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Evaluation of Soybean Resistance to Pod-Sucking Bug, *Riptortus linearis* and Performance of its Agronomic Characters

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INTRODUCTION

Indonesia's tropical climate, not only suitable for the growth of soybean plants, but also an ideal environment for the growth of various insect pests. Moreover, the largest soybean cultivation in Indonesia is in the dry season. When the rainfall is reduced, and followed by high temperatures, will further increase the explosiveness of various pests, from the juvenile phase to the maturing phase.

Pod-sucking pest, *Riptortus linearis*, is the most harmful pest among all, both for quantity and quality of soybean (Asadi, 2012; Bayu, 2015), and so far, there are no soybean varieties in Indonesia that are resistant to this pest. The pod-sucking pest problem is not only happening in Indonesia, but also in some soybean production centres in the world such as America, Japan, and Brazil (Jones & Sullivan, 1978). The yield losses due to pod sucker attacks in Indonesia was more than 70% (Tengkano *et al.*, 1988; Prayogo & Suharsono, 2005) and in Africa it was reported between 20 -100% (Singh & Allen, 1980). The amount of losses due to pod-sucking pest depends on the susceptibility of the plants and plant growth phases (Abudulai *et al.,* 2012; Bayu, 2015). In stink bug complex pests, de Godoi & Pinheiro (2009) reported that some factors play roles in the resistant of soybeans against pod-attacking stink bugs, such as the seed filling period, leaf retention, percentage index of pod damage, and percentage of spotted seeds. Thus, efforts to minimize the yield losses by using insecticides can be made before and during the growth phase.

Chiang & Talekar (1980) was grouping the soybean pests based on its attack, i.e. stem feeders, leaf feeders/foliar insects, and pod feeders. The soybean pod feeders are grouped into pod-borer (*Etiella zinckenella*) and pod-sucker (*Riptortus* sp, *Nezara viridula*, and *Piezodorus hybneri*). In Indonesia, pod-sucking pests are considered more important than other pod-attacking pests complex, because it has a widespread area, even during the rainy and dry season. In Bangladesh, it was also reported that these types of pod sucking pest, *Riptortus pedestris* (Fabricius) & *Halyomorpha halys* (Stal) were also major pod pests on soybean plants (Rahman & Lim, 2017). Losses in crop yields due to pod-sucking pest infestations not only reduce the productivity per unit area, but also reduced the seed vigor (Bae *et al.*, 2014) as a result of imperfect seed formation or seed damage.

Genetic modification to increase the resistance of soybean plants to pests, begins with an

understanding of the determinants of resistance, which is possessed by each plant. So far, there are three types of plant genetic resistance, including antibiosis, antixenosis (chemical and morphological), and tolerant. According to Smith (1989), morphological antixenosis is associated with morphological characters that could potentially be an antidote through the interruption of the process of eating and eggs laying. Various studies have shown that pod trichome characters as morphological factor were potentially be the determinants of resistance to insect pests (Hare & Elle, 2002; Traw & Dawson, 2002; Kitayama *et al.*, 2010), including on pod sucking pest (Maulidah 2006; Suharsono & Sulistyowati, 2012). The denser and longer trichomes were presumably interfered with the stylet piercing of the pod shell.

Identification of soybean resistance against pod sucking pest has been done in several production centres of soybean. In Indonesia, genotype of MLG 3032, IAC 80, and IAC 100 were reported to be resistant to pod sucking pests (Tridjaka *et al.*, 1991; Suharsono, 1996), and it was found that the resistance of IAC 80 and IAC 100 was due to the trichome factors that present in the pods wall (Suharsono, 1997). The IAC 100 genotype is also reported to be resistant to several insect species on soybean crops (Pinheiro *et al.*, 2005). In cowpea, Olatunde et al,. (2007) reported that the genotype of IT86D-716 was resistant to the pod sucking bug (*Clavigralla tomentosicollis*), and its resistance exhibited both non-preference and antibiosis resistance mechanisms. The presence of soybean resistance gene sources against pod sucking pests provides an opportunity to obtain a soybean genotype that has a resistance to pod sucking bug. As stated by Krisnawati *et al.*, (2016) that knowledge of the mechanism of resistance will doubtlessly help in the development of soybean variety with improved pest resistance, especially for *R. linearis*, and may result in reduced insecticide use. Since the soybean resistant variety to pod sucking bug is not yet available in Indonesia, the resistant genotype that may be obtained from this study will be useful as gene source in soybean breeding program, or can be released as new variety. The objective of the study was to mapping and classifying the resistance of some soybean genotypes against pod sucking pests.

