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Short Communication: Oviposition and chlorosis scoring for early screening of chili resistance to whitefly (*Bemisia tabaci*)

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Abstract. *Kamaliah TL, Syukur M, Maharijaya A, Hidayat P. 2025. Short Communication: Oviposition and chlorosis scoring for early screening of chili resistance to whitefly (*Bemisia tabaci*). Biodiversitas 26: 6410-6416.* *Bemisia tabaci* is a major pest of chili. The objective of this study was to establish a rapid screening technique for chili resistance, based on oviposition and chlorosis parameters. The research was conducted in the plastic houses of Sinar Alam Sari, Cibeureum, Bogor, Indonesia, from June to December 2021, using a randomized complete block design with three replications. Eight chili genotypes (*Capsicum annuum* cv. Adelina, Anies, C12, Kencana, Laris, Landung, Ungara, and Yuni) were infested with four non-viruliferous whiteflies per leaf in a no-choice test. The number of eggs and early instar nymphs was counted on the 7th and 10th days, respectively, while chlorosis was scored on the 21st day after infestation. Results showed significant differences among genotypes. Landung and Yuni were the most susceptible, with the highest egg counts (52.83 and 51.17 per leaf), nymph counts (48.17 and 47.50), and severe chlorosis (score 3-4). Ungara was highly resistant, with the lowest egg (9.00) and nymph counts (4.83), and minimal chlorosis (score 1-2). A strong correlation was observed between high egg/nymph numbers and severe chlorosis, which was objectively validated by RGB image analysis showing a near-perfect negative correlation between chlorosis score and the NGRDI (-0.995). The study concludes that combining egg count and chlorosis scoring—supplemented by digital RGB/NGRDI validation—provides a novel, simple, efficient, and reliable method for the early screening of chili resistance to *B. tabaci*, offering an integrated method of breeding programs.

Keywords: Antibiosis, eggs, leaf color, nymphs, whitefly

INTRODUCTION

Chili is one of the leading commodities in Indonesia's globally known horticulture subsector. Chili production from 2010 to 2022 has increased, but challenges such as pests and diseases remain (Center for Agricultural Data and Information Systems 2024). Whiteflies are one of the main pests in chili production in Asia and Indonesia (Sayekti et al. a 2021; Sandra et al. 2022). This is supported by climate change, agricultural intensification, and improper pesticide use, which contribute to developing pest resistance (Deguine et al. 2021). *Bemisia tabaci* (Gennadius, 1889) is a highly polyphagous and invasive species, making it an adept colonizer of diverse plants capable of supporting reproduction. The direct damage due to its feeding, which includes chlorosis and wilting, is compounded by indirect harm via the transmission of viral diseases (Devendra et al. 2020). Its impact is that economic assessments frequently pinpoint substantial financial losses attributable to whitefly infestations (Chaubey and Mishra 2018; Choudhary et al. 2025).

One of the mechanisms that can be explored to enhance the resistance of chili plants to whiteflies is through the antibiosis approach (Painter 1951). Antibiosis involves plant traits that can inhibit pest development, such as through oviposition and chlorosis symptoms. Oviposition can be an effective indicator for evaluating plant resistance to pest attacks (Aljbory and Chen 2018). This mechanism is a direct response of the plant to the presence of pests, where resistant plants tend to create unfavorable conditions for pests to lay their eggs (dos Santos et al. 2020; Millán-Chaidez et al. 2021). This can be manifested through changes in the physical, chemical, or physiological properties of the plant, such as the production of allelochemical compounds, changes in leaf surface structure, or the plant's immune response that inhibits the development of pest eggs or larvae (Douglas 2018).

