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Research Article

Evaluating the impact of leaf trichome on whitefly *Bemisia tabaci* (Hemiptera: Aleyrodidae) dynamics in cassava

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Abstract - Cassava (Manihot esculenta) is a vital crop in Thailand. However, its yield is significantly impacted by Cassava Mosaic Disease (CMD), caused by the Sri Lankan Cassava Mosaic Virus (SLCMV). CMD is primarily spread by whiteflies (*Bemisia tabaci*), as whitefly infestations facilitate disease transmission; therefore, understanding pest resistance in cassava is crucial. Antixenosis, a form of resistance, involves plant traits such as trichome density that deter pest colonization. This study evaluated trichome density and size across six cassava cultivars—Huaybong 60, CMR 89, Kasetsart 50, Rayong 72, Rayong 4, and Pirun 2—and assessed their influence on whitefly settling preferences. Whitefly populations were monitored for four months, while trichome density and size were analyzed using scanning electron microscopy (SEM). The results indicated significant variation in whitefly infestation among the cassava cultivars. The CMR 89, Pirun 2, Rayong 4, and Kasetsart 50 cultivars experienced the highest infestation, whereas Huaybong 60 and Rayong 72 had lower infestations. Notably, the Huaybong 60 displayed the highest trichome density, followed by Rayong 72, Pirun 2, and CMR 89, while Rayong 4 and Kasetsart 50 had lower densities. A strong negative correlation was observed between trichome density and whitefly infestation, suggesting that higher trichome density effectively reduces whitefly colonization. These findings indicate that Huaybong 60 and Rayong 72 could serve as promising genetic resources for breeding cassava cultivars with enhanced non-preference resistance (antixenosis) to whiteflies. This research highlights the importance of selecting resistant cultivars to strengthen cassava production while minimizing the impact of white-fly-transmitted diseases.

Keywords: Whitefly, cassava mosaic disease, *Bemisia tabaci*, trichome density, choice test

1. Introduction

Cassava (*Manihot esculenta* Crantz) is a starchy, nutty root vegetable native to South America. This versatile crop serves as the foundation for a wide range of products, including food, flour, animal feed, alcohol, starches for paper and textiles, sweeteners, prepared foods, and biodegradable materials. However, a significant challenge in enhancing cassava productivity is the threat posed by diseases and pests. One of the most critical diseases affecting cassava globally is cassava mosaic disease (CMD), which is particularly prevalent in Africa, India, Sri Lanka, and Southeast Asia (SEA) (Legg et al., 2015; Wang et al., 2019). In SEA, CMD has been reported in countries such as Vietnam, Thailand, and China, with the Sri Lankan Cassava Mosaic Virus (SLCMV) identified as the primary pathogen (Wang et al., 2019; Saunders et al., 2002). The disease is predominantly spread through infected cassava cuttings and the whitefly vector *B. tabaci* (Calatayud et al., 1994). Symptoms of CMD include distorted leaves, chlorotic mosaic patterns, mottling, and stunted plant growth (Chikoti, 2011), which can potentially reduce cassava yields by up to 40% or, in severe cases, by 100% (Legg & Fauquet, 2004).

Whitefly (*B. tabaci*, Hemiptera: Aleyrodidae) is a significant insect vector responsible for transmitting CMD (Legg et al., 2011) and over 200 other plant viruses, most of which belong to the Begomovirus genus family Geminiviridae (Navas-Castillo et al., 2011; Chikoti et al., 2019), the causative agents of CMD, the cassava brown streak disease, and SLCMV (Maruthi et al., 2005; Legg et al., 2011; Saunders et al., 2002). CMD can be effectively managed by propagating and distributing disease-free stem cuttings. However, breeding cassava resistant to the SLCMV via conventional methods remains a significant challenge due to the high heterozygosity and susceptibility to inbreeding depression in elite cultivars (Ntui et al., 2015). Furthermore, heavy reliance on chemical pesticides for whitefly management disrupts ecosystems and increases production costs, making it economically unviable for small-scale farmers (Abubakar et al., 2022). In response to insect pests like whiteflies, plants have evolved various defense mechanisms, including tolerance, antibiosis, and antixenosis (Punnuri et al., 2022). Tolerance enables plants to sustain growth and reproduction despite insect damage. Antibiosis directly impacts pest development by reducing fertility, size, and lifespan (Tefera et al., 2016). Antixenosis, in contrast, involves plant traits that deter pest attacks, often through the production of volatile organic compounds (VOCs) such as (E)-β-farnesene, (Z)-3-hexenal, and (E)-2-hexenal (Peterson et al., 2017).

