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Research Article

Correlation between Epidermis Thickness, Leaf Trichome Length and Density with the Whitefly *Bemisia tabaci* Population on Five Local Soybean Cultivars

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ABSTRACT

Soybeans compsumsion in Indonesia is about 2.7 million ton, while the national production is only about 0.7 million ton per year. There are some problems in the soybean production in Indonesia, including pests and diseases. Whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), is one of the important pests in soybeans causes a significant loss of yields. Developing soybean cultivars resistant to whitefly is one of method to reduce the production loss. This study aimed to determine the relationship between the physical characteristics of lower surface leaves and *B. tabaci* population on five local soybean cultivars. The experiments were conducted in the IPB greenhouse by Completely Randomized Design (RCD) using five soybean cultivars (Anjasmoro, Dena-1, Detam-3, Devon-1, and Wilis) with 3 replicates. Three plants of each cultivar were planted in a polybag, put under a cage, and after one week then infested with 5 female whiteflies. The parameters observed in this study were leaf epidermal thickness; density and length of trichome on lower leaf surface; and the number of egg, nymph, and adult of the whitefly. Observations were carried out every week, from 1−7 weeks after the plant was infested with the whitefly. The population of whitefly was affected by trichome density and length, as well as by the epidermal thickness of the lower leaf surface. The infestation of *B. tabaci* was higher in the soybean cultivars with denser and longer leaf trichomes, and thinner epidermal of the lower leaf surface. This result of this research suggests that it is necessary to include the characteristics of leaf trichome and epidermal thickness in the breeding program to produce soybean cultivars that are superior in productivity and resistant to whitefly.

Keywords: Aleyrodidae, Anjasmoro, Hemiptera, leaf characteristic, resistant

INTRODUCTION

Soybean is one of the food crops in Indonesia that has a high economic value. According to BPS (2016), the national soybean production in 2015 was only 998 thousand tons, while soybean consumption was reaching 2.2 million tons (Kementan, 2016). The realtively low soybean production in Indonesia causes by several problems including insect pests, one of them is whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae). The feeding activity of *B. tabaci* caused damage in host plants directly or indirectly (Hoddle, 2013). Loss of yield due to *B. tabaci* infestation and viruses can reach 80% (Marwoto *et al*., 2011).

Planting a resistant cultivar is one of strategy to control the pest population with environmentally friendly (Sulistyo, 2016). Berlinger (1986) stated that one of the factors affecting plant resistance to whitefly is the physical characteristics of surface leaves, i.e. leaf trichomes, leaf epidermal thickness, leaf shape, and microenvironment conditions. Until 2014, the government has released 70 superior soybean cultivars (Kementan, 2016). However, only a few cultivars are resistant to whitefly, one of which is the Tengger cultivar (Sulistyo, 2014). Therefore, developing resistant soybean cultivars to whitefly is necessary. Five local soybean cultivars have been produced by the Research Institute for Assorted Nuts and Bulbs (Balitkabi), yet the study about the physical characteristics of plants related to resistance to *B. tabaci* has not been carried out. This study aimed to determine the correlation between epidermal thickness, lowe leaf trichome density and length with populations of *B. tabaci* on five local soybean cultivars.

MATERIALS AND METHODS

The research was conducted at Bogor Agricultural University (IPB) 2017−2018 i.e. in the Cikabayan Greenhouse, Insect Biosystematics Laboratory, Department of Plant Protection - Faculty of Agriculture; Plant Ecology and Energy Laboratory, Department of Biology - Faculty of Mathematics and Natural Sciences; and the Reproduction Laboratory of LIPI Cibinong - Bogor.

Mass Rearing of Bemisia tabaci

Soybean were planted 2 seeds/polybag (30 cm × 30 cm) using 4 kg of soil and manure (1:1). Several polybags were put under the screen cage (80 cm × 80 cm) for mass rearing of *B. tabaci.* One adult female of the whitefly *B. tabaci* collected from field was infested into a cage for mass rearing for several generation (Figure 1).