Chlorosis, or leaf yellowing, is a common symptom often observed in plants infested by whiteflies (*B. tabaci*). This symptom occurs due to disturbances in the photosynthesis process, caused by the feeding activity of *B. tabaci* on the plant's phloem tissue. When whiteflies suck the plant's sap, they not only disrupt the flow of nutrients but can also inject toxic compounds or trigger stress responses in the plant, resulting in chlorophyll damage and the appearance of yellowing leaves (War et al. 2018; Selangga et al. 2023). Additionally, *B. tabaci* is also known as the main vector of gemini virus, which can exacerbate chlorosis symptoms and cause further damage to the plants (Naveed et al. 2023). Chlorosis symptoms can be an early indicator of plant resistance to pest attacks (Kaviya et al. 2024). By monitoring chlorosis symptoms, researchers can identify plant varieties that have more effective defense mechanisms against pests. This approach not only aids in the development of more resilient plant varieties but also supports sustainable and environmentally friendly pest control strategies (Baker et al. 2020).

The integration of multiple resistance indicators offers a more robust and comprehensive approach to screening plant genotypes. Although oviposition preference explains non-preference (antixenosis), the chlorosis symptom on leaves expresses the plant's physiological reaction (tolerance or antibiosis) against feeding damage. Few reports are available based on these two traits, which are crucial for early screening of plant genotypes in chili. A chlorosis scoring system was developed for cassava (Mwila et al. 2017) and sunflower (Ingale et al. 2019), but a standard rapid method for early screening based on both pre-infestation and post-infestation characters has not been reported in chili. Therefore, in this study, we determined the easy and quick characteristics of antibiosis that can be seen by using oviposition and chlorosis for early screening. This study, therefore, establishes and validates a novel, integrated screening protocol for chili. For the first time, we combine direct oviposition counts with a standardized visual chlorosis scale—specifically calibrated for chili-whitefly interactions—and further reinforce it with objective digital validation using RGB image analysis and the Normalized Green-Red Difference Index (NGRDI). This method advances existing screening techniques by simultaneously capturing both antixenosis (pest preference) and antibiosis/tolerance (plant physiological response) traits in a single, rapid, and field-applicable assay, addressing a key methodological gap in chili resistance breeding.

MATERIALS AND METHODS

Research location

Whitefly *Bemisia tabaci* were obtained from pepper plants in the field at Dramaga, Bogor, Indonesia, in June 2021. Then they were brought and reared in an insect cage in the Plastic House at Alam Sinar Sari, Dramaga, Bogor, using eggplant as host plants. The whitefly population was isolated on plants free of viruses for 2 to 3 generations. Whiteflies, used for infestation, were confirmed to be non-viruliferous (did not contain viruses) (Firdaus et al. 2012). This study was conducted from June to December 2021. The location is 192 m above sea level, with daily temperatures ranging from 25-34°C and humidity levels between 75 and 86%.

Procedures

Plant material

The genetic materials used were chili (*Capsicum annuum* L.) genotypes Adelina, Anies, C12, Kencana, Landung, Laris, Ungara, and Yuni. Seedlings were carried out in the screen house to avoid unwanted insect attacks. Plants were grown from seeds sown in a 50-cell plastic tray. Two seeds were sown in each cell of the plastic tray containing media. A month later, it was moved to a polybag (20 cm diameter × 30 cm high). Each variety was repeated 3 times, so that there were 24 experimental units, where each experimental unit consisted of three plants. Therefore, the total sample size was 72 plants. The mixture of soil, manure, and husks was used for transplanting media. During the experiment, insecticide was not applied to avoid chemical residues. The provision of plant nutrition was carried out by applying AB mix leaves (5 mL/L) once every week from 1 WAP (Weeks After Planting). Plant material used for research is 4-6 leafy plants or 4 WAP.

Testing the resistance of chili genotypes to whiteflies

Chilies were screened at the seedling stage, with 4-6 leaves or 4 weeks after planting. The resistance test was conducted in a plastic house. The leaves were wrapped in 10 cm × 7 cm modified clip plastic bags with organdy fabric. Two leaves (the third and the fourth from the top) were infested per plant. The whiteflies were infested with 4 imagos per leaf. The imago tested were 3-4 days old and taken from the rearing cage. The plants were placed in an insect cage inside a plastic house. The infested whiteflies originated from a population of whiteflies reared in an insect cage (100 cm long × 60 cm wide × 100 cm high) covered with 80 cm × 80 cm mesh organza fabric in an insect net house. The whiteflies were confined on the leaves using these clip bags for a period of 5 days. After 5 days, the adult whiteflies were collected and returned to the insect-rearing cage (Firdaus et al. 2012). The number of eggs was observed on the 7th day after infestation, and the number of early instar nymphs on the 10th day after infestation. Observation of leaf color changes was conducted on the 21st day after infestation.

