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GENOTYPIC VARIATION FOR GRAIN PROTEIN, OIL CONTENT AND YIELD RELATED TRAITS IN SOYBEAN POPULATIONS

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SUMMARY

Seed quality traits such as oil and protein contents as well as yield components are desirable characters in soybeans if it is used for food. In this study, we determined genotypic variation of grain protein and oil content as well as related traits on F3 soybean populations. Nine F3 populations of soybean originated from crosses between three male varieties and three female parents were planted in 2017. The design used was randomized block design with four replications. Several traits were observed from each entry including days to harvest (DTH), seed weight plant^{-1} (SWP), 100-grain weight (HGW), grain yield m^{-2} (GYM), grain protein (GPC) and oil content (GOC). There was large variation found among genotypes for SWP, HGW, GYM, GPC, and GOC but not for DTH. Genetic component of variance and heritability were significant (P = 0.05) for SWP, HGW, GYM, and GPC but equal to zero for DTH and GOC. Grain yield was positively correlated to HGW but negatively correlated to GPC. Seed oil content did not significantly correlated to any traits. The result indicated that SWP, HGW, GYM, and GPC may be utilized as selection criteria in a soybean breeding program. Selection response was moderately high for GYM, but relatively low for DTH, SWP, HGW, GPC and GOC.

Key words: F3 generation, genotypic variation, protein content, oil content, soybean populations

Key findings: Sufficient variability in grain yield components and grain quality found in the material under evaluation may indicate that the traits could be used as selection criteria in a breeding program. Response to selection for DTH, SWP, HGW, GPC, and GOC was relatively low, while for GYM was moderate. This inferred that selection for grain yield can be effective in early generation, while selection for other characters should be applied at later generation.

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INTRODUCTION

In Indonesia, soybean (*Glycine max* (L) Merrill) is utilized mainly as human diet, so that, seed quality related traits such as protein and oil contents are very crucial. A cultivar possessed high protein and oil content is suitable for farmers as well as for consumers to meet special food applications. These characters are considered as important economic determinant for soybean seed in the global market. Approximately 1.7 million tons of soybean seed were imported to Indonesia annually, which is more than 60% of the national demand. So that, it is important here to increase yield as well as to improve other desired characters such as seed protein and oil concentration via soybean breeding program.

In a plant breeding program, like soybean breeding, the development of variability for certain characters is important determinant in order to facilitate further selection. Variability is often obtained by crossing parents that possess specific traits to transfer into new improved varieties. Until recently, we started to concentrate our attention on the traits which related to chemical composition in soybean grain. Previous study indicated that soybean grain yield, and yield components were influenced by their genotypes and environmental condition (Rotundo and Westgate, 2009; Dukic *et al*., 2010), cultural practices such as fertilizer application (Win *et al*., 2010) and genetic by environment interaction (Kumar *et al*., 2006; Rodrigues *et al*., 2014). Further study indicated that grain protein and oil contents as well as related traits were controlled significantly by additive and dominance components of variance (Rasyad *et al*., 2016). Information on variance components and heritability is useful for further plant breeding program to predict selection response of the specific traits and to determine whether the traits would be effective to be used as selection criteria.

Protein content and oil content in grain vary among soybean populations and depend upon location where it is grown. Several studies have reported that grain protein content ranged from 25.5% to around 59% while oil contents varied from 12% to 23% (Piper and Boote, 1999; Vollman *et al*., 2000). In Turkey, genotypic variation of grain oil content ranged from 15% to 22% and protein content ranged from 34% to 40% (Arslanoglu *et al*., 2011).

Recently, Rasyad *et al*. (2016) reported an increase in grain yield was followed by a decrease in grain protein content. Similarly, Gunasekera *et al*. (2006), Li and Burton (2002), and Piper and Boote (1999) reported the same trend which reflects the presence of negative correlation between both traits. Based on recent finding, selection for both high yield potential and high protein content is difficult to achieve; however, several breeders have been attempting to select cultivars with high seed yield with comparable high grain protein content (Brim and Burton, 1979; Rodrigues *et al*., 2014). Erickson *et al*. (1981) suggests a strategy to obtain a line with high grain yield and high protein by tandem selection, wherein selection for protein content first followed by selection for yield potential.

The objectives of this study were to determine the genotypic variation of grain protein, grain oil content and their related traits in defined populations and to estimate predicted response of selection of the traits in the reference population.

