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Determination of Genetic Parameters of Seed Characteristics in Edible Soybean

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ABSTRACT

Background: Knowledge about genetic parameters can help plant breeders to determine which selection criteria are beneficial. This study aims to estimate the genetic parameters of soybean seed characteristics.

Methods: A total of 91 soybean lines from three different populations were evaluated during dry season in 2016. All genetic material used was grown following a randomized complete block design with two replicates. Soybean pods were harvested after 90% of the leaves had yellowed or fallen. The characteristics of the seeds observed included length, width, thickness and weight of 100 seeds.

Result: The results showed that there were two seed shapes (round and ellipse) and two seed sizes (large and medium). There is a broad genetic variability of soybean seed characteristics. Heritability broad sense varies from 0.42 to 0.84, classified as moderate (length-width ratio) to high (length, width, thickness, length-thickness ratio, width-thickness ration and weight of 100 seeds). This therefore shows that there is an opportunity to improve the characters of soybean seeds. The length, width, thickness, ratio of the three characters and the weight of 100 seeds can be used as selection criteria in a soybean breeding program to obtain large-seeded soybeans with a round or elliptical shape.

Key words: Genotype variance, Genotypic variability, Heritability, Phenotype variance.

INTRODUCTION

Soybeans (*Glycine max*) are the third most important food crop in Indonesia and represent an important source of vegetable protein to meet the nutritional needs of the people of Indonesia. From 2013-2015, Indonesia's national soybean production increased. This increase was mainly due to an increase in soybean cultivated land from 550,793 ha in 2013 to 613,885 ha in 2015. As a result, there has been an increase in productivity (yield) over the 2013-2015 periods of 1.41, 1.55 and 1.56 t/ha, respectively. However, domestic soybean production is only able to fulfill a small portion (33.33%) of national requirements; the remaining (66.67%) is obtained through imports (AMIS, 2015).

In Indonesia, soybean demand has increased with population growth. This soybean processing industry produces tofu, tempeh, soy sauce, soy milk, snacks, *etc* (Tahir *et al.* 2016), the vast majority (88%) turned into tofu and tempeh, 10% for other processed foods and only 2% are kept for seed stock (Swastika, 2016). The main use in tofu and tempeh makes large-seeded soybeans most popular among tempeh and tofu makers (Ginting *et al.* 2009). The Indonesian Agency for Agricultural Research and Development (IAARD) has been able to produce large-seeded varieties with weights of 14-21 g/100 seeds, similar to imported soybeans which have an average weight of 16 g/100 seeds. Superior varieties of large seeded soybeans include Argomulyo (16 g/100 seeds), Anjasmoro (16 g/100 seeds), Grobogan (18 g/100 seeds), Dena 1 (14.3 g/100 seeds), Devon 1 (15.3 g/100 seeds) and Dega 1 (21 g/100 seeds). Among the large-seeded soybean varieties, Anjasmoro and Grobogan are currently in great demand by farmers because of their large size preferred for tempeh making (Ginting *et al.* 2009).

High-yielding soybean varieties with large seeds can be developed through selective breeding. This involves selecting parents, which are used in hybridization, from local varieties, old varieties, new varieties and introduced varieties. Population formation is done through artificial crosses between selected parents, which have different characteristics followed by adopting the 'lines selection' or 'mass selection' method for developing elite lines. The success of a plant breeding program is influenced by several factors, namely the accuracy of the selection of parents, selection techniques used and lines evaluation. Genetic parameters including heritability, coefficient of genotypic variation and expected genetic advance of agronomic traits related to seed yield can be considered in soybean selection (Jain *et al.* 2018).

The success of a plant breeding program is strongly influenced by the level of genetic diversity and inheritance

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of characteristics as measured through heritability (Hapsari *et al.* 2010). For this reason, information is required on the genetic parameters of genotype variance, phenotype variance, environmental variance, heritability and expected genetic advance. The purpose of the study was to determine the genetic parameters of soybean seed characteristics.

