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Identification of soybean genotypes based on antixenosis and antibiosis to the armyworm (*Spodoptera litura*)

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Abstract. *Krisnawati A, Bayu MSYI, Adie MM. 2017. Identification of soybean genotypes based on antixenosis and antibiosis to the armyworm* (*Spodoptera litura*)*. Nusantara Bioscience 9: 164-169.* Armyworm (*Spodoptera litura* F.) is the main soybean leaf-feeding pest in Indonesia. A total of 10 soybean genotypes was identified for its resistance to armyworm through antixenosis and antibiosis tests. Genotype G100H was used as a resistant check. The experiment was conducted from April to July 2016 in Laboratory of Plant Breeding, Indonesian Legumes and Tuber Crops Research Institute, Malang, Indonesia and Laboratory of Optic, Faculty of Science and Technology, Universitas Islam Negeri Maulana Malik Ibrahim, Malang, Indonesia. Based on antixenosis resistance mechanism, two genotypes were resistant, two genotypes were moderately resistant, and six genotypes were susceptible to armyworm. Two resistant genotypes (G511H/Anj-1-4 and G100H) have short and dense trichome in the both of adaxial and abaxial positions. Those trichome orientation become a barrier for insect pests with chewing and biting type of mouth parts. Antibiosis resistance was evaluated based on armyworm growth. The lighter larval and pupal weight, and also a longer period of the larval and pupal stage were shown in two resistant genotypes which based on antixenosis test. Furthermore, the pupal weight seems to be more influential to the weight of larvae due to the mechanism of antibiosis. Hence, G511H/Anj-1-4 can be used as a source of the gene for improvement of soybean resistance to armyworm.

Keywords: Host-plant resistance, non-preference, resistance mechanisms, *Spodoptera litura*, soybean genotypes

INTRODUCTION

Soybean is an economically important crop and a staple food apart from rice produced in Indonesia. Soybean production in Indonesia in 2012 was 843.153 ton and in 2013 was 779.992 ton, there was a decrease about 63.161 ton. However, in 2014, soybean production has increased by 173.964 ton (FAOSTAT 2015). It was due to the improvement of technology, particularly in pest control management. One factor that contributes to reducing the yield and quality of soybean production is insect pests' attack. The most important pests that attack the soybean from the time they grow until physiological maturation are larvae of some species of Lepidoptera (Souza et al. 2012).

Spodoptera litura (Lepidoptera: Noctuidae) has become a serious pest on soybean in Indonesia. This pest is native to Asia but has been spread worldwide in many host plants (Baskar et al. 2012). *S. litura* attacks soybean leaves and pods which play an important role in the early reproductive phases thus resulted in crop failure. According to Punithavalli et al. (2014), in India, *S. litura* remains active from the end of July to October coinciding with a warm climate and also peak reproductive phases of soybean, causing 26-29% yield losses. Similarly, in Indonesia, armyworm is most abundant during the dry season 2 (June/July - September/October) which soybeans are mostly cultivated, and causing significant yield losses (Marwoto and Suharsono 2008). The control of *S. litura* in Indonesia and many agriculture countries has depended mostly on the application of various insecticides. The intensive application of insecticides to control this pest caused agro-ecosystem unbalance and has resulted in high levels of pest resistance to commercial insecticides including organophosphate, organochlorines, carbamates, and pyrethroids (Shad et al. 2012. Tong et al. 2013). This contributed to the difficulties in controlling this pest in a field (Ahmad et al. 2011; Muthusamy et al. 2011; Abbas et al. 2012).

Host plant resistance is an alternative and valuable strategy for controlling the herbivorous insect (Smith and Clement 2012; Oki et al. 2012; Seifi et al. 2013). The use of host plant resistance is an environment-friendly, economical, and effective method, as well as compatible with other control methods including the utilization of natural enemy (Janini et al. 2011; Jesus et al. 2014). Understanding the mechanisms of resistance is important to develop soybean varieties resistant to *S. litura*. There are three different mechanisms of plant resistance which are non-preference or antixenosis, tolerance, and antibiosis (Kogan and Ortman 1978; Smith and Clement 2012). Antixenosis is characterized by the presence of morphological character such as pubescence and presence of a chemical substance that adversely alters insect behavior. Antixenosis mechanism was used by plants to prevent or reduce colonization by insects due to insects mostly orient themselves on plants for feeding or shelter. Antixenosis is effective resistance mechanism which caused the target insect die due to lack of food.