MATERIALS AND METHODS

Nine F3 populations of soybean obtained from crosses between three male parents and three female parents randomly chosen from fourteen varieties and eight breeding lines were evaluated in 2017. The reference parents used constituted wide variability in grain protein and oil contents. The experiments were conducted at the University of Riau Agriculture experiment Station in Pekanbaru, Indonesia. Soil type in the experiment station was Inceptisol (*Fluventic dystrudepts*) characterized by low nitrogen and phosphorous contents.

Seed of each population was planted on 18 April 2017 in a plot of 3 m long and 2 m wide with a planting density of 20 cm within a row and 40 cm between rows. Two seeds were placed in every hill and keep until 15 days after planting before thinning to a single plant per hill. Fertilizers in the form of Urea, TSP and KCl at the rate of 25 kg N, 25 kg P_2O_5, 40 kg K_2O ha^{-1}, respectively were applied at planting date. Design used for the field experiment was completely randomized block design with four replications.

Days to harvest (DTH), seed weight per plant (SWP), 100-grain weight (HGW), grain yield was observed at harvest from each plot, the grain yield was then converted into grain yield m^{-2} (GYM). Crude grain protein (GPC) and oil contents (GOC) were observed by weighing 15 g of grain from each plot, and then dried to a moisture content of 130 mg g^{-1}. The grain was then ground and placed in a plastic jar until extraction. Oil extraction and calculation of grain oil concentration was done by the procedure described by Maestri *et al*. (1998). Grain protein content (GPC) was determined by converting nitrogen concentration obtained by macro-Kjeldahl methods as described by AOAC (1980).

Statistical analysis

An analysis of variance was performed for each experiment with the model: $Y_{ijk} = \mu + r_i + p_j + e_{ijk}$, in which Y_{ijk} = phenotypic value of individual population *i;* μ = the overall mean; r_i = replication effect; p_j = the effect of populations *j*; and e_{ijk} = the experimental error due to individual observation.

Genotypic variance was calculated as linear functions of mean squares from the ANOVA and the values then translating into genetic variance as outlined by Hallauer *et al.* (2010). Heritability was estimated by the following method; $h^2 = \sigma_g^2 / \sigma_p^2$, in which h^2 is heritability involving the total genetic of variance. The phenotypic variance component σ_p^2 was estimated directly from among populations mean square. Predicted selection response was calculated as the following formula; $S = i.h^2.\sigma_p$, where S is selection response, i is selection differential with selection intensity of 10 percent, h^2 is heritability of the trait, and σ_p is the standard deviation of the phenotypic variance component (Simmonds, 1979).

Phenotypic correlation was calculated by dividing the covariance of traits x and y by square root of the product of the variance of the traits x and y. Thus, $r_{x,y} = \sigma_{x,y}/(\sigma_x^2 . \sigma_y^2)^{½}$, in which $\sigma_{x,y}$ is the phenotypic covariance of traits x and y, σ_x^2 is the phenotypic variance of traits x, and σ_y^2 is the phenotypic variance of trait y.

RESULTS

Mean squares for grain quality characters and yield components are presented in Table 1. There were no significant differences among populations for days to harvest. Considerable differences were found among populations for seed weight plant^{-1}, 100-grain weight, grain yield m^{-2}, grain oil content and grain protein content. This inferred that high genotypic variation occurred among populations for most characters.

Populations mean and its standard error of the traits were presented in Table 2. On the average, days to harvest ranged from 74,67 to 92 days with a mean of 83.85 days. The earliest days to harvest was observed in family GAR and the latest genotype was in Family KKM2. Seed weight per plant varied from 15.64 g to 25.28 g with a mean of 22.77 g per plant. Variation of 100-seed weight was similar in magnitude with a range from 10.81 g to 23.85 g with the mean of 15.45 g. Three families including Fam-GAR, GKM1 and GKM2 had 100-seed weight more than 18 g so they are classified as large seed genotypes. Seed oil content slightly differed among population with a range from around 15 to 25.25% with a mean of 19.03%. This inferred that variation for both 100-seed weight and seed oil content was slightly narrow in the populations under study. Wide variation was shown for grain yield ranging from 812.44 to 1786.24 g m^{-2} with the mean of 1004.54 g m^{-2}. Grain protein content ranging from 25.31% to 46.59% with a mean of 39.91% was observed in these populations.

Estimates of genotypic variance and heritability are presented in Table 3. In this study, the genotypic variance was considered significance when its value is equal or more than twice its standard error. Genotypic variance of days to harvest and grain oil contents was equal to zero. The genotypic variance component of seed weight per plant, 100-grain weight, grain yield m-^{2}, and grain protein was significantly different from zero.

