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IDENTIFICATION OF HIGH-YIELDING AND POD-SHATTERING RESISTANCE OF SOYBEAN ELITE LINES THROUGH GENOTYPE-BY-TRAIT BIPLOT ANALYSIS

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SUMMARY

Soybean (*Glycine max* L.) genotypes that combine high yield and pod-shatter resistance are essential for improving productivity, particularly under tropical conditions. The following study evaluated the agronomic performance and pod-shattering resistance of 16 soybean genotypes, including 14 elite breeding lines and two check cultivars, across two locations in East Java, Indonesia. Significant genotype-by-environment interactions ($p \leq 0.01$) emerged for most agronomic traits, except plant height, empty pods, and seed yield. Seed yield ranged from 2.50 to 3.46 t/ha, with an overall average of 3.03 t/ha, and had a positive correlation with the number of nodes and filled pods. Four genotypes (G1, G4, G11, and G15) were highly resistant to pod shattering. Selection based on multiple traits using the GT biplot successfully identified six soybean genotypes (G1, G2, G4, G5, G8, and G15) that exhibited the best performance for filled pods, seed yield, and resistance to pod shattering. These findings demonstrate the effectiveness of multi-trait selection using the GT biplot and provide promising candidate lines for developing high-yielding, pod-shattering-resistant soybean cultivars adapted to tropical environments.

Keywords: Soybean (*G. max* L.), yield-related traits, pod-shattering resistance, genotype-environment interaction, correlation, multiple traits

Key findings: In soybeans (*G. max* L.), the seed yield proved considerably and positively correlated with the number of nodes and filled pods. The high-yielding soybean genotypes with pod-shattering resistance and desirable agronomic traits identified through genotype-by-trait biplot analysis could serve as promising genetic resources for the development of new cultivars in breeding programs.

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INTRODUCTION

Soybean (*Glycine max* [L.] Merr.) ranks as the third most important food crop, primarily serving as raw materials for tempeh and tofu in Indonesia (Harsono *et al.,* 2021). However, due to enhanced industrial uses, the domestic soybean production cannot meet the national demand. The yield gap is relatively due to low soybean productivity in Indonesia. Addressing this issue requires efforts to focus on developing stable cultivars that combine high yield with tolerance to abiotic and biotic stresses.

Soybean productivity per unit area has linkage with the genetic potential of each genotype and primary support from the plant's ability to minimize factors that lead to reduced yield. Among the factors contributing to low soybean productivity, pod shattering remains one of the most serious challenges affecting soybean production in various growing regions worldwide (Hirata *et al.,* 2022; Ngwu *et al.,* 2023). Premature splitting of mature pods can result in substantial yield losses (Kim *et al.,* 2020; Krisnawati *et al.,* 2021). The soybean cultivars prone to pod shattering can lead to yield losses ranging from 50% to 100% (Fatima *et al.,* 2020).

Efforts to enhance soybean resistance to pod shattering have had reports across the soybean-producing countries. The approach includes identifying sources of resistance and then conducting recombination through hybridization among the parental genotypes (Krisnawati *et al.,* 2019). Molecular technology has also been applicable to developing the host resistance to pod shattering in soybeans (Jia *et al.,* 2022; Seo *et al.,* 2022). A study on pod-shattering resistance and agronomic performance of soybean genotypes during the dry season in Indonesia reported soybean cultivars exhibited the highest resistance against pod shattering, which can delay the harvest up to five days in the dry season (Krisnawati and Adie, 2024).

Being a tropical region, Indonesia experiences two seasons—the rainy and dry seasons. Soybean cultivation prevails across various agroecosystems; however, they have the largest area during the dry season in lowland paddy fields, following an annual crop rotation of rice-rice-soybeans. The interaction between the genotypes and environments (GEI) is a crucial factor affecting the seed yield and its components in soybeans. This interaction also significantly influences the selection for genotypes within breeding programs (Silva *et al.,* 2022). Extensive research has explored the impact of GEI on seed yield and various agronomic traits (Li *et al.,* 2020; Mushoriwa *et al.,* 2022).

Seed yield management has a complex interplay of genetic and environmental factors, and therefore, the identification of the yield-related traits that considerably contribute to soybean productivity is crucial for optimizing overall yield. A past study suggested that pods, grains, and the 100-grain weight can serve as direct selection criteria for enhancing the soybean yield (Li *et al.,* 2020). Other studies have also identified that the number of seeds per pod plays a key role in managing the soybean yield (Silva *et al.,* 2022).

