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DIVERSITY OF MORPHOLOGICAL, AGRONOMIC, AND QUALITY TRAITS OF SOYBEAN (*GLYCINE MAX* **L.) AND THEIR POTENTIAL AS EDAMAME**

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SUMMARY

Increased consumption of edamame soybean (*Glycine max* L.) needs supplementation by production boosts through crop area and productivity expansion utilizing high-yielding varieties. This research aimed to identify the genetic diversity of germplasm soybeans. This research transpired in Leuwikopo Experimental Farm, IPB University, using a single-factor randomized complete block design with three replications. This research evaluated 28 soybean genotypes consisting of edamame and grain soybean. The results showed a diversity in morphological characters at a value of 84.62% dissimilarity level with two main groups. The edamame soybean genotype, G.AGS 439 x L Tegal-1, and grain soybean Dega-1 gave the highest weight of pods (79.677 g and 75.596 g, respectively) and the best agronomic characters, making them potential parents in crosses for yield improvement. BioMax-1 and Grobogan genotypes could serve for quality improvement with high sugar, protein, and low-fat contents.

Keywords: Dissimilarity, edamame, nutritional analysis, quality

Key findings: This study provided a high genetic variation in the soybean's morphological, agronomic, and nutritional properties. It nominated the potential genotypes of edamame soybean (*Glycine max* L.) with a high fresh pod weight, namely, G.AGS 439 x L Tegal-1 and Dega-1 as grain soybean, and the genotype of BioMAX 1 and Grobogan has favorable nutritional properties.

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INTRODUCTION

Edamame (*Glycine max* [L.] Merril), also known as vegetable soybeans, is a soybean type harvested when the pods are still green. Edamame originated from Japan, but Indonesia has been cultivating and consuming it. Edamame soybean consumption can be as a cooked vegetable or fresh as a snack after boiling. Its harvest starts when the pod is still immature but with fully developed seeds (Basavaraja *et al.*, 2005; Qing-guo *et al*., 2006; Pao *et al*., 2008). Edamame soybean harvesting commences about 35–39 days after flowering or at the R6 stage (Zeipina *et al*., 2017; Djanta *et al*., 2020). Compared to seed soybeans, vegetable soybean seeds are large and fill 80%–90% of the pod (Konovsky *et al*., 2020).

Consumers love edamame because of the pod's greenness, fluffiness, and seeds' sweet taste. In addition, edamame has higher protein and fat content, preferable to consumers. Researchers have reported that edamame consumption has increased in several countries, including the United States (Carneiro *et al*., 2020), Australia (Figueira *et al*., 2019), Europe (Hong and Gruda, 2020), sub-Saharan Africa (Djanta *et al*., 2020), and Brazil (Sentelhas *et al*., 2015). Edamame is famous in several East Asian countries such as China, Taiwan, Japan, and Korea (Xu *et al*., 2016).

Edamame contains high protein, vitamins, minerals, dietary fiber, and isoflavone levels. Johnson *et al*. (1999) reported that edamame soybeans have higher nutritional content, with every 100 g of seeds containing 582 kcal, 11.4 g protein, 7.4 g carbohydrates, 6.6 g fat, 100 mg vitamin A, 0.27 mg B1, 0.14 mg B2, 1 mg B3, 27 mg vitamin C, 140 mg phosphorus, 70 mg calcium, 1.7 mg iron, and 140 mg potassium. Edamame soybeans also have anti-nutritional factors, such as tannins, protease inhibitors, and phytic acid (Gondim-Tomaz *et al*., 2022).

Increased consumption of edamame requires an increase in its production through expansion of crop area and productivity by planting superior cultivars. Improving yield power to produce superior cultivars is the main

goal of plant breeding. Enhancing soybean yield potential is primary, with edamame soybean breeding having limited research before, especially breeding for improvement of nutritional properties, such as protein, fat, tannin, and phytic acid content.

One of the efforts to increase genetic diversity is by collecting germplasm. Prebreeding studies of edamame soybean germplasm need more focus to help develop high-yielding edamame soybean cultivars with good nourishing properties. This study has successfully collected 28 soybean germplasm. In support of high-yielding and good-quality edamame breeding activities, information is necessary on the level of diversity at the morphological, agronomic, and nutritional traits of the available germplasm. Furthermore, identifying prospective elders can ensue at the phenotype level based on agronomic and health characteristics.