Scoring of chili genotypes

Scoring of chili genotypes was conducted based on the number of eggs per leaf and the number of early instar nymphs per leaf in this study. The calculation of class width was done by looking at the smallest and largest data and then dividing them into 6 criteria (very resistant, resistant, moderately resistant, moderately susceptible, susceptible, and very susceptible).

As a standardized chlorosis scoring system for chili-whitefly interactions was not available (Table 4), we created a scale (Table 2) from synthesizing and modifying techniques detailed in the literature of various plant-pest interactions. The scoring categories and percentage ranges for chlorotic area were informed by the works of Mwila et al. (2017) on cassava, Ingale et al. (2019) on sunflower, and others, as comprehensively listed in Table 4. Our scale was specifically calibrated to capture the distinct chlorosis patterns caused by *B. tabaci* feeding damage on chili leaves.

Data analysis

Digital image analysis was performed using ImageJ software (version 1.53) to quantify leaf color changes and calculate vegetation indices. For each chlorosis score category represented in Figure 1, leaf images were cropped to isolate representative areas, and the RGB values were extracted using the "Color Histogram" tool. The Green Leaf Index (GLI) and Normalized Green-Red Difference Index (NGRDI) were then computed using the formulas GLI = (2×Green − Red − Blue) / (2×Green + Red + Blue) and NGRDI = (Green − Red) / (Green + Red), respectively. Six replicate measurements per score category were averaged to ensure consistency, providing an objective, reproducible metric to validate the visual chlorosis scoring system.

The environmental design used is a Completely Randomized Block Design (CRBD) with the host plant genotype as the treatment and three replications. Each genotype in each replicate consists of two plants. Between repetitions, they were conducted on different days. Analysis of the data used the Normality-test at the 5% level to meet the assumptions $\varepsilon ij \sim N(0, \sigma 2)$; normal spread error, middle value μ, and homogeneous variance. Furthermore, the data was tested by ANOVA (F test), and if the real treatment at the level of 5% was continued with Least Significant Difference (LSD). Software tools used were Microsoft Excel 2021, Minitab 21, and R Studio. Data on the number of eggs per plant and the number of early instar nymphs were transformed by √ (X).

RESULTS AND DISCUSSION

Number of eggs, number of early instar nymphs, and percentage of eggs hatching per leaf on whitefly infestation cannot choose leaves freely (no choice test)

It could be seen that there are significant differences in the number of eggs and the number of early instar nymphs per leaf, as well as the percentage of hatching eggs among various chili genotypes (Table 1). The number of eggs and the number of early instar nymphs per leaf were highest in the Landung and Yuni genotypes, while the lowest were found in the Ungara genotype. In this study, the percentage of eggs that hatched on the 10th day was >50%.

Change in chili pepper leaf color after 3 weeks of infestation

In the third week after the infestation of whiteflies (4 adults per leaf), there was a change in leaf color as shown in Figure 1 and Table 2. In the genotypes Landung and Yuni, the leaves turned yellow and fell off. Whereas in the genotypes Ungara and Adelina, the yellowing was minimal, and the leaves did not fall off. The leaf color scoring of the Landung and Yuni genotypes is higher than that of the Ungara and Adelina genotypes. This is suspected to be caused by the plant's response to whiteflies. The number of eggs and the number of early instar nymphs per leaf in the genotypes Landung and Yuni were higher compared to the genotypes Ungara and Adelina (Table 1).

The RGB analysis and derived vegetation indices (GLI and NGRDI) provided quantitative support for the visual chlorosis scores (Table 2). As the chlorosis score increased from 0 to 4, the GLI values generally decreased (from 0.370 to 0.361), indicating a reduction in greenness, while the NGRDI values also declined (from 0.228 to 0.042), reflecting a shift from green to green-yellow dominance in leaf color. These trends align with the progression from healthy, resistant leaves (score 0-1) to severely chlorotic, susceptible leaves (score 4), confirming that digital image analysis can objectively complement visual scoring in assessing whitefly-induced damage.