Although considerable progress has resulted in developing soybean varieties with a high-yield potential, the challenge of pod shattering remains a major constraint, particularly under tropical conditions. In Indonesia, where cultivation of soybeans is mostly during the dry season, pod shattering contributes substantially to yield losses. However, limited attention has been given to the combined improvement of yield and shattering resistance in tropical germplasm. Moreover, while the genotype-by-trait (GT) biplot analysis has succeeded in its application to visualize trait relationships and facilitate multi-trait selection in several crop species (Güngör *et al.,* 2024; Esmaily *et al.,* 2025), its operation for simultaneously identifying high-yielding and pod-shattering-resistant elite lines in tropical environments is still scarce. This lack of integrated studies creates a gap in breeding strategies, underscoring the need to identify superior lines that combine high productivity with pod-shattering resistance through the GT biplot analysis. The GT biplot analysis allows the selection of promising genotypes with desired traits (Gholizadeh *et al.,* 2023; Dadras *et al.,* 2024). The presented study aimed to evaluate the agronomic performance and pod-shattering resistance in elite soybean lines and select the promising genotypes based on multiple traits.

MATERIALS AND METHODS

Plant material and study sites

The study used 16 soybean genotypes comprising 14 soybean elite lines selected from crosses and two check cultivars (Detap 1 and Grobogan) (Table 1). The check cultivar Detap 1 is high yielding, with early maturity and pod-shatter resistance, while Grobogan is also high yielding but highly susceptible to pod shattering. The study, conducted from March to August 2024, comprised two types of activities: a) field research to assess the performance of the soybean genotypes for agronomic traits, and b) laboratory research to evaluate the genotypes' pod-shattering resistance. The field research commenced in Nganjuk and Mojokerto Regencies, East Java, which represent major soybean production areas with contrasting agroecological conditions, allowing the assessment of key genotype-environment interactions. The environmental data of each location is available in Table 2. For pod-shattering resistance, the soybean genotypes bore evaluation in the laboratory of the Purwodadi Botanical Garden, Pasuruan, East Java, Indonesia.

Experimental design and cultivation

Field research at each location used a randomized complete block design (RCBD) with 16 genotypes and four replications. In both research locations (Nganjuk and Mojokerto), the study used minimum tillage. Before planting the seeds, irrigation channels were prepared, and weeds were controlled using herbicides. The plot size was 2.4 m × 4.5 m, with a planting distance of 40 cm × 15 cm and two seeds per hill. Planting continued using a wooden dibble with planting holes approximately 2 cm deep, which succeeded in covering with organic fertilizer. The NPK fertilizer (15-15-15) application had a rate of 150 kg/ha. Pest and disease control management employed optimal use of pesticides, with mechanical weed control also performed twice.

Table 1. Soybean genotypes used in the study.

No.	Genotype code	Crossing parents	Pedigree	Remarks
1	G1	Anjasmoro × G100H	Anj/G100H-6	Advanced line
2	G2	Anjasmoro × G100H	Anj/G100H-14	Advanced line
3	G3	Anjasmoro × G100H	Anj/G100H-16	Advanced line
4	G4	Anjasmoro × G100H	Anj/G100H-21	Advanced line
5	G5	Anjasmoro × G100H	Anj/G100H-24	Advanced line
6	G6	Anjasmoro × G100H	Anj/G100H-28	Advanced line
7	G7	Anjasmoro × G100H	Anj/G100H-44	Advanced line
8	G8	Anjasmoro × G100H	Anj/IAC100-19	Advanced line
9	G9	Anjasmoro × Rajabasa	Anj/Rjbs-304	Advanced line
10	G10	Anjasmoro × Rajabasa	Anj/Rjbs-305	Advanced line
11	G11	Anjasmoro × Rajabasa	Anj/Rjbs-306	Advanced line
12	G12	Anjasmoro × Rajabasa	Anj/Rjbs-309	Advanced line
13	G13	Anjasmoro × Rajabasa	Anj/ Rjbs-311	Advanced line
14	G14	Grobogan × Anjasmoro	Grbg/Anj-2	Advanced line
15	G15	-	-	Check cultivar: Detap 1
16	G16	-	-	Check cultivar: Grobogan

Table 2. Environmental data of the experimental research locations.

Locations	Coordinates	Elevation (masl)	Soil types	Climate type
Nganjuk, East Java, Indonesia	7°35′59″S 111°53′57″E	58	Regosol	E
Mojokerto, East Java, Indonesia	7°29′15″S, 112°25′37″E	72	Grumosol	C3

masl = meters above sea level; E = wet months are less than 3 times; C3 = wet months are 5 to 6 times in a row, with the climate type based on Oldeman's climatic classification system.

Evaluation for pod-shattering resistance

The study used RCBD with four replications. When the plants reached the R8 stage (full maturity, indicated by yellowing leaves), random selection of 10 plants occurred in each soybean genotype. These plants sustained air-drying in an upright position for three days. In these 10 plants, random choosing of 30 pods incurred placement in a 15-cm diameter petri dish before placing in an oven. For soybean genotypes, the pod-shattering resistance evaluation proceeded through the oven-dry method in the laboratory (Krisnawati and Adie, 2017). In this method, the pods underwent oven-drying sequentially, starting at 30 °C for three days, followed by 40 °C for one day, 50 °C for one day, and finally, 60 °C for one day. The number of shattered pods attained recording on the seventh day, expressed as a percentage of the total pods observed.