MATERIALS AND METHODS

Plant material and experimental design

A total of 91 soybean lines were evaluated in the Indonesian Legumes and Tuber Crops Research Institute's Experimental Field, located at Probolinggo, East Java-Indonesia from August to October 2016. These soybean lines come from three different populations obtained from the crossing of medium-seeded soybeans (<14 g/100 seeds) with large-seeded soybeans (>14 g/100 seeds). Each line was planted in a plot measuring 1.6 m wide and 2.8 m long with planting spacing of 40 cm between rows and 10 cm in rows. The experiment was arranged as randomized complete block design (RCBD) with two replicates. Fertilizer was applied at planting time, *i.e.* 50 kg/ha urea, 100 kg/ha SP36 and 100 kg/ha KCl. Irrigation was done four times, *i.e.* at planting time, on 3 weeks after planting, during flowering and pods filling. Pest control was done intensively by spraying insecticide every two weeks.

Seed preparation and characterization

Soybean pods were harvested after 90% of the leaves had yellowed or fallen. The harvested pods were then dried in the sun to facilitate the spawning process. The seeds obtained were then dried again to reduce the moisture content. After that, seed inspection was carried out. Seed characteristics recorded included seed length, seed width, seed thickness and weight of 100 seeds. Measurements of seed length, width and thickness were undertaken using a digital caliper, while the measurement of the weight of 100 seeds using a digital scale. Determination of seed shape is based on the ratio between length and width of the seed. The four seed forms were assessed based on the ratio value adopted from the research by Balkaya and Odabas (2002) on chickpeas.

Statistical analysis

The data obtained was statistically analyzed using PKBT-STAT 1.0 software (developed by Center for Tropical Fruit Studies, Bogor Agricultural University, Indonesia) for analysis of variance. Genotypic variance, phenotypic variance and environmental variance were calculated using equations given by Hallauer and Miranda (2010) and Abady *et al.* (2013). Table 1 shows the equation for estimating variance components using the results of variance analysis.

Genotypic variance, phenotypic variance and environmental variance was calculated based on formulas in Table 1 as follows:

$$\text{Environmental variance } (\sigma^2_e) = Ms_e$$

$$\text{Genotypic variance } (\sigma^2_g) = \frac{(Ms_g - Ms_e)}{r}$$

$$\text{Phenotypic variance } (\sigma^2_p) = \sigma^2 g + \sigma^2 e$$

Furthermore, broad sense heritability (h_b^2) was computed as per the following formula applied by Abady *et al.* (2013):

$$\text{Heritability (broad sense) } (h_b^2) = \left(\frac{\sigma^2_g}{\sigma^2_p}\right) \times 100\%$$

A broad or narrow value of genotype characteristic variability was determined by the genotypic variance (σ^2_g) and standard deviation of the genotypic variance ($\sigma_{\sigma^2_g}$). Meanwhile, broad or narrow, as a phenotype variable, is determined by the phenotypic variance (σ^2_p) and standard deviation of the phenotypic variance ($\sigma_{\sigma^2_p}$). Genotype variability and phenotype variability were calculated using the formula suggested by Hallauer and Miranda (2010) as follows:

$$\sigma_{\sigma^2_g} = \sqrt{\frac{2}{r^2}\left\{\frac{Ms_g}{db_g + 2} + \frac{Ms_e}{db_e + 2}\right\}} \text{ and } \sigma_{\sigma^2_p} = \sqrt{\frac{2}{r^2}\left\{\frac{Ms_g}{db_g + 2}\right\}}$$

Genotype variability was broad if $(\sigma^2_g) \geq 2(\sigma_{\sigma^2_g})$ and narrow if $(\sigma^2_g) \leq 2(\sigma_{\sigma^2_g})$, meanwhile the phenotype variability was broad if $(\sigma^2_p) \geq 2(\sigma_{\sigma^2_p})$ and narrow if $(\sigma^2_p) \leq 2(\sigma_{\sigma^2_p})$.

