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DIFFERENTIAL LEVELS OF SOYBEAN RESISTANCE TO THE WHITEFLY *Bemisia tabaci* (Hemiptera: Aleyrodidae) UNDER CONTROLLED AND UNCONTROLLED ENVIRONMENTS ARE ASSOCIATED WITH PLANT AGE, DAMAGE INTENSITY, AND TRICHOME DENSITY

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Abstract

Whiteflies are a severe threat to soybean production in the tropics. This study aimed to evaluate the soybean resistance level of the whitefly *Bemisia tabaci* in controlled and uncontrolled environments that is associated with plant age, damage intensity, and trichome density. The research was conducted under two conditions: non-sprayed (NS) and sprayed (SP). This study used 50 soybean genotypes arranged in a randomized block design with three replicates. The whitefly population was derived from natural infestations. The results showed that the highest wild population of *B. tabaci* occurred at 40 days after planting (DAP), i.e., 126.08 adults/plant in the NS environment and 22.57 adults/plant in the SP environment. The peak damage intensity occurred at 50 DAP, 20.71% in the NS environment, and 17.15% in the SP environment. In the NS environment, there were six resistant genotypes (including the resistant control G100H), 25 moderate, and 19 susceptible genotypes. In the SP environment, 19 genotypes were resistant, 22 genotypes were moderate, and nine genotypes were susceptible, respectively. Six soybean genotypes showed consistent resistance to *B. tabaci* in NS and SP environments. The low density of leaf trichomes in soybean may influence the high resistance to *B. tabaci*. The resistant genotypes identified in this study could be utilized in breeding programs for *B. tabaci* resistance.

Keywords: *B. tabaci*. Breeding. Correlation. Insect population. Leaf trichome.

1. Introduction

The whitefly *Bemisia tabaci* genn (Hemiptera: Aleyrodidae) is a phloem sap-feeding polyphagous insect pest that has become the most destructive pest of various agricultural commodities worldwide (Satar et al. 2018; Manivannan et al. 2021; Naalden et al. 2021; Zhang et al. 2021), including soybean (Arnemann et al. 2019; Ayala et al. 2020; Barros et al. 2021). Previous investigations have reported that there are currently more than 1550 described species of whiteflies recorded worldwide (Martin et al. 2007), with the preferred host species for *B. tabaci* being plants from Compositae, Cruciferae, Cucurbitaceae, Solanaceae, and Leguminosae (Li et al. 2011). Due to their economic importance, whiteflies have become the most important agricultural pest and virus vectors for crops worldwide (Huang et al. 2021; Khamis et al. 2021).

Significant yield losses due to *B. tabaci* can range from 50–80% (Ellsworth and Martinez-Carrillo 2001). Populations of *B. tabaci* were found to be randomly distributed throughout soybean fields and peaked during reproductive growth stages (Suekane et al. 2018; Rodriguez et al. 2022). A study reported that *B. tabaci* infestation increased after the V1 stage, reaching 60.3% in the R1 stage (Suekane et al. 2018). Similarly, another study concluded that the critical period for *B. tabaci* control in soybean occurs around the R1 growth stage (Padilha et al. 2021). Rainfall, relative humidity, and wind speed were positively correlated with adult whitefly density in soybean crops (Rodriguez et al. 2022).

Plant damage caused by *B. tabaci* has resulted in significant economic losses, prompting the development of potential management techniques. To date, chemical insecticides have been commonly used to control *B. tabaci* (Sani et al. 2020; Wang et al. 2022). However, the high mobility of whitefly pests has caused these management efforts to be generally ineffective, in addition to the impact of pesticide use (Bernaola et al. 2021; Lykogianni et al. 2021). Therefore, a safe and environment-friendly integrated pest management (IPM) strategy is required, especially for resistant varieties.

Resistance to whiteflies is associated with leaf morphophysiological characteristics. A previous study showed that the expression of antixenosis in several soybean genotypes is related to trichome density and inclination (Baldin et al. 2017). In savoy cabbage (*B. oleracea* L. var. *sabauda*) and kale (*B. oleracea* L. var. *sabellica*), lower whitefly infestation may be associated with a greater density of leaf mesophyll cells. The layout of the cuticle, folding of epidermal cells, and stomatal size and thickness are also factors affecting whitefly susceptibility (Marasek-Ciolakowska et al. 2021). Leaf morphology, such as the density and type of trichomes, as well as leaf shape, are linked to the insect's colonization and oviposition preferences, as well as its biology (Amini et al. 2021; Mookiah et al. 2021). However, leaf trichome density has been reported to have no distinctive impact on aphids, herbivorous orthopterans, or various lepidopteran foliage feeders found in the field (Faiz et al. 2021). Environmental factors (temperature, relative humidity, sunshine, and rainfall) have also been reported to influence whitefly populations (Marabi et al. 2017).

Various indicators can be used to measure host plant resistance to whiteflies. A study on soybeans stated that the intensity of leaf damage could be used as a criterion for determining soybean resistance to whiteflies (Sari and Sulistyo 2018). Another study screened 208 soybean cultivars by the number of eggs, larvae, and pupae on the leaves, resulting in 78 highly resistant cultivars (Gulluoglu et al. 2010). Nivedita et al. (2020) found that soybean resistance to the whitefly was characterized by less oviposition and hatching, with a prolonged developmental period, high mortality, and lower survival percentage of whiteflies.

The resistance mechanism to whiteflies could be antixenosis or antibiosis (da Silva et al. 2012). The antixenosis shown by Jackson, P98Y11, and PI-229358 may be related to the characteristics of the trichomes (lower density and inclined) (Baldin et al. 2017). A study on the resistance mechanism of cucumber genotypes against *B. tabaci* revealed antixenosis in IAC-1214, antibiosis in Wellington, and antibiosis and antixenosis in IAC-1201, Campeiro, Japan, and IAC-1311 (Novaes et al. 2020). Another study found five genotypes resistant to *B. tabaci* biotype B, with antixenosis and antibiosis resistance mechanisms (Domingos et al. 2018).