Data collection

In the field research, data collection took place on days to flowering, days to maturity, plant height, the number of branches, nodes, filled pods, and empty pods, 100-seed weight, and seed yield. The pod traits' data recording also succeeded for the pod length, width, and thickness and the seed length, width, and thickness. These observations based on pod characteristics were conducted at the R8 stage. Measurements of pod characters followed the method of Krisnawati and Adie (2017), while seed characters' estimates continued according to Kibar and Öztürk (2008). Data collection used a digital caliper on 10 healthy pods randomly sampled from two representative plants in each replication. In the assessment of pod-shattering resistance in the laboratory, the percentage of shattered pods, when calculated, was operative by dividing the number of shattered pods by the total number of pods and expressed in percentage.

Statistical analysis

The data entailed subjection to a combined analysis of variance by performing the PROC GLM procedures of SAS software version 9.1.3 for Windows (SAS Institute, 2007). The degree of soybean pods resistance to shattering reached assessment using the rating scale (AVRDC, 1979). Pearson correlation analysis helped explore the relationship among the various traits of soybeans. The correlation calculation utilized the Corrplot and Hmisc packages in RStudio version 1.3.959 (R-Studio Team, 2020), following the method outlined by Singh and Chaudhary (1977). The genotype-by-trait biplot (GT), as employed, selected the promising soybean genotypes based on the multiple traits (Yan and Rajcan, 2002). The GT biplot creation used the RStudio software version 1.3.959 (RStudio Team, 2020).

RESULTS AND DISCUSSION

Genotypes' response to each environment

Each soybean genotype showed distinct agronomic responses to the two different location environments (Table 3). In location Nganjuk, the genotypes expressed significant ($p \le 0.01$) differences for all observed traits, except plant height, the number of empty pods, and seed yield. Seed yields ranged from 2.18 to 3.94 t/ha, with an average of 3.26 t/ha

Table 3. Analysis of variance for soybean agronomic traits in Nganjuk and Mojokerto.

Characters	Symbols	Nganjuk		Mojokerto	
		Replications	Genotypes	Replications	Genotypes
Days to flowering (day)	FLD	0.0572^{ns}	40.9156^{**}	0.1406^{ns}	11.7989^{**}
Days to maturity (day)	MTD	0.2916^{ns}	132.3166^{**}	0.2083^{ns}	11.4500^{**}
Plant height (cm)	PLH	251.0416^{**}	34.1500^{ns}	868.8930^{**}	139.8672^{ns}
Branches/plant	NBR	0.4322^{ns}	2.2156^{**}	0.0880^{ns}	0.7264^{ns}
Nodes/plant	NNO	2.7291^{*}	2.8958^{**}	4.6267^{ns}	3.9811^{*}
Pods/plant	NFP	24.3489^{ns}	501.3072^{**}	177.5845^{**}	75.2426^{*}
Empty pods/plant	NEP	0.6822^{ns}	1.4322^{ns}	0.6781^{ns}	0.4287^{ns}
100-seed weight (g)	SDW	0.3504^{ns}	15.9381^{**}	1.0625^{ns}	11.4958^{**}
Seed yield (t/ha)	SYD	0.4249^{ns}	1.2604^{ns}	0.0789^{ns}	0.3495^{**}

*,** = Significant at $p < 0.05$ and $p < 0.01$, respectively; Reps = Replication, NS = Nonsignificant.

Table 4. Agronomic data of 16 soybean genotypes in Nganjuk.

Genotypes	FLD (day)	MTD (day)	PLH (cm)	NBR	NNO	NFP	NEP	SDW (g)	SYD (t/ha)
1	35	83	67.25	3.00	12.00	54.50	0.00	14.75	3.75
2	37	86	67.75	3.25	10.50	48.50	1.50	15.00	3.00
3	33	78	68.25	2.75	11.50	32.25	0.75	13.00	3.48
4	38	81	69.25	3.75	9.75	46.25	0.50	12.25	3.67
5	39	87	71.25	4.00	11.25	59.25	1.75	12.75	3.94
6	33	76	70.25	3.75	11.25	23.75	1.25	15.75	3.30
7	32	70	61.50	1.75	8.75	31.50	1.75	16.43	3.54
8	37	86	70.00	4.00	11.75	49.00	0.75	14.25	2.49
9	35	77	66.50	1.75	10.50	26.00	0.50	17.50	2.18
10	37	86	66.50	2.50	10.50	47.50	1.75	14.50	2.30
11	30	72	72.25	3.50	11.50	35.00	1.00	15.40	3.68
12	37	87	68.50	2.75	10.50	42.50	2.00	13.00	3.85
13	31	73	69.00	3.25	11.25	36.75	1.00	15.80	3.69
14	31	83	65.25	2.00	12.00	34.00	0.75	18.75	3.33
15	33	76	64.75	3.50	10.75	56.00	2.00	13.75	3.19
16	29	74	72.75	2.75	10.75	28.50	1.00	18.63	2.78
Average	34	80	68.19	3.02	10.91	40.70	1.14	15.09	3.26

