Heterosis Evaluation on Chlorophyll and Carotenoid Content Characters in Crosses Soybean (*Glycine Max* L. Merril) Petek and Jayawijaya Varieties

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ABSTRACT: This study aimed to evaluate the effect of heterosis on chlorophyll and carotenoid content in a hybrid soybean. This study used two genotype of the elders, and two genotypes of F1 and F1R a hybrid varieties Petek x Jayawijaya. Petek and Jayawijaya were tolerant and sensitive to shade respectively. Observed variables were the content of chlorophyll, carotenoids, plant height, number of pods per plant, seed weight per plant and weight of 100 seeds. Heterobeltiosis value and the highest positive heterosis found in the character of pods per plant and pods per plant ranged contains 75-77% for mid parent and 41-45% for high parent, while the influence of the female elders are not present in the character of chlorophyll and carotenoid contents, plant height, number of pods per plant, seed weight per plant, seed weight per plant, weight of 100 seeds. The result of the research showed that there is no correlation between either chlorophyll or carotenoids content and other variables.

KEYWORDS: heterosis, soybean, chlorophyll and carotenoid.

1 INTRODUCTION

Soybean (*Glycine max* (L.) Merrill) is an agricultural commodity that is needed in Indonesia, because it can be consumed in a variety of processed food products such as tofu, tempeh, milk, and many other processed products. In addition, soybean is also used as an industrial raw material as well as material fresheners.

Soybean consumption in Indonesia ranked third after rice and maize, reaching about 2.2 million tons per year, while domestic production is only able to meet 35% - 40% of the total consumption. The shortage until now is fulfilled by imports of 0.8 million tones per year (Marwoto and Suharsono, 2008). This condition challenge all parties to increase domestic soybean production in order to meet consumer demand and to decrease soybean import.

One of the efforts to increase soybean production that can be done is to find the high yielding soybean varieties through plant breeding activities. The purpose of the activity of plant breeding is to obtain new varieties, such as by way of hybridization. Hybridization produces new hybrid plants that have new genetic composition which have to be selected to obtain the value of heterosis.

One way to evaluate the potential outcome is to evaluate photosynthetic capacity which reflected the rich content of chlorophyll and carotenoids as light-harvesting pigmen. Allegations of genes that influence the chlorophyll content was obtained from the results of research in studying the inheritance of flavanol glycosides as a character that affect the photosynthetic capacity (Rostini *et al.*, 2000).Soverda *et. al.*, (2009) identified several varieties of soybean plants that are tolerant of shade i.e. Petek and Ringgit varieties, and varieties that are sensitive to shade i.e. Jayawijaya and Seulewah varieties. It was also found that the content of chlorophyll and carotenoid character closely related to the efficiency of photosynthesis, other than that there were marked differences between tolerant and sensitive genotypes shade associated with chlorophyll and carotenoids (Soverda, 2011).

Purnomo (2005) in his research concluded that the levels of the carotenoid content of corn leaves unchanged by shading but have closeness (correlation) with age and varieties. Sudarmadji *et al.*, (2007) did the selection on Sesame plants by using inter-traits character correlation. His research explained that the characters that have highest seed yield per hectare also have high carotenoid content. This result inferred that there exist a correlation between grain yield and carotenoid content in Sesame plant. The character of leaf greenness level and size and density of stomata opening that has a thick palisade layer on the leaves can also be used as indirect criteria because it correlated with the physiological development of plants such as plant height, number of seeds and harvesting.

In the previous study that performed crosses breeding between Petek (soybean variety that is tolerant to shade) and Jayawijaya (soybean variety that is sensitive to shade), there has been no any information about the value of heterosis and female parents influence on levels of carotenoid content. This study aims to evaluate and analyze the correlation heterosis effect on the chlorophyll content of carotenoids in soybean varieties from crosses Jayawijaya Petek and varieties.

2 MATERIALS AND METHODS

The experiment was conducted at the Teaching Farm Faculty of Agriculture, University of Jambi, with a height of approximately 35 meters above the sea level. Research carried out for about 5 months from February to July 2012. Materials used in this study were soybean seed of Petek and Jayawijaya, F1 seed and the seed produced from cross breeding of Petek and Jayawijaya (F1R).

