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Identification of Pod Shattering Resistance and Associations between Agronomic Characters in Soybean using Genotype by Trait Biplot

M.M. Adie[1], T. Sundari[1], A. Wijanarko[2], R.D. Purwaningrahayu[1], A. Krisnawati[1]

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ABSTRACT

Background: Pod shattering has become the major problem in soybean production. The research aims to identify the pod shattering resistance and to assess the agronomic performances of 50 soybean genotypes and the association among agronomic characters.
Methods: The research materials were 50 soybean genotypes which consisted of 47 lines derived from routine crossing programs and three check cultivars. The field experiment was arranged in a randomized block design with two replications. The data were observed for yield and its component traits. The oven-dry method was performed in the laboratory to assess the pod shattering resistance.
Result: Variation among genotypes was found in the pod shattering resistance and agronomic characters. The genotype by trait biplot graph showed that pod shattering was negatively correlated with the days to maturity and plant height, but positively correlated with the seed size. Soybean genotypes of Grob/G100H-1-588 and G100H/Mhmr-4-993 were resistant to pod shattering and have a high seed weight per plant. These genotypes were potential for further varietal development or could be used as gene sources in the soybean improvement program for pod shattering resistance.

Key words: Interrelationship, Pod dehiscent, Seed loss, Yield component.

INTRODUCTION

Soybean is an important food commodity as a source of vegetable protein. Yield losses in agriculture commodities have become a global issue. In soybean, yield loss can occur during field and storage due to the pest infestation, or after the plant has reached maturity due to pod shattering. Pod shattering is the opening of the pod wall which causes the seeds to be released from the pods. The yield losses in soybean caused by pod shattering in India may reach 100% (Tiwari and Bhatnagar, 1991), whereas in Indonesia, it could reach over 70% in the susceptible cultivars (Krisnawati and Adie, 2019).

An ideal soybean variety characterized by high shatter-resistant and good agronomic characteristics that supporting yield. There is a significant opportunity to improve soybean resistance to pod shattering. Several countries have successfully obtained pod shatter resistant cultivars (Umar *et al*. 2017; Barate *et al*. 2018; Seo *et al*. 2020). Several studies have shown that pod shattering resistance is genetically controlled (Thakare *et al.* 2017; Nevhudzholi *et al.* 2020), thus the presence of a source of resistant genes has the potential to be used to improve soybean resistance to pod shattering.

A profitable breeding approach strategy involves combining pod shattering with economically important agronomic traits, such as high shatter resistance with high number of pods and seeds (Fatima *et al*. 2020). A study reported that soybean pods at the lower part of the stem had a higher shattering rate compared to the pods in the middle and upper parts of the plants (Krisnawati *et al.* 2021).

[1]Indonesian Legume and Tuber Crops Research Institute, Jl. Raya Kendalpayak Km 8, P.O. Box 66 Malang 65101, East Java, Indonesia.
[2]Indonesian Sweetener and Fiber Crops Research Institute, Jalan Raya Karangploso Km 4, Malang 65152, East Java, Indonesia.

Corresponding Author: M.M. Adie, Indonesian Legume and Tuber Crops Research Institute, Jl. Raya Kendalpayak Km 8, P.O. Box 66 Malang 65101, East Java, Indonesia. Email: mm_adie@yahoo.com

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Pod shatter resistant cultivars are advantageous not just in terms of reducing crop losses, but also in terms of postponing harvest (Lee *et al.* 2020).

To date, a variety of statistical approaches have been utilized to identify the trait relationships and the overall genotype profile of different crops. A genotype by trait (GT) biplot, a variant of the GGE biplot, has lately gained popularity as a valuable tool for studying multi-trait data (Yan and Kang, 2003). This method allows for a multi-trait cultivar evaluation by graphically presenting the interrelationships between traits and identifying superior genotypes for simultaneous improvement of many traits (Atnaf *et al.* 2017; Sharifi and Ebadi, 2018). The GT biplot has been used to investigate trait relationships and genotype evaluation

(Oliviera *et al.* 2018; Al-Naggar *et al.* 2020). However, the use of the GT biplot in soybean is still limited. Therefore, the study aims were to identify the pod shattering resistance and to assess the agronomic performances of 50 soybean genotypes and the association among agronomic characters using the genotype by trait (GT) biplot.