Heritability values ranging from 0.25 for harvest date to 0.48 for seed weight per plant were observed (Table 3). In the magnitude, the value of heritability for seed weight per plant and 100-seed weight was higher than those other characters and regarded as greater than zero. Grain yield m^{-2} and grain protein content showed heritability value of 0.38 and 0.32, respectively, and was greater than zero. The smallest value of heritability was found for days to harvest and was not different from zero. Grain oil content had considerable high value of heritability (0.33) but the value was not significantly different from zero due to the value less than twice its standard error.

Response to selection is the change in mean population when selection at certain proportion imposed to the reference population. As presented in Table 4, estimates of selection response of the traits if selection imposed at F3 were relatively low for most traits except for grain yield. The change in population mean was around 3.26% if selection was based on seed weight per pant and around 3.07% if based on 100-

Table 1. Mean square from analysis of variance of some seed quality characters and grain yield in several soybean populations.

Source of Variation	DTH (days)	SWP (g)	HGW (g)	GYM (g)	GOC (%)	GPC (%)
Replication	12.09	8.76	2.54	454.78	11.09	12.07
Population	39.71	226.01 **	64.54 **	3759.54 **	13.48 *	71.09 **
Error	14.34	17.54	12.79	373.52	3.68	14.34

DTH=days to harvest; SWP=seed weight per plant; HGW=100-grain weight; GYM=grain yield per m^2 ; GOC=grain oil contents;GPC=grain protein content; *, ** indicates significant at $P < 0.05$ and $P < 0.01$ respectively.

Table 2. Population means and their standard error of some seed characters in several soybean populations

Populations	Days to Harvest (days)	Seed weight $plant^{-1}$ (g)	100 grain weight (g)	Grain yield m^{-2} (g)	Oil content (%)	Protein content (%)
Fam MAR	82.00	19.56	12.78	1786.24	25.25	25.31
Fam MKM1	90.00	18.37	13.17	907.32	15.09	46.59
Fam MKM2	86.00	21.33	12.62	812.44	15.28	32.37
Fam GAR	74.67	15.64	20.97	1005.83	16.39	41.52
Fam GKM1	76.33	24.56	23.85	1115.45	22.93	43.74
Fam GKM2	77.33	23.43	19.77	1372.50	15.49	39.61
Fam KAR	89.00	25.28	12.68	1236.97	22.29	34.47
Fam KKM1	89.00	23.98	12.35	991.92	15.41	41.61
Fam KKM2	92.00	22.37	10.81	841.53	23.16	36.98
Means	838.1	22.27	15.45	1004.54	19.03	39.91
SE	1.75	4.98	1.80	154.411	1.60	3.41

Table 3. Estimates of Genetic variance (σ_g^2 σ_g^2), Phenotypic variance (σ_p^2), heritability (h^2), and selection response (S) for soybean yield components and grain quality.

Traits	σ_g^2 σ_g^2	σ_p^2	h^2	S (%)
Days to harvest (days)	12.13	48.53	0.25	3.06
Seeds weight $plant^{-1}$ (g)	7.12#	14.83	0.48#	3.26
100-grain weight (g)	6.62#	14.37	0.46#	3.09
Grain yield m^{-2} (g)	241.90#	635.57	0.38#	16.81
Grain oil content (%)	1.19	2.77	0.33	1.02
Grain protein content (%)	3.26#	10.19	0.32#	1.82

# indicates the value is twice its standard error

Table 4. Phenotypic correlation among several seed characters and grain yield in several soybean populations.

Traits	Seed weight ($plant^{-1}$)	100-grain weight	Grain yield (m^{-2})	Oil content	Protein content
Days to harvest	0.31**	0.23 *	- 0.20	0.19	0.12
Seed weight $plant^{-1}$	-	-0.29**	0.22 *	-0.16	-0.09
100 grain weight	-	-	0.32**	0.08	0.26*
Grain yield (m^{-2})	-	-	-	-0.03	-0.24*
Oil content	-	-	-	-	0.18

*, ** indicates correlation coefficient is significant at $p<0.05$ and $p<0.01$, respectively.

grain weight. The changes in mean were 1.32% and 1.82% for oil content and protein content, respectively when selection was based on the two traits. However, a substantial amount of change in grain yield was observed if grain yield was used as selection criterion.

Days to harvest were positively correlated to seed weight per plant and 100-grain weight but not to other characters (Table 4). Seed size represented by HGW was positively correlated to grain yield m^{-2} and GPC but negatively correlated to SWP. Positive correlation was also observed between 100-grain weight and grain protein content. There was no significant correlation between GOC and other traits.