In this research, the observed nutritious properties were sugar, protein, and fat. The nutritional properties of edamame vary during different growth stages. Identifying candidate parents at the genotype level is necessary to improve the accuracy of the work. This study aimed to obtain information on the diversity of soybean germplasm genetics and identify candidate parents for developing potential edamame cultivars with good nutritional properties.

MATERIALS AND METHODS

Experimental site and genetic materials

This research transpired at the Leuwikopo Experimental Farm of the IPB University, West Java, Indonesia. The nutritional properties of fresh edamame soybean (*Glycine max* L.) genotypes' analyses proceeded in the laboratory of Balai Besar Industri Agro, Bogor-Indonesia. The genetic material used in this study consisted of 28 soybean genotypes (Table 1). The genetic materials were from the germplasm collection of the Center for Standard Testing of Biotechnology Instruments and Agricultural Genetic Resources (BBPSI Biogen).

Note: *= Grain soybean

Morphological and agronomic characterization

Soaking the soybean seeds in warm water for one night continued for planting directly on the bed with one seed per hole at a spacing of 40 $\text{cm}^2 \times 15 \text{ cm}^2$. Inorganic fertilizers included urea at a dose of 50 kg ha⁻¹, SP36 100 kg ha⁻¹, and KCI 100 kg ha⁻¹. Harvesting of young pods occurred at the R6 - R7 stadia or around 65–68 days after sowing (DAS). The applied layout was a randomized complete block design with three replications. Morphological and agronomic characterization of edamame germplasm followed the soybean characterization guidelines (UPOV, 1998) and soybean description (IBPGR, 1984).

Nutritional properties analysis

Sugar content

Total sugar extraction employed the procedure of Yu *et al*. (2016), Hou *et al*. (2009), and Song *et al*. (2013), with some modifications. Putting about 0.15 g of fine powder from young pods into a 2.0 ml centrifuge tube received mixing with 1.5 ml of distilled water. The tube's shaking at 180 rpm at room temperature continued for 2 h, then centrifuged at 12,000 rpm for 10 min. Its 750 μl supernatant acquired 750 μl acetonitrile after its transfer into a 2.0 ml centrifuge tube.

The tubes sustained mixing and incubating at room temperature for 10 min, then centrifuged at 12,000 rpm for another 10 min. Filtering 500 μl of supernatant through a 0.45 μm membrane disc filter, sugar identification proceeded by comparing its storage time with the storage time of sugar standards. Sugar standards, at earlier prepared concentrations of 0.5, 1.0, 2.0, 3.0, and 4.0 mg/ml, provide calibration curves for measuring each.

Protein content

The protein extraction method followed the procedures of SNI 01-2891-1992 (Indonesian National Standard, 1992) in the semi-micro Kjeldahl method. The protein content extraction procedure included weighing 0.51 g of snippet and putting it into a 100 ml Kjeldahl flask. Then, add 2 g of selen mixture and 25 ml of concentrated H2SO4, then heat to boiling until the solution becomes clear greenish-green (about two hours). The diluted solution's transfer into a 100 ml volumetric flask reached up to the line mark. Next, 5 ml of the pipetted solution placed into the distiller received 5 ml of 30% NaOH and a few drops of PP indicator. Distillation continued for approximately 10 min, as a reservoir used 10 ml of 2% boric acid solution pre-mixed with indicators. Then, rinsing the cooling tip with distilled water and titrating it with 0.01 N HCl solution made a blank determination.

Calculation of protein content: $= \frac{(V_1 - V_2)x N x 0.014 x f.k x f.p}{W}$

Where: $W = weight of the sample; V1 =$ volume of 0.01 N HCl used to determine the sample; V2 = volume of HCl used to determine the blank; $N =$ normality of HCl; f.k. = protein of (food 6.25; milk and its products 6.38; peanut oil 5.46); f.p. = dilution factor.