MATERIALS AND METHODS

Plant materials

The research material was 50 soybean genotypes which consisted of 47 F_6 generation lines and three check cultivars (Table 1). The lines were generated by crossing several parental with difference traits, such as early maturity, large seed size, high yield and pod shatter-resistance. A pedigree method was used to select segregating populations (F_2, F_3, F_4 and F_5) for pod shattering and economically important agronomic traits (early maturity, large seed size and high yield).

Field research

The field experiment was conducted in Pasuruan, East Java, Indonesia (-7° 39′ 1″ S and 112°43′ 25″ E). The elevation of location was 141 m a.s.l, with climate type of C3 Oldeman (5-6 consecutive wet months and 4-6 consecutive dry months) and soil type of Entisol. The research was carried out during the dry season (August to November) 2020 in a randomized block design with two replicates. Experimental plot for each line was 1.2 m × 4.5 m, with 40 cm × 15 cm plant spacing, two plants per hill. At the time of sowing, 250 kg/ha Phonska and 100 kg/ha SP36 fertilizers were supplied per hectare. Pests, diseases, and weeding were optimally controlled throughout the growing season.

Laboratory research

The experiment in the Breeding Laboratory of Indonesian Legume and Tuber Crops Research Institute (ILETRI) was done to assess the pod shattering resistance of 50 genotypes. The shattering assessment was using oven-dry method (Krisnawati and Adie 2017). The sample plants were taken from plants at the R8 phase (full maturity, at least 95% of the pods on a plant have reached their mature color). Ten randomly plants were selected from each plot and then it dried at the room temperature for three days. Thirty pods were randomly detached from those ten sample plants and placed in the Petri dish for shattering assessment using oven-dry method, *i.e.*, the sample pods were dried for three days at 30°C and at one day at 40°C, then elevated to 50°C (one day) and 60°C (one day). The number of shattered pods was observed after being subjected to each oven temperature.

Data observation dan analysis

The parameters observed from the field experiment were days to maturity, plant height, number of branches per plant, number of nodes per plant, number of filled pods per plants, number of empty pods per plant, 100 seed weight and seed weight per plant. The pod shattering percentage was determined from the number of shattered pods per total number of sample pods, expressed as a percentage. The classification of shattering resistance was based on the shattering percentage, according to AVRDC (1979). The agronomic data were subjected to variance analysis (ANOVA). The genotype by trait biplot (Yan and Rajcan, 2002) was used to study the association among agronomic characters. The GT biplot is generated by using the RStudio software version 1.3.959 (RStudio Team, 2020).

RESULTS AND DISCUSSION

The performance of agronomic characters

Variation among genotypes was found in the performance of the agronomic characters of 50 soybean genotypes (Table 2). The days to maturity varied from 75 to 81 days (average of 78 days). In Indonesia, days to maturity is an important character in soybean development, since soybean is cultivated in a yearly planting pattern of paddy-paddy-soybean. In this study, most of the tested genotypes have early days to maturity (<80 days) (Fig 2A).

The growth characters of plant height, number of branches and number of nodes (Fig 1B, 1C, 1D) generally become the mutually agronomic supporting characters (Li *et al.* 2020). The average plant height, number of branches, and number of nodes were 57.32 cm, 1.49 branches/plant and 10.25 nodes/plant. The number of pods consisted of filled and empty pods (Fig 1E, 1F). The average of filled pods was 29.27 pods/plant, meanwhile the average of empty pods was low (1.06 pods/plant). The yield characters, namely 100 seed weight and seed yield/plant were 15.59 g/100 seeds and 18.10 g/plant (Fig 1G, 1H), respectively. In Indonesia, large-seeded soybean is important for industrial raw material for tempeh. According to Sulistyo *et al.* (2021), the length, width, thickness, the ratio of the three characters and the weight of 100 seeds can be used as selection criteria in a soybean breeding program to obtain large-seeded soybeans with a round or elliptical shape.

In this study, the range of seed yield of 50 genotypes was 6.18-18.10 g/plant. A total of 24 genotypes produced yield above the general mean (range of 10.23-18.10 g/plant). Ige *et al.* (2021) evaluate the combining ability for seed yield, obtain several genotypes as the best combiners for the number of pods/plant and seed yield/plant in soybean. In black gram, the grain yield was associated with the performance of plant height and the number of primary branches per plant (Priya *et al.* 2021).

