow in a population.

The observations on morphological and agronomical traits (Tsuciya 1987; Bara et al. 2013) consists of pod length (A), pod width (B), width at mid part of pod (D), degree of curvature (D/A), pod thickness (C), pod wall thickness (E), 100 seed weight, pod wall weight, pod weight, and seed weight. The measurements were recorded from ten healthy three-seeded pods from five representative plants in each replication (Figure 1).

Screening for pod shattering resistance was done as per oven dry method (IITA 1986; Krisnawati and Adie 2016). The evaluation of pod shattering resistance was by randomly taken a sample of 30 fully matured pods of each plot, and kept in oven at 30°C for three days, and then elevated up to 40°C for one day, elevated up to 50°C for one day, and the last was elevated up to 60°C for three days. The number of shattered pods were counted on the 7^{th} day and expressed in percentage. The genotypes were classified into five categories based on their reaction to pod shattering (IITA 1986; Krisnawati et al. 2016). The scoring rate was as follows: 1 = No pod shattering (Very Resistant); 2 = < 25 % pod shattering (Resistant); 3 = 25-50 % pod shattering (Moderately Resistant); 4 = 51-75 % pod shattering (Highly Susceptible); 5 = > 75 % pod shattering (Very Highly Susceptible)

Data on morphological and agronomical traits of pod was subjected to analysis of variance according to the procedure described by Gomez and Gomez (1984). The data on pod shattering was subjected to arcsine-square-root transformation before statistical analysis. Direct effect of pod morphological characters on pod shattering were calculated using path analysis (Singh and Chaudhary 1979).

RESULTS AND DISCUSSION

Result

Mean performance, range of value, standard deviation, and the mean square of genotype of traits studied are presented in Table 1. All traits showed significant differences. A highly significant difference also found for pod shattering, indicated the availability of high variation in shattering resistance among genotypes. Standard deviation of 10 observed traits ranged from 0.01 to 30.05. Pod length varied from 3.87 to 5.42 cm with an average of 4.57 cm, pod width ranged from 4.09 to 6.21 cm with an average of 5.45 cm, and width at mid part of the pod ranged from 0.79 to 1.01 cm with an average of 0.90 cm. The degree of curvature ranged from 0.17 to 0.24 (average of 0.90). Pod thickness varied, from 0.98 to 1.29 cm with an average of 1.11 cm, and pod wall thickness ranged from 17.65 to 23.17 µm with an average of 20.15 µm. The seed size which measured by the 100 seed weight was highly varied, from 7.91 to 23.27 g per 100 seed, with an average of 12.40 g. The percentage of pod shattering ranged from 2.50 to 100% with an average of 34.03%. Pod wall weight and pod weight ratio ranged from 26.00 to 39.30 (an average of 34.21), meanwhile seed weight and pod weight ratio ranged from 60.70 to 74.00 (an average of 65.79).

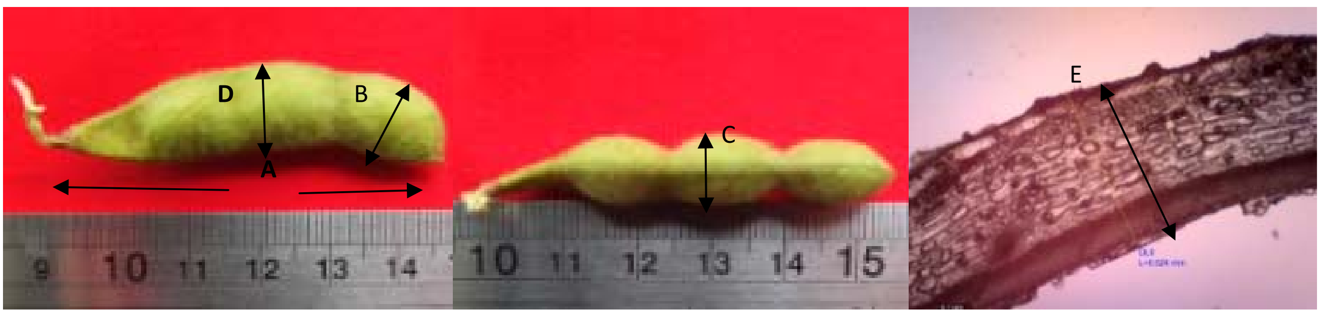

Figure 1. Measurement of the pod morphological characters. Pod length (A), pod width (B), and width at mid part of pod (D) were measured in cm; Pod thickness (C) was measured in cm; Pod wall thickness (E) was measured in µm

