



## Identification of Soybean Resistance to Pod Sucking Bug (*Riptortus linearis*) by No-Choice Test

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### Abstract

Soybean pod damaged by pod sucking bug (*Riptortus linearis*) is one of the constraint within soybean yield improvement in Indonesia. The research aimed was to identify the resistance of soybean genotypes to pod sucking pest. The experiment was conducted in Iletri's screen house from March to June 2015. The experiment was arranged in Randomized Completely Block Design using 10 soybean genotypes with three replicates. The soybean resistance was evaluated using no-choice test. Data collected on number of pod/plant, number of seed/plant, seed weight per plant, number of attacked pod/plant, and number of attacked seed/plant. The result showed that the lowest percentage both of pod and seed damage was G511H/Anjasmoro//Anjasmoro-2-8, that was 25.83 % and 19.12 %, respectively. Based on the value of the percentage of seed damage, there were five susceptible genotypes, three moderately resistant, and two resistant genotypes. Based on the value of the percentage of pod damage, showed four susceptible genotypes, five moderately resistant, and a resistant genotype. G511H/Anjasmoro//Anjasmoro-2-8 was the only resistant genotype, and it could be used as a genetic source in the improvement of soybean resistance to pod sucking bug.

### How to Cite

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## INTRODUCTION

The largest soybean cultivation areas in Indonesia are in the dry season. The abundant light and relatively stable of high temperatures in the dry season causes high infestation of pests in soybean. Pod sucking bug is a major pest in soybean production areas in Indonesia. This pest has spread over almost all provinces in Indonesia and cause considerably yield losses both in soybean quality and quantity. Pod sucking bug has the most extensive spread area and attacks compared to other soybean pests. In Ngawi (East Java), pod sucking population reported to be very high, reaching 896 adult/625 m<sup>2</sup> area (Bayu et al., 2012). Similarly, the survey population of pod sucking bug in areas of East Java and Lampung provinces showed the extent of this pest attack (Yusmani & Suharsono, 2005).

Seed damaging by pod sucking on soybean plants occurred since the pods formation until seed ripening stage (Naito, 2008). Adult of pod sucking bug *R. linearis* was reported to have a greater ability to damage the pods and seeds than the nymph (Susilo, 2007). Adult attack soybean seed by piercing the pod shell and sucking out the liquid nutrients inside the seed. Pod sucking infestation characterized by brown spots on the seeds or on the inside pod wall (Bayu & Tengkan, 2014). As a result, pods and seeds become deflated, followed by dry and fall, wrinkled seeds, black seed rot, black mottled seeds, and perforated seeds (Bayu, 2015).

The most vulnerable reproductive developmental stages of the soybean plant against pod sucking bug is the stage of R3 (pod formation), R4 (full pod formation), and R5 (beginning pod filling) (Bae et al., 2014). Attacks on seed and pod maturity phases by *Riptortus* sp. resulting in reduction the seed quality and vigor, and potentially reduce seeds germination by 5-58% (Bae et al., 2014). Other studies have also revealed that the pod sucking attack on pod maturity phase causes increased seed damage which resulting in delayed maturity (Leonard et al., 2011). The accumulation of losses caused by the pod sucking pest infestations may decreased soybean yield up to 79% (Arifin & Tengkan, 2008), and can result in crop failure if it is not well controlled (Marwoto, 2007).

Various efforts to control soybean pod sucking pest has been performed. However, in fact, the intensive use of chemical insecticides are still become main priority by farmers due to the more practical and immediate results, although it was widely known that the unwise application

can lead to several problems, such as pest resistance and resurgence, killing non-targets insects, environmental pollution, and potentially hazardous to humans (Panizzi, 2013). The use of resistant varieties is one of the mainstays of integrated pest management, and become alternative control method which is proved to be cost-effective, sustainable, and environmentally friendly.