Infestation of Bemisia tabaci *and Observation of Population*

The experiment was done using Completely Randomized Design (CRD) with five treatments (soybean cultivars) and 3 replicates. The soybean cultivars were Anjasmoro, Dena-1, Detam-3, Devon-1, and Wilis were used as treatments, designed using with 3 replicates and observed per week over 7 times. One week after planting (WAP), each soybean plant was covered. After 2 WAP, each plant was infested with 5 female adults. Observations were carried out per week (3−9 WAP) on soybean leaves to count the number of eggs, nymphs, and adults using a stereo microscope.

Measurement of Epidermal Thickness, Density and Length of Trichome

Trichome density and length of lower leaf surface was observed at plants aged 5 WAP by taking 3 leaflets per plant: at the top, middle, and bottom for each replication. Each leaflet was cut into 4 parts (1 cm × 1 cm) and 2 parts (1 cm × 1 cm) for observation of trichome density and trichome length, respectively. Trichome density was calculated using the Image Raster 3 program (PT Miconos Transdata Nusantara). Density and length of trichome were observed using Leica M.205.C microscope (PT Leica Microsystems). In addition, the epidermal thickness of the lower leaf surface was carried out at plants aged 6 WAP by taking 1 leaflet in the middle of the plant for each replicate. Leaf samples were cut across using a frozen microtome (Electro freeze MC-802A) with a size of 30 μm and stored in film tubes containing 70% alcohol as a preparation then photographed using Optilab Viewer 2.2. Epidermal thickness was measured using the Raster Image 3 program.

Figure 1. A cage for mass rearing of *Bemisia tabaci*

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Data Analysis

Data were analyzed by ANOVA using the Statistical Analysis System (SAS) program to determine whether there are any statistically significant differences between cultivars. Further analysis was carried out using Duncan's Multiple Range Test (DMRT) (α = 5%).

RESULTS AND DISCUSSION

The Morphological Characteristics of Plant

The leaf morphological characteristics of each soybean cultivar examined have different levels of trichome density, trichome length, and epidermal thickness (Table 1). The highest trichome density was Devon-1 (517 per cm^2) and significantly different from the other cultivars: Detam-3 (322 per cm^2), Wilis (272 per cm^2), Dena-1 (231 per cm^2), and Anjasmoro (188 per cm^2). Furthermore, the highest leaf trichome length was Devon-1 (0.66 mm) and significantly different from the four cultivars: Dena-1 (0.52 mm) and Anjasmoro (0.48 mm), Wilis (0.41 mm), and then Detam-3 (0.35 mm). The thickest epidermal leaf of soybean cultivars was Detam-3 (12.27 µm) and significantly different than the other cultivars: Wilis (11.18 µm), Dena-1 (0.46 µm), then Devon-1 (9.38 µm) and Anjasmoro (9.53 µm) with the thinnest epidermal.

