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Phenotypic evaluation of F10 soybean generations from Grobogan x Slamet cross for large seed size selection

Abstract. Soybeans are an important crop with high nutritional value and diverse uses. Current consumer and industry preference is for soybean varieties with large seeds and high productivity. To meet these demands, one effective approach is through hybridization. This study aims to evaluate the phenotype of F10 soybean line from a cross between Grobogan and Slamet for large-seeded and high-yield. The study was conducted in the screenhouse and Plant Breeding and Biotechnology Laboratory, Faculty of Agriculture, Jenderal Soedirman University. The experiment used a Randomized Complete Block Design (RCBD) with three replications, testing genotypes from Slamet x Grobogan crosses and three check varieties: Slamet, Grobogan, and Wilis. Observational data were analyzed using analysis of variance (ANOVA) with a 5% error rate and continued with LSD to select lines with high-performing genotype. The results of the ANOVA showed that the tested lines affected growth parameters and plant yield components. LSD analysis showed that the highest leaf length and number of leaves were in GS 7. In seed weight per plant, all lines were below Grobogan, followed by GS 7 and GS 47. In 100 seed weight, all lines were below Grobogan, but there were lines with large seed categories (> 14 g/100 seeds), namely GS7, GS 12, GS 36, and GS 39. Correlation analysis showed that the number of pods and 100 seed weight were positively correlated with seed weight per plant. Therefore, these traits can be selection indicators to identify high-yielding soybean genotype.

Keywords: Characterization · Correlation · Hybridization · Seed Size

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Introduction

Soybeans (*Glycine max*) are one of the food crops that play an essential role in meeting human nutritional needs, especially as a source of vegetable protein. In addition, soybeans are also the primary raw material in the food industry, such as tempeh, tofu, and soybean oil (Qin et al., 2022). According to data from the Central Statistics Agency (BPS), in 2024, the Central Statistics Agency (BPS) data noted that domestic soybean production in the latest year only reached around 555,000 tons, while national demand is estimated to reach 2.7 million tons per year. This indicates a fairly large deficit between soybean production and consumption in Indonesia, with a shortfall of around 2.1 million tons that must be met through imports from major soybean-producing countries such as the United States, Brazil, and Argentina (Sibuea et al., 2024). The high volume of imports reflects the lack of domestic soybean production capacity to meet market demand, which in turn requires efforts to increase domestic productivity through various innovations. Apart from its low productivity, the small size of local soybean seeds makes processed soybean food producers prefer imported soybeans with large seeds to local soybeans.

Seed size is one of the most important factors determining soybean yield. Seed size is a complex quantitative trait influenced by genetic and environmental factors during seed development. The composition and quantity of seed storage reserves directly influence seed size. Furthermore, seed size is an agronomic trait correlated with soybean oil and protein content. Therefore, increasing seed size helps increase soybean yield and likely improves seed quality (Duan et al., 2023).

One solution to increase soybean production and quality was through the hybridization method. This technique is carried out by crossing different soybean genotypes to combine the superior traits of both parents. Through this process, it is hoped that new lines can be obtained that have a better combination of characters, such as high seed yields, large seed size, resistance to pests and diseases (Lin et al., 2022), and better adaptation to diverse environments (Biliavska et al., 2021).

The parents used in this cross are Grobogan soybeans and Slamet soybeans. Grobogan soybeans are local soybeans from Grobogan Regency which have yellowish-white seeds and large seed size ranging from 16 -20 g per 100 seeds; plant productivity was quite high, ranging from 2 to 3.5 tons per ha, short plant age, the pods are large, and the level of maturity of the pods and leaves is the same, so when harvested the soybean leaves fall off (Puspitasari & Elfarisna, 2017). Meanwhile, Slamet soybeans are from a cross between Dempo and Wilis, which are resistant to leaf rust disease and tolerant to acidic soil. The average production yield can reach 2.26 tons/ha (Husen et al., 2022). So, the offspring that are expected are of high productivity, have large seeds according to consumer preferences and are resistant to acidic soil.

The diversity of soybean cross-breeding results can be seen from the differences in physical and genetic characteristics of the resulting plants, which in turn affect the production results and quality of the soybean seeds produced. In this context, the diversity of soybean cross-breeding results can be influenced by several factors, such as genetic factors from the crossed parents, the environment in which the plants grow, and the breeding techniques applied (Guo et al., 2022). This study aims to evaluate the phenotype of F10 soybean lines from a cross between Grobogan and Slamet for large-seeded and high-yield.

Materials and Methods

This research was conducted at the screenhouse of the Faculty of Agriculture and at the Plant Breeding Laboratory, Faculty of Agriculture, Jenderal Soedirman University, Purwokerto. The research was conducted in November 2020 - February 2021 during the rainy season. Daytime temperatures ranged from 26 - 30 °C, night time temperatures ranged from 24 - 26 °C, and humidity was 70 - 80%.