DISCUSSION

The main objective of soybean breeding is to develop cultivars which have high yielding ability and high grain oil and/or protein content. Present study indicated appreciable genotypic variation for some yield potential and grain quality traits which imply that these characters may be utilized for population improvement. Significant genotypic variance of seed weight plant^{-1}, 100-grain weight, grain yield and grain protein content for these populations implied the important role of genotype on the characters. This result was in agreement with several recent studies by Panthee *et al*. (2005), Chowdhury *et al*. (2015), and Chandrawat *et al*. (2017).

Hanson (1987) stated that heritability was considered as different from zero if the value is equal or more than twice its standard error. In this study, the estimate of heritability for seed weight per plant, 100-grain weight, grain yield, and grain protein content was different from zero, while heritability of days to harvest and grain oil content was equal to zero. So that, seed weight per plant, 100-grain weight, grain yield, and grain protein content can be considered as favorable indicators for population improvement through selection. Characters such as days to harvest and grain oil content were more difficult to be modified in the reference population. The values of heritability in this study were smaller than that reported by several investigators (Burton and Brim, 1981; Li and Burton 2002; Jaureguy *et al*., 2011). The smaller values of heritability obtained here may be due to the materials used under the study. We used bulked F_3 population developed from crossing between three male by three female parents, so the total phenotypic variance estimated comprised greater portion of environmental variance. Researchers stated that low heritability of the traits reflected small portion of genetic variance that may hindered the possibility of improvement through selection (Desissa, 2011; Costa *et al*., 2008). Considering this finding, it is advisable to apply selection in later generation.

Response to selection is classified by Johansson *et al*. (1955) as low when the value is less than 10%, moderate when value ranges from 10-20% and high when value more than 20%. Response to selection was relatively low for date to harvest, seed weight per plant and 100-grain weight when those traits were used as the bases of selection. Estimates response of selection were negligible when selection was based on grain oil content or protein contents which indicated that protein or oil content of the grain might not be effective as the selection criteria in these early generation populations. Moderate value of selection response obtained for grain yield m^{-1} (16.68%) inferred that selection for grain yield is effective enough when starting in early generation.

The material used in this study was bulked F3 seed which is actually early generation population. So the results may reflect that selection for traits such as DTH, SWP, HGW, GOC and GPC would be more effective if applied at later generation, while selection for GYM could be imposed at early as in the F3 generation. Costa *et al.* (2008) and Miladinovic *et al*. (2011) suggested if the value of heritability was relatively low, selection should be delayed until later generation, alternatively we may decrease the proportion of selected genotypes if the variance component of the population is sufficiently wide.

Unfortunately, negative coefficient of correlation between grain yield m^{-2} and grain protein content observed in this study, may prevent breeder to select a variety with both high yielding ability and high grain protein content. Despite of this negative correlation, however, we are still able to do a joint selection which would end up a genotype with considerably high yielding ability and high grain protein. For instances, three populations; i.e. Fam GAR, GKM1 and GKM2, produced relatively higher grain yield per m^2 and had considerable high grain protein contents. Those populations originated from the same female parent, and interestingly all could be harvested around 74 to 77 days after planting (Table 2). In the area where soybean is grown after rice-rice-soybean crop rotation in paddy soil, days to harvest of less than 80 days is desirable traits for farmers. Beside the high yield potential and protein content the three populations could be harvested earlier, so that genotypes developed from those populations may be suitable to be grown in the above environment.

Low but significantly positive correlation between protein content and 100-grain weight as also observed in other studies (Maestri *et al*., 1998; Yin and Vyn, 2005; Rasyad and Idwar, 2010). However, the value was so small that 100-grain weight might not be utilized as an alternative criterion to select high grain protein genotypes. Evaluation of grain protein content in a vast numbers of genotypes is time consuming and the method is more complicated. So, in breeding practice, alternative criteria for selecting high grain protein genotypes should be explored in the near future.

CONCLUSION

Sufficient genotypic variation for seed weight per plant, 100-grain weight, grain yield m^{-2}, crude grain protein was observed in this soybean populations. The magnitude of variation is sufficient enough that it could be employed in a soybean breeding program. Heritability estimates were low for day to harvest and oil content but considerably high for seed weight per plant, 100-grain weight, grain yield m^{-2}, and crude grain protein. Selection response was relatively low for day to harvest, oil content, seed weight per plant, 100-grain weight but moderate for grain yield m^{-2}. These findings indicated that except for grain yield m^{-2}, selection for those traits might not be effective as the selection criteria in early generation of the populations.

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