The correlation analysis underscores a clear and consistent relationship between observed chlorosis scores and the digital color values derived from leaf images (Table 3). We found that chlorosis severity closely aligns with increases in Red and Green values, while showing a notable inverse relationship with Blue. Most distinctly, the strong negative correlation with NGRDI reinforces that visual yellowing corresponds reliably to a quantifiable loss in green intensity, lending solid support to our visual assessment method.

Chili genotype scoring criteria

The standard scoring for chili resistance against the whitefly *B. tabaci* is not yet available. This scoring is based on the number of eggs per leaf and the number of early instar nymphs per leaf (Table 3). Grouping of chili plants can be done based on the response of whiteflies to the chili plants, in this case, the number of eggs and the number of early instar nymphs. Chlorosis scoring can also be used to complement the criteria for grouping chili plants. This grouping is carried out for selection considerations in the subsequent stages. Leaves on the Landung and Yuni (susceptible) genotypes have higher chlorosis scores compared to the Ungara and Adelina genotypes (Figure 1).

Table 1. The number of eggs (7 days after infestation) and the number of early instar nymphs per leaf (10 days after infestation) with an infestation of 4 adult whiteflies per leaf on chili

Genotype	Number of eggs per leaf	Number of early instar nymphs per leaf	Percentage of eggs hatching per leaf on the 10th day after infestation
Ungara	9,00±1,22e	4,83±0,62e	53,76±1,33f
Adelina	13,67±0,85d	9,00±0,71d	65,79±1,59de
C12	10,83±0,24de	7,00±0,41de	64,65±3,98e
Kencana	25,67±2,24b	20,50±2,09b	76,51±1,84b
Laris	10,50±0,71de	7,33±0,85de	69,64±3,65cd
Anies	20,83±1,93c	15,17±0,94c	73,01±2,52bc
Landung	51,17±1,03a	47,50±1,22a	92,82±0,59a
Yuni	52,83±2,09a	48,17±1,93a	91,17±0,30a
LSD critical value	3,262	2,827	4,738

Note: Values are means±SE. Means followed by different letters are significantly different based on the LSD test at the level of 5%

Table 2. Chlorosis scores on various chili leaves in the third week after infestation

Score	Chlorosis (leaf yellowing)	Criteria	Figure	RGB analysis			GLI*	NGRDI*
				Red	Green	Blue		
0	No symptom of yellowing leaves	Very resistance	1.A	40.31±2.38	64.10±3.31	18.64±0.73	0.370	0.228
1	Yellowing leaves 1-10%	Resistance	1.B	67.40±8.67	99.33±8.73	10.06±3.79	0.439	0.191
2	Yellowing leaves 11-30%	Moderate	1.C	89.89±16.21	118.17±14.79	6.29±0.88	0.422	0.136
3	Yellowing leaves 31-50%	Moderate susceptible	1.D	110.34±16.20	127.56±8.21	3.69±1.07	0.383	0.073
4	Yellowing leaves 51-80%	Susceptible	1.E	132.69±14.08	144.43±7.53	3.03±0.78	0.361	0.042
5	Yellowing leaves >80%	Very susceptible						

