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PERFORMANCE OF F2 GENERATION LINES OF SOYBEAN (*Glycine max*. L) AS BACKCROSS RESULTS OF GHJ-4 AND GHJ-5 WITH RYOKO AS A DONOR PARENT

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Abstract. *The breeding of new high-yielding varieties is one of the solutions to the low productivity of soybeans in Indonesia. Superior genotypes of 'GHJ-4 (A)' and 'GHJ-5 ' were obtained in the previous study with an average weight of 13 g/100 seeds and a potential yield of 3 tons/ha. The attempt to increase the weight and size of the seeds of 'GHJ-4' and 'GHJ-5' was continued using the backcrossing method with 'Ryoko (>35 g/100 seeds) as a parent donor. F1 plants were obtained with an average weight of 15 g/100 seeds. This study aimed to determine the performance of the F2 generation of backcross in terms of the number of filled pods, the weight of 100 seeds, yield per plant (g), yield per plot (kg), and potential yield per hectare (tons/ha), as well as the heritability value of the genotypes. In this particular study, the F2 seeds from F1 plants were then planted and observed. This study used a non-factorial randomized block design (RBD) with 11 soybean genotypes, each consisting of 3 replications. The results showed that the F2 generations (GHJ-4XRyoko, RyokoXGHJ -5, and GHJ-4XGHJ-5) are considered as prospective new high-yielding varieties with a potential yield of ≥ 3.5 tons/ha, which have successfully restored the trait of 'Ryoko' and surpassed the performance of 'GHJ-4' and 'GHJ-5' as their parents in all parameters. The heritability value of the genotypes tested belongs to the high category, on the parameters of the number of filled pods (96.937 %), yield per plant (98.102 %), the weight of 100 seeds (99.132%), yield per plot (92.605 %) and potential yield per hectare (92.257 %). For the parameters observed, the back cross yields of harvest age were 72-74 HST, the weight of 100 grains was 17-18 grams and the yield per hectare was around 4 tons/ha. The heritability value shows that the observed traits are inherited from parents on average more than 90% and the selection that will be used is pedigree selection.*

Keywords: *edamame soybean; heritability; seed size*

1. Introduction

Soybean (*Glycine max* L.) is a staple commodity of legumes in Indonesia which is a source of vegetable protein as it is rich in protein with around 40% based on its dry weight (Ali *et al*., 2020). It also has abundant oil content which consists of both unsaturated and saturated fatty acids (Szostak *et al*., 2019). Soybean is commonly used in the food industry, such as soymilk, tofu, tempeh, miso, natto, or cooking oil (Kamble *et al*., 2021). In Indonesia, about 83.7% of soybean is used as tofu and tempeh which are seen as 'the daily people's food' for its affordability and high protein content (Desmawati *et al*., 2021). Thus, optimum soybean production can advance the food industry so that it can contribute to improving national food security. However, around 56% of the soybean supply is fulfilled from imports and the national production can only fill in about 44% (Roessali *et al*., 2019). This is due to the low productivity and high production

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costs so catching up with the increasing national soybean needs is a hard task. On the other hand, the need for soybeans in Indonesia continues to increase along with the growth of the population and public awareness of consuming vegetable protein. In 2020, the national soybean production was around 632,300 tons, while the national need for soybeans was 3 million tons/year. In addition, national productivity has only reached 1.57 tons per hectare (Badan Pusat Statistik Indonesia, 2021).