Considering the great whitefly damage to soybean yield, this study evaluated the soybean resistance to whitefly *B. tabaci* in controlled and uncontrolled environments that are associated with plant age, damage intensity, and trichome density.

2. Material and Methods

Plant materials

This study used 50 soybean genotypes, which consisted of 43 genotypes resulting from the selection of crossings, five cultivated varieties (Ijen, Dega 1, Detap 1, Derap 1, and Gema), and two control cultivars (Anjasmoro and G100H). Anjasmoro was the susceptibility control, and G100H was the resistance control. This research was conducted at the Indonesian Legume and Tuber Crops Research Institute (ILETRI), Malang, East Java, Indonesia, from August to November 2020.

Soybean planting

Each soybean genotype was planted in a plastic pot (Φ = 18 cm) containing soil and manure in a 1:1 ratio. The experimental design was a randomized block with three replicates. The study was conducted under two conditions: non-sprayed (NS) and sprayed (SP). In the first environment (NS, non-sprayed), pests and diseases were not controlled until 65 days after planting (DAP). In the second environment (SP, sprayed), pests and diseases were controlled from 20 DAP until harvesting. Each genotype was fertilized with NPK fertilizer at a dose of 5 g. Weed control was performed according to the recommended practices.

Evaluation of leaf damage

Leaf damage caused by *B. tabaci* was observed at 20, 30, 40, 50, and 60 DAP under both environmental conditions (NS and SP). Leaf damage was scored based on a 0 to 4 nominal ranking scale according to leaf curling and the occurrence of blackish sooty mold on leaves (Inayati and Marwoto 2012):

0 = healthy plant, or there was no leaf curling and blackish sooty mold on leaves
1 = the occurrence of leaf curling and blackish sooty mold on leaves with an intensity > 0– 25%
2 = the occurrence of leaf curling and blackish sooty mold on leaves with an intensity > 0– 25%
3 = the occurrence of leaf curling and blackish sooty mold on leaves with an intensity > 50–75%; pods and seeds do not grow well (abnormal).
4 = the occurrence of leaf curling and blackish sooty mold on leaves with an intensity > 76%; pods and seeds do not grow well (abnormal)

Observation

The leaf damage score was used to calculate damage intensity as follows:

$$I = \frac{\sum n \times v}{N \times Z} \times 100\%$$

where:
I = damage intensity
n = number of attacked leaves at each scoring value
v = the score of leaf damage (0 to 4)
N = the total number of leaves
Z = highest score attack

The degree of resistance to *B. tabaci* was determined based on the peak of the damage intensity using the classification by AVRDC (1979) with modifications: very resistant (0%), resistant (> 0–10%), moderate (> 10–25%), susceptible (> 25–50%), and very susceptible (> 50%).

Observations were also made for the number of imago per plant and the number of leaves per plant. A number of imago was observed on the adaxial and abaxial leaf surfaces of the third node leaves per replicate at 20, 30, 40, and 50 DAP. Trichome density was also observed at 4 mm^2 on the abaxial and adaxial surfaces of leaflets (under a 40x objective) of each genotype collected from the third node at 40 DAP.

Statistical analyses

Descriptive statistics were used for data obtained from all experiments. Pearson correlation analysis (Singh and Chaudhary 1977) investigated the relationship between the damage intensity at all plant ages and trichome density. The results were visualized using the *Corrplot* and *Hmisc* packages of RStudio software (RStudio 2020).

3. Results and Discussion

The population of *B. tabaci*

Whitefly pests are well known for their high mobility. The study location was endemic to *B. tabaci*; thus, *B. tabaci* populations in soybean genotypes began to appear at 20 DAP. In the first environment (NS, non-sprayed), the average *B. tabaci* population per plant reached 3.41 adults at 20 DAP, peaked at 40 DAP (126.08 adults/plant), and then declined to 21.40 adults per plant at 60 DAP (Figure 1A). In the second environment (SP, sprayed), the population of *B. tabaci* at 20 DAP was 1.65 adults per plant, reaching the peak population at 40 DAP (22.57 adults/plant), after which the population declined at 60 DAP (9.60 adults/plant) (Figure 1B). In other studies, *B. tabaci* infestations also showed variability among genotypes (Murry et al. 2018; Kumar et al. 2019).