METHOD

The study was conducted in Ngawi (East Java, Indonesia) during the dry season (July to October) 2016. The research material consisted of 21 soybean genotypes and three check vari-

eties of Grobogan, Anjasmoro and Argomulyo. The experiment was arranged in a randomized block design with five times repetition and two types of environment. The first environment (L1) consisted of full insecticide control from 10 days after planting until the time of harvest. The second environment (L2) was controlled until 50 days after planting. The land used was wet land, formerly planted rice plants, and without the soil processing. Before planting, the land should be clean from weeds. The planting system was used individually planted (tugal) direct seeded, with 2-3 seeds per hill. Seeds were treated with insecticide to prevent bean flies. Pest and disease control was done optimally. Drainage was made to keep the soil moisture optimally. Fertilization with 250 kg Phonska and 100 kg SP_{36} or other compound fertilizers with qualized dose, that were applied after sowing the seeds. Maintenance includes weeding and watering were carried out intensively. Harvesting was done when 90% of the pods have matured.

The intensity of pod sucking attack pest was observed based on five randomly sample plants. Observations included the number of healthy pods and seeds, as well as the number of pods and seeds attacked by pod sucking pests.

The damages intensity were determined as follows :

Pod damage $(\%)$ = Number of pod damage x 100 Number of total pods Seed damage (%) = Number of seed damage x 100 Number of total seeds The grouping of pod-sucking pest resistance follows a method by Chiang & Talekar (1980): $x > \bar{x} + 2SD$ = HS (Highly Susceptible) $\bar{x} > x > \bar{x} + 2SD = S$ (Susceptible)
 $\bar{x} > x > \bar{x} - 1SD = MR$ (Moderately $= MR (Modernately Resistant)$ \bar{x} -1SD $> x > \bar{x}$ -2SD = R (Resistant)
 $x < \bar{x}$ -2SD = HR (Highly Resi $=$ HR (Highly Resistant) with $x =$ seed/pod damage \bar{x} = general mean $SD =$ standard deviation Observations of agronomic characters

observed on five sample plants which taken randomly were including the days of flowering, days to maturity, plant height, number of branches, number of nodes, and 100 seeds weight.

RESULT AND DISCUSSION

The damage intensity

The research location was a soybean production centre that cultivating soybean plants in June/July to September/October, This location is also an endemic area of pod pests especially

pod sucking pest. At the time of the study, the natural population of pod sucking pest was very high, whereas the other types of pod-damaging pests were low. The high-level of the natural population of pod-sucking pests was indicated by high levels of damage on insecticide-controlled environments. The intensity of damaged pod by pod sucking pests in insecticide-controlled environments was 41.45% and in insecticide-free environments after the plant reach 50 days after planting (second environment) reached 60.16%. The average intensity of damaged seeds in the controlled environment reached 20.10% and in a non-controlled environment after the age of 50 days after planting was 23.08% (Table 1).

The average intensity of pod sucking attacks on pod wall was higher than the intensity of damage found in seeds. Previous research also reported similar results with this study (Krisnawati *et al.*, 2006; Maulidah, 2006). The intensity of damaged pod was calculated based on the number of punctures appears on the pod wall. The insect pest had actively punctured the pod, and when there was a mechanical barrier in the pod, then each insect will move on to another pod to obtain the most appropriate feed. In the full-controlled environment, the number of punctures in the pods was 28.88%, which was higher than the number of punctures on the seeds. In the second environment, the puncture in pods was 50.36%, also higher than those of found on the seeds.

The mapping of pod damage intensity on the non-controlled environment ranged between 34.46 - 76.82% with seed damage intensity ranged between 11.72 - 42.31% (Table 1). Genotype G511H/Anj//Anj-2-8 consistently has the lowest pod and seed damage intensity in controlled environments. In non-controlled environment, genotype of G511H/Anjs/Anjs-1-3 had a lower seed damage intensity (11.72%) than G511H/ Anj//Anj-2-8 (14.88%). The pod damage intensity of G511H/Anj//Anj-2-8 and G511H/Anjs/ Anjs-1-3 were 34.46% and 39.25%, respectively. These facts indicate a difference in the resistance mechanisms of these two soybean genotypes. The resistance of the genotype G511H/Anj// Anj-2-8 may be due to physical factors present in pod wall, meanwhile the type of resistance found in G511H/Anjs/Anjs-1-3 is probably due to physical factors lying in the seed.