Since the resistance to pod sucking bug was controlled by genetic factors (Cardoso de Codoi & Pinheiro, 2009; Asadi et al., 2012), the evaluation of resistance to pod sucking bug has been performed and obtained MLG 3032, IAC 80 and IAC 100 as resistant genotypes, and has been used as genes source in soybean crosses (Adie & Krisnawati, 2009). Most of the resistant genotypes have been used as parents in the development of soybean varieties and also has produced three genotypes classified as moderately resistant (B/100-47-678-764 IAC, IAC 100/K-5-1037-1062, and K/IAC 100-997-1035T), and a resistant genotype, namely IAC 100/K-60-1092-1141 (Krisnawati & Adie, 2015). The research aimed was to identify the resistance of soybean genotypes to pod sucking pest (*Riptortus linearis*) using no-choice test.

## METHODS

Rearing of pod sucking bug *R. linearis* was in the Laboratory of Entomology, Indonesian Legumes and Tuber Crops Research Institutes (ILETRI). Imago of *R. linearis* were collected from soybean fields and reared in a rearing cages (40 × 40 × 40 cm) that was ventilated using a fine mesh on its lateral sides. Soybean plants grown in a screen house and soybean seeds were provided as food sources. Newly emerged adult *R. linearis* were collected and transferred to another cage.

The experiment was conducted in Indonesian Legumes and Tuber Crops Research Institutes (ILETRI)'s screen house from February to June 2015. The research materials consisted of ten soybean genotypes (G511H/Anjasmoro//Anjasmoro-2-8, G511H/Arg//Arg//Arg//Arg-12-15, G511H/Anj //Anj//Anj-6-3, G511H/Arg//Arg//Arg//Arg-19-7, G511H/Anjasmoro-1-7, G511H/Anj//Anj//Anj//Anj-6-7, G511H/Anjasmoro-1-4, Anjasmoro, Grobogan, and G100H). The experiment was arranged in Randomized Completely Block Design using 10 soybean genotypes with three replicates. Each genotype was planted in pot (Φ =18 cm) containing 10 kg of soil which was mixed with manure in a ratio of 4:1, two plants/pot. Planting was arranged such a way that the flowering time

of 10 soybean genotypes can be simultaneously. Each pot was fertilized with NPK fertilizer 5 g/pot. Pests and diseases were optimally controlled until flowering time, and after that the pest and disease were uncontrolled.

The resistance evaluation was use no-choice test. After 50 days after planting (DAP), each pot was enclosed in the screen cage. The screen cages were made of nylon fabric that is not translucent to adult *R. linearis*. A pair of newly emerged adult *R. linearis* were infested into each plant within screen cage at R4 stage of the soybean. Parameter measured on number of pod/plant, number of seed/plant, seed weight per plant, number of attacked pod/plant, and number of attacked seed/plant. Data were subjected to analysis of variance (ANOVA), and continued with DMRT at 5% significance level.

The general linear model test was used to find the correlation between pod characters. The damage intensity was calculated as follows:

$$\text{Pod damage} = \frac{\text{Number of pod damage}}{\text{Number of total pods}} \times 100\%$$

$$\text{Seed damage} = \frac{\text{Number of seed damage}}{\text{Number of total seeds}} \times 100\%$$

The criterion of resistance following method by Chiang dan Talekar (1980):

$x > x + 2SD$  = HS (Highly Susceptible)

$x > x > x + 2SD$  = S (Susceptible)

$x > x > x - 1SD$  = MR (Moderately Resistant)

$x - 1SD > x > x - 2SD$  = R (Resistant)

$x < x - 2SD$  = HR (Highly Resistant)

## RESULT AND DISCUSSION

Analysis of variance showed that number of attacked pod/plant, number of attacked seed/plant, pod damage, and seed damage were significantly different between genotypes (Table 1). Number of attacked pod/plant ranged from 9 to 34 pods (average 17.05 pods/plant), and number of attacked seed/plant ranged from 15 to 55 seeds, with an average 29.18 seeds/plant.

The percentage of pod and seed damage along with the resistance criteria was presented in Table 2. The pod damage of 10 soybean genotypes varied from 25.83% (resistant) to 63.80% (susceptible), with average of 40.69%. Based on the percentage of pod damage, there were four susceptible genotypes, five genotypes as moderately resistant, and one genotype categorized as resistant to pod sucking bug. Soybean genotype G511H/Anjasmoro//Anjasmoro-2-8 had the lowest percentage of pod damage, hence, it was

categorized as resistant.