***Population Development Pattern of* Bemisia tabaci**

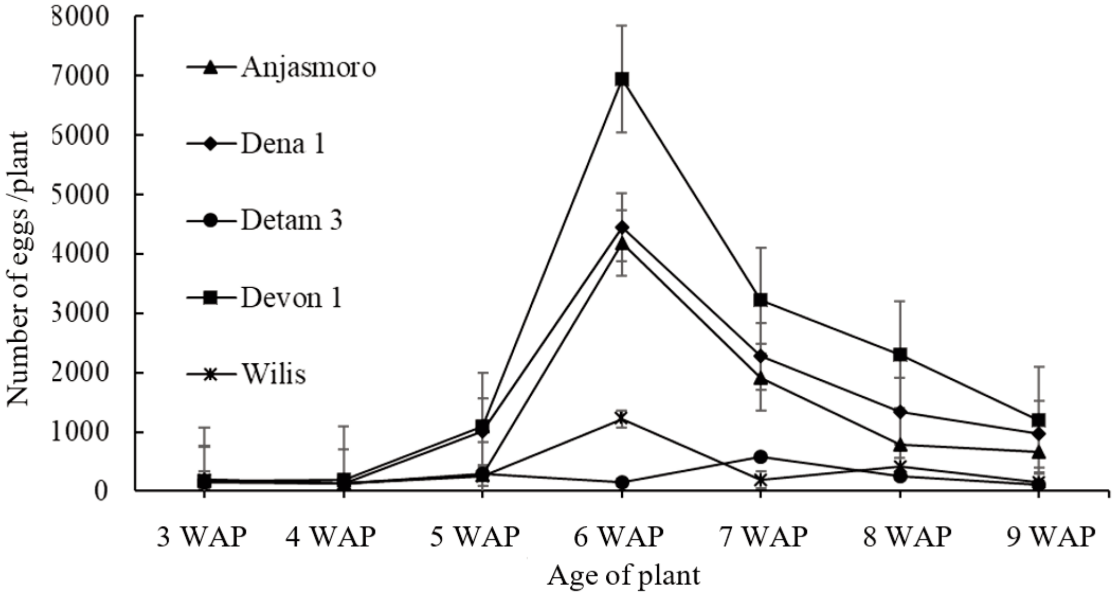
The population development pattern of the egg (Figure 2), nymph (Figure 3), and adult (Figure 4) of B. *tabaci* for each soybean cultivar were different. *B. tabaci* eggs were found from the beginning of observation (3 WAP), i.e. 192 per plant, 203 per plant, 148 per plant, 182 per plant, and 193 per plant for the cultivars of Anjasmoro, Dena-1, Detam-3, Devon-1, and Wilis, respectively. *B. tabaci* eggs began to increase from the age of 4 WAP and the peak population at 6 WAP, i.e. 4189 per plant, 4.446 per plant, 159 per plant, 6939 per plant, and 1216 per plant for Anjasmoro, Dena-1, Detam-3, Devon-1, and Wilis, respectively. The increase of *B. tabaci* eggs at 4-6 WAP was because *B. tabaci* has been in the second generation at 5 WAP, hence the number of eggs increased at 6 WAP. Takahashi *et al.* (2008) stated that the life cycle of whitefly from eggs to adult on soybean and tomato plants was 21 days. The increase in the number of eggs was influenced by the growth of the host plant, which is the bigger host plant, the more nutrient was needed. The decrease of *B. tabaci* egg began at 7 WAP until 9 WAP caused by a decrease in leaf nutrients (there was no young leaf produced), because soybean plants were in the generative growth phase. The five soybean cultivars examined have determinate growth types, the plants stop forming young leaves at 6 WAP. Gameel (1974) reported that *B. tabaci* tended to lay eggs on young leaves because the nutrients needed were easier to obtain.

Table 1. Epidermal thickness, trichome density, and trichome length of the lower leaf surface

Soybean cultivars	Epidermal thickness (µm)	Trichome density per cm^2	Trichome length (mm)
Anjasmoro	9.53a	188a	0.48c
Dena 1	10.46b	231b	0.52c
Detam 3	12.27d	322d	0.35a
Devon 1	9.38a	517e	0.66d
Wilis	11.18c	272c	0.41b

