Pertanika J. Trop. Agric. Sci. 41 (2): 825 – 832 (2018)

TROPICAL AGRICULTURAL SCIENCE

Journal homepage: http://www.pertanika.upm.edu.my/

Assessment of Soybean Resistance to Whitefly (*Bemisia tabaci* Genn.) Infestations

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ABSTRACT

The use of resistant varieties is one of the best ways to control whitefly attacks. However, to date, there is no soybean variety that is resistant to whitefly. In this study, we aimed to assess the resistance of four soybean genotypes to whitefly. Anjasmoro variety was planted as a susceptible control while G100H was used as resistant control. The study was conducted in a greenhouse using a free-choice test. All soybean genotypes were planted in polybags and arranged in a randomised completely block design with three replicates. Resistance is categorised based on the intensity of leaf damage which occurred at 45-days-old plant. The leaf damage intensity was scored using two different methods. The results showed the intensity of leaf damage by using the first method varied between 7.43% (Dena 1) and 23.93% (Anjasmoro); while that of the second method ranged between 18.03% (G100H) and 37.85% (Anjasmoro). Anjasmoro was consistently classified as highly susceptible, while Gema was consistently categorised as moderately resistant to whitefly. Dena 1 and G100H were classified as moderately resistant - resistant, while Dega 1 and Devon 1 were categorised as susceptible - moderately resistant to whitefly. Resistance of soybean genotypes tested against whitefly correlated with the density of leaf trichomes. Correlation analysis shows a negative correlation between the intensity of leaf damage and the number of leaf trichomes ($r = -0.29$, $p = 0.24$) based on method 1, thus indicating a low antixenosis mechanism in whitefly resistant genotypes.

Keywords: Antixenosis mechanism, intensity of leaf damage, leaf trichomes, soybean, whitefly

ARTICLE INFO

Article history:
Received: 18 September 2017
Accepted: 30 April 2018

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ISSN: 1511-3701

INTRODUCTION

Whitefly (*Bemisia tabaci* Genn.) is a major pest in soybean cultivation in Indonesia. The attacks of whitefly can reduce soybean yields, even crop failure and that can occur directly or indirectly. The direct damage occurs when nymphs and imago of whitefly pierce and suck the leaves liquid of the host plant causing chlorosis in the leaves (Hoodle, 2003). Honey dew excreted by the two stadia become a growth medium for the sooty mould on the leaf surface that causes disruption of the photosynthesis process (Hilje & Morales, 2008; Palumbo, 2016). The damage occurs indirectly when a virus carried by whitefly is transmitted to the host plant (Jones, 2003; Navas et al., 2011; Rodrigues et al., 2014).

One of the alternative techniques to control whitefly based on the principles of integrated pest management is to plant resistant varieties. Soybean varieties that are resistant to whitefly can be obtained through a soybean breeding programme. One important step to obtain resistant varieties is the selection of resistant plant. Planting soybean varieties resistance to whitefly infestations should consider several criteria. The density of leaf trichomes is one of morphological characters that are usually associated with resistance properties (Haq et al., 2003; Lima & Lara, 2004). In addition, the thickness of the leaves plays a role in determining antixenosis mechanism in soybean (Sulistyo & Inayati, 2016). When it is linked to the presence of pests, total population of egg, larva, pupa, and imago per leaf area can be used to determine soybean resistance to whitefly infestations (Gulluoglu et al., 2010). However, the nymph is the stage of whitefly that causes the highest leaf damage, so its presence determines the level of resistance of soybean genotype (Xu et al., 2005; Amro et al., 2009; Xu et al., 2009; and Xu, 2009).

To the best of the present authors' knowledge, there is yet to be a standard method to classify soybean genotypes according to their resistance characteristic against whitefly in Indonesia. A method often used is by calculating the intensity of leaf damage (Inayati & Marwoto, 2012) using five scores leaf damage that occurs in each leaf. Scores range from 0 (no damage symptoms) to 4 (appearance of sooty mould, abnormal pods and seeds). A Similar method is also used in black gram (*Vigna mungo*). Taggar, Gill and Sandhu (2013) scored leaf damages ranging from a score of 1 (no damage to the leaves) to a score of 5 (dry and die leaves), to determine black gram resistance to whitefly infestations. This study was aimed at developing two methods to determine the whitefly resistant soybean varieties based on their leaf damage.

MATERIALS AND METHODS

This study was conducted in a greenhouse from July to September 2016. Six genotypes of soybean were tested for resistance to whitefly infestations including Anjasmoro as susceptible control, G100H as resistant control, as well as four soybean varieties, namely Dega 1, Gema, Dena 1, and Devon 1. All genotypes were planted in polybags which 35 cm in diameter and

35 cm in height. The planting medium was used was soil and compost at the ratio 1:1. Three seeds of each genotype were planted in one polybag. NPK fertiliser with a dose of 5 g per polybag was also provided. The research was arranged in a randomised completely block design with three replicates.