As a result, developing host-plant resistance against insect vectors has emerged as a highly effective strategy for managing vector-borne viral diseases (Fereres & Moreno, 2009). Plant defenses, such as trichome density and leaf surface characteristics, play a crucial role in deterring pests (Avery et al., 2015; Heinz & Parrella, 1994; McAuslane, 1996). As hemipteran vectors of plant viruses, whiteflies rely on a sequence of behaviors to identify and select suitable host plants.

Once a plant is deemed appropriate, it initiates sustained phloem-sap feeding (Fereres & Moreno, 2009). Trichomes—small hair-like structures on plant surfaces—play a critical role in defense against insect pests by providing both toxic and deterrent effects (Skoczek et al., 2017). Increased trichome density has been shown to negatively impact insect behaviors, including oviposition, feeding, and larval development (War et al., 2012). However, the relationship between trichome density and whitefly infestation in cassava cultivars remains unclear. This knowledge gap hinders the identification and recommendation of less susceptible cultivars to whitefly damage. Therefore, this study aimed to investigate the influence of trichome density and size on whitefly infestation across different cassava cultivars.

2. Materials and methods

2.1 Plant materials

Six cassava cultivars (Manihot esculenta Crantz)—Huaybong 60, CMR 89, Kasetsart 50, Rayong 72, Rayong 4, and Pirun 2—were sourced from Nakhon Ratchasima, Thailand. DNA was extracted from the auxiliary buds at both ends of 10 cassava plants per cultivar to determine their disease-free or infected status using polymerase chain reaction (PCR) with specific Sri Lankan Cassava Mosaic Virus (SLCMV) primers. DNA extraction was performed according to the protocols described by Kollar et al. (1990) and Nakashima et al. (1993). The quality and quantity of the extracted DNA were evaluated using a spectrophotometer (Thermo Scientific™ NanoDrop™ 2000). The presence of SLCMV was detected through PCR, employing AV1 gene-specific primers (Saokham et al., 2021): AV1 forward (5′-GTT GAA GGT ACT TAT TCC C-3′) and AV1 reverse (5′-TAT TAA TAC GGT TGT AAA CGC-3′). PCR amplification was conducted in a 25-µl reaction volume, containing 1X PCR buffer (PCR Biosystems), 0.2 µM of each primer, and approximately 50 ng of DNA template. The thermal cycling conditions were as follows: an initial denaturation at 94°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 40 seconds, annealing at 55°C for 40 seconds, extension at 72°C for 40 seconds, and a final elongation at 72°C for 5 minutes. The reactions were performed using a Bio-Rad iCycler (Bio-Rad Laboratories, Hercules, CA, USA). PCR amplification results were analyzed via DNA gel electrophoresis to confirm the disease-free or infected status of the cassava samples (Supplementary data, S1). Only disease-free cassava plants were used for propagation.

The cassava stem cuttings, each measuring 20–25 cm in length, were prepared with 20 plants allocated per cultivar. The cuttings were initially planted in pots and cultivated under greenhouse conditions without the application of chemical treatments for insect pests or disease control. After one month of growth, the cassava plants were transferred to field conditions for further study. This experimental design enabled a natural assessment of plant-pest interactions, specifically evaluating the susceptibility of different cassava cultivars to whitefly infestation.

2.2 Whitefly *Bemisia tabaci* settling preference assay

The choice test experiment utilized a completely randomized design (CRD) to evaluate the preference of whitefly adults (*B. tabaci*) for six cassava cultivars: Huaybong 60, CMR 89, Kasetsart 50, Rayong 72, Rayong 4, and Pirun 2. One-month-old cassava plants were individually potted and alternately arranged in the field at Suranaree University of Technology, with a spacing of 30 cm between rows and pots. The experiment was designed with 10 replications per cassava cultivar. Whiteflies were allowed to infest the cassava plants naturally, and the number of adult whiteflies on all cassava leaves was counted at five-day intervals until the plants reached four months of age. Host preference was assessed based on the mean number of *B. tabaci* observed on each cassava cultivar. The study was conducted in field settings during 2023 to 2024 at Suranaree University of Technology (14°58′14.38″ N, 102°06′7.06″ E), Nakhon Ratchasima, Thailand.

