

## GENETIC CONTROL OF SOYBEAN RESISTANCE TO SOYBEAN POD SUCKER (*Riptortus linearis* L.)

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### ABSTRACT

*Riptortus linearis* represents the most common pod sucking pest on soybean. Genetic studies on inheritance of resistance to the pest are needed. The study used B4400 and B3802 genotypes as resistant parents, Tambora as susceptible parent. Crosses were made to form F2 population of Tambora x B4400, and Tambora x B3802. A total of 10-20 seeds each of resistant and susceptible parents, 220 F2 seeds of Tambora x B4400 crosses, and 232 F2 seeds of Tambora x B3802 crosses were grown in pots, one plant/pot. The plants were infested with adult *R. linearis* (riptortus), at the R2 stage. The results showed that the population of riptortus in each of the F2 soybean population at 7 and 15 days after infestation were >2 insects/25 plants, which enables the study of genetic resistance to soybean pod sucker. Heritability values indicated that resistance to pod sucker was controlled by genetic factors. The resistance to riptortus in B4400 and B3802 genotypes was controlled by two recessive genes located at different loci, and interacted with each other with epistatic dominant reaction. With an assumption that B and C genes were dominant, hence the resistance genes found in both soybean genotypes were *bbcc*.

Keywords: genetic resistance, soybean pod sucker *Riptortus linearis* L.

### INTRODUCTION

Currently, soybean to which the government gives priority is one of the most important food commodities after rice and maize. Soybean

consumption in Indonesia continues to increase annually, but the national production of soybean in the past 10 years (1988-2005) are still relatively low, about 1,22 million ton/year with an average productivity of about 1.18 t/ha (Sudaryanto and Swastika, 2007) although the soybean productivity based on research results reaches 3:07 t/ha (Taufiq and Wijanarko, 2007). One reason for the low productivity is the high yield losses by pests and diseases.

Pod sucker is one of the most important pests in soybean. There are three species of soybean pod suckers in Indonesia, namely *Riptortus linearis* F., *Nezara viridula* L. and *Piezodorus rubrofasciatus* (Tengkano *et al.*, 1988). All these species have piercing and sucking mouth type. These pests begin to attack soybean plant from the pods filling stage until seed ripening stage. Symptoms of the attack of the three types of soybean pod sucker are difficult to distinguish. Adult insect (imago) comes to soybean plants at flowering stage to lay eggs. Both nymph and adult pod suckers cause damage on soybean pods and seeds since the establishment of soybean pods until pod skin to dry. Signs of an attack can be seen from the former puncture of the pest mouth on the pod's skin and seed. When the pest attacked, its mouth was inserted into the pod skin and it sucked the liquid of young soybean seeds, resulting in lower soybean seed yields (Panizzi 1987; Tengkano *et al.*, 1988; Tod, 1982; Koswanudin and Djuwarso, 1997; Acle and Rolim, 1994).

Of the three soybean pod suckers, *Riptortus linearis* (riptortus) is the most important pests that can cause yield losses up to 79% (Prayogo *et al.*, 2005; Tengkano *et al.*, 1988).

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The soybean pod sucker has a similar morphology to the stinky bug of rice pest, with brownish yellow color, but it has yellowish white lines along the side of the body. This young and adult Coccinellidae attack soybean plants by sucking the liquid and the seed pod. The reproductive developmental stages of the plant defined as R3, R4 and R5 were sensitive to the pod suckers (Acle and Rolim 1994; Marwoto and Sri Hardaningsih, 2007). This insect jabbed its mouth into the pod skin and penetrated into the seed to suck the seed liquid. The pest attacks during the pod and seed development growth stage causing the pod and seed to turn flat, then dry and fall. When the attack occurs during the pod ripening stage, it generates brown spot symptoms on the pod skin and seed, resulting in losses of seed quantity and quality (Marwoto and Sri Hardaningsih, 2007).

In general, efforts to control soybean pod sucker still rely on the use of chemical insecticides. Unwise use of insecticides can cause resistance and resurgence to the insects, kill useful insects, and pollute the environments. The use of resistant varieties is an alternative to control the soybean pod sucker and they are environmentally friendly. Currently, no soybean varieties were reported resistant the soybean pod suckers. Of the 55 soybean varieties that have been released to the Indonesian farmer during the period of 1918-2009, none of them was resistant to the pest. Therefore, improvement of soybean varieties for resistance to soybean pod sucker needs to be done. In conjunction with this, knowledge about the genetics of host resistance against the pest is very important as a breeding ground in selecting the appropriate method for resistance evaluation. Currently, information on genetic inheritance of resistance of soybean to the pod sucker is still very limited.