A

B

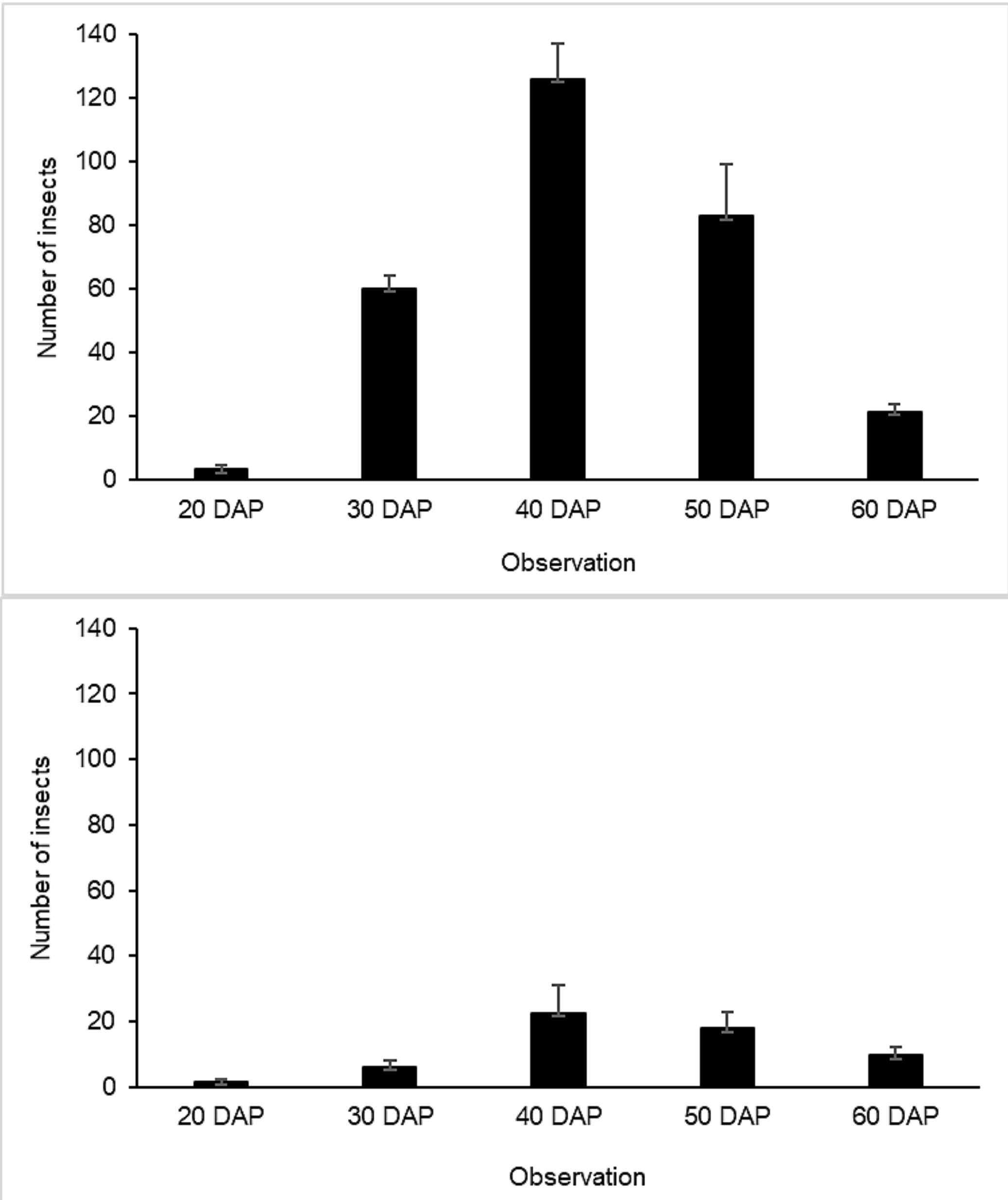

Figure 1. Average *B. tabaci* population per plant at 20, 30, 40, 50, and 60 days after planting (DAP) under A - non-sprayed and B - sprayed environments.

The variation in *B. tabaci* infestations based on plant age is shown in Figure 2 (NS) and Figure 3 (SP). This study found the highest *B. tabaci* festation in NS and SP environments at 40 DAP. The range of the *B. tabaci* population in the NS environment was 110.33 to 154.17 adults/plant, while that in the SP environment was 11.00–45.75 adults/plant. This indicates that the natural population of *B. tabaci* is relatively high, especially in the NS environment, meaning that the use of the NS environment is ideal as a selection method for soybean genotype resistance against *B. tabaci*.

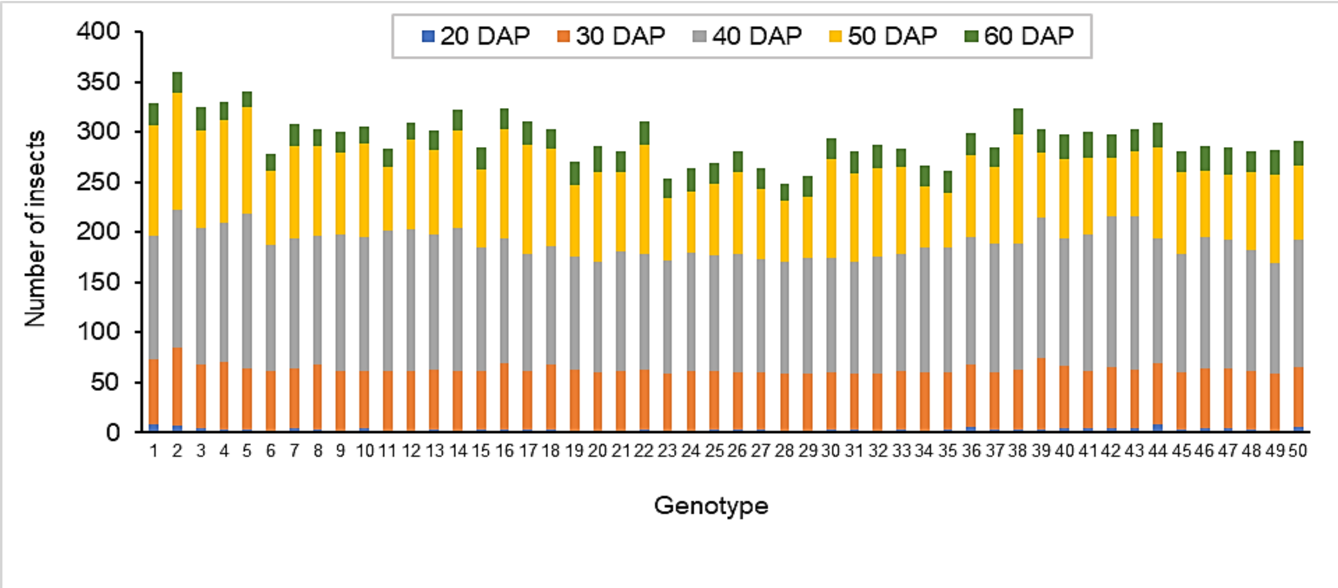

Figure 2. *B. tabaci* infestation of 50 soybean genotypes based on plant age in the non-sprayed (NS) environment.