2.3 Trichome size and density

The morphology of the abaxial surface of three-month-old cassava leaves was analyzed to explore its correlation with whitefly feeding preferences. Each measuring 5 mm² leaf samples were soaked in 37% formaldehyde for 24 hours to preserve tissue structure. Following fixation, the specimens were dehydrated in a graded ethanol series (70%, 80%, 90%, and 100%), per the protocol outlined by Schneeberg et al. (2017). The dehydrated specimens were then dried at the critical point and coated with gold using a Neo Coater. The samples' leaf surface morphology was examined using a scanning electron microscope (SEM; JEOL JSM-6010LV) at the Scanning Electron Microscope (SEM) Laboratory of the Suranaree University of Technology, with 10 replications of surface area for each cassava cultivar. Trichome density and size were assessed, measuring 10 μm^2 per surface area, 10 times per replication.

2.4 Statistical analysis

All statistical analyses were conducted using SAS software version 9.4 (SAS Institute, Inc., Cary, NC). Data on whitefly settling preferences, trichome size, and density were analyzed using Tukey's post hoc test at a significant level of 0.05, with all analyses performed using SAS® OnDemand for Academics.

For correlation analysis (Pearson correlation), similar studies by Ghaffar et al. (2011) were referenced to assess the relationships between the mean values of settling preference and biophysical factors. The mean number of whiteflies in six cassava cultivars, density, and size of trichomes were analyzed using the Pearson correlation. Differences were considered significant at a 5% probability level. Principal Component Analysis (PCA) was employed to evaluate the relationships among the six cassava cultivars. As a multivariate statistical method, PCA facilitated the identification of patterns among the cultivars based on multiple parameters, including settling preference and biophysical characteristics. PCA was performed using Origin Pro 2024 software (OriginLab Corporation, Northampton, MA, USA) (Moberly et al., 2018), allowing for the visualization and interpretation of the relationships among the cassava cultivars.

3. Results

3.1 Whitefly *Bemisia tabaci* settling preference

Analysis of variance (ANOVA) was conducted to evaluate the infestation levels of *B. tabaci* whiteflies on six cassava cultivars over 95 days. The results indicated significant differences in the number of *B. tabaci* among the cultivars at specific time points ($P < 0.05$), including on days 40, 55, 60, 65, and 95. At 40 and 55 days ($P < 0.001$ and $P = 0.027$, respectively). At 40 days, high infestation levels were observed on CMR 89 with 4.5 ± 0.39 whiteflies per plant. The high infestation levels were observed on Pirun 2 (5.9±1.62 whiteflies per plant) at 55 days, while low infestation levels were recorded on Rayong 72, with 1.6 ± 0.45 whiteflies per plant. On days 60 ($P = 0.026$) and 65 ($P = 0.018$), high infestations were observed on CMR 89, Kasetsart 50, Rayong 4, and Pirun 2, while lower levels were found on Huaybong 60 and Rayong 72. At 90 days ($P = 0.081$) and 95 days ($P = 0.019$), high infestation levels were noted on CMR 89 and Pirun 2, with 3.0 whiteflies per plant, with lower levels observed on Rayong 72 (1.1 ± 0.31 whiteflies per plant) (Figure 1). The mean number of *B. tabaci* whiteflies over the 95 days revealed significant differences among the cassava cultivars (F = 5.243; df = 5; P < 0.01). The highest infestation levels were recorded as mean ± SE on CMR 89 (45.6 ± 2.40), Pirun 2 (42.4 ± 2.23), Rayong 4 (40.9 ± 2.14), and Kasetsart 50 (38.0 ± 2.01). Conversely, the lowest infestation levels were recorded as mean ± SE on Huaybong 60 (29.8 ± 1.57) and Rayong 72 (23.0 ± 1.21). The infection levels have been detailed in Figure 2.