To study the genetics of soybean resistance to the soybean pod sucker, the genetic materials needed are resistant parents, susceptible parents, as well as F1 and F2 generations from crosses between the resistant and susceptible parents. From the previous selection of soybean germplasm for resistant to the soybean pod sucker 4 resistant and 5 susceptible parents were obtained. Cross-breeding between the two resistant parents (B4400, B3802) and a susceptible one (Tambora) have also been carried out to obtain seeds of F1

and F2 generations (Purwantoro *et al.*, 2008; Purwantoro *et al.*, 2009).

Estimation of heritability values of resistance is needed to select appropriate breeding procedures in order to obtain a genetic progress (Pettr and Frey, 1966; Allard, 1960). Heritability values are needed to estimate the genetic progress due to selection. High heritability values (>50%) indicate that the effect of environment on the inherited trait is small, so the possibility of the trait derived is also high because the contribution of genetic factors to the total variability is high (Asadi *et al.*, 2003). While, low heritability value (<50 %) indicate that that the effect of environment on the inherited trait is high, so that in the soybean breeding program for pod sucker resistance is suggested to select in early generations (F<sub>3</sub> or F<sub>4</sub>) (Cardoso de Codoi and Pinheiro. 2009)

## MATERIALS AND METHODS

The research was conducted at the ICABIOGRAD, Bogor, in 2009. The materials used in the study were soybean seeds of two resistant parent genotypes (B4400, B3802), one susceptible parent (Tambora), and F2 generations of crosses of Tambora x B4400 and Tambora x B3802. Each of the resistant and susceptible parents consisted of 10-20 seeds, while the F2 generations of crosses of Tambora x B4400 consisted of 220 seeds, and F2 generations of crosses of Tambora x B3892 consisted of 232 seeds.

Each soybean seed was planted in pots containing 10 kg of soil mixture which was mixed with manure in a ratio of 3:1, 1 plant / pot placed in the screen cages. The screen cages were made of nylon fabric that is not translucent to imago riptortus. The plants were fertilized with NPK fertilizer. Pest control was done by spraying the plants with insecticide of Decis applied two weeks after planting until the plants flowered. Adult riptortus bugs were infested into the soybean plants as much as a pair of imago/25 plants at early pod formation stage (R2). The riptortus population development was observed on day 1, day 7 and day 15 after the infestation (DAI) to assess validity of the techniques that will be used to test the soybean resistance to the soybean pod sucker. Observation of resistance to soybean pod sucker on each individual plant was done after

harvest based on the percentage of soybean pod sucker attack, which is determined by the percentage of seed damages by riptortus.

Phenotypic variability in resistance to soybean pod sucker was calculated using the formula:

$$\sigma_p^2 = \frac{\sum_{i=1}^N (X_i - \mu)^2}{N}$$

where;  $\sigma_p^2$  = standard deviation of parent;  $X_i$  = resistance score of each plant to pod sucker at observation-i,  $\mu$  = mean value of resistance score,  $N$  = Number of plant observed

Heritability in a broader sense was calculated using the following formula of Chahal and Gosal (2002):

$$H^2 = \frac{\sigma_G^2}{\sigma_G^2 + \sigma_E^2}$$

where  $H^2$  = Heritability value;  $\sigma_G^2$  = genetic variance, and  $\sigma_E^2$  = environmental variance.

While the genetic variability was calculated using the formula:

$$\sigma_G^2 = \sigma_p^2 - \sigma_E^2$$

The environment variability was estimated from the environmental variability of the parent based on the formula of Suharsono and Yusuf (2009):

$$\sigma_E^2 = \frac{n_1 \sigma_{p1} + n_2 \sigma_{p2}}{n_1 + n_2}$$

where  $\sigma_{p1}$  = variance of the parent 1, and  $\sigma_{p2}$  = variance of the parent 2,  $n$  = number of observation

Data on distribution of variability of resistance to the soybean pod sucker was analyzed to determine the form of genetic resistance. Genetic ratio of the F2 plants was calculated based on the ratio of the resistant and the susceptible plants. Model of inheritance and the number of genes controlling the resistance to the soybean pod sucker was predicted on the basis of genetic ratio fit according to Fehr (1987) and Wagner *et al.* (1980).