The plant materials were seeds, in form of 15 lines from Grobogan x Slamet, namely GS 6, GS 7, GS 12, GS 14, GS 24, GS 30, GS 36, GS 37, GS 38, GS 39, GS 41, GS 47, GS 49, GS 55, GS 58, and three check varieties, namely Slamet, Grobogan, and Wilis. In addition, present study also used urea, SP-36, KCl fertilizer, and growing media in form of inceptisol soil. Plant materials were arranged in a Randomized Complete Block Design (RCBD) with three replications. The treatments included soybean genotypes derived from Slamet and Grobogan crossess, along with three check

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varieties: Slamet, Grobogan and Wilis. The observed variables were plant height (cm), number of leaves (blades), leaf length (cm), leaf width (cm), number of nodes, number of pods, weight of 100 seeds (g), seed weight per plant (g), average number of pods per node, dry weight of the plant. Qualitative variables included hypocotyl color, flower color, seed color, leaf shape, leaf hairs, and leaf hair color.

The selection method used is the direct pedigree method based on agronomic characters, especially seed weight per plant and seed size (based on the weight of 100 seeds).

Data analysis

The observation data were analyzed using analysis of variance (ANOVA) with a 5% error rate, then continued with LSD to selecting high performance lines. The observation data to determine the relationship between quantitative characters using simple correlation analysis with the formula

$$r=\frac{n\sum xy-(\sum x)(\sum y)}{\sqrt{[n\sum x^2-(\sum x)^2][n\sum y^2-(\sum y)^2}}$$

r = Represents the correlation coefficient, which indicates the strength and direction of a linear relationship between two variables.

x and y = Represent the variables whose relationship (closeness) is being measured.

Results and Discussion

Qualitative characteristics. The results of qualitative characterization (Table 1) of the F10 soybean population derived from the Grobogan × Slamet cross revealed notable phenotypic variation in several morphological traits, including leaf shape, but uniform with the parental characters in hypocotyl color, seed color, flower color, and trichomes color. These variations indicate the presence of considerable genetic diversity within the population, which is essential for selection in breeding programs. The qualitative traits, although not directly linked to yield, serve as important indicators of genetic variability and can assist in the identification and selection of promising lines in early-generation breeding programs.

Hypocotyl color. Based on the observation results in Table 1, it can be seen that the hypocotyl color of the F10 line resulting from the cross between Grobogan soybeans and Slamet soybeans all produce purple hypocotyl colors. All hypocotyl colors in the promising lines are the same color with their parents and the comparison varieties. Hypocotyl color is related to flower color. Soybeans that have purple hypocotyl color will have purple flower color. Soybeans that have white hypocotyl color will have white flower color. This occurs because of the genetic relationship between hypocotyl color and flower color caused by the influence of pleiotropy (Wang et al., 2023).

Table 1. Qualitative observations of hypocotyl color, seed color, flower color, leaf shape, trichome density, and trichome color of F10 from Grobogan x Slamet cross

Treatment	Hypocotyl color	Seed Color	Flower color	Leaf shape	Trichome density	Trichomes color
Slamet	Purple	Yellow	Purple	Lanceolate	Not dense	White
Grobogan	Purple	Yellow	Purple	Lanceolate	Not dense	White
Wilis	Purple	Yellow	Purple	Rounded ovate	Not dense	White
GS 6	Purple	Yellow	Purple	Triangular ovate	Not dense	White
GS 7	Purple	Yellow	Purple	Lanceolate	Not dense	White
GS 12	Purple	Yellow	Purple	Triangular ovate	Not dense	White
GS 14	Purple	Yellow	Purple	Lanceolate	Not dense	White
GS 24	Purple	Yellow	Purple	Lanceolate	Not dense	White
GS 30	Purple	Yellow	Purple	Lanceolate	Not dense	White
GS 36	Purple	Yellow	Purple	Lanceolate	Not dense	White
GS 37	Purple	Yellow	Purple	Lanceolate	Not dense	White
GS 38	Purple	Yellow	Purple	Lanceolate	Not dense	White
GS 39	Purple	Yellow	Purple	Lanceolate	Not dense	White
GS 41	Purple	Yellow	Purple	Triangular ovate	Not dense	White
GS 47	Purple	Yellow	Purple	Lanceolate	Not dense	White
GS 49	Purple	Yellow	Purple	Lanceolate	Not dense	White
GS 55	Purple	Yellow	Purple	Lanceolate	Not dense	White
GS 58	Purple	Yellow	Purple	Lanceolate	Not dense	White

Seed color. It is known that soybean seeds come in various colors, but in the results of this study (Table 1), all genotypes showed yellow pigmented seeds. All soybean seed colors in these lines should have the same color as their parent varieties, namely Slamet, Grobogan, and Wilis. This character is in line with consumer preferences as stated by Jia et al., (2020), commercial soybean cultivars typically exhibit a yellow color (Choi et al., 2021). Soybean seed color was the result of a dominant allele at the I locus. This genetic factor controls the pigmentation of the seed coat, with the dominant allele causing the production of yellow pigment in the seeds. The presence of this dominant allele overrides any recessive allele at the same locus that might lead to a different color (Mau et al., 2023).