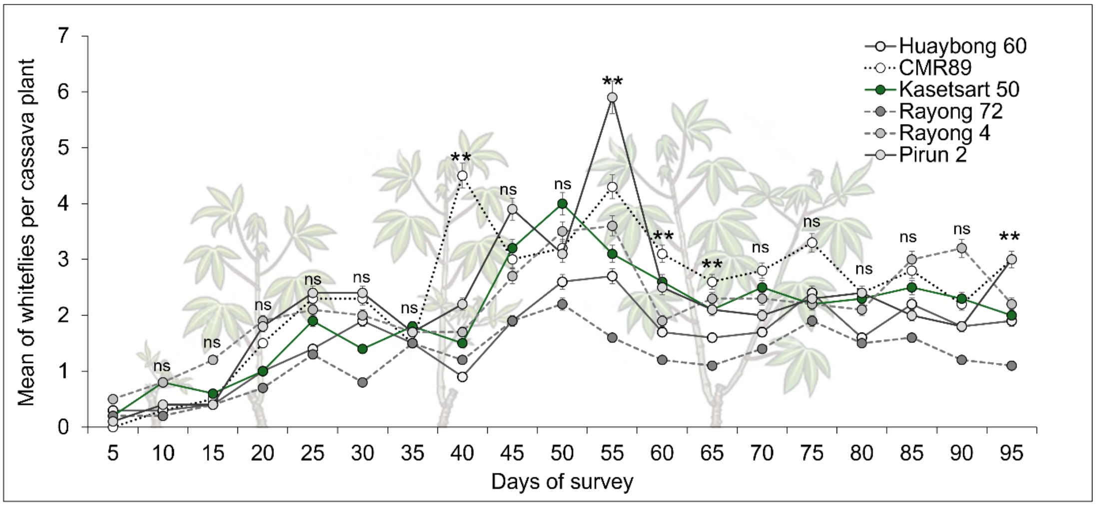

Figure 1. The mean number of *B. tabaci* whiteflies infesting six cassava cultivars over a 95-day survey period.

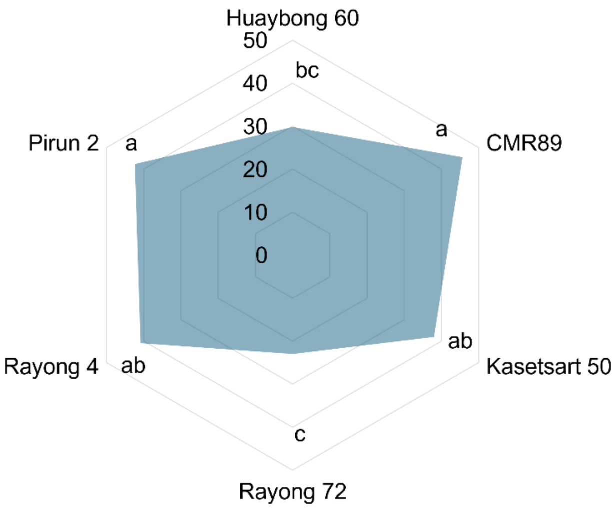

Figure 2. The mean number of *B. tabaci* whiteflies infesting six cassava cultivars over 95 days.

3.2 Trichome density and size

The three-month-old leaf morphology of the six cassava cultivars was examined using scanning electron microscopy (SEM) (Figure 3), revealing the presence of non-glandular trichomes as a characteristic feature across all cultivars. The number of trichomes per 10 µm length scale varied significantly among the cassava cultivars (F = 49.914; df = 5; P < 0.01). The Huaybong 60 cultivar exhibited the highest trichome density (79.30 ± 2.42), followed by Rayong 72 (66.00 ± 1.90) and Pirun 2 (65.50 ± 3.56). In contrast, lower trichome densities were observed in Rayong 4 (46.40 ± 3.29) and Kasetsart 50 (44.20 ± 5.17) (Table 1).

The trichome size also varied significantly among the cassava cultivars (F = 11.211; df = 5; P < 0.01). The Pirun 2 cultivar had the largest trichome size (8.18 ± 0.30), followed by Kasetsart 50 (7.42 ± 0.33), Rayong 72 (7.31 ± 0.31), Huaybong 60 (7.26 ± 0.10), and CMR 89 (7.19 ± 0.34). The smallest trichome size was recorded in Rayong 4 (6.63 ± 0.34; Table 1).

Table 1. Mean trichome density and trichome size of six cassava cultivars.