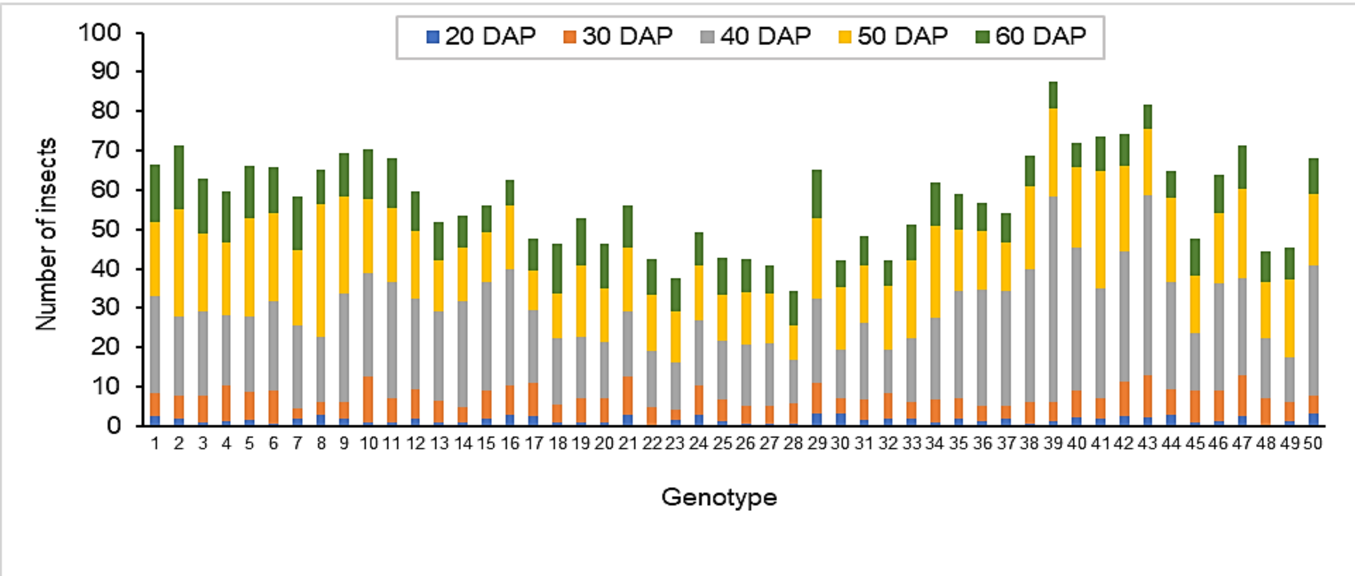

Figure 3. *B. tabaci* infestation of 50 soybean genotypes based on plant age in the sprayed (SP) environment.

Damage intensity

Plant damage intensity varied between the genotypes studied and uncontrolled (NS) and insecticide-controlled (SP) conditions. In the NS environment, the damage intensity increased from 0% (20 DAP) to 20.71% at 50 DAP, but the attack intensity did not increase significantly at 60 DAP (20.64%) (Figure 4a). A similar pattern was observed in the SP environment (Figure 4b). The damage intensity increased from 0% at 20 DAP to 17.15% and 18.24% at 50 and 60 DAP, respectively. Accordingly, the peak of plant damage occurred at 50 DAP in both NS and SP environments. The average damage intensity in the two settings was not significantly different, indicating that the opportunity to use resistant varieties was sufficient to manage *B. tabaci*. Another study concluded that choosing resistant soybean cultivars is the most effective way to control whiteflies (Xu et al. 2005).