Flower color. Based on the observation results in Table 1, it can be seen that the flower color of the F10 line resulting from a cross between Grobogan and Slamet all produced purple flowers. All flower colors in the desired lines are similar to the parents and comparison variety. Flower pigmentation in soybeans is mainly controlled by six independent loci, namely W1, W2, W3, W4, Wm, and Wp. Among the six loci that control flower color (W1, W2, W3, W4, Wm, and Wp) in soybeans, all loci encode enzymes involved in flavonoid biosynthesis, except W2 (Park et al., 2015). W1 is responsible for the synthesis of delphinidin-3-glucose and the production of purple flowers in soybean (Sundaramoorthy et al., 2015).

Leaf shape. The leaves are compound, meaning they consist of three leaflets (trifoliate), and the leaflets have a smooth or slightly serrated edge. The leaves are usually arranged alternately along the stem. Based on the observation results in Table 1, it can be seen that soybean leaves in the F10 line resulting from the cross between Grobogan and Slamet produce pointed leaf shapes like their two parents, except for accessions GS 12, GS 41, and GS 6 which have triangular, oval leaf shapes. The diversity of leaf shapes in GS12, GS41, and GS6 is possible due to the interaction between genes in Slamet, which is a progeny of Dempo with a pointed leaf shape, and Wilis with a rounded oval leaf shape with the Grobogan variety with a pointed leaf shape. The results of this cross then produce more varied leaf shapes, such as triangular ovals in GS12, GS41, and GS6. Previous studies have found that leaf shape is related to one gene: dominant homozygous genotype (LnLn) and recessive (lnln), which phenotypically show wide leaves and narrow leaves. Heterozygotes (Lnln) experience intermediate leaflets. Bernard and Weiss assigned a new gene symbol, ln, for the narrow leaf trait (Krisnawati & Adie, 2017).

Trichome Density and Color. Trichomes are small, hair-like structures that cover the surface of the leaves. These trichomes can vary in density and length depending on the soybean variety. Based on the observation results in Table 1, it can be seen that the hairs on soybean leaves have a density level that is not dense on the leaves of the F10 soybean line, which is a cross between Grobogan and Slamet. The F10 line has white leaf hairs. All soybean leaf hairs have characteristics similar to the check varieties and parents, namely the Slamet, Grobogan, and Wilis. According to Abywijaya et al., (2024) soybean leaves generally have brightly colored trichomes and their numbers vary. The density of the hairs on the surface of soybean plants is a factor that determines resistance to pests, both in the antixenosis and antibiosis types. (Adie & Krisnawati, 2017).

Quantitative Traits. Quantitative traits in plants are important characteristics that contribute directly to plant productivity and performance. These traits are controlled by many genes (polygenic). The ANOVA results showed significant differences in all characters as shown in Table 2.

The characters of plant height, number of leaves, leaf length, and leaf width showed significant variations, indicating a strong genetic influence on vegetative growth. Similarly, the characters of the number of nodes, number of pods, and the average number of pods per node also showed significant differences, reflecting the diversity of productivity potential between lines. In addition, the weight of 100 seeds and the weight of seeds per plant also showed significant differences, so they can be used as important indicators in the selection of high-yielding lines. The character of plant dry weight also showed significant differences, which illustrates the variation in biomass accumulation between genotypes. Overall, these results indicate that the Grobogan × Slamet cross lines have genetics, so they have the potential to be further developed through selection of superior agronomic characters.

Table 2. Summary of analysis of variance (ANOVA) for characters of crossed lines of Grobogan × Slamet soybeans

No.	Observation characters	Treatments	Coefficient of Variation (%)
1.	Plant height (cm)	**	4.80
2.	Leaf length (cm)	**	9.09
3.	Leaf width (cm)	*	9.47
4.	Number of leaves	**	16.51
5.	Number of segments	*	11.48
6.	Dry Weight of Plant (g)	*	16.86
7.	Number of pods per segment	**	8.56
8.	Number of pods	**	15.34
9.	Weight of 100 seeds (g)	*	16.55
10.	Seed Weight per plant (g)	**	18.21

Note: * = significantly different at the 5% level ($p < 0.05$); ** = very significantly different at the 1% level ($p < 0.01$)

Table 3. Plant height, leaf length, and leaf width of F10 from Grobogan x Slamet cross