RESULTS AND DISCUSSION

Diversity in shape and size of soybean seeds

The results of the analysis of variance showed that there were significant differences in the characteristics of seeds, including length, width, thickness, length-width ratio, length-thickness ratio, width-thickness ratio and weight of 100 seeds (Table 2). This shows that in our study soybean lines were significantly affected in all observed characters. All characters observed have a relatively low coefficient of variation, indicating that the data from each replication is relatively consistent.

The difference in seed length, seed width and seed thickness affects the ratio of the three seed characteristics. As a result, there were variations in the shape and size of seeds of 91 soybean lines tested (Table 3). Based on the ratio between length and width of the seeds, there were two observed seed shapes; namely round (69 lines) and ellipse (22 lines). While based on the weight of 100 seeds, there are two groups of seed sizes, namely 64 large seed lines (≥ 14.00 g) and 27 medium seed lines (≤ 13.99 g).

The form of soybean seeds is one of the important characters for the food industry in several countries, including in the USA and China (Salas *et al.* 2006; Xu *et al.* 2011). Consequently, selection activities in soybean breeding program abroad are more focused on the seed shape compared to the seed size. Selection of seed shapes using individual selection methods can be done in early generation populations (Cober *et al.* 1997). The form of soybean seeds is a polygenic characteristic that is controlled by many genes and shows stability in various environments

Table 1: Analysis of variance and estimation of variance components from RCBD.

Source of variation	df	Mean square	Expected mean square
Replication	r-1		
Genotype	g-1	MS_g	$\sigma^2_e + r\sigma^2_g$
Error	(r-1)(g-1)	MS_e	σ^2_e

σ^2_e= environmental variance, σ^2_g= genotypic variance, r = replication, g = genotypes.

Table 2: Analysis of variation of soybean seed characteristics.

Seed characteristics	Mean square	F-value	Coefficient variation (%)
Length	0.22	3.69**	3.34
Width	0.12	4.15**	2.72
Thickness	0.12	5.88**	2.85
Length-width ratio	0.002	2.47**	1.43
Length-thickness ratio	0.01	6.95**	1.36
Width-thickness ratio	0.004	5.62**	1.11
Weight of 100 seeds	5.47	11.09**	4.59

**Significant at P≤0.01.

(Song *et al.* 2004). This characteristic is also inherited because it has a high heritability, ranging from 0.42-0.88 (Salas *et al.* 2006; Xu *et al.* 2011).

In Indonesia, soybean farmers are more concerned with seed size than seed shape. Seed size is one of the characters used by farmers and soy-based food industries in determining which soybean varieties are to be planted. The soybean processing industry in Indonesia for tofu and tempeh prefers imported soybeans because the seed size is bigger than local soybeans (Ginting *et al.* 2009).

The soybean breeding program to obtain large seeded soybean varieties in Indonesia has a good chance of success. Our study found 64 of the 91 soybean lines tested (70.33%) belong to the large-seeded soybean group (Table 3). More of the large seeded lines than medium seeded lines were found among the soybean populations tested. This is because the three populations were obtained from crosses involving large seeded soybeans. These large varieties are Grobogan (18 g/100 seeds) and Argomulyo (16 g/100 seeds). Other studies using different populations have also found similar results. The majority of early-generation lines (98%) obtained from a cross between a Madagascar soybean variety and one from Indonesia produced large seed sizes (Sulistyo and Purwantoro, 2018).

The seed size illustrated by the weight of 100 seeds is an important factor in efforts to increase soybean production (Johnson *et al.* 2001). Seed size has been known to be genetically inherited (Brian *et al.* 2002; Kumar *et al.* 2020) and has a positive direct effect on soybean yield (El-Badway and Mehasen, 2012).

Genetic parameters

The genotypic coefficient of variation (GCV) of soybean seed characteristics is shown in Table 4. The results showed that almost all the seed characters had a low GCV (<0.10) and only one seed character was classified as moderate GCV (0.10<GCV<0.20), namely weight of 100 seeds. Previous studies gave different results. Some studies report that the weight of 100 seeds has a low GCV (Dhillon *et al.* 2005; Ghodrati, 2013) and some studies show that the weight of 100 seeds has a moderate GCV (Aditya *et al.* 2011; Chandrawat *et al.* 2017).