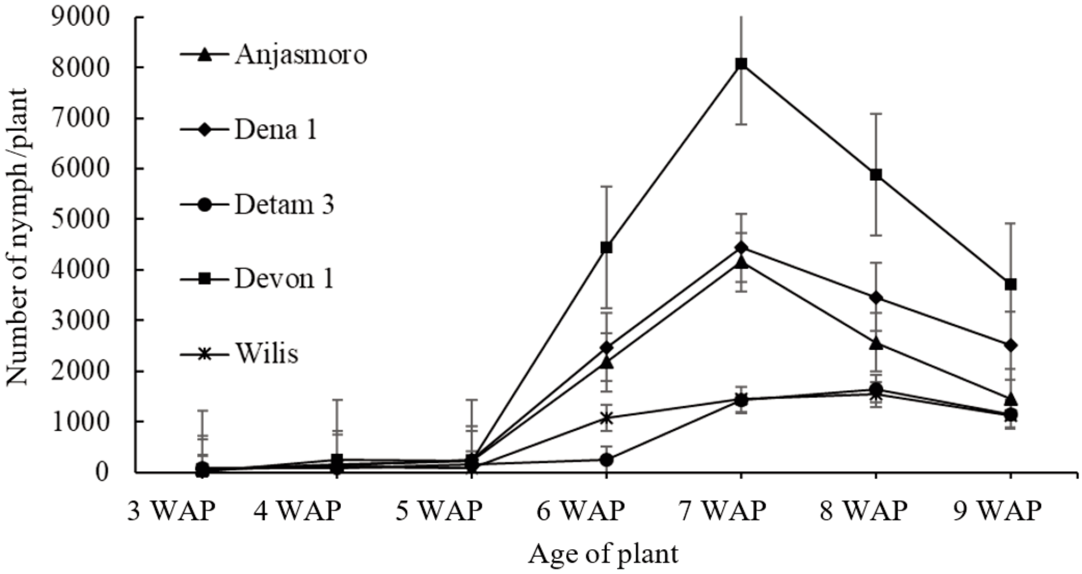
Remarks: Means followed by different letter were not significantly difference according to Duncan's Multiple Distance Test (DMRT) at α = 5%.

The nymph population (Figure 3) began to be found at 4 WAP, i.e. 165 per plant, 148 per plant, 93 per plant, 244 per plant, and 103 per plant, for Anjasmoro, Dena-1, Detam-3, Devon-1, and Wilis, respectively. *B. tabaci* nymphs began to increase at 5-7 WAP, i.e. 4156 per plant, 4441 per plant, 1429 per plant, 8066 per plant, and 1447 per plant, for Anjasmoro, Dena-1, Detam-3, Devon-1, and Wilis, respectively. The increasing of the nymphs at 5−7 WAP was caused by the whitefly has been in the second generation at 5 WAP. Because the longevity of eggs became nymphs was 6 days, hence most eggs have developed into nymphs at 7 WAP. Marwoto *et al*. (2011) reported that the longevity of *B. tabaci* egg was 6 days. The population of nymphs began to decrease at 8-9 WAP because the soybean plants have entered the phase of pod maturation, thus less in the nutrient content of the host plant. Murgianto and Hidayat (2017) stated that whitefly attack was under the economic threshold when the soybean plants have entered the pod maturation phase. Therefore, the decrease of *B. tabaci* nymphs was influenced by the plant age and the stadia of *B. tabaci*.



(WAP = week after planting)