One step that can be done to solve this problem is the use of high-yielding varieties. There are many studies reported the attempt of producing or assessing the potential of high-yielding soybean lines both conventional (Arsyad & Asadi, 2011; Badiaraja *et al.*, 2021; Jan *et al.*, 2022; McNeece *et al.*, 2021; Mustikawati & Endriani, 2020; Vieira & Chen, 2021) and molecular attempts (Gao *et al.*, 2022; Yuhong *et al.*, 2021). In the same attempt to produce high-yielding varieties of soybean, Sjamsijah and Poerwoko (2010) experimented with the crossing between 'Ryoko' and 'Wilis'. 'Ryoko' edamame is known to have a large seed size (<35 g/100 seeds) but has a small amount of pods (Jiang *et al.*, 2018). On the other hand, 'Wilis' is known to have a lot of pods but has a small seed size. In the hope of combining the two superior traits, 'UNEJ 2' which has a seed size of 15 g/100 seeds was produced. Since 'UNEJ 2' still has a relatively long harvest age, it was crossed with 'Malabar' which has an early maturity trait. The results of this crossing are called GHJ-4 and GHJ-5.

After several years of careful selection, the F6 generation of GHJ-4 and GHJ-5 were obtained with the characteristics of: 1) have a high productivity of more than 3.5 tons/ha, 2) have early maturity that can allow farmers to plant twice a year, 3) resistant to the main disease of soybeans, namely leaf rust disease. These obtained lines have been adapted in eight soybean centers in East Java. However, the seed size of 'GHJ-4' and 'GHJ-5' were smaller than 'UNEJ 2' with only 13 g/100 seeds.

In the attempt to restore the parental trait of large seed size, backcross was conducted. A backcross method is a form of repeated crossing with one of its parents for generations that aims to transfer or add superior traits from the donor parent to their descendant genotypes. In doing the backcross, it is necessary to be repeated until the desired donor parent's trait is obtained, but still retaining the other superior traits. The F1 generation of backcrosses between 'GHJ-4' and 'GHJ-5' with 'Ryokko' as the donor parent showed that the performances of the backcrossed genotypes were increased with an average seed size of 15 g/100 seeds (Sjamsijah *et al.*, 2020). As a continuation of the previous studies, this study aimed to determine the performance of the F2 generation of backcross in terms of the number of filled pods, the weight of 100 seeds, yield per plant (g), yield per plot (kg), and potential yield per hectare (tons/ha), as well as the heritability value of the genotypes.

2. Methods

This research was carried out from August to November 2020 at the experimental field of the State Polytechnic of Jember, at 89 m above sea level with an average temperature of 23° C – 32° C and rainfall of 1,968 – 3,394 mm/year. The equipment used in this study include hoes, sprayers, roll measuring tape, stakes, sickles, pruning scissor, rulers, stationeries, scissors, analytical scales, moisture testers, winnow, and label papers. The materials used in this study included three parental soybean seeds namely GHJ-4 (A), GHJ-5 (B), and Ryoko (C) which produced 3 new genotypes namely: A x C, B x C, C x B, as well as comparative lines, namely GHJ-4, GHJ-5, Ryoko, Malabar, and Argomulyo. Fertilizers used in this study were urea, KCl, SP-36, Phonska, and foliar fertilizers (Gandasil B and D), whereas the pesticides used were insecticides (Marshal, Furadan 3G, and Decis) and fungicides (Dithane). This study used a non-factorial Randomized Block Design (RBD) with 8 soybean genotypes, each consisting of 3 replications and 7 samples per experimental unit. The statistical method of randomized group design according to (1).

$$Yij = \mu + \sigma i + \alpha j + €ij \qquad (1)$$

where Yij is the response or observational value of the *i-th* block and *j-th* genotype, μ is the general middle value, σi is the influence of the *i-th* block, αj is the influence of the j-th genotype and $€ij$ is the effect of the experiment error from the i-th block and the j-th genotype. From the results of these calculations, if there is a significant difference, it is further tested with Duncan's multiple range test (DMRT) with a 5% error level.