Fat content

The fat extraction method followed the procedure of SNI 01-2891-1992 (Indonesian National Standard, 1992) in the hydrolysis method (Weibull). The fat content extraction procedure carefully weighed 1 g - 2 g of snippet into a glass cup, then added 30 ml of 25% HCl, 20 ml of water, and a few boiling stones. After that, filter the solution in hot conditions by washing it with hot water until no acid reactions occur. Next, dry the filter paper with its contents at a temperature of 100 °C -105 °C, then put it in a filter paper wrapper (paper thimble) and extract with hexane or other fat solvents for 2 - 3 h at a temperature of approximately 80 °C. Then, distilling the hexane solution and drying the fat extract applied a 100 °C – 105 °C temperature, continued by cooling, weighing, and repeating the drying process until it reached a fixed weight.

> Calculation of fat content: $=\frac{W_1-W_2}{W}x 100\%$

Where: $W = weight of sample, q; W1 = weight$ of fat flask after extraction, g; $W2 = weight of$ fat flask before extraction, g.

Data analysis

Morphological and agronomic data underwent cluster analyses using the PBStat.com tool, while agronomic data processing continued through ANOVA, followed by genetic parameter estimation and cluster analysis. ANOVA followed the general linear model with SAS software version 9.0. The estimation of variance component values relied on separating the expected mean square value for each source of variance.

RESULTS

Climatic conditions per month during the experiment from October to December 2021 had an average temperature of 26.2 °C, average rainfall of 312.6 mm, and average humidity of 84% (Meteorology, Climatology and Geophysics Agency, 2022). Sumarno and Manshuri (2007) explained that the optimum conditions for soybean cultivation are rainfall ranging from 120–135 mm/month, an average temperature of 22 °C–27 °C, air humidity ranging from 70%–90%. Weather conditions from the time of the R1 growth phase to the R8 phase are relatively suitable for soybean growth, except for rainfall.

Morphological character diversity of soybean germplasm

The morphological performance of the 28 edamame soybean (*Glycine max* L.) genotypes is available in Table 2. The observed morphological characteristics showed high diversity, except for hypocotyl and flower colors and leaf size. The clustered analysis showed that the tested germplasm can be in two groups at a level of 84.62% dissimilarity (Figure 1). Group I further broke into four subgroups, with the first subgroup consisting of Wasemame, Shiromame GS.439, G.12930, G.AGS 439 x L Tegal-2, Edamame Lokal Petani, and AGS.438. Genotypes in the first subgroup showed similarities in color in hypocotyl, hair stem, branch trichomes, seed coat, and leaf and seed size. The second subgroup consists of Biosoy-1, Biosoy-2, Ginjiro, G.12936, BioMax-1, and G.AGS.439 x L-Tegal-1. The second subgroup materialized because of common growth type, leaf size, leaf texture, and seed color. The third subgroup consists of BioMax-2, AGS.432, AGS.440, Kedelai Taiwan, and Singapore A, including the fourth subgroup. Genotypes in the third subgroup own similarities in growth habits, leaf texture, and seed shape traits.

Figure 1. Dendrogram results of cluster performance based on morphological characteristics of 28 soybean genotypes using the software PBStat-CL.com. WSM=Wasemame, SRM=Shiromame, G.12930, Biosoy-1, Biosoy-2, LT2=G.AGS 439 x L-Tegal-2, Grobogan, KCL=G. Ked.Cina x L-Tegal, BMX1=BioMax1, BMX2=BioMax2, Panderman, G.10428, ELP=Edamame Lokal Petani, GlySo=Glycine soja, Lokal Tegal, Dega, Ginjiro, ANJAS1=G.AGS(GH)12930 x Anjasmoro-1, AGS.438, G.12936, Ryocoh, BET=Black Early Type, AGS.440, SING-A=Singapore A, KTW=Kedelai Taiwan, AGS.432, LT1=G.AGS 439 x L-Tegal-1, AGS.439.

Group II cuts into two subgroups, with the first subgroup being G7 (Grobogan), G8 (G.Ked Cina x L Tegal), G16 (Dega-1), G18 (G.AGS[GH].12930 x Anjasmoro-1), (Panderman), G12 (G.10428), and G15 (Lokal Tegal). Genotypes in the first subgroup were similar in growth type, growth habit, hypocotyl, hair stem, branch trichome color, leaf texture, and leaf shape. The second subgroup consisted of G14 (Glycine soja), G21 (Ryocoh), and G22 (Black early type), showing similarities in flower, seed, and seed coat colors.

