

The Second International Conference on Genetic Resources and Biotechnology

Harnessing Technology for Conservation and Sustainable Use of Genetic Resources for Food and Agriculture

Bogor, Indonesia • 24–25 May 2021

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Rerenstradika Tizar Terryana and Dani Satyawan



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Preface: The Second International Conference on Genetic Resources and Biotechnology

The Second International Conference on Genetic Resources and Biotechnology, which is the continuation of the first event held in 2018, focuses on topics related to advances in biotechnology to create more opportunities for effective conservation and sustainable utilization of genetic resources for food and agriculture. This year conference's theme is Harnessing Technology for Conservation and Sustainable Use of Genetic Resources for Food and Agriculture. The conference was organized by Indonesian Agency for Agricultural Research and Development (IAARD), Ministry of Agriculture, Indonesia, in collaboration with Indonesian Biotechnology Consortium and held on 24th-25th of May 2021 virtually due to the pandemic of COVID-19.

The conference aims to share and exchange current scientific information and technological developments on biotechnology and their applications for conservation and sustainable use of genetic, to encourage and promote quality, efficiency, and modernization of management and utilization of genetic resources, and to facilitate national and international collaboration among participants. There are five scopes discussed in this conference. They are effective management of conservation and sustainable use of genetic resources for food and agriculture, application of genomics and molecular markers for genetic resource conservation and crop adaptation to climate change, application of innovative crop improvement techniques for conservation and sustainable use of plant genetic resources for food and agriculture, plant cell and tissue culture for conservation and effective utilization of genetic resources, and the use of microbial genetic resources as biological control agents of agricultural pests and diseases, and for soil bioremediation.

Five speakers from the United States of America, Japan, India and Indonesia were invited to discuss about their expertise and knowledge on relevant subjects in the plenary sessions. This conference was attended by more than 100 participants including 75 presenters and 44 listeners worldwide. They came from diverse governmental, private, or academic institutions and also scientific communities. The presented materials have undergone peer review processes and only qualified papers were selected. Furthermore, all papers were subjected to double blind peer-review and expected to meet the scientific criteria of significance and academic excellence to be published in a conference proceedings indexed in a well-known, reputable service.

We would like to express our sincere gratitude to our speakers, presenters and all participants for their contributions in this conference. We would also like to express our appreciation for the generosity of our sponsors that support this conference: PT CropLife, PT ITS Science Indonesia, PT Fajar Mas Murni and PT Prima Instrument Analitika. Lastly, special thanks to all committee members for their exceptional work and contributions in the conference and publication.

Chair of Organizing Committee

Dr. Toto Hadiarto

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Variation in Pod Shattering Resistance among Black Soybean Genotypes Associated with Agronomic Traits

Ayda Krisnawati^{a)}, Titik Sundari and M. Muchlish Adie

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Jln. Raya Kendalpayak Km. 8, Malang 65101, East Java, Indonesia*

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Abstract. Increasing the availability of the improved black soybean cultivars in Indonesia could be done by exploring the black soybean genotypes with high yield and pod shattering resistance. This study aimed to evaluate the pod shattering resistance of black soybeans associated with agronomic traits and to identify the best genotype based on multiple traits. A total of 16 black soybean genotypes was evaluated for their pod shattering resistance and agronomic performances. The field experiment was conducted in Madiun, East Java Province, Indonesia from July to October 2020, with four replications. Pod shattering evaluation used the oven-dry method. The identification of pod shattering using oven drying was effective to classify the shattering resistance of black soybean genotypes into resistant (5 genotypes), moderate (3 genotypes), susceptible (4 genotypes), and highly susceptible (4 genotypes). Seven genotypes yielded above the mean yield with a range of 10.13–12.51 g per plant, and three of them were resistant to pod shattering. Genotype by trait biplot indicated that seed yield per plant was positively correlated with days to maturity, plant height, number of nodes, number of branches, and number of pods. The G7 and G14 showed pod shatter resistance with the highest yield of 12.51 g and 12.07 g, respectively. These identified superior lines could be beneficial to increase black soybean yield productivity in Indonesia.