Pod-shatter resistance

Pod shattering resistance of 50 soybean genotypes varied among genotypes (Table 1). The use of gradual temperature in the oven-dry method showed that the soybean pods were remaining unshattered after subjected to 30°C dan 40°C. Pods began to shatter after subjected to 50°C and were increased after 60°C.

Table 1: List of genotypes used, the shattering percentage and the resistance criteria.

Pedigree	Pod shattering at 50°C		Pod shattering at 60°C		Remark
	%	Criteria	%	Criteria	
Dega/G100H-1-71	100	HS	100	HS	Breeding lines
IAC100/Dega-1-89	100	HS	85	HS	Breeding lines
Anjas/G100H-1-171	0	HR	5	R	Breeding lines
G100H/Anjas-1-177	5	R	37	S	Breeding lines
G100H/Anjas-2-179	2	R	20	M	Breeding lines
Anjas/IAC 100-1-183	0	HR	33	S	Breeding lines
Anjas/IAC 100-2-185	100	HS	100	HS	Breeding lines
Grob/G100H-3-293	100	HS	100	HS	Breeding lines
Grob/G100H-5-310	50	S	100	HS	Breeding lines
Grob/G100H-7-338	0	HR	7	R	Breeding lines
Grob/G100H-8-342	100	HS	100	HS	Breeding lines
Grob/G100H-10-364	100	HS	100	HS	Breeding lines
Grob/G100H-13-375	100	HS	100	HS	Breeding lines
Grob/G100H-14-403	0	HR	7	R	Breeding lines
Mahameru/IAC100-1-421	3	R	20	M	Breeding lines
Mahameru/IAC100-2-426	0	HR	7	R	Breeding lines
Mahameru/IAC100-3-427	32	S	93	HS	Breeding lines
IAC100/Mahameru-1-434	90	HS	95	HS	Breeding lines
Dega/G100H-1-480	0	HR	95	HS	Breeding lines
Grob/G100H-1-588	0	HR	2	R	Breeding lines
G100H/Anjas-1-602	25	M	95	HS	Breeding lines
G100H/Anjas-2-603	100	HS	100	H	Breeding lines
Anjas/IAC100-1-613	2	R	3	R	Breeding lines
Grob/G100H-1-857	55	HS	27	S	Breeding lines
Grob/G100H-3-881	50	S	100	HS	Breeding lines
Mahameru/IAC100-1-925	0	HR	100	HS	Breeding lines
Mahameru/IAC100-3-937	100	HS	100	HS	Breeding lines
Mahameru/IAC100-4-939	50	S	100	HS	Breeding lines
Mahameru/G100H-1-955	50	S	100	HS	Breeding lines
G100H/Mahameru-1-961	0	HR	83	HS	Breeding lines
G100H/Mahameru-2-962	50	S	100	HS	Breeding lines
G100H/Mahameru-3-975	85	HS	100	HS	Breeding lines
G100H/Mahameru-4-993	0	HR	3	R	Breeding lines
Dega/IAC100-1-1102	2	R	95	HS	Breeding lines
Dega/IAC100-2-1103	100	HS	100	HS	Breeding lines
Dega/IAC100-3-1109	100	HS	100	HS	Breeding lines
Dega/IAC100-4-1116	90	HS	92	HS	Breeding lines
Dega/IAC100-5-1129	55	HS	100	HS	Breeding lines
Dega/IAC100-7-1135	50	S	100	HS	Breeding lines
Dega/IAC100-8-1138	100	HS	100	HS	Breeding lines
Grob/G100H-2-1162	50	S	100	HS	Breeding lines
Grob/G100H-4-1195	0	HR	100	HS	Breeding lines
Grob/G100H-9-352	0	HR	100	HS	Breeding lines
Grob/G100H-2-861	20	M	45	S	Breeding lines
Grob/G100H-5-901	52	HS	100	HS	Breeding lines
Grob/G100H-3-1170	100	HS	90	HS	Breeding lines
Dega 1	0	HR	95	HS	Check cultivar
Detap 1	10	R	11	M	Check cultivar
Devon 1	100	HS	100	HS	Check cultivar
Devon 2	20	M	60	HS	Check cultivar

HR= Highly resistant, R= Resistant, M= Moderately resistant, S= Susceptible, HS= Highly susceptible.