Treatment	Plant height (cm)	Leaf length (cm)	Leaf width (cm)	Number of leaves	Number of segments	Dry Weight of Plant (g)
Slamet	66.73 def	9.36 a	6.23 bc	72.73 a	6.33 abc	20.77 ab
Grobogan	55.15 g	8.26 ab	6.51 b	57.16 bcd	8.00 ab	17.23 bc
Wilis	49.45 h	6.60 b	5.53 c	31.00 f	8.66 ab	15.01 c
GS 6	72.85 bc	8.93 ab	6.10 bc	52.83 cde	8.66 ab	17.87 bc
GS 7	75.50 b	9.70 a	6.06 bc	74.00 a	8.00 ab	20.34 bc
GS 12	82.65 a	9.13 ab	6.68 b	47.00 de	4.66 c	21.11 ab
GS 14	69.63 cde	8.25 ab	5.80 bc	52.00 cde	9.33 a	18.81 bc
GS 24	74.90 bc	8.48 ab	6.21 bc	53.50 cde	9.33 a	20.81 ab
GS 30	65.50 def	9.26 ab	6.23 bc	70.33 ab	7.33 abc	20.23 bc
GS 36	64.33 ef	9.78 a	7.82 a	61.50 abcd	6.83 abc	26.20 a
GS 37	75.16 b	8.81 ab	5.98 bc	55.00 cde	6.50 abc	20.44 bc
GS 38	70.85 bcd	8.51 ab	5.95 bc	46.50 de	6.83 abc	19.77 bc
GS 39	64.86 ef	8.75 ab	5.90 bc	61.66 abcd	6.83 abc	20.01 bc
GS 41	63.73 f	9.45 a	5.96 bc	60.00 abcd	8.66 ab	17.97 bc
GS 47	65.80 def	8.03 ab	6.08 bc	51.00 cde	6.83 abc	19.47 bc
GS 49	72.93 bc	8.35 ab	5.83 bc	47.83 cde	6.16 abc	19.04 bc
GS 55	67.13 def	9.13 ab	6.23 bc	62.50 abc	5.83 bc	18.07 bc
GS 58	75.93 b	8.20 ab	6.23 bc	40.00 ef	8.50 ab	17.09 bc

Note: Means followed by the same letter in a column indicate no significant difference based on LSD at $\alpha < 0.05$.

The cross between soybean varieties Slamet and Grobogan exhibited a range of quantitative traits (Table 3) that are valuable for breeding and selection. This hybrid combination showed variability in pod formation and seed development, with certain individuals demonstrating higher seed weight and overall biomass compared to the parent lines. The transgressive segregation observed in some traits suggests the potential for selecting superior progenies.

Plant height. Plant height is a significant indicator of a plant's vigor and its ability to compete for resources such as light, water, and nutrients. Taller plants often have a more extensive root system and better access to sunlight, which enhances their growth. The results of the LSD analysis (Table 3) showed that the plant height of all lines exceeded that of the Grobogan and Wilis varieties. Lines with plant heights exceeding the three check varieties were GS 12 (82.65 cm), GS 7 (75.50 cm), GS 37 (75.16 cm), GS 49 (72.93 cm), GS 58 (75.93 cm), GS 6 (72.85 cm), GS 24 (74.90 cm), and GS 38 (70.85 cm). Taller plants have a better ability to capture sunlight because their higher canopy position allows them to access sunlight more directly and avoid shade from other plants. This can support more efficient photosynthesis. Tivoli et al., (2013) also stated that increasing plant height contributes to better air circulation within the canopy, as the more open canopy structure allows for optimal air circulation between the leaves and stems. This helps reduce micro-humidity levels around leaf surfaces, which typically create an ideal environment for the growth of fungi and other pathogens.

Leaf length. The results of the LSD analysis (Table 3) showed that the lines with the longest leaves were GS 7 (9.70 cm), GS 36 (9.78 cm), GS 41 (9.45 cm) which were not significantly different from the Slamet variety, besides that GS 6, GS 12, GS 14, GS 24, GS 30, GS 37, GS 38, GS 39, GS 47, GS 49, GS 55, and GS 58 lines were not significantly different from the Grobogan variety. All lines showed longer leaf lengths than the Wilis variety. Plants will develop and grow well if the leaves which function in the photosynthesis process have the appropriate number and size. A larger leaf surface allows more chloroplasts to be exposed to light, enhancing the plant's ability to absorb sunlight and convert it into energy through photosynthesis. Photosynthesis produces primary metabolites used for plant metabolism that support essential physiological functions, including cell division, elongation, and differentiation, which drive overall plant growth and development. Therefore, plants with larger leaves can maintain higher metabolic activity, resulting in optimal biomass accumulation and yields (Raza et al., 2022).