INTRODUCTION

Black soybeans have nutritional benefits, particularly in terms of protein content and antioxidant activities, in addition to being useful as raw materials in a variety of industries. To maximize the genetic potential of black soybeans with high yield, it is important to identify black soybean genotypes with high yield potential capability and their ability to minimize the yield losses as a result of the pod shattering incidence.

The pod shattering incidence occurs when the seeds are released from the soybean pod wall after the pods reach maturity and might reduce soybean yield. Yield losses up to 50% have been reported in susceptible varieties to pod shattering [1], even other soybean production centers reported yield losses reached 100% [2–4]. In the cover crop, such as hairy vetch, pod dehiscence reduces seed yield and causes weed problems in subsequent crops [5]. In soybean, the shattering rate had the greatest effect on yield changes during the delayed harvest [6]. The pattern of pod shattering was reported to be related to the different weather patterns, especially temperature and rainfall in each year [7]. The significant yield losses due to pod shattering require a strategy to reduce or suppress yield loss, and the most reasonable solution is to provide soybean varieties resistant to pod shattering.

In the tropics, soybean cultivation would be more susceptible to pod shattering. In Indonesia, the largest soybean cultivation is during the dry season with the seed filling period to harvest will occur at the peak of the dry season. A hot and dry environmental condition could be the main cause of high pod shattering incidence [8, 9]. Some studies found several factors affecting the shattering, such as relative humidity, pod water content, pod attributes, and environmental condition [7, 10, 11]. According to a study on *Brassica napus* L., it appears that pod wall weight and water content could be used as the soybean resistance determinants for pod shattering. A chemical analysis was also presented, with the conclusion that the hemicellulose content was the most important factor in pod shattering resistance index (SRI) [12]. Liu *et al.* [13] suggested studying the pod wall characters to gain a better understanding

of the resistance to pod shattering behavior. Another study suggested the use of visual dehiscence scores as an efficient screening attribute in the early breeding process and the force of dehiscence during advanced stages of selection [5].

The availability of gene sources and an understanding of the determinants of resistance are both needed in developing soybean varieties resistant to pod shattering. A study for evaluating soybean genotypes for lodging and pod shattering resistance in Ghana obtained four resistant lines to lodging and shattering [14]. In India, a total of nine soybean shatter-resistant was successfully obtained [15]. Furthermore, soybean SJ2 from Thailand was successfully used as a source of resistant genes to develop pod shatter-resistant soybean varieties in Japan, and the Hayahikari and Yukihomore varieties were obtained [16].

In Indonesia, the soybean variety of Detap 1, which is resistant to pod shattering and has a high yield, is selected from the cross between shatter-resistant and shatter-susceptible parents [17]. Since there are many genetic sources available worldwide, it is a high possibility to improve soybean resistance to pod shattering. The valuable asset of diverse soybean germplasm and breeding program would provide a source of resistant genes and allowed researchers to trace the determinants of soybean resistance to pod shattering from a morphological, anatomical, and chemical perspective. The study in the pod shattering of black soybeans is still limited, as well as the relationship with the agronomic traits. This study aimed to evaluate the pod shattering resistance of black soybeans associated with agronomic traits and to identify the best genotype based on multiple traits.

MATERIALS AND METHODS

Sixteen black soybean genotypes were used in this study consisting of 14 genotypes selected from crossing, and 2 black soybean cultivars (Malika and Detam 4) as check varieties, belonging to Indonesian Legume and Tuber Crops Research Institute (ILETRI), Indonesian Agency for Agricultural Research and Development. The field experiment was carried out in Madiun, East Java Province, Indonesia located at 7°29'15" S, 111°36'18" E, 295 m a.s.l., with Oldeman climate type was E1 and soil type of Alfisol. The study was conducted during the dry season, from July to October 2020.