Table 1. Mean, range, and standard deviation for observed characters of 30 soybean genotypes in 2015

Parameter	Mean	Min	Max	SD	Mean Square of genotype
Pod length (A) (cm)	4.57	3.87	5.42	0.41	0.66734**
Pod width (B) (cm)	5.45	4.09	6.21	0.53	1.12100**
Width at mid part of pod (D) (cm)	0.90	0.79	1.01	0.04	0.00753**
Degree of curvature (D/A)	0.90	0.17	0.24	0.01	0.00074**
Pod thickness (C) (cm)	1.11	0.98	1.29	0.06	0.01339**
Pod wall thickness (E) (µm)	20.15	17.65	23.17	1.18	5.52824**
100 seed weight (g)	12.40	7.91	23.27	2.89	33.4137**
Pod shattering (%)	34.03	2.50	100	30.05	25.3269**
Pod wall weight and pod weight ratio	34.21	26.00	39.30	2.50	24.9153*
Seed weight and pod weight ratio	65.79	60.70	74.00	2.50	24.9153*

Note: Min = minimal value; Max = Maximal value; SD = standard deviation, * = significant at 1 % probability level ($p < 0.01$), ** = significant at 5 % probability level ($p < 0.05$), ns = not significant

Table 2. Direct and indirect effects of pod morphological traits with pod shattering percentage in soybean in 2015

Charac-ters	X_1	X_2	X_3	X_4	X_5	X_6	X_7	r
X_1	**3.020**	-1.236	-0.261	-1.160	1.237	-0.341	-1.179	0.415
X_2	-1.236	**2.420**	-0.777	0.003	-0.417	-0.087	0.298	0.141
X_3	-0.261	-0.777	**2.517**	-0.372	-0.147	-0.801	-0.721	0.115
X_4	-1.160	0.003	-0.372	**3.698**	-0.968	-1.207	1.962	0.226
X_5	1.237	-0.417	-0.147	-0.968	**1.719**	-0.255	-0.637	0.247
X_6	-0.341	-0.087	-0.801	-1.207	-0.255	**2.621**	-0.018	0.255
X_7	-1.179	0.298	-0.721	1.962	-0.637	-0.018	**2.353**	0.231

Note: X_1 = pod length, X_2 = pod width, X_3 = pod thickness, X_4 = pod wall thickness, X_5 = 100 seed weight, X_6 = pod wall weight and pod weight ratio, X_7 = seed weight and pod weight ratio, r = correlation

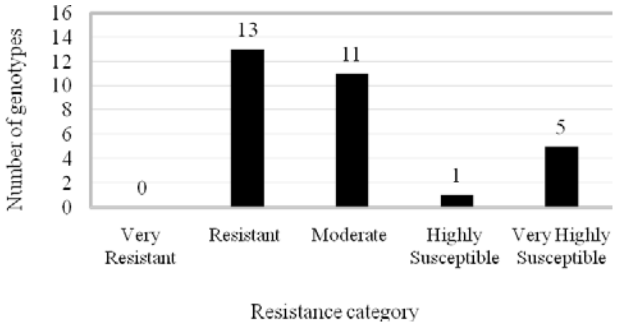

Figure 2. Pod shattering resistance of 30 soybean genotypes in 2015

Soybean genotypes were classified based on their reaction to pod shattering into five scoring rate (Figure 2). The results showed that the very resistant genotype (no shattered pod) was not found in this study. However, a total of 13 genotypes were found to be resistant, 11 genotypes were moderately tolerant one genotype was highly susceptible, and five genotypes were very highly susceptible to pod shattering.

Table 2 showed the direct and indirect effects of pod morphological traits associated with pod shattering percentage in soybean. The highest correlation coefficient was X_1 (pod length), that is r = 0.415.The direct effects of the pod length (X_1) and pod wall thickness (X_4) on shattering percentage as indicated by path coefficient were the highest (3.020 and 3.698, respectively), while other causal effects were small. This is indicated that those characters were equally important within pod shattering resistance. The seed size (measured 100 seed weight) were found to be the least important character.