Diverse agronomic characteristics of soybean germplasm

The edamame soybean trait of plant height had a data interval of 15.87–46.09 cm, and grain soybean had a data interval of 26.93–49.70 cm. The trait number of young pods of edamame soybeans in the data interval is 8.44–41.89, and the number of young pods of three-seed soybeans has a data interval of 19.75–34.25. The data showed the young pods harvested at the R6 stage (35–39 days) are

prevalent in both types of soybeans, indicating the potential for selection for high-yielding soybeans.

The number of young pods of threeseed edamame in the data interval is 0.89 – 14.50 pods, while the grain soybean has a data interval of 1–9.29. The weight of young edamame pods in the data interval is 14.97– 79.68 g, while grain soybeans have a data interval of 33.42–75.59 g. Additionally, the weight of pods also has the potential as a selection criterion for high yield. Therefore, the genotype with the best agronomic characteristics in edamame is the G. AGS 439 x L Tegal-1, while in grain soybeans, Dega-1.

Genetic diversity is a key factor in plant breeding. Influences on the diversity levels in the population can come from genetic and environmental diversity as measured by the value of heritability. The separation of the mean square value of expectation shows that the heritability value of all characteristics in soybean germplasm belonged in the high category, with a range of values between 63.65%–100.00% (Table 3).

Notes: Values followed by the same letter in the same column do not differ according to Tukey's 5% test; PH = Plant height (cm), NL = Number of leaves; NB = Number of branches; NFB = Number of fertile books; NYP = Number of young pods; NYPT = Number of young pod three seeds; WYP = Weight of young pods (g); NDP = Number of dry pods; NDPT = Number of dry pod three seeds; $WDP = Weight of dry pools (q)$.

Heritability values can help breeders select based on phenotypic appearance. Malek *et al.* (2014) reported that high heritability values for the traits, plant height, number of branches and pods per plant, and 100-seed weight could considerably be favorable characteristics for soybean improvement through effective phenotypic selection. It agrees with the research results with each character having a heritability value with high criteria (Table 4).

The dendrogram of agronomic performance based on quantitative characters observed in 28 genotypes of edamame soybean appears in Figure 2. The dendrogram is an analysis using the Euclidean distance method on numerical variables, thus obtaining a cophenetic distance. The assessment showed a dissimilarity coefficient of 10.85% with four

large groups. Group I, divided into four subgroups, had the first subgroup consisting of seven genotypes with high average value similarity in plant height, the number of young pods, the weight of young pods, and the weight of dry pods. The second subgroup consisted of three genotypes, the third subgroup of two genotypes, and the fourth subgroup contained one genotype (*Glycine soja*). The three subgroups had higher mean values on the weight of young pods. Group II comprised two subgroups, with the first subgroup consisting of 12 genotypes, and the second subgroup consisting of two genotypes with similarities in the mean values of each character, especially in the weight of young pods (79.68 g) and weight of dry pods (27.33

Figure 2. Dendrogram results of cluster performance based on agronomy traits of 28 soybean genotypes using the software PBStat-CL.com. WSM=Wasemame, SRM=Shiromame, G.12930, Biosoy-1, Biosoy-2, LT2=G.AGS 439 x L-Tegal-2, Grobogan, KCL=G. Ked China x L Tegal, BMX1=BioMax1, BMX2=BioMax2, Panderman, G.10428, ELP=Edamame Lokal Petani, GlySo=Glycine soja, Lokal Tegal, Dega, Ginjiro, ANJAS1=G.AGS(GH)12930 x Anjasmoro-1, AGS.438, G.12936, Ryocoh, BET=Black Early Type, AGS.440, SING-A=Singapore A, KTW=Kedelai Taiwan, AGS.432, LT1=G.AGS 439 x L-Tegal-1, AGS.439.

Notes: σ^2 _e = variance error; σ^2 _g = variance genetic; σ^2 _p = variance phenotype; h²_{bs} (%) = broad sense heritability.

g). Group III consisted of two genotypes (Dega-1 and Shiromame) with similarities in the mean values of the weight of young pods (75.59 and 70.74 g). Group IV contained genotypes (G. Ked.Cina x L-Tegal) with the lowest mean values of plant height, weight of young pods, and weight of dry pods.