The field study was arranged in a randomized block design with four replications. The plants were planted in lowland after the rice planting with no-tillage. Each genotype was planted in a plot size of 2 m × 4.5 m, with plant spacing of 40 cm × 15 cm, two plants per hill. At the time of sowing, the plants were fertilized with 50 kg/ha urea, 100 kg/ha SP36, and 75 kg/ha KCl. Intensive monitoring was used to control pests and diseases.

The resistance of black soybeans to pod shattering was investigated at the Breeding Laboratory of ILETRI, Malang. Ten sample plants from each combination of the genotype and replication were randomly taken at the R8 stage (the pods reached full mature color or had turned brown) and then were dried at room temperature for 3 days. Thirty pods were randomly selected, put in Petri dishes ($\varnothing = 15$ cm), and were placed in an oven. The oven-dry method of 60°C for one day was used to evaluate the plants' resistance to pod shattering [18].

The observations were made on the agronomic traits (days to flowering, days to maturity, plant height, number of branches per plant, number of nodes per plant, number of filled pods per plant, number of empty pods per plant, 100-seed weight, and seed weight per plant) and the pod shattering percentage (the number of the shattered pods divided by the number of sample pods) [17]. The classification of the resistance was based on the shattering percentage [19]: very resistant (0%), resistant (1–10%), moderate (11–25%), susceptible (26–50%), and highly susceptible (>50%).

The agronomic traits and the pod shattering were subjected to analysis of variance (ANOVA). The relationship among agronomic traits and pod shattering was investigated using the genotype by trait biplot [20]. The Genotype by Trait (GT) biplot was generated with version 1.3.959 of the R Core Team [21].

RESULTS AND DISCUSSIONS

Variation of Pod Shattering Resistance and Agronomical Traits

The mean square from the analysis of variance for the 10 traits measured revealed that the genotype effects were highly significant ($p < 0.01$) for all measured traits, except the number of branches and number of empty pods (Table 1). These results imply the availability of genetic variability among black soybean genotypes, suggesting further steps could be performed via a breeding program by identifying desirable soybean genotypes [4, 22].

TABLE 1. Mean square for agronomic traits and pod shattering in black soybean genotypes.

No.	Trait	Symbol	Mean square	
			Replication	Genotype
1	Days to flowering	DTF	0.0002	10.6666*
2	Days to maturity	DTM	0.0005	10.4000**
3	Plant height (cm)	PTH	10.1058	69.7870**
4	Number of branches	NOB	4.3877	0.8620 ns
5	Number of nodes	NON	77.6429	14.0443*
6	Number of filled pods	NFP	837.3905	183.8611**
7	Number of empty pods	NEP	2.3861	0.7709 ns
8	100-seed weight	SWG	1.8033	3.4190**
9	Seed weight per plant (g)	WST	45.0775	7.6958*
10	Pod shattering	PSH	3.5821	30.0847**

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

The evaluation of pod shattering resistance using the oven-dry method revealed a wide range of resistance to pod shattering with significant differences among black soybean genotypes (Table 1). The response of black soybean genotypes to pod shattering varied from resistant to very susceptible. The pod shattering degree of 16 black soybean genotypes was classified as 5 resistant, 3 moderate, 4 susceptible, and 4 very susceptible genotypes (Fig. 1). The information in the pod shattering resistance of black soybeans in Indonesia is still limited. A previous study which investigated the pod shattering resistance of black, green, and yellow soybean seeds had successfully classified the 97 genotypes into 20 very resistant, 17 resistant, 15 moderate, 9 susceptible, and 36 very susceptible genotypes. Furthermore, the pod shattering in soybean between seed colors with a light color (green or yellow) and dark color (black) that was not significantly different was reported [23]. Notably, the resistance to pod shattering was mainly due to the genetic background, pod and seed attributes, and environmental conditions [12, 16, 24, 25]. The genotypes tested in this study were generated from the selection of progenies derived from the cross of Anjasmoro as one of the parents, harboring pod shattering resistance [26, 27]. In this study, five resistant genotypes to pod shattering had successfully identified and suggested that the genetic makeup plays a significant role in increasing soybean resistance to pod shattering.