The highest pod damaged was showed by Anjasmoro variety. The susceptibility of Anjasmoro to pod sucking bug has been confirmed in previous researches (Hendriwal et al., 2013; Krisnawati & Adie 2015). Although it was indicated susceptible to pest attacks, however, Anjasmoro is one of the popular variety that are currently gained much attention and have high seed demand by farmers, due to its high-yielding and suitable for raw materials of tofu and tempeh. Grobogan variety is also popular variety, and categorized as moderately resistant to pod sucking bug with pod damage 29.12%, but it was reported as susceptible to rust disease (Inayati & Yusnawan 2016). So far, G100H genotype had been considered as resistant to various soybean foliar insect pest and pod feeders (Adie et al., 2003; Maulidah 2006; Bayu 2015), but in this study, G100H showed to be to be susceptible to pod sucking bug, with the damage pod as 52.85%. Presumably, the genotypes used in this research have higher resistance than G100H. Study of resistance to pod sucking bug using no-choice test by Adie and Krisnawati (2016), has obtained percentage of damage pods ranged from 23.92% to 73.61%.

The percentage of seed damage ranged from 19.12% to 7.33% (Table 3). Based on seed damage, a total of five genotypes categorized as susceptible to pod sucking bug, three genotypes categorized as moderately resistant, and two genotypes as resistant.

The highest seed damage were found on Anjasmoro and G100H, which are 7.33% and 50.03%, respectively. The lowest seed damage was found on G511H/Anjasmoro//Anjasmoro-2-8 (19.12%). Another resistant genotype was G511H/Arg//Arg//Arg//Arg-19-7, with the seed damage 23.35%. A study on the resistance of soybean germplasm to pod sucking bug by Bayu (2015) obtained the range of seed attack from 0.54-70.78%. Other study reported a high variability on seed and pod attacks, ranging from 19-73% for pod damage, and 11-56% for seed damage (Asadi 2009). Pod sucking bugs cause injury to soybean seeds as they insert the stylets through the pod wall into the seed for feeding on plant juices.

In this study, if the pod sucking bug resistance is based on the intensity of damage pods and seeds, then the consistently resistant genotype was G5111/Anjasmoro//Anjasmoro-2-8. Evaluation of soybean resistance to pod sucking bug *R. linearis* by Krisnawati & Adie (2015) obtained three moderately resistant genotypes and a resistant genotype. Variations in the severity

**Table 1.** Analysis of variance on pod characters and pod sucking attack of 10 soybean genotypes. 2015.

Parameter	Mean Square		CV (%)
	Replication	Genotype	
Number of pod/plant	180.658333 <sup>ns</sup>	277.130556 <sup>ns</sup>	31.05
Number of seed /plant	722.633333 <sup>ns</sup>	1108.52222 <sup>ns</sup>	31.05
Seed damage	3.565290 <sup>ns</sup>	554.647826 <sup>**</sup>	32.96
Pod damage	65.020543 <sup>ns</sup>	520.790074 <sup>*</sup>	34.44
Seed weight/plant	3.30596083 <sup>ns</sup>	6.90660222 <sup>ns</sup>	14.13

CV= coefficient of variation; \* = significant at 1 % probability level ( $p < 0.01$ ), \*\* = significant at 5 % probability level ( $p < 0.05$ ), ns = not significant

**Table 2.** Percentage of pod damage and resistance criteria of 10 soybean genotypes. 2015.

Soybean genotype	Pod damage (%)	Resistance criteria
G 511 H/Anjasmoro//Anjasmoro-2-8	25.83 c	Resistant
G 511 H/Arg//Arg///Arg///Arg-12-15	34.58 bc	Moderately Resistant
G 511 H/Anj//Anj///Anj-6-3	48.19 ab	Susceptible
G 511 H/Arg//Arg///Arg///Arg-19-7	28.64 bc	Moderately Resistant
G 511 H/Anjasmoro-1-7	30.96 bc	Moderately Resistant
G 511 H/Anj//Anj///Anj///Anjs-6-7	38.58 abc	Moderately Resistant
G 511 H/Anjasmoro-1-4	54.39 ab	Susceptible
Anjasmoro	63.80 a	Susceptible
Grobogan	29.12 bc	Moderately Resistant
G100H	52.85 abc	Susceptible
Average	40.69	

Numbers followed by the same letter in the same column are not significantly different at DMRT 5%.