This study was implemented by using a randomized block design (RBD) with one factor that genotypes with repeat 5 times. Genotypes tested are: Varieties Petek (G1), Varieties Jayawijaya (G2), F1 crosses Petek x Jayawijaya (G3), and F1 crosses Jayawijaya x Petek (G4). The variables observed were: total chlorophyll (mol / g), carotenoids (mol / g), plant height (cm), number of pods per plant (pod), weight of seeds per plant (g), and weight of 100 seeds (g). To test wether there was the difference among the two genotypes, the data was analyzed using analysis of variance and LSD test with the α level of 5%.

To test the positive heterosis in each character can be observed by using the formula:

Mid-parent Heterosis $=\frac{F1-MP}{MP} X 100\%$ High-parent Heterosis $=\frac{(F1-HP)}{HP} X 100\%$

F1 = appearances from cross breeding, MP = average appearance of the two elders = (P1 + P2)/2 and HP = best appearance of elders.

To test the correlation between chlorophyll and carotenoid content with other characters analysis of covariance was used according to Singh and Chaundry (1979).

$$Kov_{\cdot g.xy} = \frac{\mathbf{K}_2 - \mathbf{K}_3}{\mathbf{r}}$$

$$Kov_{\cdot p.xy} = Kov_{\cdot g.xy} + MSP2$$
(1)

K₁ = MSP_b (Mea1n Sum of Product for blocks / replications)

K₂ = MSP_t (Mean Sum of Product for treatment)

Kova

Phenotypic and genotypic correlation coefficient between the characters X and Y are:

$$r_{gxy} = \frac{Kov. g_{xy}}{\sqrt{(\sigma_{gx}^2)(\sigma_{gy}^2)}}$$

$$r_{pxy} = \frac{Kov. p_{xy}}{\sqrt{(\sigma_{gx}^2)(\sigma_{gy}^2)}}$$
(2)
(3)

Where *r* is correlation, r_{gxy} is genetic correlation, r_{pxy} is phenotypically correlation, Kov._{g.xy} is genetic covariance, Kov._{p.xy} is phenotype covariance, σ_{gx}^2 is variance of genotype variables 1, σ_{gy}^2 is variance of genotype variables 2, σ_{px}^2 is variance of

phenotype variables 1, σ_{py}^2 is variance of phenotype variables 2. Furthermore, to test the significance of the correlation, t-test according to the Student's t-test Gasperz (1995) as follows:

$$t - test = \frac{r_{xy}\sqrt{(n-2)}}{\sqrt{1 - r_{xy}^2}}$$
(4)

If the t-test is greater than $t_{(\alpha = 0:05, n-2)}$, then reject $H_{0.}$

The effect of female parents (maternal effect) for each variable was evaluated by using analysis of variance followed by LSD test at α =5% to analyze the difference between the value of F1 genotype and F1R.

3 RESULTS AND DISCUSSION

3.1 RESULTS

The Chlorophyll Content

Results of analysis of variance revealed that genotype have a very significant effect on the character of the chlorophyll content. The chlorophyll content is presented in Table 1.

Table 1. The chlorophyll content and heterosis and heterobeltiosis values

Genotypes	Average	Heterosis (%)	Heterobeltiosis (%)
Petek	2.14 a		
Jayawijaya	2.31 b		
Petek x Jayawijaya (F1)	2.35 c	-2,73	-4,22
Jayawijaya x Petek (F1R)	2.50 d	3,77	3

Remarks: Values followed by the same letters in the same column are not significant at the 5% LSD.

Table 1 above shows that the chlorophyll content of genotypes were significantly different. Petek variety have the highest chlorophyll content 2.50 μ mol/g, while the chlorophyll content of Jayawijaya was the lowest (2.14 μ mol/g).

The carotenoids content

The analysis of variance indicated that the genotype has a very significant effect on the character of the carotenoid content. The LSD of the character of carotenoid content is presented in Table 2.