Cassava cultivars	Trichome density per 10 (µm²)				Size of trichome (±m²)			
Huaybong 60	79.30	±	2.42	a	7.26	±	0.10	b
CMR 89	60.90	±	1.90	b	7.19	±	0.34	b
Kasetsart 50	44.20	±	5.17	c	7.42	±	0.33	b
Rayong 72	66.00	±	6.03	b	7.31	±	0.31	b
Rayong 4	46.40	±	3.29	c	6.63	±	0.34	c
Pirun 2	65.50	±	3.56	b	8.18	±	0.30	a

Note: Values in the same column followed by different superscript letters are significantly different (P < 0.05) according to one-way ANOVA followed by Tukey's post hoc test.

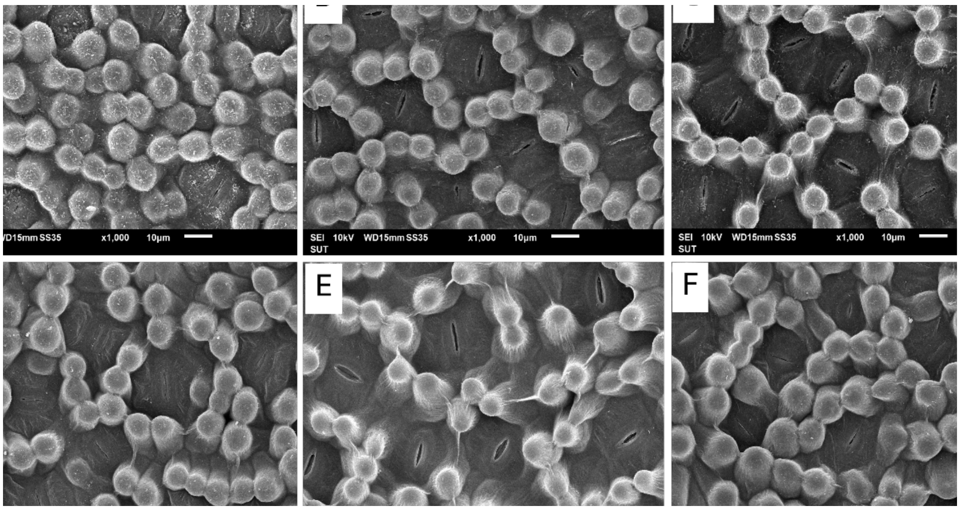

Figure 3. Trichomes of six cassava cultivars were observed through scanning electron microscopy (SEM) at 1000x magnification. Images show the leaf surface of (A) Huaybong 60, (B) CMR 89, (C) Kasetsart 50, (D) Rayong 72, (E) Rayong 4, and (F) Pirun 2.

3.3 Correlation analysis (Pearson correlation)

The number of whitefly infestations at various time intervals demonstrated strong negative correlations with trichome density at three-month-old, indicating an inverse relationship between the two variables. Specifically, at 10, 15, 40, 55, and 90 days, strong negative correlations (dark blue color) with trichome density per 10 μm^2 were observed: r =-0.86, r =-0.70, r =-0.75, r =-0.82, and r =-0.70, respectively (Figure 4). Moreover, the trichome density negatively correlated with the number of whitefly infestations at 5, 20, 25, 60, 65, 80, and 85 days of the observed period. (blue color). The result suggests that higher trichome density on cassava leaves is associated with lower whitefly infestations.