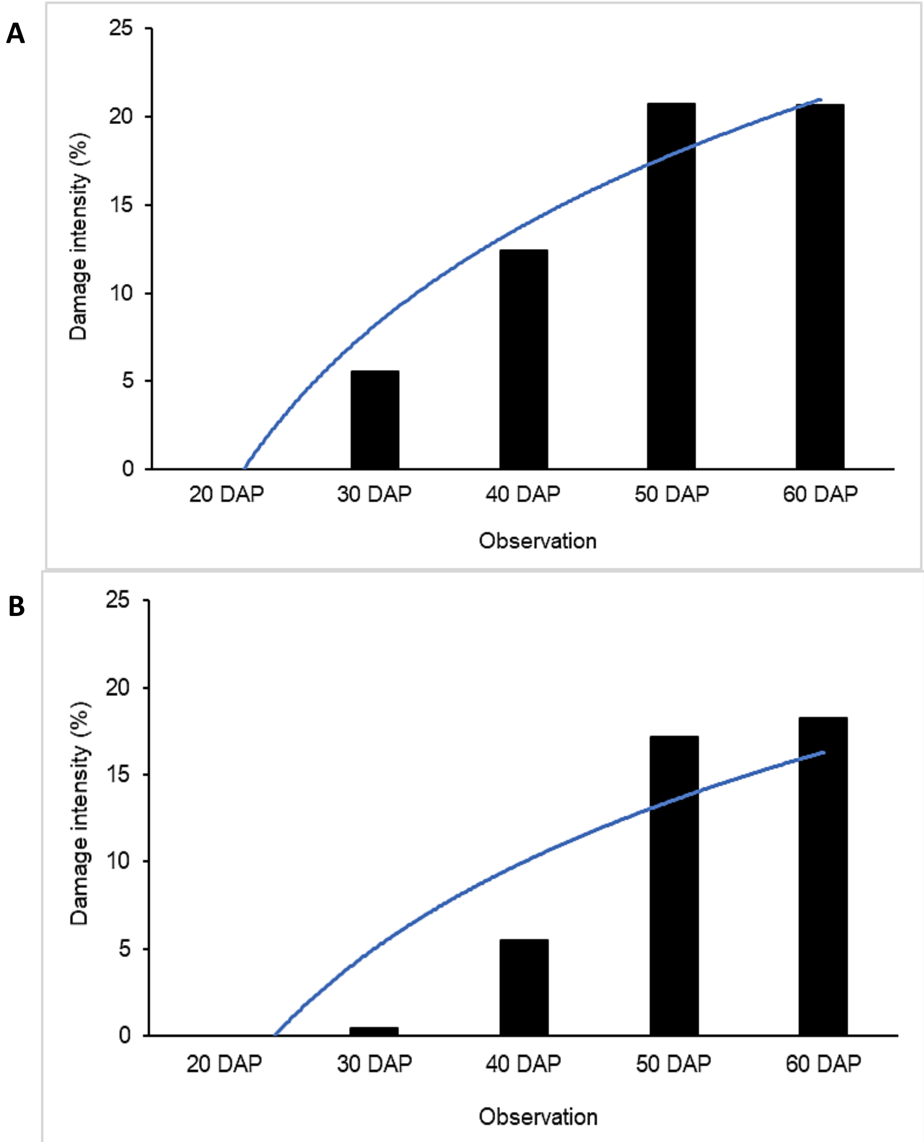

Figure 4. Average damage intensity: A - under non-sprayed (NS) and B - sprayed (SP) conditions at different plant ages.

Damage intensity varied among the soybean genotypes. The range of damage intensity at 50 DAP in the NS environment was 6.34–33.02% (Figure 5). The susceptible control cultivar (Anjasmoro) had a damage intensity of 19.66%, whereas the resistant control cultivar (G100H) had a damage intensity of 9.87%. Several genotypes demonstrated a lower damage intensity than that of G100H. The lowest damage intensity at 50 DAP was shown by SPL-20-1, and its resistance remained consistent after 30 DAP.

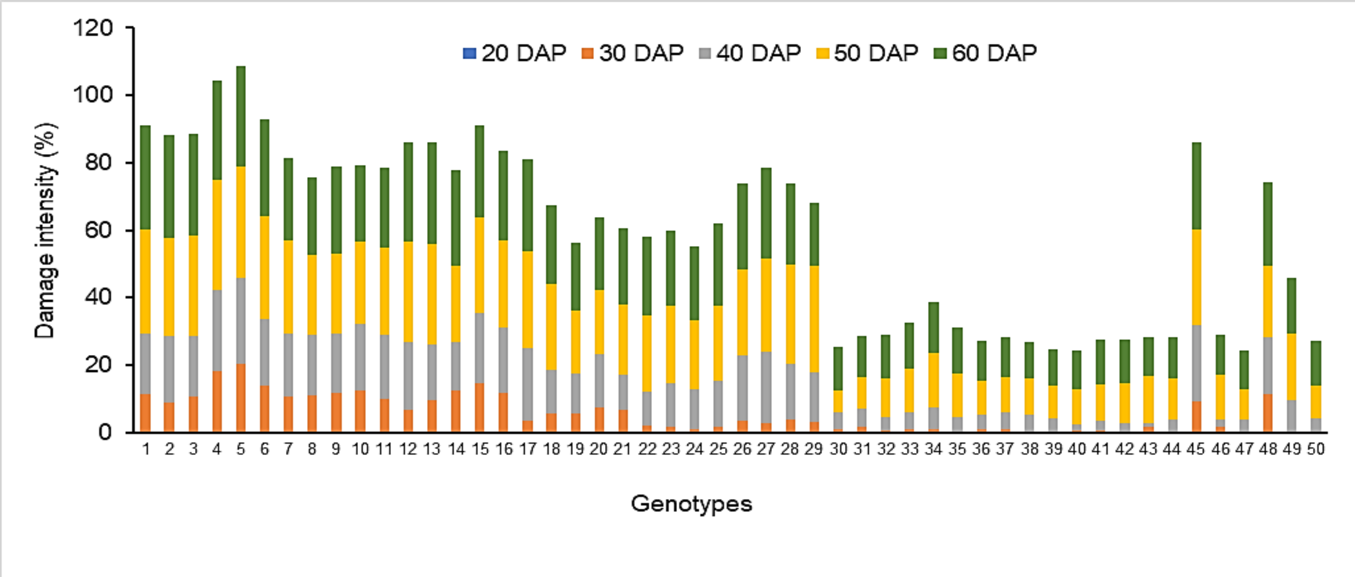

Figure 5. Damage intensity of 50 soybean genotypes at different plant ages under non-sprayed (NS) environment.

In the SP environment (Figure 6), the range of the damage intensity at 50 DAP was 1.67–45.49%. SPL-20-11 exhibited the lowest damage intensity, followed by SPL-20-1 (4.35%). The damage intensity of the control cultivars (Anjasmoro and G100H) was 24.21 and 5.69%, respectively.