Note: *GLI: Green Leaf Index, **NGRDI: Normalized Green-Red Difference Index

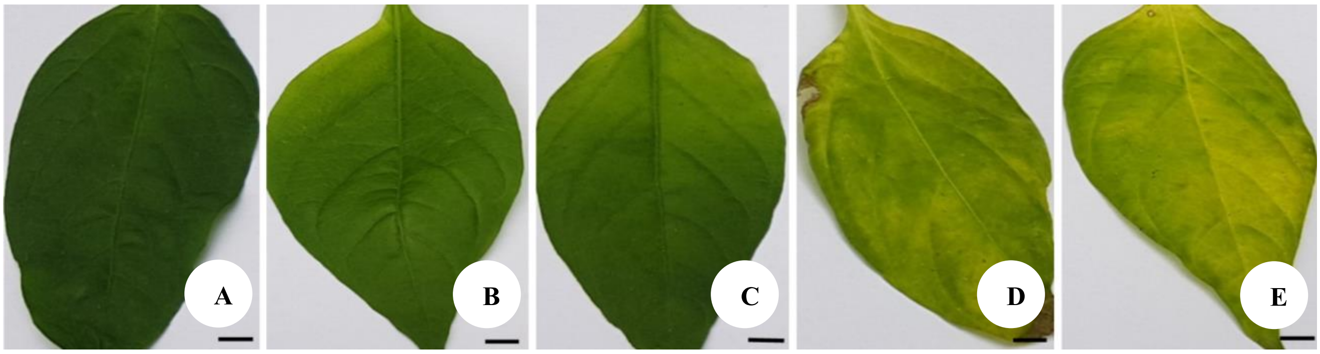

Figure 1. Scoring the color of chili leave s in the 3rd week after the infestation of *Bemesi a tabaci*. A. Score 0, B. Score 1, C. Score 2, D. Score 3, E. Score 4. Scale bar: 5 mm

Table 3. Correlation between chlorosis scores and RGB analysis

Correlation	Red	Green	Blue	GLI	NGRDI
Chlorosis Score	0.993	0.972	-0.931	-0.345	-0.995

Discussion

Ungara is a moderately resistant genotype to pepper yellow leaf curl virus, while Yuni is a highly susceptible genotype (Sayekti et al. b 2021), with few trichomes (<3 per 10 mm^2), a thick epidermis (>20 µm), and a leaf thickness of 166 µm (Kamaliah et al. 2022). The number of eggs in the Ungara genotype using the antixenosis method was 7.67 per plant (Kamaliah et al. 2022). Oviposition data is used as an indicator of plant resistance to pests (Padilha et al. 2021).

Combining oviposition data and chlorosis scores is a useful and quick way to test for chili resistance to *B. tabaci* early on. Our results show that genotypes like Ungara and Adelina have few eggs and very little chlorosis (score 1-2). These genotypes had stronger mechanisms for antibiosis and tolerance than genotypes that were more susceptible, like Landung and Yuni. This two-part method shows how the pest first chooses the plant and how the plant reacts to damage from constant feeding. It gives a better overall resistance profile than just one trait (dos Santos et al. 2020; Peñalver-Cruz et al. 2020).

The strong link between high egg numbers and severe chlorosis highlights chlorosis as a clear sign of plant stress. This method is especially helpful for breeding programs with limited resources because it doesn't need special equipment and can be used quickly in the field or greenhouse (Yadav et al. 2022). Chlorosis scoring connects to photosynthetic efficiency and plant health, allowing breeders to identify genotypes that maintain their physiological integrity under pest pressure, a trait often overlooked in screening methods that focus only on insect counts (Mwila et al. 2017).

The newly developed chlorosis scale (Table 2) was adapted from other crop-pest interaction scales like cassava (Mwila et al. 2017) and sunflower (Ingale et al. 2019). It was, however, specifically calibrated for application in screening interactions between whiteflies and chili. The development of this scale was informed by comprehensive review of existing chlorosis scoring systems used for various pest insects on different host plants (Table 5). This comparative analysis confirmed that while the specific percentage ranges and symptom descriptions may vary, the principle of using visual chlorosis as a quantitative resistance indicator is well-established across pathosystems. Our scale contributes to this body of knowledge by providing a standardized tool specifically validated for chili-whitefly interactions, filling a previously existing methodological gap.