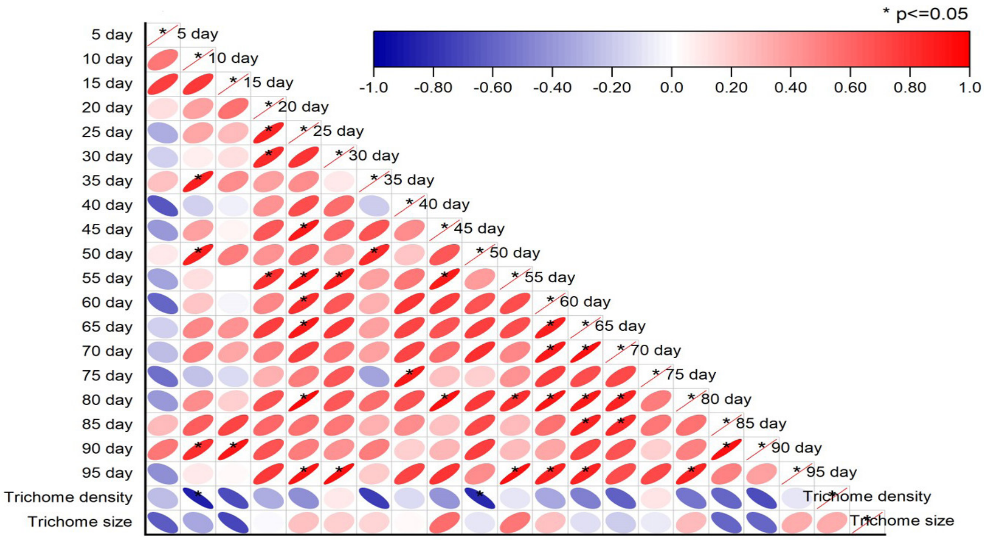

Figure 4. Correlation analysis of the number of whiteflies infested in a 5 to 95-day-old cassava with trichome density and size. (Blue means negative correlation, and red indicates positive correlation).

3.4 Principal component analysis

The first two principal components (PCs) accounted for 94.01% of the total variability in the data, with PC1 contributing 64.34% and PC2 contributing 29.67%. Through PCA, cassava cultivars were classified into two distinct groups. Group A included CMR 89, Kasetsart 50, Rayong 4 and Pirun 2. Group B comprised Huaybong 60 and Rayong 72 cultivars (Figure 5). Group A has a lowertrichome density than Group B, which also experienced fewer whitefly infestations.

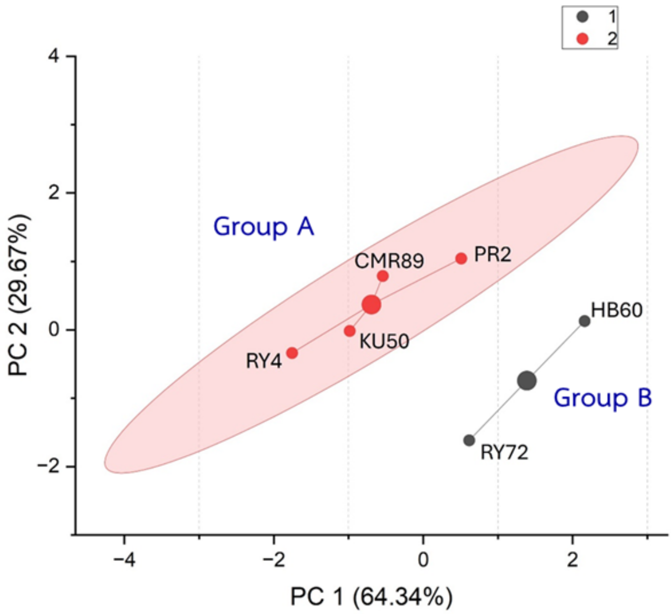

Figure 5. PCA (PC1 and PC2) of the number of whiteflies infested at different times with trichome density and size from six cassava cultivars.

4. Discussion

Whitefly infestations varied significantly among the six cassava cultivars, with notable differences identified through analysis of variance (ANOVA). These findings align with PCA results, categorizing cassava cultivars into two groups. Cultivars such as CMR 89, Kasetsart 50, Rayong 4, and Pirun 2 experienced higher whitefly infestations, while Huaybong 60 and Rayong 72 exhibited lower infestation rates. A correlation analysis revealed a positive and a strong negative relationship between whitefly numbers and trichome density. In a positive relationship, trichome density increases, and whitefly infestation increases. In a negative relationship, as trichome density increases, whitefly infestation decreases, underscoring the critical role of trichomes in enhancing plant resistance. In Arabidopsis thaliana, trichome development is mainly regulated by genes like GLABRA1 (GL1) rather than the plant's age. GL1 and other genes, such as GL3 and TTG1, form a complex that initiates trichome formation. This suggests that trichome numbers are genetically determined and not directly affected by plant age (Pattanaik et al., 2014). The density of trichomes is a physical barrier that obstructs whitefly movement, feeding, and oviposition. Whiteflies' small and delicate bodies struggle to navigate through densely packed trichomes, limiting their access to the plant's surface for feeding and reproduction (Oriani & Vendramim, 2010). In cassava of Brazil, the reduced susceptibility of cassava cultivars to *B. tuberculata* infestation may be associated with lower trichome density, increased light reflectance, and elevated chroma values (Pastório et al., 2023). Similar findings have been reported in other plants. For instance, tomato plants with higher trichome density have shown reduced infestations of whiteflies *B. tabaci*, as trichomes physically block whiteflies from establishing feeding sites (Simmons et al., 2003). Additionally, dense trichomes influence whitefly behavior by discouraging them from settling in areas with high trichome density, resulting in fewer eggs and reduced population growth