**Table 3.** Percentage of seed damage and resistance criteria of 10 soybean genotypes. 2015.

Soybean genotype	Seed damage (%)	Resistance criteria
G 511 H/Anjasmoro//Anjasmoro-2-8	19.123 c	Resistant
G 511 H/Arg//Arg///Arg///Arg-12-15	24.927 bc	Moderately Resistant
G 511 H/Anj//Anj///Anj-6-3	39.610 abc	Susceptible
G 511 H/Arg//Arg///Arg///Arg-19-7	23.357 bc	Resistant
G 511 H/Anjasmoro-1-7	24.550 bc	Moderately Resistant
G 511 H/Anj//Anj///Anj///Anjs-6-7	38.703 abc	Susceptible
G 511 H/Anjasmoro-1-4	44.637 ab	Susceptible
Anjasmoro	57.330 a	Susceptible
Grobogan	20.203 c	Moderately Resistant
G100H	50.030 a	Susceptible

Numbers followed by the same letter in the same column are not significantly different at DMRT 5%.

of damage depends on various factors, such as the pest population density, plant growth stages, pest developmental stage, plant genetic/response of plants to pests, and pest control management (Ewete & Joda 1996; Lourençao et al., 2010; Depieri & Panizzqi 2011). Kim & Lim (2010) reported that the timing and pattern of pod sucking bug occurrence in soybean is closely related to

the plant stage, and reached the maximum density when soybeans were in R4–R5 stage and the R5–R6 stage (Lee et al., 2004; Bae et al., 2015). The economic threshold (ET) different between countries. The current recommended ET for controlling sucking bugs in Brazil is 2 insects per meter for seed production and only 1 per meter for crop seeds (Bueno et al., 2013). In Mississippi,

the ET is higher than the one adopted in Brazil, at 3.3 per meter (Catchot, 2008). In Indonesia, Susilo (2007) stated that the population density of four adults of *R. linearis* at plant stage of R3-4, R5-6 and R7-8 cause the highest pod and seed damage, and also produced the lowest soybean yields. Therefore, the presence of adult *R. linearis* before reaching four adults in soybean planting area, already need attention before it reaches the economic threshold.

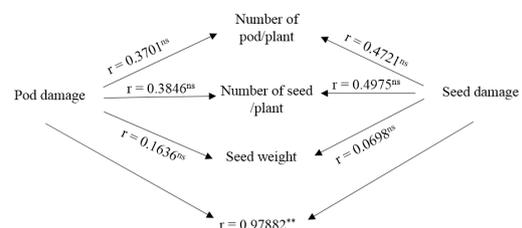
Referring to the intensity of pod damage and seed damage, G511H/Anjasmoro//Anjasmoro-2-8 was consistently resistant. Genotype which classified as susceptible based on pod damage, was also susceptible based on seed damage. Genotype G511H/Arg//Arg//Arg//Arg-19-7 classified as moderately resistant based on pod damage, but showed resistant reaction based on seed damage. This indicated that G511H/Arg//Arg//Arg//Arg-19-7 has antibiosis resistance, whereas the resistance of G511H/Anj//Anj//Anj//Anjs-6-7 is antixenosis.