FLD = Days to flowering, MTD = Days to maturity, PLH = Plant height, NBR = Branches/plant, NNO = Nodes/plant, NFP = Pods/plant, NEP = Empty pods/plant, SDW = 100-seed weight, and SYD = Seed yield.

in 16 genotypes (Table 4), indicating diverse responses to the local environment. The check cultivars Detap 1 (3.19 t/ha) and Grobogan (2.78 t/ha) performed well, suggesting location Nganjuk was favorable for soybean production. Notably, seven genotypes exceeded 3.5 t/ha seed yield, showing the highest adaptability and performance. These results align with previous studies highlighting relevant yield variability across environments, influenced by factors like soil fertility, climate, and genotypic potential (Li *et al.,* 2020; Abebe *et al.,* 2024).

In Nganjuk, the 16 soybean genotypes exhibited wide variation in yield components (Table 4). Flowering occurred between 29 and 39 days, and maturity between 73 and 87 days. Plant height varied from 61.50 to 72.75 cm, with branches (1.75–4.00) and nodes (8.75–12.00) influencing pod number and productivity (Xu *et al.,* 2021). The number of filled pods ranged from 26.00 to 59.23, and 100-seed weight ranged from 12.25 to 18.75 g, classifying the genotypes as medium- to large-seeded. The genotype G11 had the

Table 5. Agronomic data of 16 soybean genotypes in Mojokerto.

Genotypes	FLD (day)	MTD (day)	PLH (cm)	NBR	NNO	NFP	NEP	SDW (g)	SYD (t/ha)
1	34	80	53.43	2.75	9.85	36.00	0.67	17.00	3.03
2	33	82	58.75	1.93	9.24	29.42	0.75	16.50	3.23
3	32	79	47.18	2.85	7.83	24.42	0.50	17.50	2.61
4	32	80	49.85	1.85	8.42	28.17	0.25	17.00	2.89
5	35	81	44.08	1.65	7.75	23.75	0.59	16.00	2.14
6	34	82	58.68	2.43	9.25	32.33	0.67	16.50	2.75
7	35	80	49.78	2.25	8.50	29.17	0.58	16.50	3.07
8	35	79	58.25	2.08	11.33	30.42	0.33	14.00	2.66
9	33	78	53.35	2.50	8.00	27.00	1.09	17.00	2.83
10	32	79	52.40	2.50	8.92	27.75	0.67	19.00	2.74
11	33	82	48.58	1.50	8.83	22.92	0.08	16.50	2.81
12	32	80	52.83	2.25	8.33	29.00	0.50	17.50	2.69
13	35	81	41.50	2.33	7.92	22.33	0.83	16.00	3.23
14	35	82	38.35	2.58	8.67	27.00	1.50	14.50	2.77
15	35	84	50.65	1.85	7.67	25.17	0.75	17.50	2.29
16	29	79	46.10	1.50	7.25	17.75	0.58	21.50	2.95
Average	33	81	50.24	2.18	8.61	27.04	0.65	16.91	2.79

FLD = Days to flowering, MTD = Days to maturity, PLH = Plant height, NBR = Branches/plant, NNO = Nodes/plant, NFP = Pods/plant, NEP = Empty pods/plant, SDW = 100-seed weight, and SYD = Seed yield.

shortest maturity period (72 days) and a higher 100-seed weight (15.40 g) and also demonstrated the highest seed yield (3.68 t/ha). The results suggested that G11 could be an ideal choice for the farming community, as its preference leans toward high productivity combined with early maturity (Yusron *et al.,* 2023).

In the location of Mojokerto (Table 5), seed yields ranged from 2.14 to 3.23 t/ha (average 2.79 t/ha), suggesting this region's conditions may be less favorable for soybean production than the location of Nganjuk. However, the genotypes G2 and G13 were the highest-yielding (3.23 t/ha), indicating that specific genotypes can perform well under these conditions. This aligns with past studies showing that genotype performance can vary widely, depending on the environment and genotypes adaptability (Sritongtae *et al.,* 2021; Rani *et al.,* 2023). Substantial variation was evident in yield components among the genotypes (Table 5). Days to flowering ranged from 29 to 35 days, and maturity from 78 to 84 days, while plant height varied between 41.50 and 58.75 cm. The number of branches (1.50–2.75) and nodes (7.25–11.33) fluctuated modestly, whereas filled pods (17.75–36.00) showed greater divergence among genotypes. Filled pods ranged from 17.75 to 36.00, while empty pods were few. Seed size, indicated by the 100-seed weight, ranged from 14.00 to 21.50 g, classifying the genotypes within the large-seeded category, and the higher values are potentially important for seed markets and consumer preferences (Kuswantoro *et al.,* 2023).

Overall, the soybean genotypes were notable with higher values for average seed yield, days to flowering, maturity, and plant height at Nganjuk than in Mojokerto, which may refer to the more favorable growth conditions in Nganjuk. However, despite the lower seed yield in Mojokerto, the seed size was larger, suggesting that environmental factors in Nganjuk may favor the overall productivity, and those in Mojokerto may encourage the development of larger seeds. These findings were consistent with previous studies, which emphasized that environmental conditions also affect seed size in soybeans (Rani *et al.,* 2023; Abebe *et al.,* 2024).