(McAuslane, 1996). This phenomenon has been documented in other crops, such as geraniums and cotton, where higher trichome densities correlate with fewer whitefly eggs and lower pest populations (Oriani & Vendramim, 2010). Similarly, in tomatoes, it was found that the inheritance of trichome density is associated with resistance to whiteflies. The report of crossbreeding between *Solanum lycopersicum* and *S. galapagense* showed that plants with higher densities of type IV glandular trichomes exhibited resistance levels comparable to those of the resistant wild accession (Andrade et al., 2017). Moreover, the report found that increasing the density of trichomes in tomatoes enhances resistance to whiteflies. The study suggested that this trait can be introduced from wild species into cultivated varieties through breeding programs (Escobar-Bravo et al., 2016). A negative correlation was observed in tobacco between whitefly resistance and trichome density (Li et al., 2014). In cotton, increased trichome density is associated with the colonization and oviposition preference of *B. tabaci* (Jindal & Dhaliwal, 2011).

Moreover, high-density trichomes impede whitefly feeding and reduce reproductive success, resulting in fewer mature whiteflies and a decline in infestation over time. Similar to tobacco plants, it was found that high trichome density diminishes feeding efficiency and reduces reproduction rates in whiteflies (McAuslane, 1996). Similarly, increasing trichome density in soybean plants has been linked to higher mortality rates in whitefly nymphs, thereby further alleviating pest pressure (McAuslane, 1996). While trichome density significantly deters whiteflies, trichome size exhibited positive and negative correlations with infestation levels. Studies suggest that trichome density is more influential than size in reducing insect infestations. For example, research on tomato plants demonstrated that higher trichome density effectively reduces colonization by whiteflies and aphids, regardless of trichome size (Simmons et al., 2003). Similarly, in Arabidopsis thaliana, high-density trichomes were more effective at impeding insect movement and feeding than size alone (Mauricio & Rausher, 1997). Research on cotton plants confirmed that higher trichome density, rather than size, plays a crucial role in reducing infestations of whiteflies. (Jindal & Dhaliwal, 2011). Additionally, the non-glandular trichomes observed in the six cassava cultivars act as a protective barrier, limiting insect access and delaying damage to the plant's epidermal layer (Kariyat et al., 2019). This physical defense mechanism enhances cassava's ability to resist insect attacks by impeding whitefly colonization and feeding. However, although the principal components (PCs) were classified into two distinct groups, group A included CMR 89, Kasetsart 50, Rayong 4, Pirun 2, and CMR 89. Group B comprised Huaybong 60 and Rayong 72 cultivars; however, the results show that Rayong 72 does not significantly differ from CMR89 in trichome density and size. This PC result divided cassava cultivars into two groups based on the number of insect infestations and trichome density, with 22 parameters that may have other factors, such as the presence of certain chemical compounds or allelochemicals, play a crucial role in plant–insect interactions (Ibanez et al., 2012; Tlak Gajger & Dar, 2021).

5. Conclusion

In conclusion, trichome density is vitalfor defending cassava cultivars against whitefly infestations. Higher trichome densities provide excellent protection against pest pressures, particularly incultivars such as Huaybong 60 and Rayong 72 which possess high-density trichomes demonstrate enhanced resistance to whiteflies by obstructing their movement. Consequently, cassava cultivars with higher trichome densities experience significantly lower whitefly infestations and sustain less damage than those with lower trichome densities. This underscoresthe importance of trichome density as a key trait for breeding insect-resistant cassava cultivars. Enhancing trichome density can improve crop resilience against whitefly pests, contributing to more sustainable and resilient cassava cultivation.

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