Figure 2. The population of *Bemisia tabaci* eggs per plant

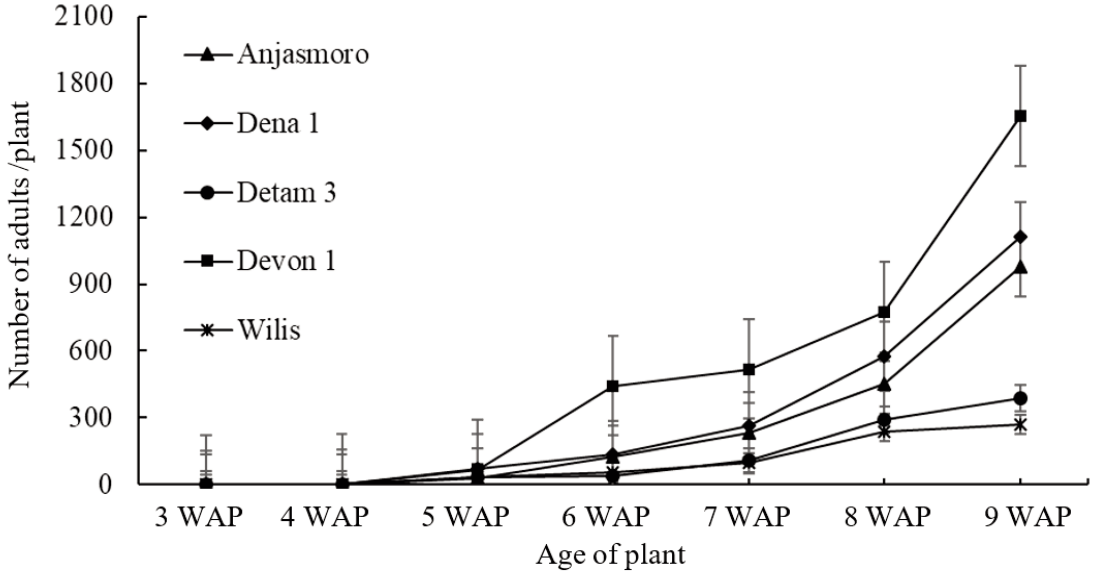


(WAP = week after planting)

Figure 3. The population of *Bemisia tabaci* nymphs per plant

B. tabaci adults has a different developmental pattern than the nymph and egg (Figure 4). The adults began to be found at 5 WAP, i.e. 29 per plant, 74 per plant, 33 per plant, 68 per plant, and 35 per plant for Anjasmoro, Dena-1, Detam-3, Devon-1, and Wilis, respectively and began to increase at the 9 WAP, i.e. 981 per plant, 1113 per plant, 390 per plant, 1655 per plant, and 270 per plant for Anjasmoro, Dena-1, Detam-3, Devon-1, and Wilis, respectively. The number of adults continued to increase because of *B. tabaci* has been in the third generation at 8 WAP hence the adults increased at 9 WAP.

Those results revealed that the high development of *B. tabaci* populations was found in Devon-1. Devon-1 has the physical characteristics of the lower leaf surface that may provide the suitable habitat for *B. tabaci* to grow and develop, i.e. the high of trichome density and length with a thin epidermal layer.



(WAP = week after planting)

Figure 4. The population of *Bemisia tabaci* adults per plant

Valle *et al*. (2012) stated that the higher trichome density and the longer of leaf trichome, the more suitable habitat for *B. tabaci* eggs and nymphs to develop. The interaction between density, length, and slope angle of the leaf trichome will determine the genotype characteristic resistance of soybean cultivar (Figure 5). The lowest development of *B. tabaci* was found in the Detam-3. Because Detam-3 has the physical characteristics of the lower leaf surface that might not support the development of *B. tabaci*, such as the thick epidermal layer (Figure 6) and short trichome length. The thick epidermal layer might prevent the nymph from getting the needed nutrients hence the number of the *B. tabaci* nymphs were low. Sulistyo and Inayati (2016) reported that the soybean cultivar with the characteristic of very and medium resistant to *B. tabaci* has a genotype of thick leaves. The short leaf trichome might make easier for eggs and nymphs to be carried away by the wind and be attacked by the natural enemies.

***The Relationship between the Leaf Morphological Characteristicss and the Development of* B. tabaci**

The epidermal layer of the lower leaf surface of soybean plants has a negative relationship with the development of *B. tabaci* (Figure 7). The high correlation coefficient value of eggs, nymphs, and adults (r = -0.854, -0.799, -0.803) showed that the thicker of the leaf epidermis layer, the less of the development of *B. tabaci*. Because the thin leaf epidermis layer will provide a suitable habitat for the nymphs to find vascular bundles. Cohen *et al.* (1996) reported that more than 98% of the salivary sheath of *B. tabaci* nymphs was found to be connected with the vascular bundle and to be attached with the minor vein (either single or double strand) to obtain nutrients carried by xylem and phloem tissue.