The grouping of resistance

The resistance of 24 soybean genotypes to pod sucking pest is grouped into highly resistant, resistant, moderate, susceptible, and highly susceptible. The resistance measurement in each resistance group are shown in Table 1 and the number of genotypes in each resistance group is shown in Table 2.

Based on the pod damage intensity in a non-controlled environment, it was successfully obtained a highly resistant genotype (G511H/ Anj//Anj-2-8) and a resistant genotype (G511H/ Anjs/Anjs-1-3) to pod sucking bug. In full controlled environment, it was obtained two genotypes classified as resistant genotypes (G511H/ Anj//Anj-2-8 and Grobogan). The G511H/ Anj//Anj-2-8 genotype was consistently resistant to pod sucking bug in both environments. Grobogan was classified as resistant genotype in controlled environment and genotype G511H/Anjs/ Anjs-1-3 was classified as resistant genotype in non-controlled environment.

The grouping of genotype resistance based on the seed damage intensity in a non-controlled environment showed four genotypes classified as resistant genotypes, and in the controlled environment, there were three resistant genotypes. Based on seed damage intensity, there were two genotypes (G511H/Anj//Anj-2-8 and G511H/ Anjs/Anjs-5-5) consistently resistant in the both environments.

Plant resistance to herbivory insects is divided into two forms of defense: direct and indirect defense. The direct defense are in form of structural components (e.g. pod wall thickness, trichomes), production of primary metabolites (e.g. protease inhibitors, antioxidant enzymes) and non-volatile secondary metabolites. The indirect defense group is related to the production of volatile organic compounds (Dicke & Hilker, 2003; Courtois *et al.*, 2009). A research by Wang *et al.*, (2007) found that characters of pod thickness have an important role in controlling soybean resistance against pod pests. In addition to the thickness of soybean pod wall, the characters of heavy pod wall and the ratio of pod wall to total pod also need to be considered as a determinant factor of soybean resistance to pod sucking pest (Guang-Yu el al., 2011). Different studies have reported that a shorter filling periods, a small percentage of seeds affected, and a small seed size are likely to be a measurement of soybean resistance to pod sucking pests (Moura & Pinheiro, 2002; Pinheiro *et al.*, 2005). In a leaf pests, it was reported that the morphological character of the leaf area and the density of trichome on the leaves were also reported to be the important characters of resistance in a leaf pest complexes (Ul-Haq *et al.*, 2003).

Based on the intensity of pod and seed damage, it appears that the genotype of G511H/ Anjasmoro//Anjasmoro-2-8 is consistently resistant in the non-controlled environment as well as in the full controlled environment. Meanwhile, the genotype of G511H/Anjs/Anjs-1-3, showed consistent resistance in the non-controlled environment. Thus, both soybean genotypes are considered to be resistant to pod sucking bug.

Figure 1. Soybean seed and pod damages (%) in controlled (L1) and non-controlled environments (L2).

Figure 2. Soybean attacked by pod sucking bug: (a) soybeans' damaged pod in the field, (b) soybean pod and pod sucking bug, *Riptortus linearis*, F, (c) healthy and damaged soybean seed, $X =$ damaged seed, $Y =$ healthy seed.

Agronomic characters

Farmers' preferences for the characteristics of soybean varieties in addition to having high yields, resistant to certain biotic or abiotic stresses, is having agronomic characteristics in accordance with Indonesia's tropical agroecology.

The days to flowering of 24 genotypes in the controlled environment ranged from 30-34 days (an average of 32 days) and in a non-controlled environment ranged from 28-34 days (an average of 31 days). The days to maturity ranged from 77 to 85 days (an average of 80 days) in controlled environments and between 77 - 87 days (average 81 days) in an environment without any control. The effect of controlling with insecticides did not affect the maturity of the tested soybean genotype.

Nowadays, one of the user preference for soybean in Indonesia is variety with early days to maturity (<80 days). The G511H/Anj/Anj-2-8 and G511H/Anjs/Anjs-1-3 which are considered as resistant to pod sucking bug, had average days

Table 1. Percentages of pod damage and seed damage by pod sucking bug in controlled and noncontrolled environments on 24 soybean genotypes. 2016.