The shattering resistance after subjected to the oven-dry temperature of 50°C was classified into highly resistant (13 genotypes), resistant (six genotypes), moderately resistant (three genotypes), susceptible (eight genotypes) and highly susceptible (20 genotypes). Furthermore, after subjection to the oven-dry temperature of 60°C, none of the soybean genotypes were highly resistant to shattering. The shattering resistance was grouped into resistant (seven genotypes), moderately resistant (three genotypes), susceptible (4 genotypes) and highly susceptible (36 genotypes) (Fig 1).

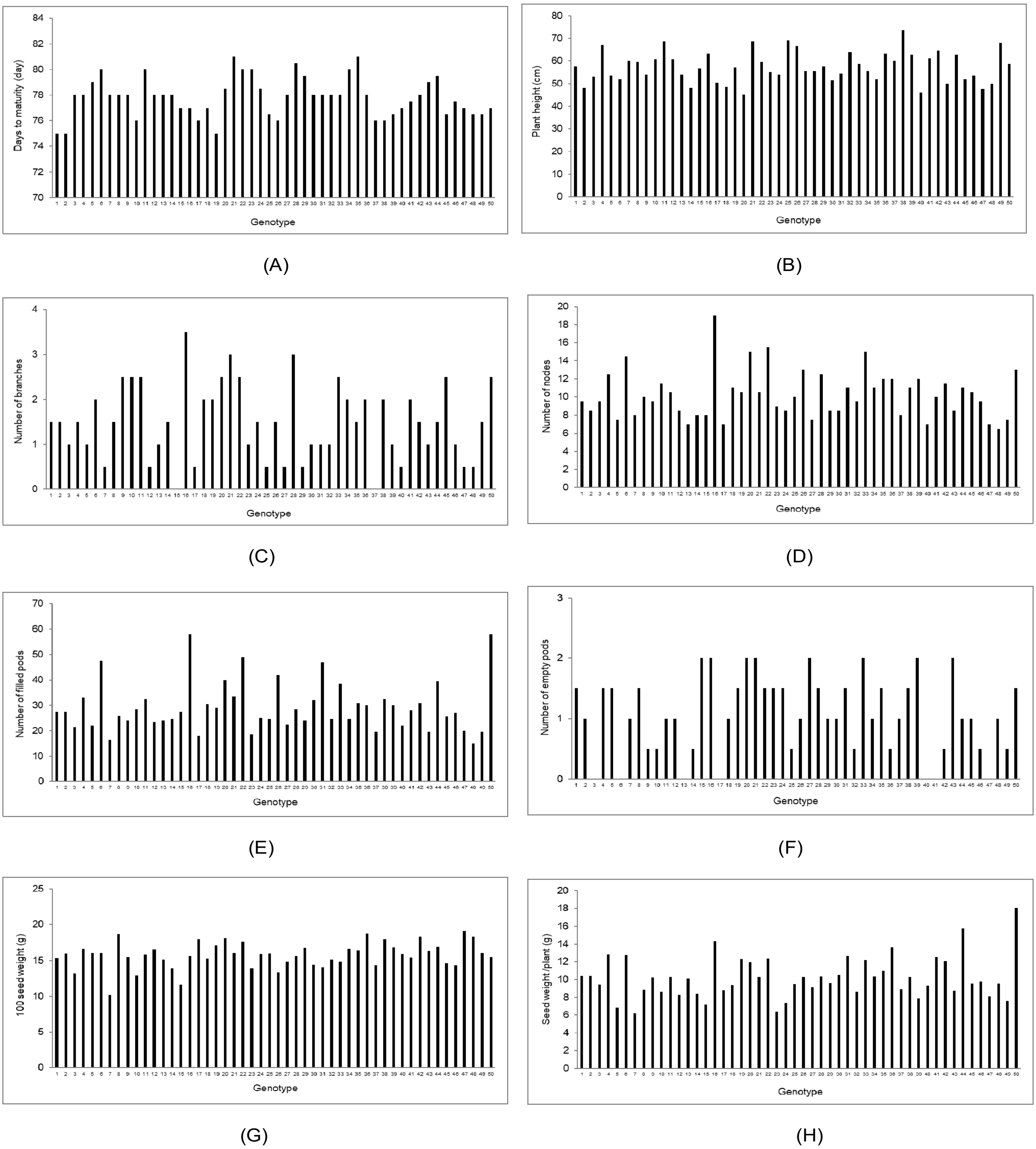

Fig 1: The performance of agronomic characters from 50 soybean genotypes.
(A= Days to maturity, B= Plant height, C= Number of branches, D= Number of nodes, E= Number of filled pods, F= Number of empty pods, G= 100 seed weight, H= Seed weight/plant).