Table 6. Combined analysis of variance of soybean genotypes at the two locations.

Characters	Symbols	Mean Squares			
		Reps/L	Locations (L)	Genotypes (G)	L × G
Days to flowering (day)	FLD	0.0989^{ns}	12.5000**	26.8479**	25.8666**
Days to maturity (day)	MTD	0.2500^{ns}	24.5000**	66.9000**	76.8666**
Plant height (cm)	PLH	559.9673**	10315.8657**	87.4107^{ns}	86.6065^{ns}
Branches/plant	NBR	0.2601^{ns}	22.6969**	0.8611^{ns}	2.0809**
Nodes/plant	NNO	3.6779**	168.8203**	4.4688**	2.4080*
Filled pods/plant	NFP	100.9652^{ns}	5977.0711**	322.0806^{ns}	244.4638**
Empty pods/plant	NEP	0.6801^{ns}	7.8457**	0.8752^{ns}	0.9858^{ns}
100-seed weight (g)	SDW	0.7064^{ns}	105.1250**	15.2811**	12.1528**
Seed yield (t/ha)	SYD	0.2519^{ns}	6.9704**	0.7632^{ns}	0.8466^{ns}

*,** = Significant at $p < 0.05$ and $p < 0.01$, respectively; Reps = Replication, NS = Nonsignificant.

G × E interaction and seed yield determinants

The performance of 16 soybean genotypes in two different environments revealed a genotype-by-environment interaction (GEI) for all agronomic traits, except for the number of empty pods and seed yield (Table 6). This indicates the agronomic traits of each genotype vary across different environments. The environments significantly ($p \leq 0.01$) affected all agronomic traits, while the genotypes had a significant ($p \leq 0.01$) effect on days to flowering, days to maturity, the number of nodes, and 100-seed weight. The GEI plays an important role in influencing the complex quantitative traits, such as soybean yield (Rani *et al.,* 2023). This interaction occurs when different genotypes respond uniquely to varying environmental conditions, resulting in differences in agronomic performance, including seed yield (Döttinger *et al.,* 2023).

Across the different environments, the seed yield of 16 genotypes ranged from 2.50 to 3.46 t/ha, with an average of 3.03 t/ha (Figure 1). Genotype G13 achieved the highest seed yield (3.46 t/ha), followed by G1 (3.39 t/ha). Both these promising genotypes also outperformed the two check cultivars (Detap 1 and Grobogan), which produced lower yields than the overall average. These findings highlight the potential of particular soybean genotypes, such as G13 and G1, to provide the topmost yields under tested conditions, indicating their suitability for environments where maximized productivity is a priority. Past studies enunciated similar results, and the specific genotypes showed superior performance compared with the local and commercial cultivars, particularly in regions with favorable growing conditions (Sritongtae *et al.,* 2021; Adie *et al.,* 2022b; Abebe *et al.,* 2024).

For maturity (Figure 2), the days to maturity for genotypes ranged from 77 to 84 days, with an average of 80 days at both locations. In Indonesia, the maturity period is a crucial trait due to the cropping system, which follows the annual paddy-paddy-soybean rotation (Krisnawati *et al.,* 2019). Soybean cultivars with a shorter maturity period are highly preferred, as they allow the farmers to maximize the growing season and efficiently transition between crops. In the presented study, the genotype G13 revealed the shortest maturity period (77 days), while also performing exceptionally well for seed yield and seed size. It further supported the preference for early-maturing cultivars in Indonesian agriculture. In this study, the average seed size was 16.00 g/100 seeds, which falls under the large category (Krisnawati *et al.,* 2019). Both genotypes, G13 and G1, were the highest-yielding and exhibited large-seed sizes, which further add to their attractiveness for the farming community in Indonesia, as seed size becomes a significant consideration in cultivar selection (Kuswantoro *et al.,* 2020).

The conduct of a correlation analysis assessed the relationships among the various agronomic traits of the soybean genotypes

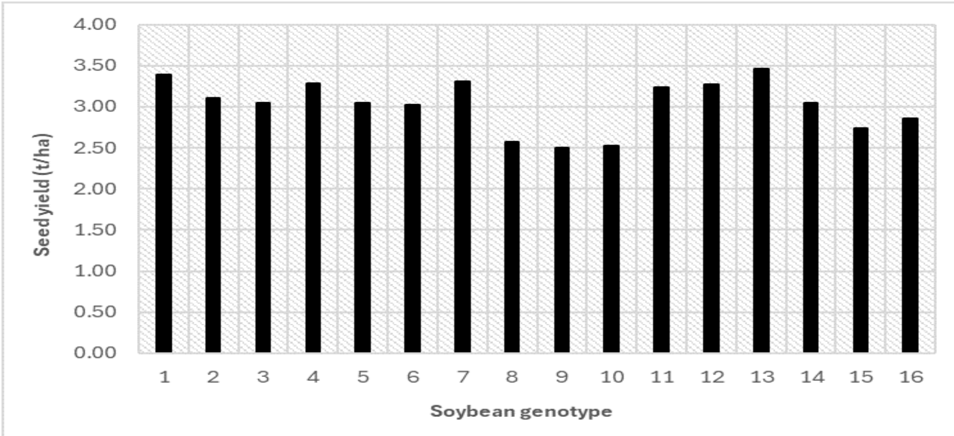

Figure 1. Seed yield of 16 soybean genotypes across locations.