All of the genotypes mentioned were grown in the field at the same time and their performances were observed in terms of the number of filled pods, the weight of 100 seeds, yield per plant (g), yield per plot (kg), and potential yield per hectare (tons/ha). Heritability value was also estimated with (2) (Ibrahim *et al*., 2019).

$$h^2 = \frac{\sigma^2 g}{\sigma^2 g + \frac{\sigma^2 e}{r}} \qquad (2)$$

where h^2 is the heritability value, $\sigma^2 g$ is the variety of genotypes, $\sigma^2 e$ is the variety of environments, and r for replication.

3. Results and Discussion

The development of new plant varieties on some genotypes can be done by the backcross method. This method can give rise to the good traits of the parent to improve the combination of traits. This parent trait does not occur at the beginning of the crossing so it requires a repetition of the backcross to form the desired trait The result of the Analysis of Variance can be seen in

Table 1 below.

Table 1. Recapitulation of F-values from ANOVA in all observation parameters

Parameters	F-value		F-table 1%
Number of Filled Pods	95.933	**	4.28
Yield per plant	156.049	**	4.28
Weight 100 Seeds	343.765	**	4.28
Results per plot	38.568	**	4.28
Potential Yield per Hectare	36.746	**	4.28

Description: ** = significant difference at a 1% of error level

All of the genotypes studied (three genotypes of backcross result, three parents, and three comparative varieties) resulted in a very significant difference on all parameters tested based on analysis of variance. The data were then analyzed with Duncan's Multiple Range Test (DMRT) at a level of 5%. The test recapitulation is presented in Table 2.

Table 2. Recapitulation of 5% DMRT results in all observation parameters

Genotypes	Number of filled pods	Yield per plant (g)	Weight of 100 seeds (g)	Yield per plot (kg)	Yield Potential per hectare (tons/ha)
GHJ-4	43.12 cd	11.38 d	15.51 d	1.69 d	3.39 d
GHJ-5	45.70 a	12.36 d	16.70 c	1.85 cd	3.57 cd
Ryoko	32.96 f	20.73 a	31.75 a	3.10 a	6.20 a
Agromulyo	41.37 e	8.37 e	13.80 e	1.26 e	2.51 e
AXC	42.17 de	13.62 c	17.21 c	2.05 bc	4.10 bc
BXC	44.00 bc	14.20 c	17.50 c	2.13 bc	4.27 b
CXB	44.50 ab	15.52 b	18.85 b	2.32 b	4.65 b
Malabar	41.34 e	8.98 e	14.27 e	1.35 e	2.69 e

Remarks: The number followed by the same letter in the same column indicates a non-significant difference based on DMRT at a 5% error level
AXC = crossing result between GHJ-4 and Ryoko
BXC = crossing result between GHJ-5 and Ryoko
CXB = crossing result between Ryoko and GHJ-5

3.1 Number of Filled Pods

The number of filled pods from the eight genotypes of soybean plants ranged from 32.96 to 45.70. Table 2 showed that there was no significant difference between the GHJ-5 genotype and RyokoXGHJ-5 genotype, but showed a significant difference from GHJ-5XRyoko, GHJ-4, GHJ-4XRyoko, Agromulyo, Malabar, and Ryoko genotypes. The RyokoXB genotype was not significantly different from the GHJ-5XRyoko genotype but was different significantly from the GHJ-4, GHJ-4XRyoko, Agromulyo, Malabar, and Ryoko genotypes. The GHJ-5XRyoko genotype was not significantly different from the GHJ-4 genotype but it was significantly different from the GHJ-4XRyoko, Agromulyo, Malabar, and Ryoko genotypes. The GHJ-4 genotype was not significantly different from the GHJ-4XRyoko genotype but it was significantly different from the Agromulyo, Malabar, and Ryoko genotypes. The GHJ-4XRyoko genotype was not significantly different from the Agromulyo and Malabar genotypes but it was significantly different from the Ryoko genotype. The GHJ-5 genotype tends to produce the highest filled pod number, which is 45.70 pods. The RyokoXGHJ-5 genotype is a crossed genotype that has the highest average number of filled pods (44.5 pods) compared to other crossed genotypes (GHJ-5XRyoko and GHJ-4XRyoko). The selection results for the number of pods per plant can still be maintained by increasing seed size. This is very beneficial for researchers because the number of filled pods does not decrease even though a back cross is carried out.