	Pod damage (%)			Seed damage (%)		
Genotype		L2	Average	L1	L2	Average
G511H/Anjs/Anjs-2-13	36.05	56.60	46.32	15.50	24.90	20.20
G511H/Anjs-1-1	37.65	67.74	52.70	17.35	24.36	20.85
G511H/Arg//Arg///Arg-30-7	40.71	63.10	51.90	16.82	15.64	16.23
G511H/Kaba//Kaba///-4-4	41.13	66.92	54.03	20.15	23.00	21.58
G511H/Kaba//Kaba///Kaba////Kaba 16-2	68.40	56.08	62.24	39.79	25.47	32.63
G511H/Anjs/Anjs///Anjs-3-3	33.89	60.53	47.21	17.08	19.97	18.53
G511H/Anjs/Anjs///Anjs-6-13	45.84	65.70	55.77	25.93	22.53	24.23
G511H/Anjs/Anjs-1-2	37.25	58.75	48.00	16.85	24.13	20.49
G511H/Anjs/Anjs-5-5	35.59	52.06	43.83	13.41	16.70	15.06
G511H/Anjs/Anjs///Anjs-6-11	40.92	56.30	48.61	18.99	21.26	20.12
G511H/Anjs/Anjs///Anjs-8-1	38.84	55.24	47.04	19.36	22.51	20.93
G511H/Anjs/Anjs-1-3	33.48	39.25	36.36	17.11	11.72	14.41
G511H/Anjs/Anjs///Anjs-6-12	44.15	99.88	72.02	20.54	42.31	31.42
G511H/Anj//Anj///Anj////Anjs-6-8	35.09	69.44	52.27	16.37	23.90	20.14
G511H/Anj//Anj-2-8	31.81	34.46	33.14	10.88	14.88	12.88
G511H/Arg//Arg///Arg///Arg-12-15	45.13	48.24	46.69	22.86	19.94	21.40
G511H/Anj//Anj///Anj-6-3	41.07	48.40	44.74	18.46	24.99	21.73
G511H/Arg//Arg///Arg///Arg-19-7	39.21	68.85	54.03	18.29	21.71	20.00
G511H/Anjasmoro-1-7	47.89	68.35	58.12	24.35	28.05	26.20
G511H/Anj//Anj///Anj////Anjs-6-7	44.71	58.31	51.51	24.41	25.42	24.92
G511H/Anjasmoro-1-4	49.67	58.38	54.03	24.10	27.45	25.78
Grobogan	32.11	62.78	47.44	14.21	19.35	16.78
Anjasmoro	59.87	76.82	68.35	31.61	35.44	33.53
Argomulyo	34.43	51.65	43.04	17.89	18.26	18.08
Average	41.45	60.16	50.81	20.10	23.08	21.59
Standard deviation	8.31	12.37	8.39	5.91	6.14	5.19

 $\overline{L1}$ = full insecticide control;

 $L2 =$ insecticide control up to 50 days after planting

 $L1 = \text{full}$ insecticide control;

 $L2$ = insecticide control up to 50 days after planting

Resistance criteria	Pod damage $(\%)$		Seed damage (%)		
	T .1	ΤЭ		I .2	
Highly resistant					
Resistant	2				
Moderately resistant	15	11	12	g	
Susceptible	6			q	
Highly Susceptible					

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Table 3. Number of genotype on each resistance criteria to pod sucking bug. 2016.

 $L1 = \text{full}$ insecticide control,

 $L2$ = insecticide control up to 50 days after planting

Table 4. Days to flowering and days to maturity of 24 soybean genotypes. 2016.