Furthermore, antibiosis is a mechanism of resistance in which feeding on the plant causes mortality or the inhibition of growth, development, and physiological activity in the insect.

The use of soybean resistant against *S. litura* has been studied in many countries and showed the effectiveness for controlling *S. litura*. Himeshirazu and Sodendaizu are known as Japanese soybean cultivar which indicated resistant against *S. litura* (Komatsu et al. 2004). Komatsu et al. (2010) reported that Kyushu 155 exhibited resistance to *S. litura*. Bae et al. (2005) found that the soybean cultivar namely Bay showed a high level of antibiosis against *S. litura* when larvae were reared on whole plants. The other author, Souza et al. (2012) reported that IAC 100 have a high degree of nonpreference for feeding to the larva of *Spodoptera* species based on free choice and nonchoice tests. In Indonesia, there are few number of soybean genotypes resistant against *S. litura*. Bayu et al. (2013) evaluated the resistance of soybean genotypes tolerant to acid soil and drought against *S. litura*. The author recorded that IAC 100 have a low feeding preference by *S. litura* based on the free choice test. Moreover, they found G100H which is the result of crossbreeding between IAC 100 with Himeshirazu showed resistance against *S. litura* through antixenosis mechanism.

The information about the resistance of some genotypes through antixenosis mechanism are useful in a breeding program to obtain resistant donor parents. Moreover, the selection of more resistant soybean genotypes against *S. litura* becomes highly required to help soybean producers to minimize the pest attack and furthermore, increase the yield of soybean. Therefore, the objective of this study was to identify soybean resistance to armyworm.

MATERIALS AND METHODS

Research materials consisted of ten soybean genotypes. The experiment was conducted from April to July 2016 in the Laboratory of Plant Breeding, Indonesian Legumes and Tuber Crops Research Institute (ILETRI), Malang, Indonesia, and Laboratory of Optic, Faculty of Science and Technology, Maulana Malik Ibrahim State Islamic University, Malang, Indonesia. The first instar larvae of armyworm were collected from a soybean field in Kendalpayak Research Station (Malang) and was reared in Laboratory of Plant Breeding (ILETRI).

Antixenosis test

For the antixenosis test, we grew 10 soybean genotypes at the ILETRI's greenhouse. Each genotype was grown in plastic pots (25 cm diameter × 20 cm height) which were filled with mixed soil and manure (2:1). The experiment was arranged in completely randomized design with four replicates. An antixenosis test was performed in air-conditioned room maintain at 24 ± 1°C. The paired-comparison tests of the feeding preferences of the larvae were carried out in petri dishes. The fully expanded leaflets of similar age and size from tested genotypes (test plant) and resistant check plant (G100H) were collected and used in this antixenosis test. Two leaflets from each genotype and resistant check, respectively; were excised, and washed with running water, drained, then cut to round shape with 1.5 cm in diameter. Excised round leaflets (leaf disc) from each genotype were placed in petri dishes (12 cm diameter × 2 cm height). The bottom of each petri dish was covered with moist filter paper to maintain the humidity. The third instar larva of *S. litura* was placed on the filter paper between the leaf discs. Each treatment has three replications. The experiment was run until 100% area of leaf disc of tested or check genotype was consumed by *S. litura* larvae, and then the visual defoliation was assessed based on preference index (C) according to Kogan and Goeden (1970).

The preference index (C) was calculated as follows:

$$C = \frac{2A}{M + A}$$

Where,
A = Feeding on the test plant
M = Feeding on the check plant

C < 1 = more resistant (less preferred) than a check, indicates the test plant has higher antixenosis resistance than the check plant.

C = 1 = the feeding on the test plant has equaled the feeding on the test plant.

C > 1 = more susceptible (more preferred) than a check, indicates a preference for the test plant.

Evaluation of the leaf pubescence length and density were conducted by collecting a fully expanded leaflet (third leaf below the apical meristem of the one-month-old plant) from each genotype. The density of trichomes of each genotype was counted under a binocular microscope by placing small paper of 5 × 5 mm above the leaflet. Thus, the density of trichomes stated as a number of trichomes per 25 mm^2. The length of trichomes (µm) was also measured under a binocular microscope. The both of density and length of trichomes were measured on the adaxial and abaxial surface of leaves, respectively. All the data were presented as average values.