Leaf width. The wide leaf morphology indicates that the leaf is capable of optimal photosynthesis. The results of the LSD analysis (Table 3) showed that the line with the widest leaves, namely GS 36 (7.82 cm), exceeded the three check varieties, while the line that had a leaf width that was not significantly different from Grobogan was the GS 12 line (6.68 cm), and all lines had leaf widths that were wider than the Wilis variety. Wider leaf sizes tend to have more surface area to absorb sunlight. This is important because the wider the leaf surface area, the more leaf cells can contain chloroplasts, which contain chlorophyll. Increasing the amount of chlorophyll in leaves can increase photosynthesis efficiency because more light can be absorbed and converted into chemical energy. These energy-rich compounds are essential for carbon fixation during the Calvin cycle, which results in greater production of sugars and other primary metabolites that support plant growth and development. (Zhou et al., 2023).

Number of leaves . The leaves of soybean plants are essential to their structure and function, which is the main part of photosynthesis. The results of the LSD analysis (Table 3) showed that the line with the highest number of leaves was GS 7 (74.00), which was not significantly different from Slamet (72.73), several lines showed a higher number of leaves than Grobogan, namely GS 30 (70.33), GS 36 (61.50), GS 39 (61.66), GS 41 (60.00), and GS 55 (62.50), in addition, all lines showed a higher number of leaves than the Wilis variety. The number of leaves is important, because the increase in the number of leaves, the light needed for plant photosynthesis will increase. The increase in seed weight per plant and yield (tons/ha) are also related to the rise in soybean leaves. This can happen because the light that can be captured increases with the increase in the number of leaves. Hence, it has the opportunity to increase the photosynthesis process, and the potential for assimilates translocated to the seeds will also be greater. However, the disadvantage of having many leaves is that the chance of overlapping will increase, so little light is received (Chiozza et al., 2024). However, the more leaves there are, the greater the possibility that the leaves will overlap so that the light the leaves receive becomes limited. The limited light received by the leaves will cause the leaves to be inefficient in producing photosynthate because the photosynthesis process is not optimal (Burgess et al., 2017).

Number of segments. The segments are essentially the internodal sections between leaf nodes, play a key role in determining the overall plant height and the distribution of reproductive structures such as flowers and pods. In soybean, a higher number of segments often correlates with increased potential for branching and pod formation, which can ultimately influence yield. The results of the LSD analysis (Table 3) showed a greater number of segments than Grobogan and Slamet, namely GS 14 (9.33) and GS 24 (9.33). In addition, there were numbers of segments that were not significantly different from Grobogan and Wilis, namely GS 6 (8.66), GS 7 (8.00), GS 41 (8.66), and GS 58 (8.50). There were lines that were not significantly different from Slamet, namely GS 30, GS 36, GS 37, GS 38, GS 39, GS 47, and GS 49, and other lines had a lower number of segments than the three check varieties. Therefore, number of segments determines of a plant's reproductive potential, as each node can produce inflorescences that produce flowers and pods. Increasing node number generally results in a higher capacity for flower and pod formation, which positively contributes to overall seed yield.

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This trait is influenced by genetic factors and environmental conditions that regulate vegetative growth and node differentiation.

Dry Weight of Soybean. The dry weight of soybean plants is the amount of organic matter produced by the plant. Based on Table 2, the dry weight of the F10 line exceeded the Slamet variety; some exceeded the Grobogan variety, some exceeded the Wilis variety, and some were less than check varieties. The results of the LSD analysis (Table 3) showed that the GS 36 line (26.20 g) had a higher dry plant weight compared to the other lines and comparison varieties, namely Slamet (20.77 g), Grobogan (17.23 g) and Wilis (15.01 g) varieties. These results indicate that the GS 36 line has better dry biomass formation potential than the three comparison varieties. This advantage is caused by genetic factors that support more considerable plant growth. Li et al. (2024) stated that plant lines exhibiting high dry matter weight tend to have more efficient nutrient uptake and utilization capacities. This increased efficiency is associated with a well-developed root system that increases access to nutrient uptake, as well as more effective internal transport and metabolic systems for distributing and assimilating nutrients throughout the plant. Higher dry matter weight generally reflects optimal plant physiological performance, including photosynthetic activity, nutrient utilization efficiency, and biomass allocation.

Number of pods per segment. The number of pods per soybean plant node can vary depending on genetic factors, environment, and agronomic treatments applied. The results of the LSD analysis (Table 4) on the number of pods per segment (Table 4) showed that the lines with the high number of pods per segment were GS 6 (7.58), GS 38 (7.58), and GS 58 (7.25) which was higher than Grobogan (5.66 pods) and Wilis (4.83 pods) but lower than Slamet (8.25 pods). Each soybean line has a different genetic potential for pod formation at each stem segment. However, none of the selected lines has inherited the characteristic of high pod number per segment, which is comparable to the Slamet variety. The number of pods per segment is an important quantitative trait in determining the yield potential of soybean plants. This trait is controlled by several genes (polygenic), which act additively and are influenced by genetic interactions and environmental factors. Therefore, it is not a single gene that determines pod number, but rather a combination of many genes, each making a small contribution to the expression of the trait. This polygenic trait leads to wide variation among genotypes, as seen in the results of this study (Kumar et al., 2023).