Discussion

Soybean variety with resistance to pod shattering resistance is most important factor for the improvement of soybean, especially in tropics. The Indonesian tropical climate is characterized by abundant sunshine, which provide an ideal environment for soybeans production. The soybean in Indonesia is mostly cultivated during the second dry season (June/July to September/October) after paddy, which is characterized by high temperature and low humidity, hence will increase in pod shattering and resulting in significant yield losses. Therefore, it is important to develop soybean varieties resistant to pod shattering to improve soybean production in Indonesia.

Morphological and physiological characteristics of the pods were reported to be associated with resistance to pod shattering, indicated that those characters could be used as an indicators for pod shattering selection. In this research, characteristics of pod were studied to recognize the significant affecting factors. Ten pod characters showed significant variation among genotypes in the degree of pod shattering. Similar findings were reported by Bara et al. (2013), which also found significant variation among soybean genotypes in pod length, pod width, width at mid part of pod, degree of curvature, pod thickness, pod wall thickness, and 100 seed weight. But this result is not in agreement with Tsuciya (1987).

Evaluation of shattering resistance showed that between 30 soybean genotypes consists of resistant, moderate, highly susceptible and very highly susceptible to pod shattering. Range of percentage of resistance from 2.50% (G511H/Argom//Argom-2-1) to 100% (Grobogan) (Figure 3). In this research, Grobogan, a popular variety among farmers due to its large seed size and suitable for tempeh industry (ILETRI 2015) showed a very high susceptibility to pod shattering. Some level of resistance to pod shattering was reported by Gadde (2006) and Khan et al. (2013). However, Krisnawati et al. (2016; data not showed) found eight very resistant genotypes.

A B

Figure 3. Pod shattering in soybean genotypes: A. G511H/Argom//Argom-2-1; B. Grobogan

Other researchers also found significant variation in pod shattering resistance among genotypes. Zhang and Bellalloui (2012) studied soybean shattering resistance 4 weeks after maturity (WAM), and showed that average shattering score of the examined varieties at the four WAM was 13.3% with a range of shattering scores from 0% to 40%. Krisnawati et al. (2015) screened 68 soybean genotypes in the laboratory using the oven method, and the degree of shattering ranged from zero to 80%. A total of 23 genotypes were very resistant, 31 resistant, and eight moderately resistant, and the rests were highly susceptible to pod shattering. Furthermore, Krisnawati and Adie (2016) using oven method and field observations to evaluate the degree of shattering and found the pod shattering the field varies between 1.3 to 42.3 pods, with an average of 18.9 pods, whereas number of shattered pods in the laboratory ranged from 7 to 26 pods (22.2% to 87.2%), with an average of 19.3 pods (64.3%). The least shattered pods showed by genotypes derived from crossing combination of 'G511H × Anjs-6-5' (6.8 pods or 22.2%) and 'G511H × Anjs-8-1' (8.8 pods or 29.4%). Furthermore, a study by Bara et al. (2013) found the shattering percentage of 69 soybean genotypes ranged from 0.6730 to 67.05% with mean of 19.11%; and suggested the shattering tolerant genotypes of 'JS 335', 'JSM 170', 'MAUS 61-2' as donor of shattering resistance in breeding programs. According to Bara et al. (2013), the rate of seed shattering will accelerated after 7 days, and then enhanced with the age of the matured pod. This is in agreement with Krisnawati and Adie (2016) which stated that the rate of pod shattering increase linearly with days to maturity, and showed a significant two weeks after days to maturity.