Table 4. Scoring the grouping of chili plants

Genotype	Eggs score	Nymphs score	Total score	Criteria	Chlorosis scoring
Ungara	1	1	2	VR: Very Resistance	1-2
Adelina	2	1	3	R: Resistance	1-2
C12	2	1	3	R: Resistance	2-3
Laris	2	1	3	R: Resistance	2-3
Anies	3	2	5	R: Resistance	2-3
Kencana	3	3	6	M: Moderate	2-3
Yuni	6	6	12	S: Susceptible	3-4
Landung	6	6	12	S: Susceptible	3-4

Note: *Determination of criteria based on the score of the number of eggs per leaf and the number of early instar nymphs per leaf. *Resistance category criteria (based on total score): Very Resistant (VR): 1-2, Resistant (R): 3-5, Moderate (M): 6, Moderately Susceptible (MS): 7-8, Susceptible (S): 9-11, Very Susceptible (VS): ≥12

Table 5. Chlorosis scoring of pest attacks on various plants

Pests	Crop	Chlorosis scoring	References
Whitefly/*Bemisia tabaci* (Hemiptera: Aleyrodidae)	Cassava	1 - no apparent symptoms; 2 - mild chlorotic blotches on <10% of leaves; 3 - moderate chlorotic blotches on 11-30% of leaves; 4 - yellowing or chlorosis on 31-50% of leaves; and 5 - yellowing and deformation of upper leaves, chlorosis on >50% of leaves and plant stunting	Mwila et al. (2017)
Leafhopper/*Amrasca biguttula biguttula* (Homoptera: Cicadellidae) and Whitefly/*Bemisia tabaci* (Hemiptera: Aleyrodidae)	Sunflower	0 - free from leaf hopper injury 1 - slight yellowish on edges of leaves up to 30% 2 - yellowing and curling up to 40% leaves 3 - yellowing and curling up to 60% leaves 4 - yellowing and curling up to 80% leaves 5 - maximum yellowing, cupping, and curling up to 100% leaves	Ingale et al. (2019)
Whitefly/*Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae)	Tomato	0 - there are no signs or symptoms of the disease; 1 - mild mottling 2 - mottling on the leaf area/light downward cupping 3 - pronounced downward or upward leaf chlorosis leaf mottling 4 - severe mosaic/leaf distortion/crinkled leaf/plant stunting/leaf bunching 5 - severe leaf distortion/necrosis/ narrowed or shoes-string leaf	Paweer et al. (2025)
Russian wheat aphid/*Diuraphis noxia* (Hemiptera: Aphididae)	Wheat	1 - no cholorotic spots 2 - chlorotic spots 3 - chlorotic area <15% of total leaf area 4 - chlorotic area <25% of total leaf area 5 - chlorotic area <40% of total leaf area 6 - chlorotic area <55% of total leaf area 7 - chlorotic area <70% of total leaf area 8 - chlorotic area <85% of total leaf area 9 - chlorotic area >85% of total leaf area	Ni and Quisenberry (1997)
Aphid (Hemiptera: Aphididae)	Brassicaceae	0 - free from aphid infestation 1 - normal growth, no yellowing of the leaves, except only a few aphids along with little or no symptoms of injury. 2 - average growth, curling, and yellowing of the leaves on some branches 3 - growth below average, curling, and yellowing of the leaves on some branches 4 - very poor growth, heavy curling and the yellowing of leaves, stunting the plants, heavy aphid colonies on plants 5- heavy stunting plants; curling, crinkling, and yellowing of almost all the leaves, plants full of aphids	Panwar et al. (2023)
Tea green leafhopper/ *Empoasca onukii* (Homoptera: Cicadellidae)	Tea	0 - no damage 1 - light damage 2 - heavy damage	Yorozuya et al. (2021)
Leafhopper/*Amrasca biguttula biguttula* (Homoptera: Cicadellidae)	Okra	1 - entire foliage free from crinkling/curling with no yellowing, bronzing, browning, and drying of leaves 2 - crinkling, curling of a few leaves, mostly in the lower portion of a plant, and little yellowing of leaves 3 - crinkling, curling, and yellowing of leaves, almost all over the plant, plant growth noticeably hampered 4 - extreme crinkling, curling, yellowing, bronzing, and drying of leaves and progressive defoliation, plant growth remarkably stunted	Prithiva et al. (2019)