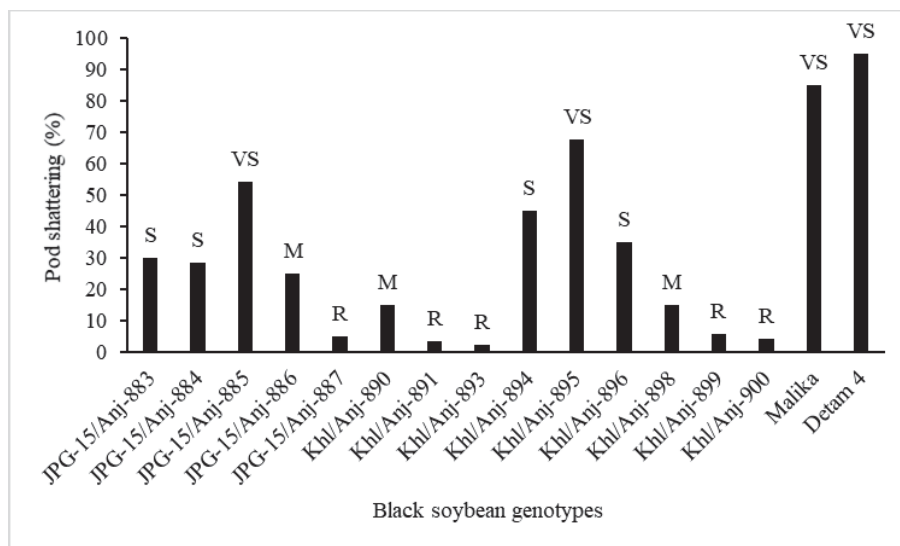
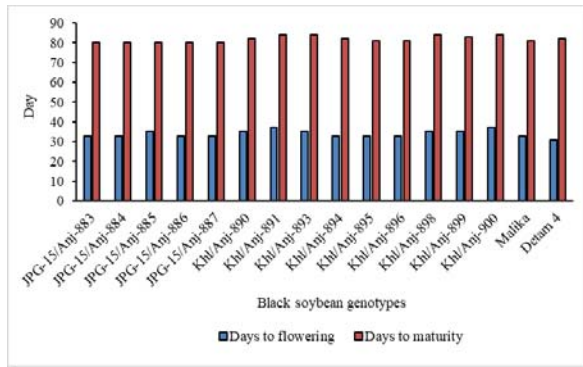
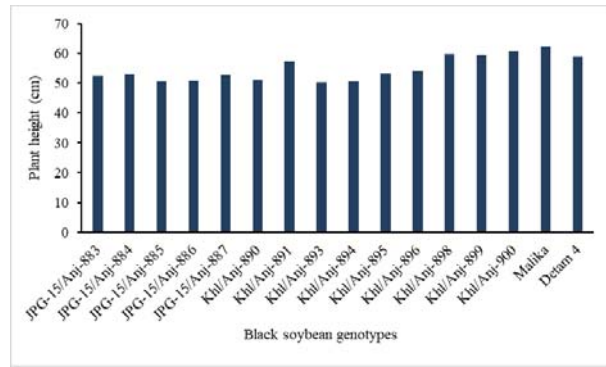


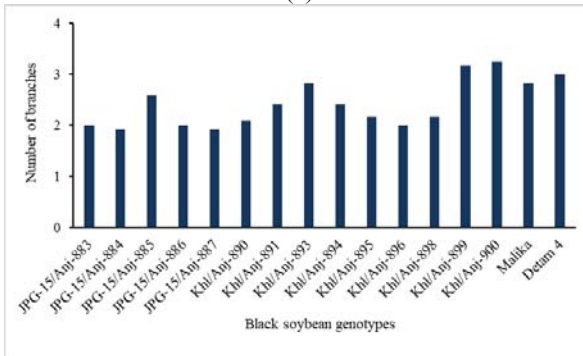
FIGURE 1. Pod shattering resistance of 16 black soybean genotypes. R = resistant, M = moderate, S = susceptible, VS = very susceptible.