Table 4. Number of pods per segment, total number of pods, weight of 100 seeds (g), seed weight per plant (g) of F10 from Grobogan x Slamet cross

Treatment	Number of pods per segment	Number of pods	Weight of 100 seeds (g)	Seed Weight per plant (g)
Slamet	8.25 a	70.33 a	13.19 abc	11.31 bc
Grobogan	5.66 fg	59.83 abc	15.63 a	17.71 a
Wilis	4.83 g	29.33 f	12.47 abc	7.30 d
GS 6	7.58 ab	56.66 abcd	11.31 bc	9.34 cd
GS 7	6.83 bcde	51.00 bcde	14.09 abc	13.51 b
GS 12	7.08 bcd	41.83 ef	14.83 ab	12.63 bc
GS 14	7.16 bcd	57.50 abcd	14.21 abc	12.37 bc
GS 24	7.41 abc	52.16 bcde	10.73 c	11.50 bc
GS 30	6.91 bcde	63.16 ab	11.19 c	12.08 bc
GS 36	6.66 bcde	59.66 abc	14.19 abc	12.45 bc
GS 37	7.16 bcd	47.16 cde	12.71 abc	10.80 bcd
GS 38	7.58 ab	54.66 bcde	13.74 abc	11.78 bc
GS 39	6.50 cdef	44.33 de	14.26 abc	9.98 bcd
GS 41	6.33 def	46.83 cde	12.84 abc	10.26 bcd
GS 47	6.00 ef	64.66 ab	13.26 abc	13.48 b
GS 49	6.33 bcde	61.83 ab	11.84 bc	11.89 bc
GS 55	6.66 bcd	55.66 bcd	13.53 abc	12.60 bc
GS 58	7.25 ab	54.66 bcde	12.91 abc	12.71 bc

Note: Means followed by the same letter in a column indicate no significant difference based on LSD at α < 0.05.

Number of pods. The LSD analysis (Table 4) results showed that the Slamet variety had the highest number of pods (70.33). This was followed by the GS 30 (63.16), GS 47 (64.66), and GS 49 (61.83) lines, each with a higher number of pods than Grobogan (59.83). All tested lines had a higher number of pods than Wilis. The diversity in the number of pods reflects the genetic potential of each line in producing pods. The GS 30, 47, and 49 lines can be categorized as fairly productive, considering the number of pods they produce is higher than the other two comparison varieties (Grobogan and Wilis) but have not reached the maximum potential like the Slamet variety. This indicates that the number of pods in soybeans is controlled by a complex and quantitative group of genes (Tayade et al., 2023). Because it is controlled by many genes, each with a relatively small effect, the process of genetic segregation in the resulting cross-breeding generation results in a wide variety of phenotypes, encompassing trait values that can be found between the two parents. This diversity reflects the varying combinations of alleles of the genes responsible for the trait. In addition, interactions between genes and interactions between genes and the environment also contribute to expanding the observed phenotypic diversity.

Weight of 100 seeds. The weight of 100 seeds indicates how large or small a soybean seed is. In Indonesia, soybean seed sizes are classified into three categories: small (< 10 g/100 seeds), medium (10-14 g/100 seeds), and large (> 14 g/100 seeds) (Krisnawati & Adie, 2015). The Grobogan variety was included in the large-seeded category, and the Slamet variety was included in the medium-seeded category. The results of the LSD (Table 4) analysis on the weight of 100 seeds per plant (Table 4) showed that the line with the highest value was the Grobogan variety (15.63), followed by GS 12 line (14.83 g per 100 seeds), but several lines had large seed sizes, namely GS7 (14.09 g), GS 12 (14.83 g), GS 36 (14.19 g), and GS 39 (14.26 g). Besides that, there were the diversity in each genotype; some were superior to the Slamet variety, some were superior to the Wilis variety, some lines were under three comparison varieties, and all lines were below the Grobogan variety. Large soybean seeds are a desirable trait in the tempeh industry, as they align with consumer preferences for firmer, more textured, and higher-quality tempeh. In Indonesia, a shift in preference among soybean farmers and the tempeh industry from medium-sized seeds to larger soybean seeds (14 g/100 seeds) has been a significant factor in driving genetic improvement of soybean varieties (Kuswantoro et al., 2020).

Seeds Weight per Plant. The results of the LSD analysis (Table 4) on seed weight per plant (Table 4) The highest yield was found in the Grobogan variety (17.71 g), while the line with the high seed weight, namely GS 7 (13.51 g) and GS 47 (13.48 g), but still below Grobogan's yield and higher than the comparison varieties, namely Slamet and Wilis. Besides that, there were variations in the results obtained in the seed weight variable per plant; 11 lines exceed the seed weight above the Slamet variety and all lines have seed weights higher than the Wilis variety. Tayade et al., (2023) stated that the difference in seed weight is due to the genetic characteristics of the plant related to the size and number of seeds. Genetically, some plants have a tendency to produce large seeds with higher mass, while others produce seeds in greater numbers but with relatively small sizes such as the Slamet variety. This variation reflects differences in the regulation of genes involved in embryo development, seed filling, and the efficiency of photosynthates allocation to reproductive organs. The GS 7 line has the highest weight compared to other lines. This is supported by the weight of 100 seeds, which is included in the large seed category.