The frequency of observation values (scores) of resistance in the F2 plant population was determined based on the percentage of seed damages using a scoring of 0-4 (Table 3), which were grouped into two, three, or four classes as follows: the grouping frequencies of susceptibility (score 0-4) in the two-class grouping resulted in score 0 = resistant and scores 1-4 = susceptible; the three-class grouping resulted in score 0 = resistant, while score 1-4 was divided into two susceptible groups, i.e., scores 1-2 = susceptible group 1 and scores 3-4 = susceptible group 2; In the four-class grouping, score 0 = resistant, and score 1-4 was divided into three susceptible groups, i.e., score 1 = susceptible group 1, score 2 = susceptible group 2, and scores 3-4 = susceptible group 3.

Suitability of genetic ratio was tested by Chi-square formula according to Gomez and Gomez (1984): Based on the genetic ratio, the number and characteristics of the controlling resistance to the soybean pod sucker can be predicted.

## RESULTS AND DISCUSSION

### Population of Soybean Pod Sucker

The population growth of riptortus in each of the F2 plant populations from the two-cross combinations of Tambora x B-4400 and Tambora x B-3802. The plants infested with riptortus and observed at 1, 7, and 15 DAI are presented in Table 1. The population of riptortus on each F2 plant populations increased sharply in each week after infestation. Pest population reached more than two insects per 25 plants, which are considered above the economic threshold. The populations of riptortus from 7 to 15 DAI was high enough, thus met the requirement as a basis for resistance evaluation to soybean pod sucker. According to Ewete and Joda (1996) the severity of seed damage of soybean is closely related to riptortus density. A population of two to eight insects per plant caused significant damage on two soybean varieties TGX 536-02D and TGX 849-294D, when compared to the non-infested check.

### Heritability

Heritability is the proportion of phenotypic variance attributable to genetic variations, which

means the amount or portion of a character is controlled by its genetical factor (Allard, 1966). The heritability value can be used to determine a selection method, and whether the selection needs to be conducted at early generation using pedigree or at late generation using bulk or SSD selection method.

In this study, the heritability value of the two cross combinations between the susceptible parent and the resistance parents, was 0.99, which is very high (Table 2). The heritability value  $>0.5$  is considered high, which means that the effect of environment on the variability of score of resistance is very low. It indicated that resistance of the soybean genotypes to the soybean pod sucker was controlled by genetic factors (resistance genes). The high heritability values indicated that the evaluation of the soybean breeding materials for resistance to the soybean pod sucker might have been done at early generation (F2 generation) by using pedigree method.

#### F2 Populations of Tambora x B4400 and Tambora x B3802

Scoring for resistance of the individual plant of the F2 soybean generation to the soybean pod

sucker was done after harvest. The frequency distribution of the F2 soybean plant populations of Tambora x B 4400 and Tambora x B 3802 which was scored based on the percentages of the soybean pod sucker was shown in Table 3, while the morphological performances of the F2 soybean plants were shown in Figure 1 and 2.

Frequency values of both F2 soybean populations based on Chi-square analysis fit to the two class grouping with ratio of 15 susceptible : 1 resistant (Table 4). Genetic ratio of 15 : 1 in the F2 soybean populations indicated that the resistance of both B4400 and B3802 soybean genotypes was controlled by an interaction of two genes which were located at different loci with a duplicate epistatic dominance. The dominant allele at both two loci masked the expression of recessive alleles at the other two loci. If the dominant resistant genes to the pest were *B* and *C*, while the recessive genes were *b* and *c*, then the interaction was gene *B* epistatic to genes *C* and *c*, and gene *C* epistatic to genes *B* and *b*. Hence, the probabilities of the combination of resistance genes in both soybean genotypes are: *B\_C\_* = 9 (susceptible), *B\_cc* = 3 (susceptible), *bbC\_* = 3 (susceptible), *bbcc* = 1 (resistance).

Table 1. Development of *Riptortus* populations in two F2 populations of soybean crosses in the screen house at 1, 7, and 15 DAI

F2 Plant Population of:	Number of <i>Riptortus linearis</i> (insects/25 plant) at:		
	1 DAI	7 DAI	15 DAI
Tambora x B-4400	8	21	20
Tambora x B-3802	7	20	47

Remarks: DAI: days after infestation.