3.2 Weight of 100 Seeds

The weight of 100 seeds in eight soybean genotypes showed an average range of 13.80 g to 31.75 g. Based on Table 2, Ryoko achieved the highest weight of 100 seeds with an average of 31.75 g, followed by CXB (18.85 g) which showed significant differences from each other. The other crossed genotypes as well as the two other parents, GHJ-5 and GHJ-4 resulted in a significantly lower weight of 100 seeds compared to CXB. The lowest weight of 100 seeds was shown in the two comparative varieties: Agromulyo and Malabar.

The difference in the weight of 100 seeds between each genotype and the comparison variety is influenced by the size of the seed. Nilahayati and Putri (2015) stated that seed conditions and genetic factors during the filling process determine the maximum seed weight and seed size. The size and weight of the seeds can also be determined by the size of the pods. The size of soybean seeds has three criteria: small (< 10 g/100 seeds), medium (10-14 g/100 seeds), and large (> 14 g/100 seeds).

However, other countries may have different criteria. In America and Japan, for example, seeds that weigh less than 25g/100 seeds are categorized as small while those with more than 25g/100 seeds are categorized as large. In this research, the seeds produced from the crossed genotypes are categorized as large seeds and showed an increase compared to the other parents: GHJ-4 and GHJ-5.

The size of 100 soybean seeds can also be influenced by genetic traits in each of their genotypes, but seed size can also be influenced by the conditions of the cultivation environment. Each line has a different genotype response to environmental or climatic conditions resulting in a difference in the weight of 100 seeds (Donkor *et al*., 2022). The weight of 100 seeds is one of several components that are used as a basis for selection reference (Kuswantoro *et al*., 2020).

3.3 Yield Per Plant

Table 2 shows that yield per plant was achieved by Ryoko with an average of 20.73 g, followed by CXB 15.52 g which is significantly different from Ryoko. The other crossed genotypes resulted in a significantly lower yield per plant, followed by the two other parents, GHJ-5 and GHJ-4. The lowest yield per plant was shown in the two comparative varieties, Agromulyo and Malabar.

The low yield per plant can be due to the low number of filled pods. The increase in yield is also greatly influenced by the number of pods filled with crops, where the more pods contained per plant, the higher the weight yield per plant. The yield character is a complex character that is heavily influenced entirely by the character of the components of the yield gene. The yield components are controlled by many genes in which expression is heavily influenced by the environment (Nilahayati *et al.*, 2022). The weight of seed per plant is one of the yield characteristics that can increase the productivity of the overall soybean yield in addition to other yield characteristics. Tabasum *et al.* (2010) reported that the weight of seeds per plant had a positive correlation with the number of pods and the number of branches. The weight of seeds per plant is also determined by the number of seeds so that it has a close relationship with the number of filled pods. The number of filled pods is also influenced by plant height. Anggraeni *et al.* (2010) stated that a high plant height causes an even distribution of light throughout the leaves leading to more efficient photosynthesis. This photosynthesis efficiency has a positive effect on yield (De Souza *et al.*, 2023).

3.4 Yield per Plot

Similar to yield per plant, in terms of yield per plot, Table 2 indicates that Ryoko resulted as the highest with an average of 3.10 kg, followed significantly by CXB with 2.32 kg. However, CXB is not significantly different from other crossed genotypes, AXC and BXC. Meanwhile, GHJ-5 and GHJ-4 gave a lower yield per plot compared to the crossed genotypes, followed by Agromulyo and Malabar as the lowest. The yield per plot is equivalent to the yield per plant since the yield per plot is obtained from summing the total yield per plant in one plot. The yield per plot can be used to find out how much the genotype or variety can produce in the existing environment. Therefore, the yield per plot can be the basis to determine whether the genotype has a high yield potential. Seed weight is one of the determinants of the productivity of soybean plants. The higher the weight of soybean seeds produced, the better the level of productivity. Seed weight is related to photosynthesis results as a major source in seed weight suppliers.