However, the trichome density and length of the lower leaf surface have a positive relationship to the development of *B. tabaci* with the correlation coefficient value of eggs, nymphs, and adults were 0.485 and 0.979; 0.645 and 0.988; and 0.600 and 0.972, respectively (Figure 8 and 9) showed that the higher density and the longer of leaf trichomes, the more number of *B. Tabaci* was found. Trichome density is a main morphological characteristic related to plant resistance to sucking pests and has a positive correlation with the colonization of sucking pests (Aslam & Saeed, 2004). Previous research by Indrayani and Sulistyowati's (2005) also reported that the leaves with a high trichome density were positively correlated with the increase of *B. tabaci* population compared to leaves with fewer trichomes. High correlation coefficients of the *B. tabaci* eggs, nymphs, and adults showed that the leaf trichomes

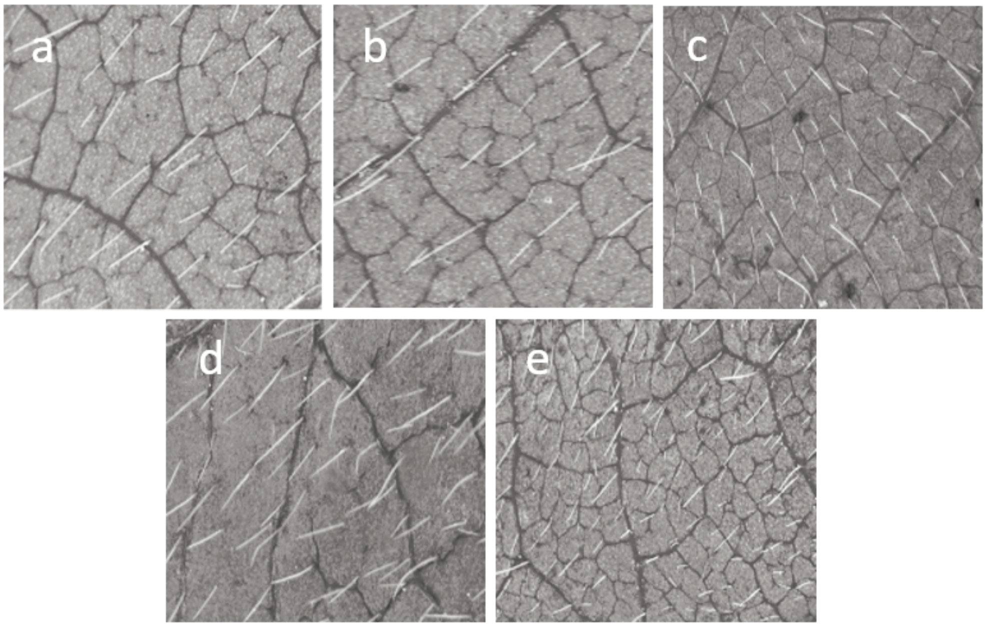


Figure 5. The trichome of lower leaf surface of soybean cultivars (50 mm × 50 mm); (a) Anjasmoro, (b) Dena-1, (c) Detam-3, (d) Devon-1, and (e) Wilis