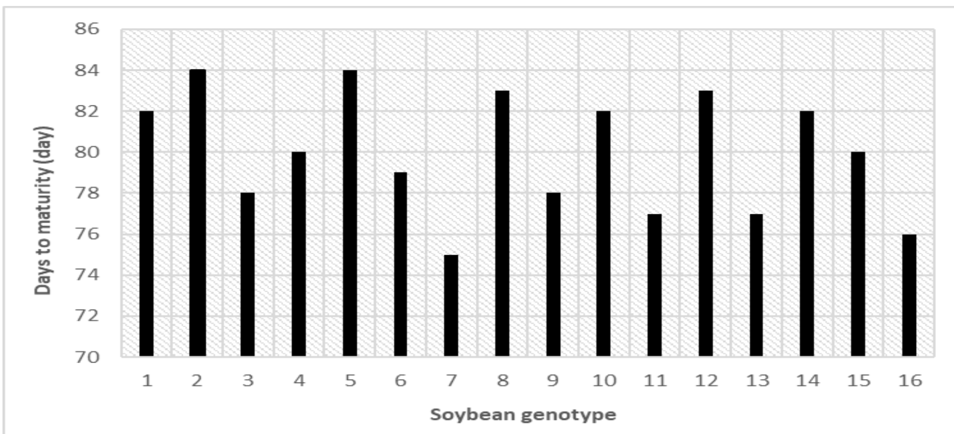

Figure 2. Days to maturity of 16 soybean genotypes across locations.

(Figure 3). The analysis disclosed that soybean seed yield had a significantly positive correlation with the number of nodes and filled pods. This suggested that an increase in the number of nodes and filled pods could contribute to a higher seed yield, which aligns with previous studies stating a demonstration of a positive association between the seed yield and its components (Prathima *et al.,* 2022; Silva *et al.,* 2022; Contardi *et al.,* 2024). These traits proved critical for determining the reproductive success of the plant and its ability to produce more pods, which directly influences the overall productivity. However, the seed yield showed a considerable negative correlation with the 100-seed weight, implying that genotypes with larger seeds tend to produce lower yields. This negative relationship between seed yield and seed size has also been part of reports in previous soybean studies (Rani *et al.*, 2023). These results imply that to achieve the highest soybean productivity, more nodes and filled pods are essential, while greater seed size will eventually decrease the productivity.

Pod-shattering resistance

The analysis of variance evaluated at temperatures of 50 °C and 60 °C for pod-shattering resistance, as well as for pod and seed traits, provided significant ($p \leq 0.01$) differences among the tested soybean genotypes (Table 7). In the laboratory analysis

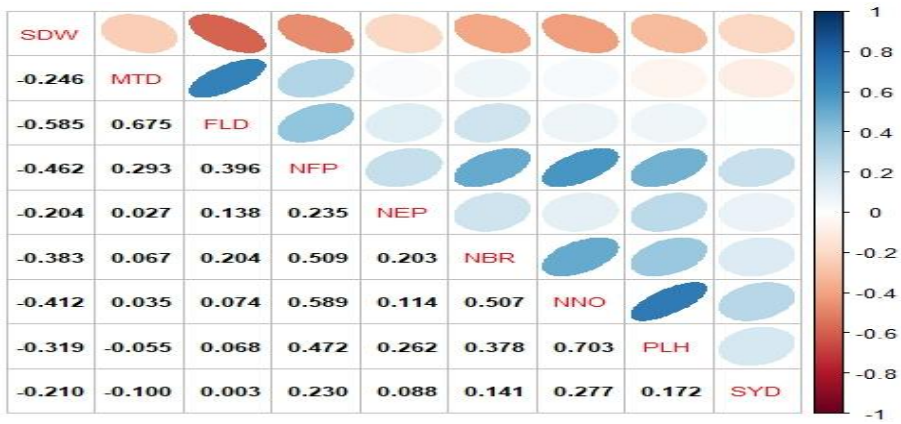

Figure 3. Correlation matrices of soybean agronomic traits. Blue represents positive correlations, while red signifies negative correlations. The color intensity corresponds to the strength of the correlation, with darker shades indicating stronger correlation. SDW: 100-seed weight, MTD: days to maturity, FLD: days to flowering, NFP: number of filled pods, NEP: number of empty pods, NBR: number of branches, NNO: number of nodes, PLH: plant height, and SYD: seed yield.