Soybean plants differ in resistance to insect pests, and expressed as a combination of antibiosis (toxicity) and antixenosis (nonpreference) (Moraes 2005). The mechanism of resistance in G511H/Arg//Arg//Arg//Arg-19-7 was antibiosis, that is insect resistance in which feeding on this genotype will results in mortality or disruption of growth, development, or physiology in the insect. Antibiotic effects on insects can vary widely, and may result from morphological and chemical plant factors (Smith, 2005). Although chemicals are the most common causes for antibiotic effects, plant structures like trichomes may also directly affect the physiology of insects in a negative way (Traw & Dawson, 2002; Handley et al., 2005). Dicke & Hilker (2003) reported that the plant defense against insect herbivores consists of direct defense which include trichomes (hairs on the plant), production of primary metabolites (protein inhibitor and an antioxidant enzyme), and the production of secondary metabolites (phenolic acids and isoflavones). In addition, the plant has indirect defense is through the production of volatile organic compounds such as methyl salicylate and cis-jasmone to attract natural enemies thus insect will avoid that plant (Courtois et al., 2009; Michereff et al., 2011).

Meanwhile, the G511H/Anj//Anj//Anj//Anjs-6-7 was showed antixenosis, or nonpreference, that is resistance in which the insect is either repelled from or not attracted to its normal host plant. Antixenosis can be considered as the first defensive line in plants against insect damage (Smith, 2005), and considered as

an important component of resistance in soybean against pod sucking bug, as this can deter or delay sucking bug colonization and reduce the potential of infestations reaching economically injurious levels (Kennedy et al., 1987; Webster & Inayatullah, 1988). According to Smith and Clement (2012), Antixenosis is effective mechanism where the target insect will dead because they are lack of food. In contrary, Herrera (2012) stated that antixenosis might be a poor defense for plant in current agricultural practice where monoculture predominates, since insects deprived of their preferred host may eventually accept a less preferred one. However, previous research found three soybean lines (PI 171451, PI 227687, and PI 229358) showed resistance to multiple pests through antixenosis and antibiosis (Van Duyn et al., 1971).

Morphology character of soybean pod was reported to play a role in resistance mechanisms against pod sucking bug *R. linearis*. Correlation between seed and pod components with damage intensity illustrates in Figure 1. Both of seed and pod damage were not affected by the number of pods, number of seeds and seed weight. This is in contrast with the results of research by Maulidah (2006) which obtain a relatively high correlation between the damage intensity with seed weight, in which a higher the damage intensity will tend to decrease the seed weight.



**Figure 1.** Correlation between pod and seed characters and damage intensity.

$r$  = coefficient of correlation, ns = not significant, \*\* = significant at 5 % probability level ( $p < 0.05$ ).

The positive correlation of these two variables indicate that as the intensity of damage pods increases, the intensity of seed damage increases. Various studies have reported that the morphological characters of pods play an imperative role in soybean plant defense against pests (Ha-qul et al., 2003; Suharsono & Sulityowati, 2012). Stated pod characteristics (wall thickness, hairiness, and hardness) may directly affect stink bug *R. linearis* feeding. Furthermore, the pod thickness had significantly able to reduce the attacking level of pod sucking pest. According to Norris & Kogan (1980), morphological and physiological fac-



**Figure 2.** Screening of soybean resistance to pod sucking bug: (a). Soybean plants in screenhouse with different sowing time for simultant flowering time; (b) Soybean plants before enclosed in screen cage; (c) Screening soybean to pod sucking bug using no-choice test.

tors in plants may interfere with the mechanism of host selection, feeding, ingestion, digestion, mating, and oviposition and used insect pest. However, in this study, it appears that the morphology of pods did not significantly affect on pod sucking bug's attack, which is indicated by a positive correlation between the intensity of pod damage with the intensity of seed damage. But, this fact gives the possibility that the selection of soybean resistance to pod sucking bug is could be performed based on the intensity of pod damage.

## CONCLUSION

The morphological pod characters such as number of pod/plant, number of seed/plant, and seed weight, were may not contribute to the soybean resistance to pod sucking bug. Soybean resistance to pod sucking bug may exhibit antibiosis, and or antixenosis resistance. Genotype G511H/Anjasmoro//Anjasmoro-2-8 is resistant to pod sucking bug *Riptortus linearis* based on both of pod and seed damage.

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