Table 2. The content of carotenoids and heterosis and heterobeltiosis values

Genotypes	Average	Heterosis (%)	Heterobeltiosis (%)
Petek	0,91a		
Jayawijaya	0,94a		
Petek x Jayawijaya (F1)	0,90a	-2,68	-4,42
Jayawijaya x Petek (F1R)	0,97a	4,87	3

Remarks: Values followed by the same letters in the same column are not significant at the 5% LSD

Table 2 above shows that the carotenoid content was not significantly different between the varieties of Petek, Jayawijaya, Petek x Jayawijaya and Jayawijaya x Petek. The positive value of heterobeltiosis and heterosis was found at Jayawijaya x Petek (F1R).

Plant Height

The analysis of variance showed that the genotype has a very significant effect on the plant height. The LSD of the carotenoid content of character presented in Table 3.

Genotypes	Average plant height (cm)	Heterosis (%)	Heterobeltiosis (%)
Petek	67,83a		
Jayawijaya	78,08b		
Petek x Jayawijaya (F1)	77,83b	6,68	-0,32
Jayawijaya x Petek (F1R)	78,37b	7,42	0,37

Table 3. Plant height, Heterosis and Heterobeltiosis value

Remarks: Values followed by the same letters in the same column are not significant at the 5% LSD

Table 3 shows that the plant height of Petek is significantly different from those of other varieties. While the value of heterosis of both cross breeding are positive, but the value of heterobeltiosis is positive only on the cross breeding between Jayawijawa and Petek (F1R).

The number of pods per plant

The analysis of variance shows that the varieties significantly affect the number of pods per plant, but the effect is different among varieties. The average number of pods per plant is presented in Table 4.

Table 4. The number of pods per plant, Heterosis and Heterobeltiosis values

Genotypes	Average Number of pods	Heterosis (%)	Heterobeltiosis (%)
Petek	299,25a		
Jayawijaya	479,5b		
Petek x Jayawijaya (F1)	414,16b	6,37	-13,62
Jayawijaya x Petek (F1R)	479,25b	27,7	3,7

Remarks: Values followed by the same letters in the same column are not significant at the 5% LSD

Table 4 shows that the number of pods per plant of Petek is significantly lower in comparison with Jayawijaya, cross breeding of Petek and Jayawijaya and cross breeding of Jayawijaya and Petek. While the number of pods per plant of Jayawijaya, breeding of Petek and Jayawijaya and cross breeding of Jayawijaya and Petek is not significantly different. The positive values heterosis and heterobeltiosis are found in the both cross breeding (F1 and F1R). heterobeltiosis is positive only on the cross breeding between Jayawijawa and Petek (F1R).

Seed weight per plant

Analysis of variance showed that the varieties did not significantly affect the seed weight per plant. The results of LSD test at α =5% is presented in Table 5.

Table 5. The seed weight per plant, Heterosis and Heterobeltiosis values

Genotypes	Average seed weight (g)	Heterosis (%)	Heterobeltiosis (%)
Petek	65,53a		
Jayawijaya	74,49a		
Petek x Jayawijaya (F1)	62,79a	-10,31	-15,7
Jayawijaya x Petek (F1R)	72,72a	3,87	-2,37

Remarks: Values followed by the same letters in the same column are not significant at the 5% LSD

Table 5 shows that the seed weight per plant is not different significantly among all varieties. Positive vaue of heterosis is found only at cross breeding of Jayawijaya and Petek (F1R).

Weight of 100 seeds

Results of analysis of variance indicates that the varieties significantly affect the weight of 100 seeds per plant. The average weight of 100 seeds after further tested by LSD test level of 5% and the value of heterosis and heterobeltiosis presented in Table 6.