Principal Coordinate Analysis. Figure 1 showed that the result of Principal Coordinate Analysis (PCoA) which illustrates the genetic relationship between soybean genotypes resulting from a cross between the Grobogan and Slamet varieties. Based on the analysis results, the genotypes are divided into two main groups. The first group (in green) consists of genotypes GS 7.n, GS 12.n, GS 14.n, GS 36.n, and GS 39.n which are genetically close to each other. This closeness is based on qualitative traits and also the main character that is the goal of this study, namely large seed size and high production. Meanwhile, the second group (in red) includes other genotypes such as GS 6.n, GS 24.n, GS 30.n, and GS 58.n which are lines with medium seed size (less than 14 g/100 seeds). However, GS 7 is located at different coordinates, this shows that apart from having large seed size, GS 7 is also a high-yielding line. This indicates the presence of the main traits that are the purposes of this soybean breeding, namely large seeds and higher

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production compared to other lines. Increasing seed size is a crucial way to increase soybean yield. Seed composition and reserve content directly determine this. Soybeans with larger seeds have higher oil content, while soybeans with smaller seeds typically have lower oil content than cultivated soybeans. However, seed protein content does not increase in large-seeded soybean cultivars. Therefore, soybean quality improvement involves increasing seed size, which correlates with oil accumulation and possibly accompanying changes in protein content (Wang et al., 2020).

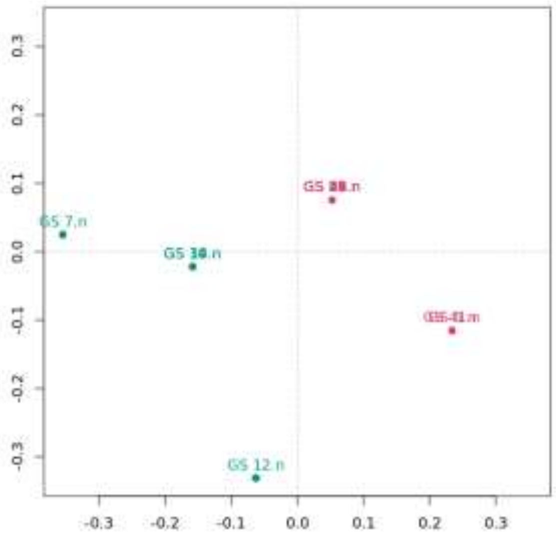

Figure 1. Principal Coordinate Analysis of F10 Lines of Grobogan x Slamet Cross

Correlation Analysis. Growth components are closely related to yield. The relationships between different plant traits are a common phenomenon and understanding these correlations is valuable, particularly as a basis for selection programs. The type of correlation used is Pearson correlation. Correlation analysis is useful for identifying the relationships between two or more variables; however, it cannot precisely distinguish the direct and indirect effects of each trait (Khomphet et al., 2022).

The results of the correlation analysis in Table 5 showed that not all characters are correlated with each other. The analysis results show that the variable plant height is related to leaf length and the number of pods per segment. The level of correlation between plant height, leaf length, and pods per pod is strongly positive, with coefficient figures of 0.621 and 0.695. This correlation is indirect, as taller plants tend to have more stem segments, which could produce more pods overall or, through more optimal photosynthesis, could increase the number of pods per segment. According to Miao et al. (2024), plant height is an important agronomic trait related to apical dominance, plant architecture, cultivation, harvesting, yield, and lodging. Yu et al. (2020) adding that ideal plant height will open up space to obtain more sunlight than non-ideal plants. With more exposure to light, plants can photosynthesize more efficiently, converting light energy into chemical energy stored in sugar. This sugar provides the energy needed for growth, reproduction, and pod formation. So tall plants allow for high yields.