Antibiosis test

Antibiosis test was measured based on the biology of *S. litura*. One fully expanded young leaf (third leaf below the apical meristem of one-month-old plants) was randomly collected and placed in plastic petri dishes (20 cm diameter × 3 cm height with a hole in its center) lined with moistened filter paper. Then, one neonate *S. litura* larva was transferred into each petri dish. During the study, fresh leaflets were supplied every day and the filter papers were wetted. The fresh leaflets and the consumed leaflets were weighed every day. It was aimed to determine the quantity of the consumed leaf each day in grams. The following biological parameters were evaluated: the weight of 7, 11, and 15 days old larvae; pupal weight, pupal length, and pupal diameter. The experiment was set in a completely randomized design, with ten treatments (genotypes) and five replicates. Each replicate consisted of one neonate larva as the experimental unit.

RESULTS AND DISCUSSION

Antixenosis

Antixenosis is one of plant resistance mechanisms against the insect pests. Antixenosis resistance resulted from the physical barrier of the plant that reduces the interaction between the host plant and insects. In this study, the degree of antixenosis resistance was evaluated based on preference test, which was calculated by comparing the defoliation rate between the tested genotype and the check genotype (G100H).

Preference index ranged from 0.57 to 1.61 (Table 1). Test genotypes with preference index less than 1.0 indicated that those genotypes have higher resistance than the check genotype (G100H). On the contrary, preference index higher than 1.0 indicated that those genotypes were more susceptible than the check. Based on antixenosis mechanism, six of them were susceptible, one genotype was moderate, and one genotype (G511H/Anj-1-4) was resistant (Table 1).

The dense leaf pubescence has been found to be an effective antixenosis defense. The length and density of trichome from ten soybean genotypes were presented in Table 2. The length of leaf trichomes on the adaxial (upper) surface tended to be longer than that on the abaxial (lower) surface. On the contrary, the density of leaf trichomes on the adaxial was higher than that on abaxial surface. Based on trichome length, G511H/Anj-1-6 had the longest trichome at adaxial as well as abaxial surface, but this genotype was categorized as susceptible. Based on trichome density, genotype of G100H (check genotype) showed the highest density, followed by G511H/Anj-1-4. These two genotypes were categorized as resistant. Thus, based on those facts, it was suggested that the trichome density seemed to be more play important role than trichome length within antixenosis resistance in soybean.

Antibiosis

Antibiosis resistance mechanism involved physical or chemical characteristic that inhibits the growth, development, and physiological process of insect (Oki et al. 2012). The average larval weight at seven days after infestation (DAI) was 0.0649 g, and increased into 0.1536 g at 11 DAI and 0.4665 g at 15 DAI (Table 3). The antibiosis effect was found on larval growth at 7 and 11 DAI. At 7 DAI, the lower larval weight was found when *S. litura* fed on G511H/Anj-1-6 and Ijen (0.0202 g and 0.0338 g, respectively). However, at 11 DAI, a lower larval weight was found when larvae fed on G100H, Grobogan, and G 511 H/Anj-1-4.

The highest consumed leaf by larva was found on G511H/Anj-1-4 (0.1775 g/day), and the lowest consumed leaf was found on G511H/Anj//Anj-5-1 (0.0922 g/day). The longest larval duration was found when larvae fed on Grobogan, G100H, and G511 H/Anj-1-4 (Table 4).

Weight and length of pupa were different among genotypes, but the pupal diameter was not affected by host plant (Table 5). The larvae fed on G100H resulted in the lowest weight and low pupal length. The pupal duration varied from 6 - 11 days. Our results indicated that genotype with antibiosis resistance tends to have long larval duration. Based on those facts above, each soybean genotype expresses different resistant mechanism to armyworm.

Table 1. Preference index and the antixenosis resistance criteria of 10 soybean genotypes. 2016

Genotype	Preference index	Criteria
G 511 H/Anj//Anj///Anj-11-2	1.60	Susceptible
G 511 H/Anj-1-6	1.43	Susceptible
G 511 H/Anj//Anj///Anj-7-1	1.61	Susceptible
G511 H/Anj//Anj-5-1	1.15	Susceptible
G 511 H/Argom//Argom-2-1	1.35	Susceptible
G 511 H/Anj-1-4	**0.57**	**Resistant**
G 511 H/Anj-1-2	1.67	Susceptible
Ijen	1.09	Moderate
G100H (check genotype)	0.77	Resistant
Grobogan	1.06	Moderate