Table 2. Variance of parents, environment, phenotype, genetic, and heritability value in two F2 populations of soybean crosses

F2 Populations	$\sigma^2_{p1}$	$\sigma^2_{p2}$	$\sigma^2_E$	$\sigma^2_{ph}$	$\sigma^2_G$	$H^2$
Tambora x B4400	29.155	18.880	4.697	1186.901	1182.204	0.996
Tambora x B3802	29.155	11.191	4.372	477.645	473.273	0.991

Remarks:  $\sigma^2_{p1}$  = variance of parent 1;  $\sigma^2_{p2}$  = variance of parent 2;  $\sigma^2_E$  = variance of environment;  $\sigma^2_{ph}$  = variance of phenotype;  $\sigma^2_G$  = variance of genetic;  $H^2$  = heritability value

Table 3. Frequency distribution of F2 populations of Tambora x B 4400 and Tambora x B 3802 based on scores of resistance to the soybean pod sucker.

Score	Plant reaction	Pest infestation (%)	Individual number of F2 population of:	
			Tambora x B 4400	Tambora x B 3802
0	Resistant	0 – 20	16	14
1	Moderately resistant	21 – 40	19	16
2	Moderately susceptible	41 – 60	36	33
3	Susceptible	61 – 80	70	78
4	Highly susceptible	>80	78	85
<b>Total</b>			<b>219</b>	<b>226</b>

Table 4. Frequency ratio of F2 population of Tambora x B4400 and Tambora x B3802 cross combinations

Phenotypic grouping	Calculated $\chi^2$ of crosses:		$\chi^2$ table	
	Tambora X B4400	Tambora X B3802		
Two-class grouping:			P = 0.05	P = 0.01
3 : 1	18.28**	21.3**	3.84	6.63
9 : 7	103.43**	113.33**		
13 : 3	7.89**	8.76**		
15 : 1	<b>0.04<sup>ns</sup></b>	<b>0.00015<sup>ns</sup></b>		
Three-class grouping:				
9 : 3 : 4	18.09**	30.11**	5.99	9.21
12 : 3 : 1	6.72**	<b>1<sup>ns</sup></b>		
9 : 6 : 1	14.34**	27.54**		
10 : 3 : 1	20.92**	23.12**		
Four-class grouping:				
<b>9 : 3 : 3 : 1</b>	<b>17.30**</b>	<b>28.60**</b>	<b>7.81</b>	<b>11.34</b>

Remarks : \* : significant; \*\* : highly significant; tn : not significant, at 5% level of significance

In addition that the F2 frequency values of the F2 populations of Tambora x B 4400 and Tambora x B 3802 were suitable for the two-class grouping (15 : 1), Tambora x B 3802 crossed was also suitable for the three-class grouping (12:3:1). Based on the probability values from the calculated Chi-square ( $\chi^2$ ), however, the  $\chi^2$  values

of the two-class grouping were lower than those of the three-class grouping (Table4). This means that the genetic inheritance of the soybean crosses of Tambora x B3802 and Tambora x B 3802 were controlled by the interactions of two epistatic dominant genes (15:1) located at different loci.



**F2: Tambora x BB440**



**F2: Tambora x BB3802**

Figure 2. Morphological performances of F2 plants of Tambora X B4400 and Tambora X B3802 in the study of resistance to the soybean pod sucker, *Reptortus linearis*. (Pod damages attacked by *riptortus* were shown by white arrows)

## CONCLUSIONS

Populations of *Riptortus linearis* in each of the F2 population of Tambora x B4400 and Tambora x B3802 cross combinations were considered sufficient to separate resistance and susceptible plant segregation.

Heritability value of the two soybean cross combination (Tambora x B4400 and Tambora x B3802) was high (0.99), meaning that the resistance to the soybean pod sucker was controlled by the genetic factors. Selection for soybean resistance to pod sucker could be done at early generation of soybean crosses.

The resistance of soybean genotypes B4400 and B3802 to pod sucker was controlled by the interaction of two epistatic dominant genes located at different loci. With an assumption that B and C genes were dominant causing susceptible to pod sucker, hence the resistance genes found in both soybean genotypes were *bbcc*.

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