Table 5. Correlation matrix between soybean characters

Karakter	PH	LL	LW	TNS	NP	NL	W100S	SWP	NPS	DWP
PH	1	0.621**	0.089	-0.270	0.122	0.074	-0.132	-0.154	0.695**	0.344
LL		1	0.223	-0.202	0.424*	0.746**	-0.158	0.068	0.693**	0.488*
LW				-0.295	0.290	0.278	0.316	0.323	0.139	0.746**
TNS				1	-0.123	-0.165	-0.313	-0.036	-0.113	-0.389
NP					1	0.493*	-0.065	0.386	0.438*	0.324
NL						1	0.097	0.192	0.380	0.476*
W100S							1	0.553**	-0.189	0.135
SWP								1	-0.182	-0.017
NPS									1	0.404*
DWP										1

Note: PH = Plant height; LL = Leaf length; LW = Leaf width; TNS = Total number of segments; NP = Number of pods; NL = Number of leaves; W100S = Weight of 100 seeds; SWP = Seed weight per plant; NPS = Number of pods per segment; DWP = Dry weight of plant; ** = Highly significant difference; *= Significant difference

The leaf length character is related to the number of pods, the number of leaves, the number of pods per segment, and the dry weight of the plant. The level of closeness of the relationship between leaf length and the number of pods is weakly positive, with a correlation of 0.424, but the number of pods per segment is strongly positive, with a correlation of 0.693. In addition, there is a weakly positive relationship with a correlation of 0.488 against the dry weight of the plant. The character of leaf width is related to the dry weight of the plant. The level of closeness of the relationship between leaf width and plant dry weight is strongly positive, with a correlation of 0.746. The number of leaves is related to the plant's dry weight; the relationship's level of closeness is 0.476. Leaf length is indirectly correlated with pod number in soybean plants. This is because leaf length is a vegetative morphological trait related to the plant's ability to capture light for photosynthesis. Optimal photosynthesis can increase growth and yield, including pod number. However, leaf length does not directly increase pod number; instead, it supports plant physiological processes contributing to yield increases. Therefore, the relationship between leaf length and pod number is indirect through intermediary mechanisms such as photosynthetic efficiency and biomass accumulation (Shi et al., 2019).

There is a positive correlation between the number of pods and the number of pods per segment. However, the strength of this relationship is weak, with a correlation coefficient of 0.438. The total number of pods per plant reflects the plant's capacity to produce pods. This number is influenced by the distribution of resources, as the products of photosynthesis, along with available water and nutrients, must be shared among a greater number of pods (Zewide & Ademe, 2025).

The number of pods per segment is related to the plant’s dry weight. However, the strength of this relationship is only weakly positive, with a correlation coefficient of 0.404. The indirect correlation between the number of pods per segment and the dry weight of the plant shows that in plants with a high number of pods, larger photosynthetic organs likely supply them, which indicates that the dry weight of the plant is greater. Each pod formed on the plant requires energy and nutrients produced through photosynthesis. A higher number of pods per segment is typically supported by the abundant supply of energy and resources provided by the plant through its photosynthetic organs. Plants with a greater number of pods per segment generally also have more biomass, which is reflected in a higher dry weight (Vogel et al., 2021).

The weight of 100 seeds shows a strong positive correlation with the total seed weight per plant, with a correlation coefficient of 0.553. The relationship between 100-seed weight and total seed weight per plant is a direct correlation. This is because 100-seed weight reflects the average seed size, one of the main components determining the total seed yield per plant. Assuming the number of seeds is relatively constant or does not vary significantly between plants, increasing the weight per 100 seeds will directly increase the total seed weight per plant. In this context, the relationship between the two characteristics is causal and linear: the larger the seed size (100-seed weight), the greater the total seed weight produced by the plant. According to Wang et al. (2025), the weight of 100 seeds largely depends on seed size. This was also supported by Baek et al. (2020), who explained that while the maximum potential seed size is genetically determined, the actual size of the seeds is influenced by environmental conditions during the seed-filling stage. Similarly, Mai et al. (2023) found that seed production per plant is correlated with the weight of 100 seeds. The total seed weight per plant reflects the plant's ability to utilize assimilates during seed filling, which is also related to the number of pods produced. So, the higher 100-seed weight tends to result in a higher total seed weight per plant.

The GS 7 line is a line that meets the criteria for breeding objectives, namely having a seed weight per plant that exceeds the comparison variety Slamet and is also the highest among other lines, which is 13.51 g. In addition, GS 7 also has a seed size that is included in the large category, with a weight of 100 seeds exceeding 14 g. However, the dry weight of the GS 7 plant is not higher than the Slamet variety. This condition indicates that GS 7 has a higher efficiency in translocating the results of photosynthesis to generative organs, especially pods, compared to vegetative parts or others biomass. This efficiency is an important indicator in plant breeding because it shows the ability of plants to optimize the final result without having to increase the total biomass.

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Conclusion

The morphological characteristics of the F10 lines resulting from the Grobogan × Slamet soybean cross showed traits namely flower color, hypocotyl color, seed color, leaf hairiness, and leaf hair color that were consistent with those of the parent varieties. There was diversity in quantitative traits, but GS 7 line was potential as a candidate variety due to its leaf length and number of leaves, so that had high seed weight per plant (13.51 g), high number of pods (51 pods), and large seed size, with a 100-seed weight of 14.09 g. However, there was no strain that has a higher number of seeds per plant and a weight of 100 seeds than Grobogan. There were also correlations observed among several traits, notably between the number of pods and 100-seed weight with the seed weight per plant.

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