Since the anatomical and morphological character of pod was considered have important role in resistance to pod shattering in soybean, a path coefficient analysis was used to quantify the relation between pod shattering with pod characters. The highest direct effects were observed with pod length and pod wall thickness, suggesting that these characters were the most important contributors to the pod shattering resistance. These results were supported by earlier findings by Tiwari and Bhatia (1995). Overall, three anatomical characters (bundle cap length, pod wall thickness, and bundle cap thickness) were strongly related with predictive value for resistance to pod shattering. In addition, Bara et al. (2013) stated that pod wall thickness was more reliable as a trait for selection than character of pod length, by simple selection procedure. Pod wall thickness was considered as an important factor in pod shattering resistance. According to Tiwari and Bhatia (1995), thick wall was developed from the parenchyma cells of the outer most and peripheral side of the vascular bundle at the dorsal and ventral sutures of soybean pod, resulting a structure which referred as bundle cap. Larger and thicker bundle caps would impart more physical strength to the pod, and also result in shortened parenchyma at the center of the sutures, thus limiting the degree to which the clefts may enlarge prior pod shattering.

In Indonesia, soybean variety with pod shattering resistance is not available. The existing varieties resistant to pod shattering were derived from the adaptation trials. Hence, it is important to develop soybean varieties with pod shattering resistance to prevent yield losses. The initial step in breeding for pod shattering resistance is identification of sources of resistance for use in hybridization program, as well as the identification of characters that may use as selection indices.

To conclude, the findings of the present study indicated that pod wall thickness and pod length are two key factors associated with pod shattering resistance. These traits could potentially serve as selection criteria to pod shattering resistance. Moreover, soybean resistance to pod shattering could be enhanced by increasing thickness of the pod wall.

ACKNOWLEDGEMENTS

The author wish to thank Dr. Rao C. N. Rachaputi from The University of Queensland, Australia who assisted in the proof-reading of the manuscript and also for his valuable comments that greatly improved the manuscript. We also thank Arifin for helping in the field research. This research was supported by the Indonesian Agency for Agricultural Research and Development (IAARD) Ministry of Agriculture, Jakarta.

REFERENCES

Adeyeye AS, Togun AO, Akanbi WB, Adepoju IO, Ibirinde DO. 2014. Pod shattering of different soybean varieties, *Glycine max* (L) Merrill, as affected by some growth and yield parameters. Int. J. of Agric. Policy and Res. 2 (1): 010-015.

Agarwal AP, Salimath PM, Patil SA. 2003. Inheritance of pod shattering in soybean [*Glycine max* (L.) Merrill]. Indian. J. Genet., 63 : 265-266.

AGRA. 2014. Grow New Soybean Varieties for Better Yields. AGRA (Growing Africa's Agriculture). http: //www.soybeanafrica.com/Commercial-soybean-varieties-Uganda.pdf

Agrawal AP, Basarkar PW, Salimath PM, Patil SA. 2002. Role of cell wall-degrading enzymes in pod shattering process of soybean. *Glycine max* (L) Merrill. Research Communication 58, Current Science 82 (1).

Bara N, Khare D, Srivastava AN. 2013. Studies on the factors affecting pod shattering in soybean. Indian J. Genet. 73 (3): 270-277.

Bhor TJ, Chimote VP, Deshmukh MP. 2014. Inheritance of pod shattering in soybean [*Glycine max* (L.) Merrill]. Electric J. of Plant Breeding 5 (4): 671-676.

Carpenter JA, Fehr WR. 1986. Genetic variability for desirable agronomic traits in populations containing *Glycine soja* germplasm. Crop Sci. 26: 681-686.

Caviness CE. 1969. Heritability of pod dehiscence and its association with some agronomic characters in soybeans. Crop Sci 9: 207-209.4

Esau K. 1977. Anatomy of Seed Plants. 2nd ed. John Wiley & Sons, New York.

Gadde, PM. 2006. Genetic investigation in soybean (*Glycine max* (L.) Merrill). [Thesis]. University of Agricultural Sciences, Dharwad.

Gomez KA, Gomez AA. 1984. Statistical Procedures for Agricultural Research. John Wiley & Sons, New York. 680 p.

Gulluoglu L, Halis A, Mehmet A. 2006. Effects of some plant growth regulators and nutrient complexes on pod shattering and yield losses of soybean under hot and dry conditions. J Plant Sci 5: 368-372.

IITA [International Institute of Tropical Agriculture]. 1986. A laboratory method for evaluating resistance to pod shattering in soybeans. Annual Report. 58-59. IITA, Ibadan, Nigeria.