The resistance of six soybean genotypes to whitefly was tested using a free choice test. Each replicate is placed in bamboo cage covered with tile fabric in order to prevent the whitefly from flying to one replicate to the other , but still allows it to move from one genotype to another according to its preference. The bamboo cages were 200 cm in height x 150 cm wide x 350 cm long. Whitefly infestation was done on 21-day-old plants by placing 10 imago whiteflies to leaf surface of each individual plant (Mansaray & Sundufu, 2009).

The leaf damages were observed on plants 45 days after planting. They (the damages on the leaf) were scored based on two different methods. The first scoring method was based on Inayati and Marwoto (2012) the second method was adapted from Taggar et al. (2013). The resistance category of soybean genotypes was tested using Chiang and Talekar's formula (1980). The leaf trichomes and leaf thickness were studied on 49-day-old plants to determine whether there was an antixenosis mechanism present. The fifth leaf from the above was used as a reference in calculating leaf trichomes and leaf thickness. The observations were performed under a light microscope.

RESULTS AND DISCUSSION

The Intensity of Leaf Damage and Resistance Category

Table 1 shows results of leaf damage using method 1. The intensity of leaf damage of six soybean genotypes varies between 7.43% and 23.98%. Anjasmoro which served as a susceptible control was the most severe with leaf damage reaching 23.98%. Among the four tested genotypes, none of which showed leaf damage more than the susceptible control. Meanwhile, G100H that served as a resistant control showed 10.91% of leaf damage. Better resistances in Dena 1 and Gema were observed with the intensities of leaves damage at 7.43% and 8.49% respectively, compared with resistant control-.

Using method 1 (Table 1) on four genotypes, it was found no soybean genotypes was highly resistant (HR). However, one resistant (R) genotype (Dena 1), one moderately resistant (MR) genotypes (Gema), and two susceptible (S) genotype (Dega 1 and Devon 1) were observed. The resistant control (G100H) was categorised as moderately resistant genotype. The susceptible control (Anjasmoro) was categorised as highly susceptible. These results indicate that the first method was effective in distinguishing between resistant and susceptible genotype of soybean to whitefly.

Table 1 shows leaf damage intensity of six soybean genotypes calculated using method 2 showed higher values, compared with method 1. These values ranged between 18.03% and 37.85%. In method 1, Anjasmoro used as a susceptible control, showed the highest leaf damage with LDI (Leaf Damaging Intensity) reaching 37.85%. Meanwhile, G100H used as a resistant control showed the lowest LDI at 18.03% that was lower than those of the other soybean genotypes. Method 2 was slightly different from the method 1 because there were no genotypes with lower intensity of leaf damage than G100H. The four tested soybean genotype had higher LDI than G100H but lower than that of Anjasmoro.

Table 1
The intensity of leaf damage and resistance category of six soybean genotypes using two methods

Genotype	LDI method 1	Resistance category	LDI method 2	Resistance category
Anjasmoro	23.98[a]	HS	37.85[a]	HS
Dega 1	14.63[abc]	S	26.21[b]	MR
Gema	8.49[bc]	MR	25.32[b]	MR
Dena 1	7.43[c]	R	26.56[b]	MR
Devon 1	17.96[ab]	S	26.14[b]	MR
G 100 H	10.91[bc]	MR	18.03[c]	R
LSD 5%	9.53		6.07	

Note. Means within a column and followed by the same letter(s) are not significantly different based on LSD at 5%, LDI = leaf damage intensity, HS = highly susceptible, S = susceptible, MR = moderately resistant, R = resistant

Based on LDI using method 2, six genotypes were divided into three categories of resistance, namely highly susceptible genotype (Anjasmoro), resistant genotype (G100H), and moderately resistant genotypes (Dega 1, Gema, Dena 1, and Devon 1). Compared with the first method, there was a change in the degree resistance in some of the genotypes in the second method. Changes in the resistance category were highly visible for Dega 1 and Devon 1. In method 1, the two genotypes were classified as susceptible, while in method 2, these were categorised as moderately resistant. Therefore, different methods used for calculation of leaf damage intensity showed different resistance category of soybean genotypes.

In this study, the two methods provided relatively similar results in terms of determining the resistance category of the susceptible control. Anjasmoro was classified as highly susceptible to whitefly in both methods. Yield losses in Anjasmoro variety may reach 80% (Inayati & Marwoto, 2012). Sulistyo and Inayati (2016) found that Anjasmoro had a high sensitivity against whitefly. Whitefly populations in small amounts on Anjasmoro can already lead to a decrease in its yield.