Table 2. The trichome length and density of 10 soybean genotypes. 2016

Genotype	Trichome length (µm)		Trichome density/ 25 mm^2	
	Adaxial	Abaxial	Adaxial	Abaxial
G 511 H/Anj//Anj///Anj-11-2	1209.8 b	1098.6 c	24.3 f	45.3 h
G 511 H/Anj-1-6	1290.3 a	1240.1 a	19.0 g	47.0 g
G 511 H/Anj//Anj///Anj-7-1	1037.5 c	1063.9 d	24.0 f	51.0 f
G 511 H/Anj//Anj-5-1	1286.7 a	1142.3 b	13.7 h	26.7 i
G 511 H/Argom//Argom-2-1	1120.6 b	827.3 h	31.3 e	51.3 f
G 511 H/Anj-1-4	953.1 d	854.6 g	**53.3 b**	**105.3 b**
G 511 H/Anj-1-2	1019.9 c	936.0 e	31.0 e	66.3 e
Ijen	928.0 d	886.2 f	43.0 c	101.3 c
G100H (check genotype)	888.8 d	889.9 f	**57.0 a**	**122.3 a**
Grobogan	889.2 d	825.5 h	37.0 d	82.7 d

Note: Numbers with the same letter in the same column are not significantly different according to DMRT test ($p<0.05$)

Table 3. The weight of *S. litura* larvae in 10 soybean genotypes. 2016

Genotype	**Weight of larvae (g)**		
	7 hsi	**11 hsi**	**15 hsi**
G 511 H/Anj//Anj///Anj-11-2	0.0417 f	0.2332 b	0.5828
G 511 H/Anj-1-6	0.0202 h	0.0685 g	0.4446
G 511 H/Anj//Anj///Anj-7-1	0.0779 a	0.1989 d	0.6134
G 511 H/Anj//Anj-5-1	0.0771 b	0.1531 e	0.3965
G 511 H/Argom//Argom-2-1	0.0586 c	0.2099 c	0.3823
G 511 H/Anj-1-4	0.0467 e	0.0589 h	0.4515
G 511 H/Anj-1-2	0.1942 a	0.3851 a	0.7153
Ijen	0.0338 g	0.1140 f	0.5730
G100H	0.0464 e	0.0602 h	0.2609
Grobogan	0.0522 d	0.0539 i	0.2447
Average	0.0649	0.1536	0.4665

Note: Numbers with the same letter in the same column are not significantly different according to DMRT test ($p<0.05$)

Table 4. Leaf consumed and duration of *S. litura* larvae in 10 soybean genotypes. 2016

Genotype	**Consumed leaf/day (g)**	**Leaf duration (days)**
G 511 H/Anj//Anj///Anj-11-2	0.1275 c	15
G 511 H/Anj-1-6	0.1120 e	17
G 511 H/Anj//Anj///Anj-7-1	0.1115 f	16
G 511 H/Anj//Anj-5-1	0.0922 h	15
G 511 H/Argom//Argom-2-1	0.1071 g	14
G 511 H/Anj-1-4	0.1775 a	18
G 511 H/Anj-1-2	0.1248 cd	15
Ijen	0.1044 g	17
G100H	0.1219 d	22
Grobogan	0.1615 b	23
Average	0.1240	17

Note: Numbers with the same letter in the same column are not significantly different according to DMRT test ($p<0.05$)

Table 5. Weight, length, and diameter of pupa and duration of *S. litura* pupa in 10 soybean genotypes. 2016

Genotype	**Pupa**			
	Weight (g)	**Length (cm)**	**Diameter (cm)**	**Duration (days)**
G 511 H/Anj//Anj///Anj-11-2	0.2092 ed	1.42 bc	0.34	6 d
G 511 H/Anj-1-6	0.2750 ab	1.58 abc	0.40	7 d
G 511 H/Anj//Anj///Anj-7-1	0.2134 d	1.54 bc	0.34	8 c
G 511 H/Anj//Anj-5-1	0.2724 a	1.60 abc	0.34	11 a
G 511 H/Argom//Argom-2-1	0.2510 c	1.52 bc	0.34	11 a
G 511 H/Anj-1-4	0.2640 b	1.66 a	0.40	9 c
G 511 H/Anj-1-2	0.2786 a	1.62 ab	0.42	11 a
Ijen	0.2043 e	1.60 ab	0.36	10 bc
G100H	0.1610 f	1.30 c	0.34	11 a
Grobogan	0.2513 c	1.58 abc	0.40	11 a
Average	0.2380	1.54	0.37	9.5