Table 2: Analysis of variance and descriptive data for agronomic characters of 50 soybean genotypes.

Character	Code	Mean square		Minimum value	Maximum value	Average	Standard deviation
		Replication	Genotype				
Days to maturity (day)	DTM	0.0400*	4.6685**	75	81	78	1.53
Plant height (cm)	PHG	585.6400**	90.6277^{ns}	45.00	73.50	57.32	6.66
Number of branches/plant	NOB	0.0900^{ns}	1.3977^{ns}	0.00	3.50	1.49	0.83
Number of nodes/plant	NON	0.0100^{ns}	13.4744^{ns}	6.50	19.00	10.25	2.57
Number of filled pods/plant	NFP	132.2500^{ns}	191.2083^{ns}	15.00	58.00	29.27	9.68
Number of empty pods/plant	NEP	3.2400^{ns}	0.8293^{ns}	0.00	2.00	1.06	0.64
100 seed weight(g)	SWH	14.6765^{ns}	6.4696**	10.18	18.78	15.58	1.88
Seed weight/plant (g)	SWP	66.9124*	10.9249*	6.18	18.10	10.20	2.31

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

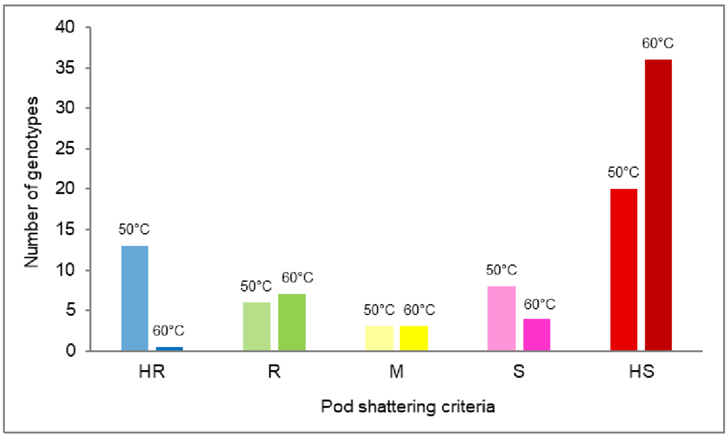

Fig 2: The pod shattering resistance of 50 soybean genotypes after subjection to oven-dry temperature of 50°C and 60°C. (HR= Highly resistant, R= Resistant, M= Moderately resistant, S= Succeptible, HS = Highly succeptible).

This study showed that the use of the oven-dry method of 60°C provides a higher pressure than 50°C, thus the selected resistant genotypes have a chance of being resistant in the field condition.

Interrelationship among characters

The interrelationship among yield, yield components, and pod shattering was evaluated using the genotype by trait (GT) biplot. The GT biplot of mean performance of soybean genotypes explained 52% of the total variation. The low goodness of fit reflects the complexities of the relationships among characters (Yan and Rajcan, 2002; Sharifi and Ebadi, 2018). Nevertheless, the biplot could still capture the fundamental patterns among the characters (Atnaf *et al.* 2017).

In the GT biplot (Fig 3), an acute angle (90°) indicates a positive correlation, an obtuse angle (>90°) indicates a negative correlation and a right angle indicates no correlation (Yan and Tinker, 2006). Thus, the pod shattering (PSH) was positively correlated with 100 seed weight (SWG) and plant height (PHG), but negatively correlated with other agronomic characters. A larger seed size and a higher plant height causing an increase on the pod shattering percentage. The positive association between pod shattering and seed size was also reported in previous studies (Bara *et al.* 2013; Krisnawati *et al.* 2020). On the other hand, seed yield was positively correlated with seed weight per plant (SWP), number of branches (NOB), number of filled pods per plant (NFP) and number of nodes per plant (NON). In this study, the days to maturity was positively correlated with NEP, NOB, NFP and NON. The interrelationship between yield and yield components and the use of for selection criterion in soybean have been extensively studied (Yahaya and Ankrumah, 2016; Kumar *et al.* 2020).