Table 7. Analysis of variance for pod-shattering evaluation and pod and seed physical traits in Nganjuk.

Characters	Symbols	Mean Squares	
		Replications	Genotypes
Pod shattering at 50 °C (%)	PS50	0.2204	28.8077**
Pod shattering at 60 °C (%)	PS60	0.7535	49.5665**
Pod length (cm)	PDL	0.0756	0.4300**
Pod width (cm)	PDW	0.0006	0.0127**
Pod thickness (cm)	PDT	0.1006	0.0035^{NS}
Seed length (cm)	SDL	0.0005	0.0157**
Seed width (cm)	SDW	0.0011	0.0053**
Seed thickness (cm)	SDT	0.0038	0.0038**

** = significant at the p < 0.01, NS = not significant.

using the oven-dry method, all soybean genotypes appeared resistant to pod shattering at 30 °C and 40 °C. However, at 50 °C, eight out of 16 tested genotypes achieved a highly resistant (HR) classification, three genotypes were moderately resistant (M), two genotypes were susceptible (S), and three genotypes arose as highly susceptible (HS). By increasing the temperature to 60 °C, four soybean genotypes acquired the HR category, four genotypes were resistant (R), and eight genotypes were HS. Krisnawati and Adie (2017) reported significant (p ≤ 0.01) differences among the soybean genotypes for pod-shattering resistance, emphasizing the importance of selection for pod-shattering resistance through breeding programs.

In this study, four genotypes, G1, G4, G11, and G15, were successfully identified as consistently exhibiting HR at the highest temperatures (50 °C and 60 °C) (Table 8). Past studies have also successfully obtained several HR genotypes for pod shattering in soybeans (Krisnawati *et al.,* 2019; Fatima *et al.,* 2020; Adie *et al.,* 2022a, b). These promising genotypes could be valuable for soybean cultivar improvement, especially in regions prone to high temperatures, as they

Table 8. Pod-shattering percentage of soybean genotypes and their resistance criteria at 50 °C and 60 °C in Nganjuk.

No.	Genotypes	Pod-shattering percentage at different temperature and resistance criteria					
		30 °C (%)	40 °C (%)	50 °C (%)	Resistance criteria at 50 °C	60 °C (%)	Resistance criteria at 60 °C
1	G1	0	0	0	HR	0	HR
2	G2	0	0	0	HR	2	R
3	G3	0	0	32	S	100	HS
4	G4	0	0	0	HR	0	HR
5	G5	0	0	0	HR	7	R
6	G6	0	0	75	HS	100	HS
7	G7	0	0	32	S	59	HS
8	G8	0	0	0	HR	2	R
9	G9	0	0	25	M	60	HS
10	G10	0	0	65	HS	100	HS
11	G11	0	0	0	HR	0	HR
12	G12	0	0	0	HR	4	R
13	G13	0	0	20	M	69	HS
14	G14	0	0	20	M	55	HS
15	G15	0	0	0	HR	0	HR
16	G16	0	7	63	HS	100	HS
Means		0	0.44	20.75		41.13	

HR = highly resistant, R = resistant, M = moderately resistant, S = susceptible, and HS = highly susceptible.

can contribute to increased harvest efficiency by reducing yield losses due to pod shattering (Krisnawati *et al.,* 2022). In this study, the 16 soybean genotypes exhibited considerable variability for the pod and seeds' physical traits (Table 7), highlighting the diverse physical characteristics of the genotypes, which can influence crucial agronomic traits such as pod-shattering resistance.

The correlation analysis revealed that pod-shattering resistance had a substantial ($p \leq 0.05$) influence from seed thickness (SDT), with a positive correlation (r = 0.568*), indicating that genotypes with larger seed thickness tend to be more susceptible to pod shattering. The results suggested seed size, particularly seed thickness, plays a vital role in pod integrity, as thicker seeds may exert more pressure on the pod wall, increasing the likelihood of pod-shattering. These results agree with previous research, which has also emphasized the role of pod physical traits in determining the pod-shattering response in soybeans (Prathima *et al.,* 2022; Fatima *et al.,* 2024).

Selection of promising genotypes

A which-won-where biplot derived with genotype-by-trait (GT biplot) analysis helped facilitate the selection of promising genotypes based on multiple traits, particularly those associated with high seed yield and pod-shattering resistance. The GT biplot analysis indicated that the first two principal components explained 36.72% and 21.68% of the variability in the standardized data, respectively, totaling 58.4% (Figure 4).

The polygon view is the most important feature of the GT biplot that helps identify genotypes with the maximum values for one or more traits. The vertex genotype within each sector represents the superior genotype for the tested traits. In the biplot, the genotypes located in a sector associated with one or more specific traits exhibited strong performance concerning that trait. Meanwhile, the genotypes located in the opposite direction to the trait's position were the genotypes recognized with a lower performance for the said trait (Adie *et al.,* 2022a, b).