Genotypes	Average	Heterosis (%)	Heterobeltiosis (%)
Petek	9,98b		
Jayawijaya	6,72a		
Petek x Jayawijaya (F1)	6,41a	-23,19	-35,726
Jayawijaya x Petek (F1R)	6,88a	-17,6	-31,05

Table 6. The weight of seeds per plant, the value of Heterosis and Heterobeltiosis

Remarks: Values followed by the same letters in the same column are not significant at the 5% LSD

Table 6 it appears that the variable weight of 100 seed varieties Petek weight of 100 seeds showed significantly larger in comparison with varieties Jayawijaya, Petek x Jayawijaya, Jayawijaya x Petek. While varieties Jayawijaya, Petek x Jayawijaya, Jayawijaya x Petek not significantly different. The variable weight of 100 seeds, heterosis and heterobeltiosis are negative value.

Correlation

Correlation coefficient of genotype and phenotype between chlorophyll content characters and other characters who observed are presented in Table 7.

Characters	r _g	r _f	
High Plant	-0.4796 ^{ns}	-0.3559 ^{ns}	
The number of pods per plant	-0.2698 ^{ns}	-0.1871 ^{ns}	
The weight of seeds per plant	-0.0336 ^{ns}	-0.0166 ^{ns}	
Weight of 100 seeds	0.5008 ^{ns}	0.4944 ^{ns}	

Remarks: ns = not significantly different, rg = genotype correlation coefficient, rf = phenotype correlation coefficient.

Table 7 shows that there is no correlation between the genotype and phenotype of chlorophyll content and the character plant height, number of pods per plant, the weight of seeds per plant and 100-seed weight.

Characters	r _g	r _f
High Plant	0,27ns	-0,01ns
The number of pods per plant	0,42ns	-0,02ns
The weight of seeds per plant	0,71ns	0,04ns
Weight of 100 seeds	-0,12ns	-0,13ns

Remarks: ns = *not significantly different, rg* = *genotype correlation coefficient, rf* = *phenotype correlation coefficient.*

From Table 8, the results of analysis of covariance showed no correlation between carotenoid content with all the characters are observed. However, look at the variable grain weight per plant showed a greater number than the value of

another variable, the variable weight of seeds per plant is the only component results show figures close correlation with carotenoid that is with a value of 0.71.

Heterosis

Mid-parent heterosis values and High parent for the characters observed in the presented in Table 9

Table 9. Value heterosis characters observed

Characters	MP (%)	HP (%)
Chlorophyll content	-5.74	-5.74
Carotenoids content	-6.54	-6.53
High plant	9.54	1.44
The number of pods per plant	74.93	41.38
The weight of seeds per plant	22.59	20.16
Weight of 100 seeds	-27.59	-41.25

In Table 9 shows that the Mid-parent heterosis values and high positive parent present in plant height, number of pods per plant and seed weight per plant.

3.2 DISCUSSION

Soybean is self-pollinated crops thus have the homozygous genotype. Homozygous gene pair will always be homozygous when self-pollinated, self-pollination leads to an increase in homozygosity from generation to generation (Syukur *et al.*, 2002). Chlorophyll content of Petek Variety, Jayawijaya, Petek x Jayawijaya, and Jayawijaya x Petek showed significant differences with each other. F1 genotype chlorophyll content and F1R not follow the two parents. However, it appears that the genotype Petek x Jayawijaya (F1) and Jayawijaya x Petek (F1R) has an approximate value for its mother. Character chlorophyll content is controlled by the genes contained in the cytoplasm. This is in line with the statement of Rostini (2000), chlorophyll is a photosynthetic pigment found in the cytoplasm. In the character of chlorophyll suspected there was an interaction between the genes contained in the cytoplasm by genes contained in the core.

Character number of seeds per plant, plant height, and weight of 100 seeds showed that genotype Jayawijaya, Petek x Jayawijaya, and Jayawijaya x Petek was not significantly different, but the genotypes were significantly different with genotype Petek. Allegedly, character number of seeds per plant, plant height, and weight of 100 seeds is controlled by genes core with a dominant gene action perfectly. The dominant genes are thought to genotype Jayawijaya while genes contained in Petek is recessive. Another study showed that a dominant gene from one of his elders found by Susanto and Muchlis (2008), that the dominant form of taper leaf to leaf oval shape so that offspring F1dan F1R all leafy taper.