3.5 Yield per Hectare

The yield per hectare of eight genotypes ranged from 2.51 tons to 6.20 tons. As seen in Table 2, the highest yield per hectare was achieved by Ryoko with 6.20 tons/ha which was significantly different from all other genotypes. After Ryoko, the three crossed genotypes, CXB, BXC, and AXC, followed by 4.65, 4.27, and 4.10 tons/ha, respectively, with no significant difference from each other. Meanwhile, GHJ-5 and GHJ-4 resulted in a lower yield per hectare compared to the crossed genotypes with 3.57 and 3.39 tons/ha, followed by Malabar and Agromulyo as the lowest at 2.69 and 2.51 tons/ha, respectively.

Yield per hectare is closely related to yield per plant and yield per plot as they have a positive correlation. Accordingly, stated that the weight of the fruit per plant has a positive correlation with the weight per hectare. In addition, Sjamsijah (2015) stated the importance of including yield per ha in the variety description to ease the farmers in calculating the yield of their cultivation.

3.6 Heritability

The heritability value shows that the observed traits are inherited from parents on average more than 90% and the selection that will be used is pedigree selection. Heritability is a genetic parameter used to measure the ability of a genotype in several plant populations to pass on its characteristics. Syukur *et al.* (2015) classified heritability values into three categories, namely: high ($h^2 > 0.5$), medium ($0.2\ h^2\ 0.5$), and low ($h^2 < 0.2$). According to Barmawi *et al.* (2013), a high heritability value indicates that the influence of genetic factors plays a more important role in controlling a character than environmental factors. The estimated value of heritability in the broad sense (h^2) can be determined by dividing the total genotypic variance by the total genotypic variance plus the total environmental variance. This heritability shows the extent to which these traits can be passed on to subsequent descendants.

Table 3. Results of Heritability broad sense tests on all observation parameters

Parameters	Heritability/h^2 (%)	Category
Number of Filled Pods	96.937	High
Yield per Plant	98.102	High
Weight 100 Seeds	99.132	High
Yield per plot	92.605	High
Potential Yield per Hectare	92.257	High

Description:
Heritability category: High ($h^2 > 75\%$), Medium ($20\% \leq h^2 < 75\%$), Low ($h^2 < 20\%$)

Table 3 shows that the genotypes tested have high heritability values, which are more than 75% in all parameters. This indicates that genetic influence plays a greater role than environmental influences, so selection in this population is considered to be efficient and effective because it hopefully would result in a great genetic gain. The high heritability value of the trait is controlled by dominantly addictive genes so that it will be inherited from the parents to the progeny (Priyanto *et al.*, 2023). When the heritability value is close to 100%, the phenotypic trait that appears is considered the best index in improving the trait by providing great genetic gain for selection (Nugroho *et al.*, 2021).

4. Conclusions

Based on the results and discussion, it can be concluded that the F2 generation of CXB (crossing of female parent Ryoko and male parent GHJ-5) was the best one amongst the other crossed genotypes in terms of the number of filled pods, the weight of 100 seeds, yield per plant, yield per plot and potential yield per hectare. However, all of the crossed genotypes are considered prospective new high-yielding varieties with a potential yield of ≥ 3.5 tons/ha, which has successfully restored the trait of Ryoko and exceeded the performance of GHJ-4 and GHJ-5 as their parents in all parameters. The heritability value of the genotypes tested belongs to the high category, on the parameters of the number of filled pods (96 %$), yield per plant (98.102 %), the weight of 100 seeds (99.132%), yield per plot (92.605 %) and potential yield per hectare (92.257 %).

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