Variation of nutritional properties of edamame soybean germplasm

Genotypes tested for quality attributes were those with high yield potentials. Analysis of variance showed that genotypes significantly affected the sugar, protein, and fat contents

Table 5. Variance analysis on quality traits of germplasm consisting of edamame and grain soybean.

MS = Mean square; CV = Coefficient variance; $**$ = significant at the level of 1%; $*$ = significant at the level of 5%.

Table 6. Quality traits of germplasm consisting of edamame and grain soybean.

Notes: Values followed by the same letter in the same column do not differ according to Tukey's 5% test.

(Table 5). It indicates variations in the sugar, protein, and fat contents among the genotypes tested. This diversity can be due to genetic and environmental factors. The mean, minimum, and maximum values for the three are available in Table 5.

The yield performance of quality features of nine soybean genotypes is visible in Table 6. Similar to quantitative characteristics, quality traits break into two types of soybeans, namely, edamame soybean (vegetable soybean) with six genotypes and a grainsoybean type with three genotypes. The edamame soybean genotype with the highest sugar and protein quality is BioMAX 1 (23.42% and 13.02%), with 6.29% fat quality. It aligns with the standard nutritional value of edamame soybeans (Tsou and Hong, 1991; Masuda, 1991) that the nutritional value of sugar in edamame ranges from 6.0%–14.0%, protein is about 13.6%, and fat is about 6.32%. In addition, this study found that the quality of seed soybeans was higher than in edamame

soybeans. It is a fact that the genotype Grobogan has the superior quality of sugar (31.52%), protein (15.43%), and fat (6.36%). Based on the average value and quality performance of edamame soybean genotypes, the quality values of protein and fat for all test genotypes have met the standards. The sugar content in edamame is higher than in soybean seeds, with a range of 16.60–31.52.

Genotype Grobogan has the highest sugar and protein quality values but has the second lowest fat quality after genotype G.AGS 439 x L Tegal-1. However, the Grobogan genotype's fat quality score (6.36) was within the edamame nutritional value standard. It indicates that the Grobogan genotype meets the edamame nutritional standard values of sugar, protein, and fat. Edamame soybeans have about 40% protein by dry weight, making edamame a good source of vegetable protein. In the study of Yu *et al*. (2022), the protein content ranged between 39.1% and 43.9%. The high protein content in the study is very

| Quality Characters | e ັ | ັ | ັ | $^{\prime}$ % $h_{\rm b}$ | Criteria |
|---------------------------|--------|-------|-------|------------------------------|----------|
| Sugar | 0.06 | 37.48 | 37.54 | 99.84 | High |
| Protein | 0.02 | 4.20 | 4.22 | 99.53 | High |
| Fat | 0.01 | 1.42 | 1.43 | 99.30 | High |

Table 7. Estimation of genetic parameters of selected soybean germplasm.

Notes: $\sigma_{\rm e}^2$ = variance error; $\sigma_{\rm q}^2$ = variance genetic; $\sigma_{\rm p}^2$ = variance phenotype; $h_{\rm bs}^2$ (%) = broad sense heritability.

different from the quality test conducted. This is because the research depended on dry weight, while the quality test relied on fresh weight.

The heritability value of the quality of edamame soybean genotypes passed the high category on all characteristics, with a range of values between 99.30%–99.84% (Table 7). With a high heritability value, it is promising for the traits of sugar, protein, and fat contents to pass to their offspring. Thus, genotypes with high sugar, protein, and fat contents will be favorable as parents if used in crosses.

DISCUSSION

High demand for soybeans (*Glycine max* [L] Merr.) in Indonesia to reach high import values must partner with the development of superior soybean genotypes. Developing superior soybeans can succeed by selecting prospective crossbred elders with the best characteristics, both qualitative and quantitative, and assessing quality (biochemical) attributes. Currently, a way has progressed to facilitate the selection of soybean germplasm by observing various traits. The observed traits depend on the qualitative, quantitative, molecular, and biochemical characteristic analysis of soybean genotypes.