Note: Numbers with the same letter in the same column are not significantly different according to DMRT test ($p<0.05$)

Discussion

The armyworm (*S. litura*) is a serious leaf-feeding insect of many agricultural crops, including soybean. Almost no soybean variety is resistant to armyworm, hence the management of armyworm was mainly performed using chemical insecticides. The unwise use of insecticide has exhibited insects' resistance to various classes of insecticides (Ahmad et al. 2008). In Indonesia, armyworm has been reported resistant to insecticides of monocrotophos, endosulfan, decametrin, and metomil (Hadiyani 1995; Marwoto and Bedjo 1997). In Hunan province (China), *S. litura* was reported to have developed high resistance against a wide variety of insecticides including organophosphate, carbamate, and pyrethroids (Tong et al. 2013). Hence, the use of insect-resistant crop variety is a valuable strategy in crop protection to reduce the negative effects of insecticides.

The resistance mechanisms of soybean genotype to armyworm are usually divided into antixenosis, antibiosis, and tolerance; hence could even be a combination of various mechanisms of resistance. Antixenosis, or referred to as non-preference, involves the morphological resistance in which the insect is either repelled by or not attracted to the host plant, either as feed as well as for eggs-laying (Oki et al. 2012; Smith and Clement 2012). In this study, based on preference index, G511H/Anj-1-4 has an antixenosis resistance mechanism. Its degree of resistance was similar to those of G100H that have been considered as resistant line to armyworm. De Souza et al. (2014) reported that soybean lines of IAC 100 and IAC 17 have non-preference resistance mechanism. The significant factor of antixenosis resistant was trichome characters. Trichome as an insect defense mechanism plays important role by negatively affects the ovipositional behavior, feeding, and larval nutrition of insect pests (Al Ayedh 1997; Handley et al. 2005; War et al. 2012). Thus, the presence of trichome potentially affects the decrease of weight and feeding of insects. Gannon and Bach (1996) studied the relationship between leaf trichome density (dense, normal, and glabrous) and soybean resistance to Mexican leaf beetle, and obtained that the larval mortality was 2.5-5 times greater on the densely pubescent lines compared to those of the glabrous and the normal lines. Furthermore, the leaf defoliation rate was 3-13 times greater on the glabrous lines than that on the normal and dense lines. These indicate that leaf trichome plays important role by providing resistance to the plants against insect herbivores. The current result revealed that the density of trichome provides higher antixenosis than the length of trichome for armyworm which has a type of biting and chewing mouth.

Besides antixenosis, soybean genotypes also exhibit antibiosis resistance to armyworm. Antixenosis resistance is a mechanism in which host-expressed such trait that has adverse effects on insect behavior. These involves the inhibition of growth, development, or physiological processes in the insects, increased mortality or reduced longevity and reproduction of the insect (Kogan 1975; Smith 1989; Oki et al. 2012). The antibiosis resistance was evaluated through biological processes of armyworm. Different host-plants influence the weight of larvae. The antibiosis effect is usually evaluated through the weight of larvae, the weight of pupa, and duration of larvae or pupa (Lambert and Killen 1984). The genotype of G511H/Anj-1-6 resulted in the lowest weight of larvae at 7 DAI and showed consistently up to 11 DAI, revealed that this genotype exhibit a high antibiosis. Genotypes G511H/Anj-1-4, G100H, and Grobogan exhibit intermediate antixenosis. Ijen variety, which one parent derived from Himeshirazu (resistant to common cutworm) (Oki et al. 2012), exhibit antibiosis on larval stage until 7 DAI. Antibiosis was not significantly affected the weight of larvae at the late duration (15 DAI). This may be due to the larva will enter prepupal stage which indicates the reduction of consumed leaf or even stop eating the leaves. The lowest weight of pupa and the longest duration of pupa was found on *S. litura* that fed G100H during the larval stage.

Each genotype exhibits different resistance mechanism to armyworm. In Indonesia, soybean is cultivated throughout the season, and the host-plants of armyworm are abundant in the field. In such conditions, antibiosis resistance seems to be less significant since the larvae have the ability to recover their body when the feed does not contain antibiosis. In contrast, antixenosis resistance will prevent or reduce the interaction between the armyworm with host-plants.

Thus, from this study, we can conclude that each soybean genotype has different resistance mechanism to armyworm. Genotype G511H/Anj-1-4 was resistant to armyworm, hence can potentially be used as a source of the gene for improvement of soybean resistance to armyworm.

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