On the character number of pods per plant showed significant differences between genotypes Petek and Jayawijaya, F1 and F1 Resiprok (F1R), while F1 with F1R not significantly different. F1 genotype and F1R showed better performance than the two parents. Allegedly genes contained in F1 genotype and F1R is heterozygous at the locus-locus. The characteristics that appear on the genotype of the F1 is not a characteristic of one parent but a mixture of the two parents. On the character number of pods per plant, and pods per plant contains no dominant and recessive alleles, two alleles interact to produce a new phenotype different from the two parents (Syukur *et al.*, 2002).

F1 genotype and F1R have a high increase for the character number of pods per plant, and pods per plant that contains the results of this cross is good to use as a parent for the assembly of hybrid soybean. The increase in production variables characters are also present in Setiostono study (2008), states that there is an increase in production variables were high compared to the average of the two parents in character ear length, number of grains per ear, and weight of 1000 seeds of maize plants from crosses .

Positive heterosis values seen on plant height, number of pods per plant, number of pods per plant lists, number of seeds per plant and seed weight per plant. The highest heterosis values is in character number of pods per plant and number of pods per plant which contains increased by 77.39% MP for the character number of pods per plant and contains 74.93% for the character number of pods per plant. The highest value is also contained in the HP character number of pods per plant contains increased by 45.01%, and the character number of pods per plant were experiencing enhancements to HP value that is equal to 41.38%.

The high value of heterosis and heterobeltiosis the character number of pods per plant, number of pods per plant contains, and weight of 100 seeds showed that the results Petek x Jayawijaya has the potential to stem hybrid soybean crop. This is in line with the research of Hakim (2010), that the number of pods per plant is the main component that directly influence the outcome of green beans.

The emergence of heterosis effect caused accumulation of dominant genes, while heterobeltiosis not be separated from a more dominant effect (over-dominant) in the character of high heterosis values also showed gene action on the character of production per plant so that the hybridization technique is very useful to explore the potential for production in plants (Perez *et . al.*, 2009). The amount of heterosis in character number of pods per plant and pods per plant contains also expected to have a high affinity values on the result of crossing Petek x Jayawijaya, so support for selection in F2 generation and the next generation.

This is in line with the opinion of Frankel, (1983) in Wahyudi *et al.*, (2006) that heterosis is defined as a hybrid priorities better than the average of the two parents. Heterobeltiosis heterosis values and the character number of pods per plant and number of pods per plant contains high indicates that there are genetic differences between the two elders is large enough. According to Shing and Jain (1970) in Sujiprihati *et. al.*, (2007), a large genetic differences among elders is one of the factors that determine the expression of heterosis.

Characters of chlorophyll and carotenoids in genotypes in the test did not correlate with other characters observed, such as plant height, number of pods per plant, number of pods per plant contains, seed weight per plant and 100-seed weight. Allegedly, the absence of correlation between chlorophyll and carotenoids with yield and yield components because there is a high interaction on gene action in the cytoplasm and nucleus of cells. It can be said that the increase in chlorophyll content had no effect on yield and yield components. According to Lestari (2006), correlations between the characters can be a means of indirect selection on the main character. The chlorophyll and carotenoid character cannot be used as criteria for indirect selection on soybean genotypes from crosses Petek x Jayawijaya.

4 CONCLUSIONS AND SUGGESTIONS

4.1 CONCLUSIONS

Crosses soybean varieties of Petek x Jayawijaya and Jayawijaya x Petek has positive heterosis and heterobeltiosis values and in comparison with the parent. Crosses which has value of heterosis and the highest positive heterobeltiosis found in variety of Jayawijaya x Petek (F1R). There is no effect of the female parents on chlorophyll and carotenoid content. The content of chlorophyll and carotenoids are not correlated to all observed variables. The result showed that the grain weight per plant has higher values when compared with the value of another variable that is equal to 0.71. Grain weight per plant is the only variable that shows the approximate value correlation with carotenoids.

4.2 SUGGESTIONS

The study should be continued with varying cross in order to obtain the properties of a new plant that has a high heterosis and heterobeltiosis.

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