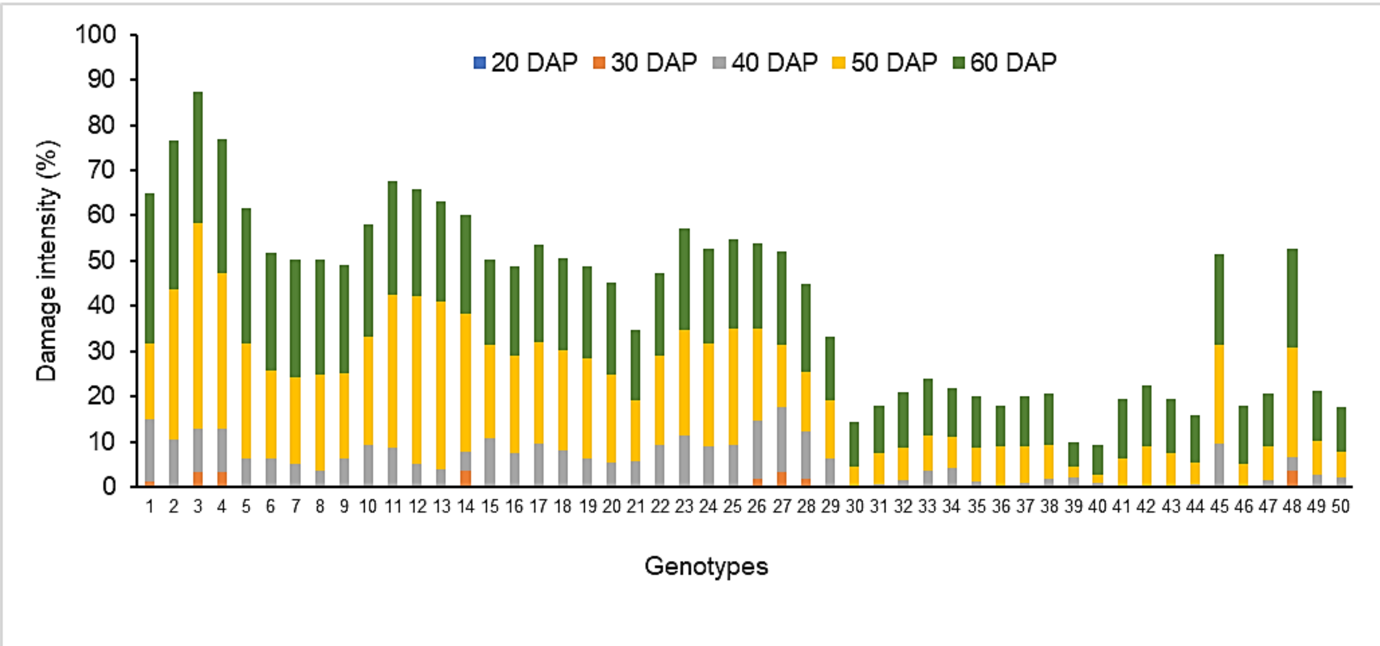

Figure 6. Damage intensity of 50 soybean genotypes at different plant ages under sprayed (SP) environment.

Classification of soybean resistance to *B. tabaci*

The resistance classification of 50 soybean genotypes against *B. tabaci* was based on the peak of the damage intensity (50 DAP). In the NS environment, there were six resistant genotypes (including the resistant control G100H), 25 moderate, and 19 susceptible genotypes. In the SP environment, 19 resistant, 22 moderate, and nine susceptible genotypes were obtained (Figure 7). Highly resistant and susceptible genotypes were not found in NS or SP environments. However, other studies have found that soybeans resistant to *B. tabaci* ranges from highly susceptible to highly resistance (Gulluoglu et al. 2010; Cruz & Baldin 2016; Murry et al. 2018). Each genotype has a unique mechanism of resistance to whiteflies. The resistance mechanisms in soybean genotypes are reported to be antixenosis, antibiosis, or a combination of the two (Vieira et al. 2011; da Silva et al. 2012). The resistant genotypes obtained from this study can be used against whiteflies in the IPM program or as a source of resistance in plant breeding programs.

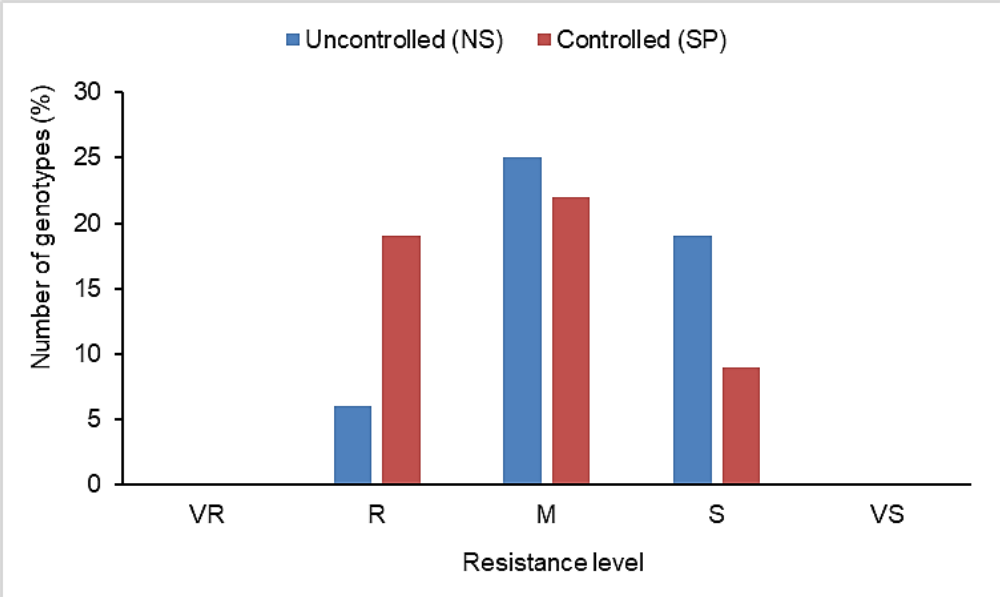

Figure 7. Resistance classification of 50 soybean genotypes against *B. tabaci* (VR, very resistant; R, resistant; M, moderate; S, susceptible; VS, very susceptible).