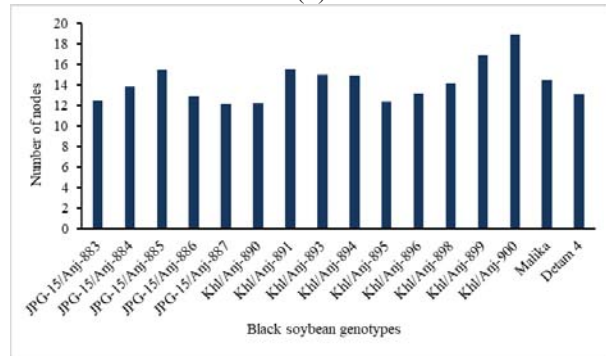
(a)



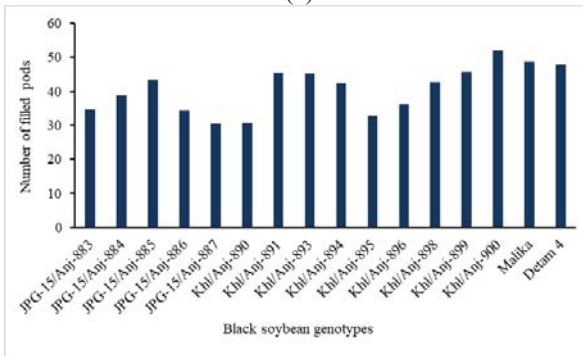
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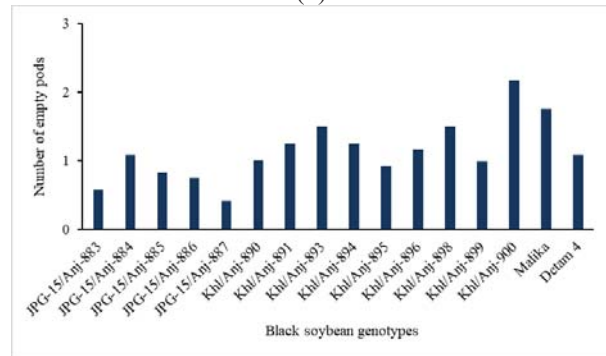
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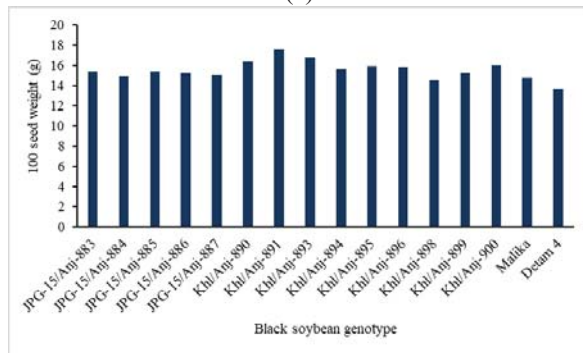
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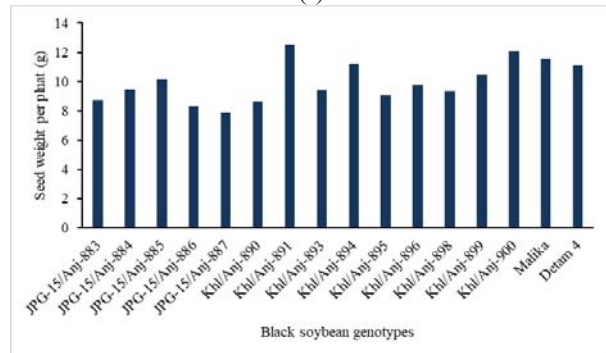
(e)



(f)



(g)



(h)

FIGURE 2. The performance of the agronomic traits of 16 black soybean genotypes. (a) Days to flowering and 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 per plant.

The performance of agronomic traits varied among black soybean genotypes, except for the number of branches and the number of empty pods (Table 1). The range of the days to flowering was 31–37 days with an average of 34 days (Fig. 2a). Meanwhile, the range of days to maturity was 80–84 days with an average of 82 days (Fig. 2a). In Indonesia, soybean days to maturity are classified into late maturity (>90 days), medium maturity (80 to 90 days), and early maturity (<80 days) [18]. Thus, five genotypes showed early maturity, and the rests have medium maturity.