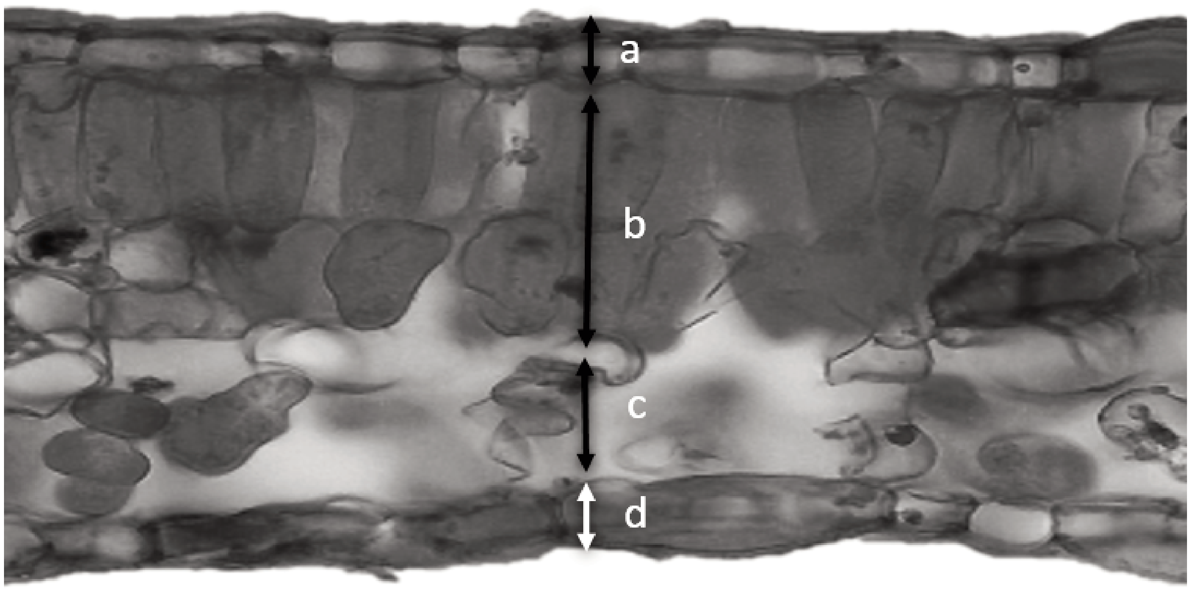


Figure 6. Epidermal cross-section of the lower leaf surface of Detam-3 cultivar; (a) upper leaf epidermis, (b) palisade tissue, (c) spongy tissue, (d) lower leaf epidermis

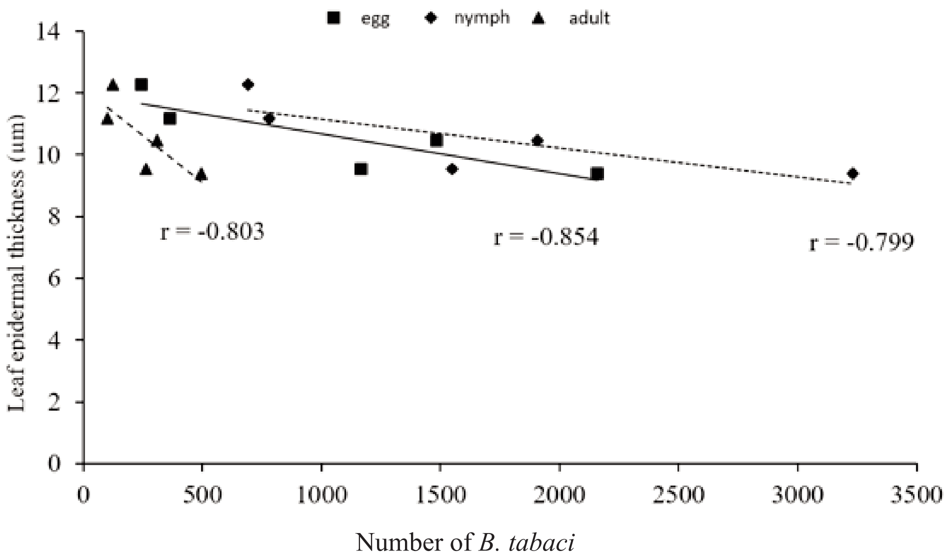


Figure 7. The relationship between the epidermal thickness of the lower leaf surface and the number of *Bemisia tabaci*