Character profiles of genotype

In Fig 4, a set of perpendicular lines divides the biplot graph into several sectors to characterize the genotypes. In Fig 4, only two quadrants in the biplot containing genotype points and agronomic character points. The genotypes located in the biplot vertex (vertex genotypes) perform best in one or more characters. Accordingly, in the first quadrant, G33, G16 and G50 as vertex genotypes demonstrated the best performance on the NEP, DTF, NON, NFP, NOB and SWP characters. In the second quadrant, G36 as the vertex genotype indicates that the genotype has the highest value for SWG, PHG and PSH characters. The genotypes located at the biplot vertices are very useful as candidates for parents in the breeding programs to develop varieties responsive to the traits of interest (Yan and Rajcan, 2002; Paramesh *et al.* 2016).

In the evaluation of the pod shattering character, genotypes that formed an acute angle with the pod shattering

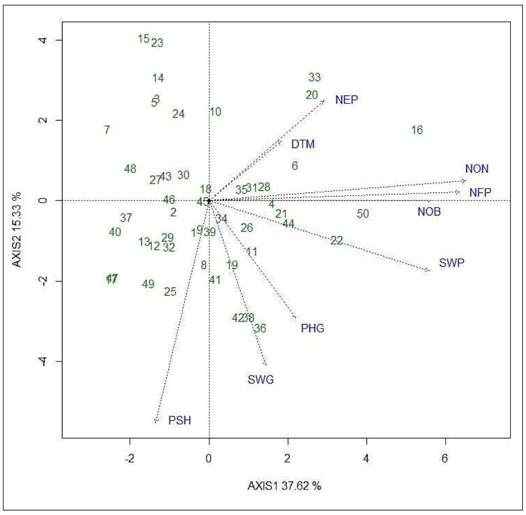

Fig 3: Biplots showing the association among different characters for 50 soybean genotypes. The code of genotypes and characters were according to Table 1 and Table 2, respectively.

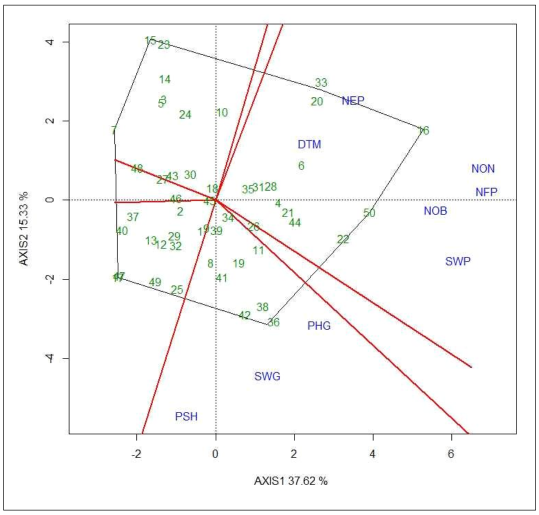

Fig 4: Polygon view of the soybean genotype-by-trait biplot for visualizing the character profiles of the genotypes. The code of genotypes and characters were according to Table 1 and Table 2, respectively.

vector revealed genotypes with an above-average performance (high pod shattering). The genotype that forms an obtuse angle with the character vector (located in the opposite direction to the character's position) is the genotype with lower performance for the character. Based on Fig 4, G20 (Grob/G100H-1-588) and G33 (G100H/Mhmr-4-993) were identified as high shatter-resistant and high yield. These genotypes have the potential to be developed as superior varieties as well as a source of genes to improve soybean pod shattering resistance.

CONCLUSION

The days to maturity was negatively correlated with pod shattering, while the plant height and seed size had a positive correlation with pod shattering. The days to flowering was positively related to the character of the number of nodes, the number of branches and the number of filled pods. Two soybean genotypes (Grob/G100H-1-588 and G100H/Mhmr-4-993) were identified as resistant to pod shattering and produced a high seed weight per plant, hence could utilize for further varietal development, or could be used as gene sources in the soybean improvement for pod shattering resistance.

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