In general, all soybean genotypes grew normally, as indicated by the plant height trait, which averaged 54.84 cm (Fig. 2b), and this trait supported the performance of other agronomic traits. The averages number of branches (Fig. 2c) and the number of nodes (Fig. 2d) per plant were 2.42 and 14.21, respectively. Furthermore, the average number of filled pods per plant was 40.69 (Fig. 2e), while the average number of empty pods per plant was 1.14 (Fig. 2f). The grouping of soybean seed size in Indonesia consists of small seed (<10 g per 100 seeds), medium (10–14 g per 100 seeds), and large size (>14 g per 100 seeds) [28]. Thus, the seed size of 16 soybean genotypes consists of medium (1 genotype) and large (15 genotypes) seed size (Fig. 2g). The average seed weight per plant was 9.98 g, and seven black soybean genotypes had seed yields that exceeded the average of all genotypes (Fig. 2h). Three out of seven genotypes were resistant to pod shattering (Khl/Anj-891, Khl/Anj-899, and Khl/Anj-900). A previous study in yellow soybeans also successfully obtained two of eight very resistant genotypes (G511H/Anj//Anj///Anj////Anj-6-11 and G511H/Anj//Anj///Anj////Anj-5-4) that have high yield [18]. Similarly, a study in yellow soybeans also obtained two very resistant genotypes (G42 and G15) with high yield [23]. The incorporation of pod shattering resistance and high yield is an ideal combination to maintain soybean yield productivity.

The Relationship among Agronomic Traits

The relationship among agronomic traits was investigated using GT biplot [29]. In the present study, the GT biplot accounted for 78% of the total variation. According to Yang *et al.* [30], the first two PCs should explain more than 60% of the variation in the data. In this study, the first two PCs explained most of the total variation. It suggested that the biplot charts effectively represented data variability, which allowed for a safe and clear interpretation [31].

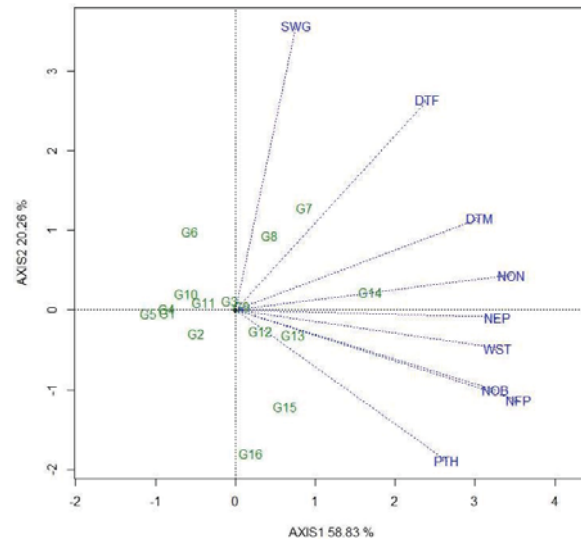


FIGURE 3. The interrelationships among agronomic traits of 16 black soybean genotypes are shown in a vector view of the GT biplot. The correlation coefficients between two traits are approximated by the cosine of the angle between their vectors. The symbol of traits is presented in Table 1. The name of genotypes is according to Fig. 1.

The cosine of the angle between the vectors is used to estimate the correlation coefficient between any two traits (Fig. 3). An acute angle (<90°) indicated a positive correlation; an angle close to 90° revealed unrelated traits, and an obtuse angle close to 180° showed a strong negative relationship [32]. Accordingly, the WST has a positive association with the NON, NOB, NFP, NEP, and DTM, as indicated by the small obtuse angles among their vectors.

These results indicated that the higher the values of these agronomic traits, the more the seed yield. Another study also found a strong positive correlation between soybean yield with days to flowering, the number of branches, and the number of pods per plant [33]. The yield components that have a strong relationship with seed yield can be utilized for indirect selection to improve seed yield [34]. Thus, the characters showing a positive correlation with yield could effectively be utilized in a crop improvement program to develop new high yielding soybean varieties.