In this study, Gema, Dena 1, and G100H soybean genotypes were classified as resistance – moderate resistance to whitefly. Therefore, it is suggested that Gema, Dena 1, and G100H consist of antixenosis that are resistant to whitefly. Sulistyo and Inayati (2016) reported that antixenosis resistance of Gema against whitefly correlates with the density and length of leaf trichomes, as well as leaf thickness. This may also explain why the resistance category of Gema is consistent despite using two different scoring methods.

Dena 1 is progeny of a cross between Argomulyo and IAC 100, while G100H is soybean genotype obtained through crosses between IAC 100 and Hymmeshirazu. The resistance of Dena 1 and G100H against whitefly are allegedly derived from IAC 100. Previous studies have pointed to IAC 100 as one of the soybean germplasms that can be used as a source of gene resistance against various pests (Piubelli et al., 2003; Pinheiro, Vello, Rossetto, & Zucchi, 2005; Suharsono, 2006; Suharsono & Adie, 2010). The IAC 100 has a mechanism of antibiosis against whitefly by extending the period of nymphs and reducing the appearance of imago up to 80% (Lima & Lara, 2004), thus, showing symptoms of reduced damage (Vieira et al., 2011).

Leaf Trichomes and Leaf Thickness

Table 2 contains an analysis of variance that shows significant differences in the character of leaf trichomes among six soybean genotypes. Dena 1 and Dega 1 had the highest leaf trichomes at 88 and 82 respectively. The lowest number of trichomes were found on G100H and Anjasmoro, 45 and 47 trichomes respectively. It can be concluded that analysis of variance on the leaf thickness character showed no differences in leaf thickness among the six soybean genotypes.

Table 2
Leaf trichomes and leaf thickness of six soybean genotypes

Genotype	Leaf trichomes	Leaf thickness (μm)
Anjasmoro	47.0[c]	0.43[a]
Dega 1	82.7[ab]	0.43[a]
Gema	64.7[bc]	0.40[a]
Dena 1	88.7[a]	0.44[a]
Devon 1	51.00[c]	0.44[a]
G 100 H	45.3[c]	0.43[a]
LSD 5%	22.43	0.07

Note. Means within a column and followed by the same letter(s) are not significantly different based on LSD at 5%

The resistant ability of six soybean genotypes to whitefly may be related to the characteristics of their leaves. Therefore, the study attempted to find correlation between number of leaf trichomes and resistance of six soybean genotypes and found a negative correlation based on the number of leaf trichomes using method 1 (r = -0.294, *p* = 0.237)). Table 3 indicates a negative correlation between the intensity of leaf damage and its (leaf) thickness using method 1 (r = -0.037, p = 0.883), as well as in method 2 (r = -0.085, p = 0.737). Although the value of the correlation coefficient is low, the results may support occurrence of antixenosis mechanism on tested soybean genotypes.

Table 3
Correlation analysis of leaf damage intensity with leaf trichomes and leaf thickness

	LDI method 1	LDI method 2
Leaf trichomes	-0.294 (p = 0.237)	0.057 (p = 0.823)
Leaf thickness	-0.037 (p = 0.883)	-0.085 (p = 0.737)

Note. LDI = leaf damage intensity

The mechanism of the host plant resistance against pests could be in the form of antixenosis, antibiosis, and tolerance (Emden, 2002). Although the population of whitefly per leaf area was not observed in this study, the results of correlation analysis points to a relationship between leaf trichomes and leaf thickness in terms of intensity of damage on leaf. This indicates a low level of antixenosis mechanism on soybean genotypes tested. Haq et al. (2003) found leaf trichomes as a character of leaf morphology that affect the level of soybean resistance to whitefly infestations . Sulistyo and Inayati (2016) added that the leaf thickness influenced the resistance of soybean to whitefly. According to Silva et al. (2012), the least number of eggs laid on resistant soybean leaves with dense trichomes showed the function of antixenosis mechanism.

CONCLUSION

Based on the results, it can be concluded Method 1 and Method 2 used to calculate the intensity of leaf damage provides relatively consistent results in distinguishing the resistance of soybean genotypes to whitefly infestation. The consistency can be seen in Anjasmoro that was classified as highly susceptible, as well as in Gema, Dena 1, and G100H as moderately resistant and resistant to whitefly. The intensity of leaf damage can be used as a criterion in determining soybean resistance to whitefly. This study also showed occurrence of a low antixenosis mechanism that correlates with leaf trichomes density of the soybean genotypes tested.

ACKNOWLEDGEMENT

The authors acknowledge with gratitude the grant provided by Ministry of Research, Technology and Higher Education through INSINAS scheme in 2016 (grant number RD-2016-0185).

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