Iletri [Indonesian Legumes and Tuber Crops Research Institute]. 2015. Variety Grobogan and Anjasmoro as favorite varieties for tempeh industry in Malang. http: //balitkabi.litbang.pertanian.go.id/kilas-litbang/2071-varietas-grobogan-dan-anjasmoro-sangat-diminati-pengrajin-kripik-tempe-malang.html [Indonesian]

Khan MH, Tyagu DS,Dar ZA. 2013. Screening of soybean (*Glycine max* (L.) Merrill) genotypes for resistance to rust, yellow mosaic and pod shattering. Intech, Chapter 7, www.intechopen.com/download/pdf/42600.

Krisnawati A, Adie MM, Harnowo D. 2015. Variability of physical characters of pod related to the pod shattering resistance. ILETRI National Seminar Proceedings 2014. In press.[Indonesian].

Krisnawati A, Adie MM. 2016. Pod shattering resistance in different soybean genotypes. Proceedings of ILETRI National Seminar. ILETRI, IAARD, Malang. [Indonesian]

Mohammed H. 2010. Genetic analysis of resistance to pod shattering in soybean (*Glycine max*. (L) Merrill). [Thesis]. The Department of Crop and Soil Sciences, Faculty of Agriculture of the College of Agriculture and Natural Resources, Kwame Nkrumah University of Science and Technology, Kumasi.

Peterson M, Sander L, Child RD, Van Onckelen HA, Ulvskov P, Borkhardt B. 1996. Isolation and characterization of a pod dehiscence zone specific polygalacturonase from *Brassica napus*. Pl Mol Biol 31: 517-527.

Philbrook B, Oplinger ES. 1989. Soybean field losses as influenced by harvest delays. Agron J 81: 251-258.

Singh RK, Chaudhary BD. 1979. Biometrical Method in Quantitative Genetic Analysis. Kalyani Publisher, New Delhi.

Srivastava M, Manglik P, Bhatia VS, Guruprasad KN. 1998. Phenilalanine ammonialyase in shattering and shattering genotypes of soybean. Soybean Genet Newslett 25: 80-82.

Sujata B, Basavaraja GT,Salimath PM .2012. Analysis of variability in segregation of soybean (*Glycine max* L Merill). Karnataka J Agric Sci 25 (2): 176-178.

Tefera H, Bandyopadhyay R, Adeleke RA, Boukar O, Mohammed I. 2009. Grain yields of rust resistant promiscuous soybean lines in the Guinea Savanna of Nigeria. Afr Crop Sci Confer Proc 9: 129-134.

Tiwari S, Bhatia VS. 1995. Characterization of pod anatomy associated with resistance to pod-shattering in soybeans. Ann Bot 72: 483-485

Tiwari S, Bhatnagar P. 1989. Minimizing pod shattering in soybean. Indian Farming 39: 23-24.

Tiwari S, Bhatnagar P. 1991. Pod shattering as related to other agronomic attributes in soybean. Trop Agric (Trinidad) 68: 102-103.

Tsuciya T. 1987. Physiological and genetic analysis of pod shattering in soybeans. Japan Agric Res Quart 21 (3): 166-175.

Tukamuhabwa P, Dashiell KE, Rubaihayo PR, Adipala E. 2000. Inheritance of resistance to pod shattering in soybean. Afr Crop Sci J 8 (3): 203-211.

Tukamuhabwa P, Dashiell KE, Rubaihayo PR, Nabasirye M. 2002. Determination of field yield loss and effect of environment on pod shattering in soybean. Afr Crop Sci J 10 (3): 203-209.

Upadhyay PC, Paradkar VK. 1991. Pod shattering in soybean (*Glycine max* (L.) Merill.). J Oilseeds Res 8 (1): 121-122.

VCIA [Virginia Crop Improvement Association]. 2014. Glenn Soybean Variety Description. VCE (Virginia Cooperative Extension). http: //www.virginiacrop.org/vcia.soyvar.htm

Zhang L, Bellalloui. 2012. Effects of planting dates on shattering patterns under early soybean production system. Amer J Pl Sci 3 (1): 119-124.

Zhang L, Boahen L. 2010. Evaluation of critical shattering time of early-maturity soybeans under early soybean production system. Agric Biol J North Amer 1 (4): 440-447.