Performance of Black Soybeans Based on Multiple Traits

The GT biplot of the “which-won-where” graph was used to compare genotypes based on multiple traits and to distinguish genotypes that are especially good in some aspects and thus can be used as parents in soybean breeding [35]. A series of perpendicular lines divide the biplot map into several sectors to classify the genotypes (Fig. 4). The winning genotype for each sector is the one located on the respective vertex. The vertex genotype is the genotype that occupied the vertex location in the biplot. In each sector, the vertex genotypes were considered the genotypes with the highest value of the traits within the sector.

In the present study, the biplot was divided into six sectors with 10 traits fall into three sectors. The black soybean genotypes G16, G15, G14, and G7 were regarded as vertex genotypes that exhibited superior performance for the traits allocated within the sector. The G15 and G16 as the vertex genotypes of the first sector exhibited the greatest value for PSH, thus these genotypes have the highest percentage of pod shattering than the other genotypes. The G14 as the vertex genotypes showing the best performance in the most agronomic traits (PTH, DTM, NOB, NON, NFP, NEP, and WST). The G7 exhibits the best performance on the DTF and SWG. The G7 and G14 were positioned at the opposite side with the PSH, hence these genotypes were categorized as resistant to pod shattering (have a low percentage of pod shattering).

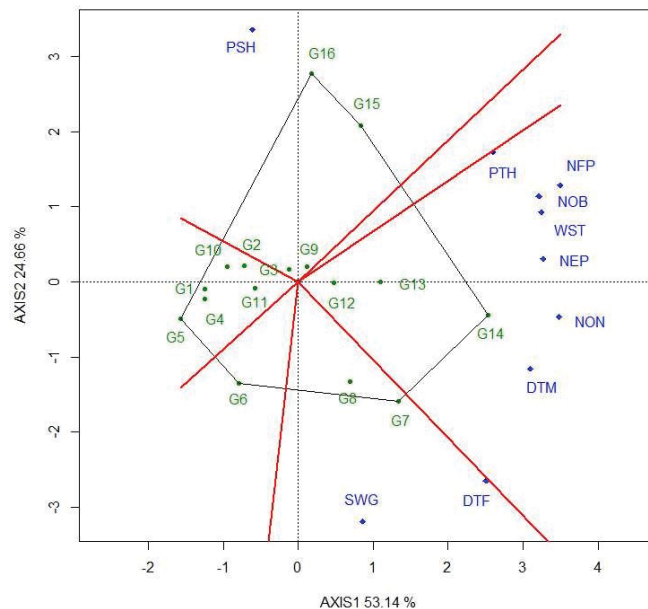


FIGURE 4. The GT biplot representing the “which-won-where” graph.

Based on the biplot in Fig. 4, the black soybean genotype G7 is the most resistant to pod shattering and has a large seed size. Meanwhile, G14 also showed the best yield and other yield components, and resistant to pod shattering. According to these results, those genotypes could be selected as parents in the black soybean breeding program, or they could be developed for producing new improved black soybean cultivars with pod shattering resistance. A previous study in yellow soybeans has also obtained three genotypes with high yield, large seed size, and medium maturity that potentially be used as parental lines to improve soybean shattering resistance in the breeding program [12]. The availability of pod shatter-resistant cultivars could minimize the yield losses or increase the yield productivity of black soybeans in Indonesia.

CONCLUSION

The pod shattering degree of 16 black soybean genotypes was classified as 5 resistant, 3 moderate, 4 susceptible, and 4 very susceptible genotypes. Seven genotypes yielded above the mean yield, and three of them were resistant to pod shattering. The seed yield per plant was positively correlated with days to maturity, plant height, number of nodes, number of branches, and number of pods. Based on the GT biplot, the G7 and G14 could be used as parents in the breeding program or developed for obtaining new improved black soybean cultivars with pod shattering resistance.

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