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The density of leaf trichomes

The leaf trichome is a plant structure that plays a vital role in resistance to insect herbivores. In this study, the leaf trichome density varied among the 50 soybean genotypes and the range was 17.00 to 79.75 trichomes/4 mm^2, with an average of 45.76/4 mm^2 (Figure 8). The resistant control cultivar G100H, the progeny of IAC 100 × Himeshirazu (Adie et al., 2020), had a dense trichome (38.00/4 mm^2). A total of 22 soybean genotypes had a trichome leaf density over the general mean, ranging from 47.75–79.75/4 mm^2.

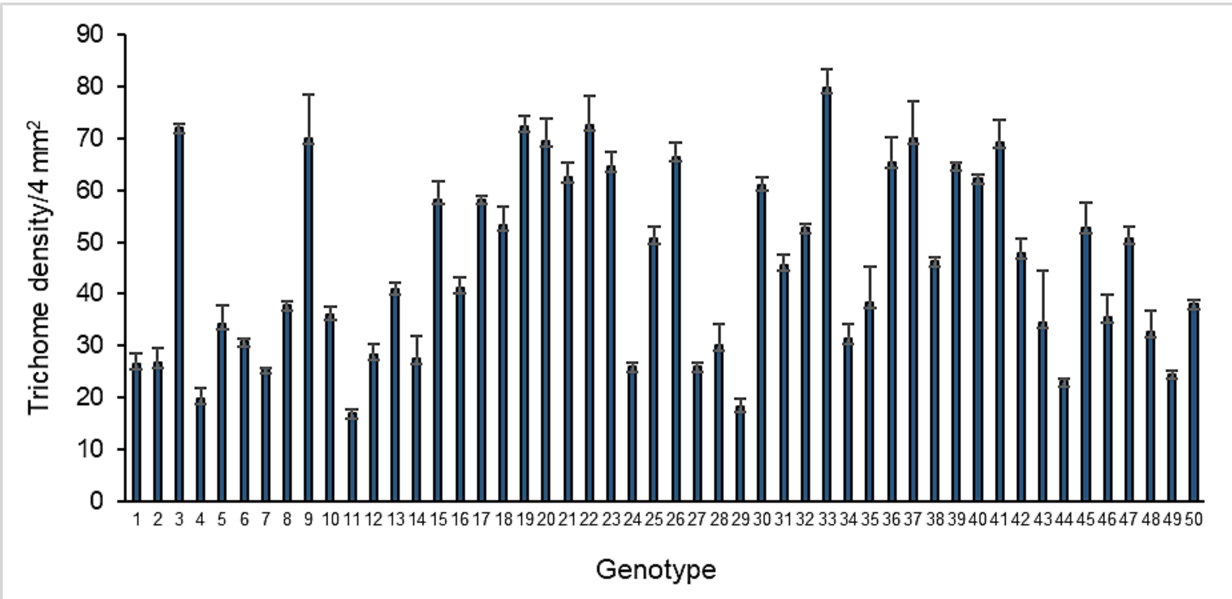

Figure 8. The density of leaf trichomes of 50 soybean genotypes at 40 days after planting (DAP) was observed on the abaxial and adaxial surfaces of the leaflets.

Another study reported that plant defense against insect herbivores is associated with leaf surface and morphological characteristics, such as trichome orientation, leaf thickness, and color (Goiana et al. 2020; de Oliveira et al.,2020). The type of insect determines the study of leaf morphology concerning pest resistance and the genetic makeup of the genotype used.

Association among characters

Investigations of the association among characters in determining soybean resistance to the *B. tabaci* were conducted between the damage intensity at 50 DAP with the *B. tabaci* population per plant at 20, 30, 40, 50, and 60 DAP and the leaf trichome in the NS (Figure 9a) as well as the SP environment (Figure 9b). The damage intensity at 50 DAP was used for correlation analysis because the highest (peak) average damage intensity occurred at 50 DAP. The highest (peak) whitefly infestation in the NS environment as well as in the SP environment occurred at 40 DAP (followed by 50 DAP), which also had an impact on the high damage intensity in the next ten days (50 DAP).