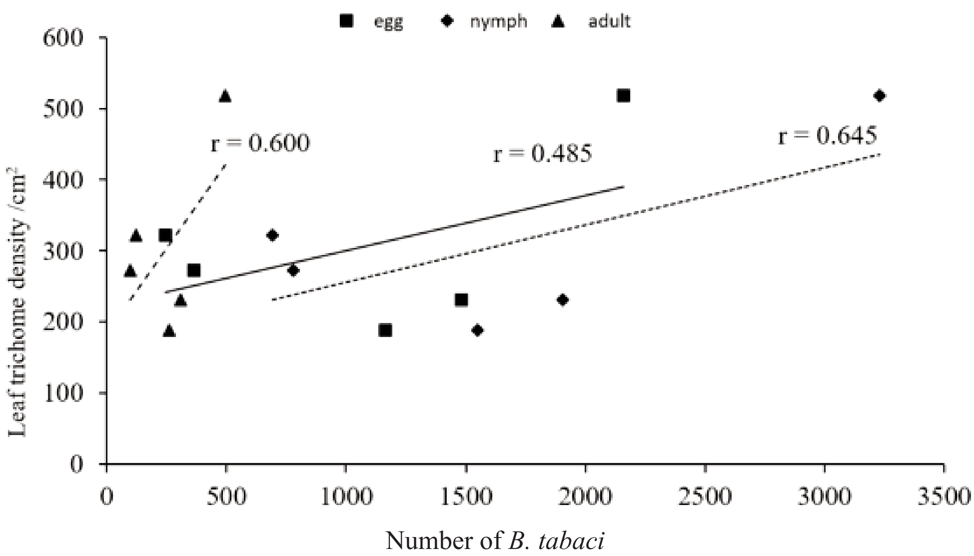


Figure 8. The relationship between the trichome density of lower leaf surface and the number of *Bemisia tabaci*

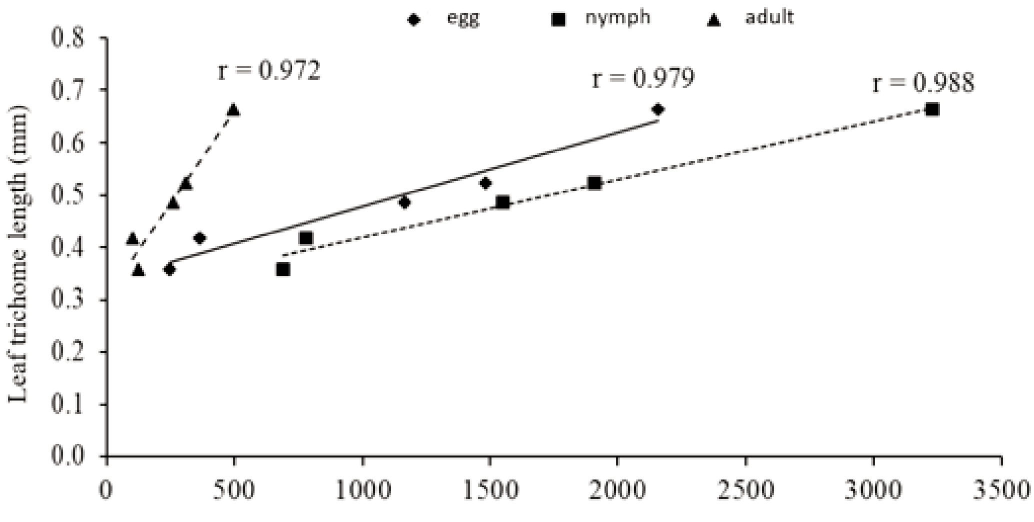


Figure 9. The relationship between the trichome length of the lower leaf surface and the number of *Bemisia tabaci*

influenced the development of *B. tabaci*. The leaves with the long trichomes might provide the suitable habitat for *B. tabaci* to lay eggs on it. A similar result also reported by Vieira *et al*. (2011) that the long trichome might prevent the eggs to be carried away by the wind.

CONCLUSION

The population of whitefly was affected by trichome density and length, as well as by the epidermal thickness of the lower leaf surface. The infestation of *B. tabaci* was higher in the soybean cultivars with denser and longer leaf trichomes, and thinner epidermal of the lower leaf surface. This result of this research suggests that it is necessary to include the characteristics of leaf trichome and epidermal thickness in the breeding program to produce soybean cultivars that are superior in productivity and resistant to whitefly.

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