Qualitative soybean germplasm scrutiny is manageable by environmental factors and plant genetics. This selection is an effective way to distinguish between genotypes in soybean germplasm collections. Genetic diversity of edamame soybean germplasm genotypes has succeeded using qualitative characters. This study found 28 soybean germplasms dominated by indeterminate growth types (18) versus determinates (10). It affects the genotype with good quantitative attributes, especially in yield, because the

indeterminate type has a strong growth capacity, which can accommodate the number of soybean fruits/pods. In addition, the determinate type has a massive sunlightcapturing power. Hence, it can channel more photosynthates to the soybean fruits/pods. The genotype G.AGS 439 x L Tegal-2 had the highest number of young pods, and G.AGS 439 x L Tegal-1, besides having the highest number of young pods' three seeds and the weight of young pods, also had good quantitative/agronomic traits.

Moreover, genotypes Ginjiro, Ryocoh, and G.10428 had the maximum number of dry pods, number of dry pod three seeds, and weight of dry pods. The number of pods per plant character can become a selection criterion in obtaining high-yielding soybean genotypes (Asadi, 2009). Therefore, G.AGS 439 x L Tegal-1 as edamame soybean and Dega-1 have the potential as crossing elders in yield improvement.

Based on the results of variance analysis, which includes plant height, the number of leaves, the number of pods, the number of pod three-seeds, and the weight of pod bore influences from genotypes. However, genetic x environmental interactions can also influence them, resulting in the genotypes tested not being able to display relatively the same performance in various environments. It is a suspicion that Dramaga, Bogor, located in the highlands, has relatively high rainfall and temperature. This condition causes a decrease in the soybean fruit/pod yield level. Khatab *et al*. (2016) reported that genetic relationships between genotypes based on agromorphological data do not always match genetic proximity based on parentage and origin. It is because soybean genotypes generally materialize through conventional breeding, which proceeded by hybridization with parents with a narrow genetic background

(Bhartiya *et al*., 2012; Rasyad *et al*., 2018; Kuswantoro *et al*., 2020).

In general, fresh green soybean seeds (physiological stages R6-R7) contain 22%– 32% protein, 16%–22% oil, and oligosaccharides on a dry weight, while soybean seeds on a dry weight basis (stage R8) contain about 40% protein, 20% oil, 35% carbohydrates, and 5% ash. However, the protein content observed in this study is lower than the findings by Rao *et al.* (2002) in their work, which ranged from 333.2 to 386.0 g kg-1. This difference was attributable to the two studies' varied seed development stages and moisture content.

The edamame soybean genotype BioMAX 1 has the best quality of sugar, protein, and fat, while the grain soybean genotype is the Grobogan genotype based on nutritional value standards. Liu *et al*. (2022) reported that one important difference between the two types of soybeans (edamame soybeans and seed soybeans) is that edamame soybean seeds are much bigger and sweeter than grain soybean seeds. Takahashi and Ohyama (2011) conducted a study on the nutritional content of edamame compared to soybean grains, peas, and mung beans, showing edamame contains more protein (41.3%) than soybean (35.3%). Reports on using fertilizers, such as N-P-K in formulations of 30-80-38 kg ha-1, also improved the edamame's flavor, sugar content, and seed size (Zeipiņa *et al*., 2017). These outcomes agree with the research of Obatolu (2006) on protein content ranging from 11.6% to 15.3%. The low values observed for the protein content of immature soybean seeds have shown to be higher than most cereal and root crops (Johnson *et al.* 1999). Shurtleff and Aoyagi (1991) also reported that fresh green soybeans containing 12.7% protein are among the most nutritious of all green vegetables. With consumer demand for edamame soybeans that are sweet with high protein content but still have a low-fat content, the BioMAX 1 genotype as edamame soybeans and the Grobogan genotype as seed soybeans have the potential as parents for quality improvement in these three biochemical characteristics.

CONCLUSIONS

Genetic diversity emerged based on the edamame's morphological, agronomic, and biochemical characteristics. Genotypes that can benefit as parents in crosses for yield improvement are G.AGS 439 x L Tegal-1 and Dega-1 genotypes with the highest weight of pods and good agronomic traits. The study recommended BioMAX 1 and Grobogan for quality improvement.

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