Genotype		Days to flowering (day)		Days to maturity (day)		
		L2	Average	L1	L2	Average
G511H/Anjs/Anjs-2-13	33	31	32	77	81	79
G511H/Anjs-1-1	32	31	32	81	85	83
G511H/Arg//Arg///Arg-30-7	31	32	31	78	83	80
G511H/Kaba//Kaba///-4-4	31	31	31	83	87	85
G511H/Kaba//Kaba///Kaba////Kaba 16-2	31	31	31	83	84	84
G511H/Anjs/Anjs///Anjs-3-3	30	30	30	78	79	79
G511H/Anjs/Anjs///Anjs-6-13	31	32	31	78	80	79
G511H/Anjs/Anjs-1-2	34	34	34	80	82	81
G511H/Anjs/Anjs-5-5	31	31	31	84	87	85
G511H/Anjs/Anjs///Anjs-6-11	32	33	32	79	79	79
G511H/Anjs/Anjs///Anjs-8-1	32	32	32	79	82	81
G511H/Anjs/Anjs-1-3	31	31	31	81	82	82
G511H/Anjs/Anjs///Anjs-6-12	32	31	32	83	83	83
G511H/Anj//Anj///Anj////Anjs-6-8	33	33	33	80	80	80
G511H/Anj//Anj-2-8	30	30	30	78	77	78
G511H/Arg//Arg///Arg///Arg-12-15	32	31	32	79	81	80
G511H/Anj//Anj///Anj-6-3	29	28	29	81	80	81
G511H/Arg//Arg///Arg///Arg-19-7	33	31	32	83	82	83
G511H/Anjasmoro-1-7	33	32	32	79	81	80
G511H/Anj//Anj///Anj////Anjs-6-7	34	34	34	78	78	78
G511H/Anjasmoro-1-4	33	33	33	79	80	79
Grobogan	30	29	30	77	79	78
Anjasmoro	34	34	34	85	85	85
Argomulyo	32	32	32	77	78	78
Average	32	31	32	80	81	81

 $\overline{L1}$ = full insecticide control,

 $L2$ = insecticide control up to 50 days after planting

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Table 5. Plant height and number of branches of 24 soybean genotypes. 2016.

 $\overline{L1}$ = full insecticide control,

 $L2$ = insecticide control up to 50 days after planting

to maturity of 78 and 82 days, respectively. Thus based on the maturity classification of soybean maturity in Indonesia, then the G511H/Anj// Anj-2-8 was classified as genotype with early maturity

Plant height in the controlled environment ranged from 25.43 - 41.87 cm (an average of 35.02 cm) and in a non-controlled environment was between 26.37 - 44.90 cm (an average of 35.13 cm). The character of number branches per plant ranged from 1.27 - 4.30 branches (an average of 2.92 branches) in a controlled environment while in non-controlled environment between 1.86 - 4.63 branches (average 3.09 branches) (Table 5). Soybean cultivation in July - October is at the peak of high temperatures causing less optimal

growth. Insecticide control does not affect plant height as well as number of branches.The similar pattern also showed in number of node per plant. This is because the maximum formation process of number of branches and number of nodes has generally ended at the time of the formation of flowers or when the plant enters the generative phase.

Seed size, as well as the days to maturity, becomes a consideration for soybean users in Indonesia in determining the choice of superior varieties. The effect of insecticides does not affect the size of the healthy seeds (not attacked by pod-sucking bugs) between controlled and non-controlled environments. The average of 100 seeds weight in controlled environment was

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Table 6. Number of node and 100 seed weight of 24 soybean genotypes. 2016.

 $\overline{L1}$ = full insecticide control,

 $L2$ = insecticide control up to 50 days after planting

14.91 g, and in non-controlled environment was 14.82 g (Table 6). The genotypes which identified as resistant to pod sucking pest (G511H/Anj// Anj-2-8 and G511H/Anjs/Anjs-1-3) have an average 100 seeds' weight of 15.57 g and 14.22 g, respectively. This means the two genotypes were classified as having large seed size according to seed size classification in Indonesia. However, when combined with the days to maturity, then only G511H/Anj//Anj-2-8 was more suitable for development in Indonesia, in addition to its resistant to pod sucking pests, it also has large seed size and classified as genotype with early days to maturity.

The resistant genotype obtained from this

study (G511H/Anj//Anj-2-8) is useful for gene source in the soybean breeding program for resistance to pod sucking bug, or can be recommended to be released as new soybean variety. Moreover, this genotype is having such characteristics that in accordance with farmers' preferences (early days to maturity and large seed size), hence it potential to be developed in tropical area of Indonesia for minimizing the yield losses caused by pod sucking pest.

CONCLUSION

The damage intensity caused by pod sucking pest on pod wall was higher than those of on the seed. The improvement of soybean resistance to pod sucking bug is more effective by increasing the resistance of pod rather than by increasing the resistance of seed on soybean plants. Genotype G511H/Anj//Anj-2-8 is classified as resistant to pod sucking bug, with high yielding and suitable to be developed in a tropical region of Indonesia, due to early days to maturity (< 80 days) and large seed size.

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