Although all of the seed characteristics observed had a low GCV, but all of the seed characters have a wide genetic diversity (Table 4). The wide genetic diversity of length, width, thickness, length-width ratio, length-thickness ratio, width-thickness ratio and weight of 100 seeds are caused by different genetic backgrounds of the three populations tested.

Table 3: Classification of 91 soybean lines based on their seed characteristics.

Population	Number of lines	Seed shape		Seed size	
		Round	Ellipss	Large	Medium
G100H/9305//IAC100///Grob	35	32	3	31	4
G100H/9305//IAC100///Arg	47	33	14	29	18
Kaba/IAC100//Brg///Grob	9	4	5	4	5
Total	91	69	22	64	27

Table 4: Genotypic coefficient of variation (GCV) of soybean seed.

Seed characteristics	GCV	σ^2_g	$\sigma^2_{\sigma g}$	$2\sigma^2_{\sigma g}$	Category
Length	3.87	0.08	0.02	0.04	Wide
Width	3.42	0.05	0.01	0.02	Wide
Thickness	4.46	0.05	0.01	0.02	Wide
Length-width ratio	2.45	0.0008	0.0002	0.0004	Wide
Length-thickness ratio	4.70	0.0046	0.0008	0.002	Wide
Width-thickness ratio	3.39	0.0017	0.0003	0.0006	Wide
Weight of 100 seeds	10.30	2.49	0.42	0.85	Wide

GCV = Genotypic coefficient of variation, σ^2_g = Genotypic variation, $\sigma^2_{\sigma g}$ = Standard deviation of genotypic variation.

Tabel 5: Broad sense heritability of soybean seed characteristics.

Seed characteristics	h^2	Criteria
Length	0.57	High
Width	0.61	High
Thickness	0.71	High
Length-width ratio	0.42	Moderate
Length-thickness ratio	0.75	High
Width-thickness ratio	0.70	High
Weight of 100 seeds	0.84	High

An estimate of the values of heritability for each seed characteristic is shown in Table 5. The results show that the weight of 100 seeds had the highest heritability (0.84), while the ratio of seed length to width had the lowest heritability (0.42). Based on the classification of heritability proposed by McWhirter (1979), then almost all the characteristics of soybean seeds in our study were classified as high heritability ($h^2 \geq 0.50$). The moderate heritability ($0.20 \leq h^2 \leq 0.50$) is only found in the ratio of seed length to width.

The estimated value of heritability obtained in our study is similar to previous studies. The heritability of soybean seed shape (width-length ratio and thickness-length ratio) is relatively high, respectively with values of 0.49 and 0.76 (offspring-parent method) and 0.59 and 0.79 (narrow sense heritability) (Cober *et al.* 1997). Higher heritability of seed characteristics, which is between 0.92 and 0.98, was reported by Hu *et al.* (2013). The results of our study indicate that soybean seed characteristics are inherited, because genetic factors play a role more than environmental factors which are reflected in high heritability. Inheritance of the length, width and thickness of the seeds is reportedly controlled through the cytoplasmic gene (Liang *et al.* 2005).

The heritability values of soybean seed characteristics obtained in our study showed that the seed size reflected from weight of 100 seeds has the highest heritability (Table 5). This is likely influenced by the length, width and thickness of the seeds which also have high heritability. These three seed characters reportedly have a positive correlation with the weight of 100 seeds (Liang *et al.* 2016). However, the shape of seed does not determine the seed size. The results of correlation analysis showed that there was no significant correlation between the ratio (length-width, length-thickness and width-thickness) and the weight of 100 seeds. This is because the shape and size of soybean seeds are controlled by different genes (Hu *et al.* 2013).

CONCLUSION

Genetic variability of soybean seeds characteristics are classified into a wide variety. Heritability is classified as moderate (length-width ratio) to high (length, width, thickness, length-thickness ratio, width-thickness ratio and weight of 100 seeds). This shows that there is an opportunity to improve the character of soybean seeds. The length, width, thickness, ratio of the three characters and weight of 100 seeds can be used as selection criteria to produce larger seeded varieties.

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