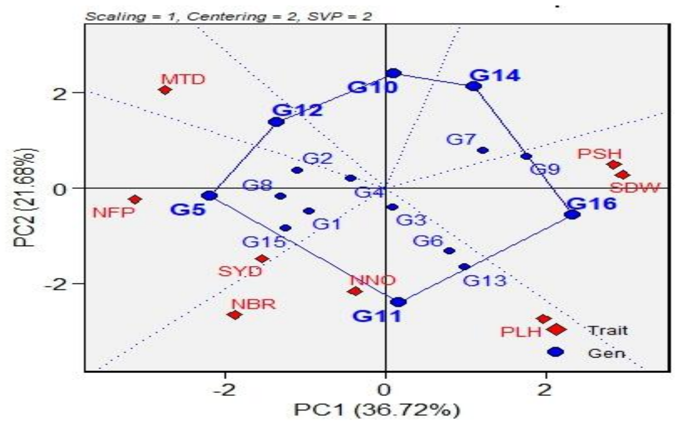

Figure 4. Polygon view of the soybean genotype-by-trait biplot showing which genotype had the highest values for which traits. Genotypes are represented by blue circular symbols with genotype codes (G1-G16), while traits are indicated by red diamond shapes with traits' codes. MTD: days to maturity, PLH: plant height, NBR: number of branches, NNO: number of nodes, NFP: number of filled pods, SDW: 100-seed weight, SYD: seed yield, and PSH: pod-shattering percentage.

As shown in Figure 4, the vertex genotypes included G5, G10, G11, G12, G14, and G16. Genotype G5 and the other genotypes in this sector (G1, G2, G4, G8, and G15) demonstrated the best performance regarding the number of filled pods and seed yield. These genotypes showed positions in the opposite direction of the pod-shattering trait (PSH), indicating the highest resistance to pod-shattering, as characterized by a low percentage of shattering. Genotype G11 exhibited the highest values for plant height (PLH), the number of branches (NBR), and the number of nodes (NNO). The other genotypes (G3, G6, G13) within the same sector also displayed superior performance for these traits. In the adjacent sector, the genotypes G16 and G9 showed the larger seed size (SDW); however, they gave a low yield (SYD) and high percentage of pod-shattering (PSH), indicating susceptibility to shattering. The genotype G12 has the highest value for days to maturity (MTD). The vertex genotypes G10 and G14 did not perform well for any of the measured traits, highlighting their overall inferior performance compared with other genotypes.

The application of biplot analysis to multi-trait data allowed for a visual comparison of the genotypes and helped in the selection process, with the GT biplot serving as an effective graphical tool. In this study, selection based on multiple traits using the GT biplot has resulted in six genotypes (G1, G2, G4, G5, G8, and G15) exhibiting excellent performance for the number of filled pods, seed yield, and pod-shattering resistance (Figure 3). Reports on the use of pod-shattering-resistant cultivars, combined with the highest seed yield, stated to effectively enhance the productivity (Krisnawati *et al.,* 2021; Ngwu *et al.,* 2023). By utilizing the GT biplot analysis, similar findings also emerged in several studies and have successfully identified the soybean cultivars with high pod-shattering resistance and seed yield (Adie *et al.,* 2022a, b). These promising genotypes could play an essential role in further varietal development and can serve as valuable genetic resources in soybean breeding programs focused on enhancing resistance to pod shattering.

The findings of this study have critical implications for soybean breeding. The identified genotypes with superior yield and pod-shattering resistance (G1, G2, G4, G5, G8, and G15) can become valuable parental lines in future breeding programs aimed at developing high-yielding, resilient cultivars for tropical environments. Among the evaluated traits, filled pods and seed yield emerged as key productivity determinants, while seed thickness showed a strong association with pod-shattering resistance, underscoring their importance as selection criteria. Beyond phenotypic evaluation, integrating these results with molecular approaches, such as marker-assisted selection or genomic selection, could accelerate the identification and deployment of pod-shattering-resistance alleles while ensuring a stable yield performance (Kim *et al.,* 2020; Seo *et al.,* 2022). Nevertheless, this study has some limitations, including its evaluation across only two locations and one growing season, as well as the lack of molecular validation. Future studies involving multi-environment trials across diverse seasons, combined with molecular characterization, will be essential to validate these findings and broaden their applicability for soybean improvement programs.

CONCLUSIONS

Soybean genotypes showed varied responses to each environment, as reflected by significant differences among genotypes in seed yield and agronomic traits. The seed yield had a significantly positive correlation with the number of nodes and filled pods, indicating these traits are key determinants of productivity. Selection based on multiple traits using the genotype-by-trait biplot successfully identified six genotypes (G1, G2, G4, G5, G8, and G15) that exhibited superior performance for the number of filled pods, seed yield, and the highest resistance to pod shattering. These superior genotypes can directly serve as parental genotype in breeding programs or entail further evaluation in advanced yield trials. These findings have meaningful implications for the Indonesian lowland cropping system, providing breeders with promising materials to develop high-yielding, pod-shattering-resistant soybean varieties adapted to tropical conditions and guide breeding strategies for the region.

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