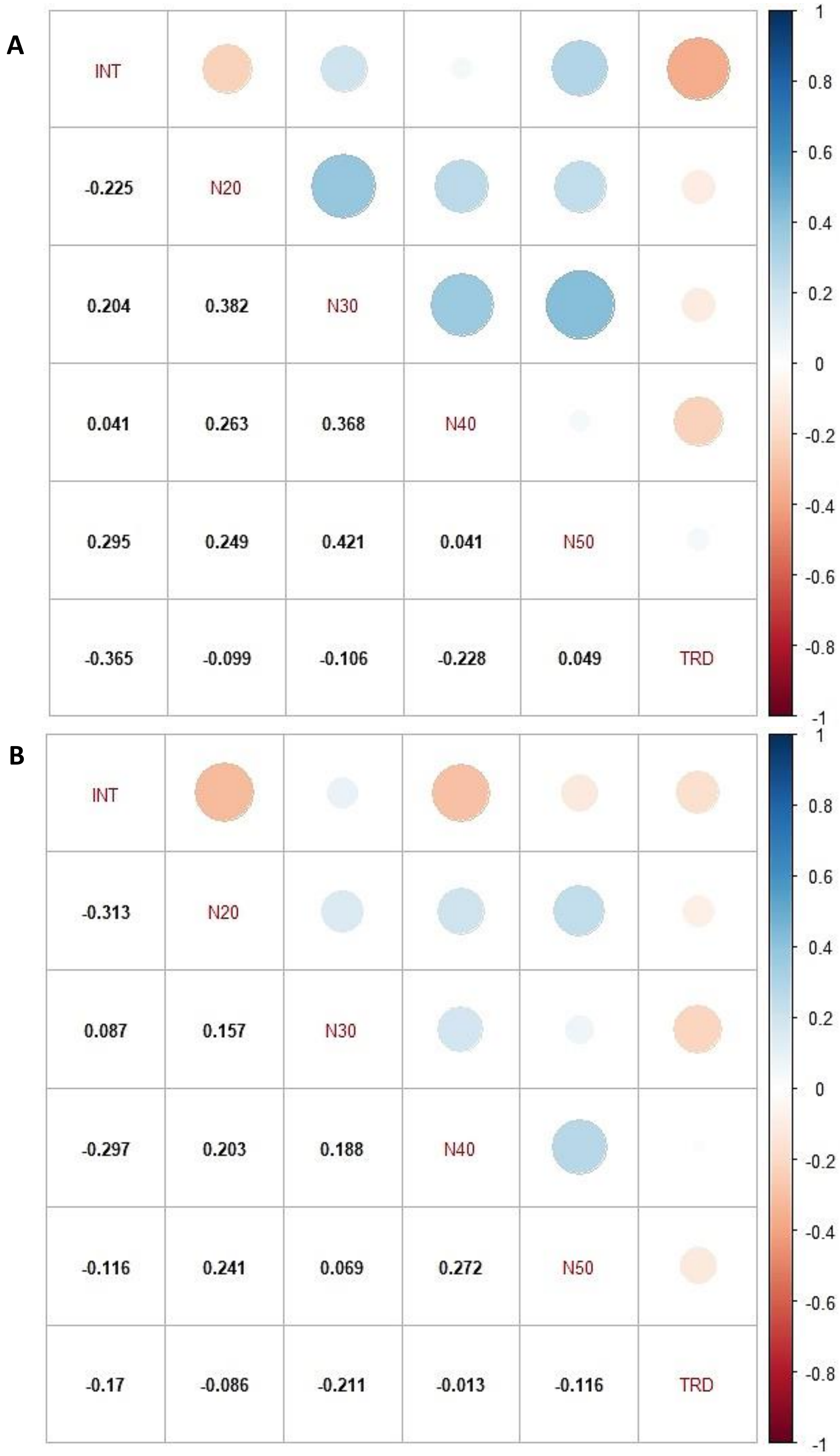

Figure 9. Association among characters using the Pearson correlation in the: A - non-sprayed (NS) and B - sprayed (SP) environments.

In the NS environment (Figure 9A), leaf damage intensity showed a significant positive correlation with the insect pest population at 50 DAP (r = 0.295*). The intensity of leaf damage was not significantly correlated with the peak insect pest population (40 DAP), which may be due to the leaf damage that occurred several days after the whitefly infestation (Wang et al. 2019). A study by Patil et al. (2021) showed that the highest whitefly population resulted in higher MYMV disease in the following week. Furthermore, the damage intensity was significantly negatively correlated with the density of leaf trichomes (r = –0.365**). A high number of trichomes resulted in high damage intensity. This study is in line with that of Sari and Sulistyo (2018), who found a negative correlation between the intensity of leaf damage and the number of leaf trichomes. The high density of trichomes found on leaves is essential for whitefly colonization because it allows individual insects to stay on the surface of leaves and avoid being swept away by the wind (Vieira et al. 2011). Da Silva et al. (2012) obtained a positive correlation between trichome density and oviposition site. They suggested that trichome density encourages or stimulates the permanence and oviposition of the insect on the plant. Other theories have proposed that genotypes with a high number of trichomes can create a microclimate that is more conducive to oviposition by *B. tabaci* (Butter and Vir 1989).

The association patterns were different in the SP environment (Figure 9B). The leaf damage intensity at 50 DAP was negatively correlated with the insect pest population at 20 DAP (r = –0.313*) and 40 DAP (r = –0.297*). This result could be attributed to the *B. tabaci* control mechanism (sprayed with insecticide). The number of insect populations could be high, but it could not cause high damage intensity due to the effect of a controlled environment. This also affects leaf trichome density, not associated with leaf damage intensity.

The six resistant genotypes in the NS (and SP) environment (Table 1) had trichome densities ranging from 38–65 trichomes/4 mm2. Sulistyo and Inayati (2016) found that genotypes resistant to *B. tabaci* have a trichome density range of 44–67 trichomes. Based on the results above, we can see that the trichome density could become an important characteristic that plays a vital role in the soybean's resistance to *B. tabaci*. According to da Silva et al., (2012), the antixenosis mechanism is demonstrated by the lowest number of eggs on resistant soybean leaves with less dense trichomes. Several studies have reported that antixenosis resistance is associated with the characteristics of trichomes (Baldin et al. 2017) and the luminosity of leaflets (Santos et al. 2022).

4. Conclusions

The highest natural population of *B. tabaci* occurred at 40 DAP, which caused the peak of leaf damage intensity at 50 DAP. Based on the damage intensity at 50 DAP, there were six resistant genotypes, 25 moderate, and 19 susceptible genotypes in the NS environment. In the SP environment, there were 19 resistant genotypes, 22 moderate, and nine susceptible genotypes. The six soybean genotypes in the NS and SP environments showed consistent resistance to *B. tabaci*. The correlation between damage intensity and the density of leaf trichomes indicated that the low density of leaf trichomes might strongly influence the high resistance of *B. tabaci*. The resistant genotypes identified in this study could be utilized in breeding programs for *B. tabaci* resistance.

Authors' Contributions: KRISNAWATI, A.: conception and design, acquisition of data, analysis and interpretation of data and drafting the manuscript; ADIE, M.M.: conception and design and drafting the manuscript; KRISDIANA, R.: analysis and interpretation of data and drafting the manuscript; PRAYOGO, Y.: analysis and interpretation of data and drafting the manuscript; SOEHENDI, R.: critical review of important intellectual content; BALIADI, Y.: critical review of important intellectual content. All authors have read and approved the final version of the manuscript.

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Ethics Approval: Not applicable.

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