A valuable tool for rapid and precise screening in plant breeding programs uses RGB image analysis for phenotyping. Osuna-Caballero et al. (2023) developed an RGB image-based application for phenotyping rust disease in pea foliage. An analysis of differential coloration in leaf images to estimate disease severity was done. Image-based phenotyping can accelerate the understanding and development of plant resistance to insect pest attacks (Gothe et al. 2024). The employment of an equivalent methodology to evaluate the chlorosis reaction in these chili plants would improve the screening for resistance, through fast, cheap and objective plant health evaluations under insect pressure. Red, green, and blue values, known as RGB, work along with the green leaf index and the normalized green-red difference index. These serve as key parameters in digital image analysis. They help quantify leaf conditions, as shown in Table 2. That focus stays on chlorosis in particular. Chlorosis shows up as yellowing in leaves. It often points to problems in photosynthetic processes. Those issues come from biotic stress, like pest infestations. RGB values pick up the strength in each color channel. The shift from green to yellow indicates the degradation of chlorophyll. The GLI and NGRDI were calculated from RGB values using existing formulas. GLI and NGRDI values were assumed to correlate with chlorosis.

When chili plants face whitefly issues, like with *B. tabaci*, these indices do a solid job at showing the physiological stress and feeding damage. Resistant genotypes often hold onto higher GLI and NGRDI values. That points to stronger leaf greenness and photosynthetic function staying intact. Susceptible genotypes show lower values instead. Those tie directly to heavier chlorosis and tissue harm. The whole quantitative setup provides a sharper and more consistent option. Those can feel subjective at times. They also miss finer shifts in leaf color pretty easily.

These robust correlations between our visual chlorosis scores and the image-based indices offer more than just validation; they highlight a practical convergence of qualitative and quantitative evaluation. The clear decline in NGRDI with increasing chlorosis scores confirms that the visual symptoms reflect measurable physiological deterioration. This relationship thus facilitates a two-tiered approach to screening, where a rapid visual scoring can be trusted to screen selections in the field, with the digital data providing an independent, objective reference to verify phenotyping when necessary. This combined method increases the validity of early screening, without sacrificing the throughput necessary for practical breeding operations.

Combining traditional biological parameters such as egg counts and nymph numbers with RGB-derived indices like GLI and NGRDI creates a robust framework for evaluating antibiosis and tolerance mechanisms in plants. This integrative phenotyping strategy not only captures the pest's preference and plant damage response but also streamlines early selection of resilient genotypes in breeding efforts. By leveraging digital phenotyping, breeders can improve the efficiency and accuracy of resistance screening, fostering sustainable crop production with reduced chemical inputs (Baker et al. 2020; Li et al. 2023). Further studies should be conducted at several locations and with chili varieties with the aim of confirming its broad applicability as well as perfecting the scoring system.

In conclusion, the combined use of oviposition count and chlorosis scoring provides a rapid, low-cost, and effective method for the early screening of chili resistance to *B. tabaci*. This approach is further strengthened by incorporating RGB image analysis and the NGRDI, which provide an objective, digital validation of the visual chlorosis scores, ensuring greater consistency and accuracy in phenotypic assessment. Dual-parameter approach efficiently distinguishes resistant genotypes, such as Ungara and Adelina, from susceptible ones like Landung and Yuni, based on both antixenosis and antibiosis/tolerance traits. For practical application, this protocol can be directly adopted in breeding programs to accelerate the selection of promising lines at the seedling stage, reducing dependence on chemical controls. Future work should focus on validating these criteria across diverse environments and chili species to ensure their robustness and wider applicability.

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REFERENCES

Aljbory Z, Chen M-S. 2018. Indirect plant defense against insect herbivores: A review. Insect Sci 25 (1): 2-23. DOI: 10.1111/1744-7917.12436.

Baker BP, Green TA, Loker AJ. 2020. Biological control and integrated pest management in organic and conventional systems. Biol Control 140: 104095. DOI: 10.1016/j.biocontrol.2019.104095.

Center for Agricultural Data and Information Systems. 2024. Analysis of red chili trade performance. Ministry of Agriculture, Jakarta. [Indonesian]

Chaubey AN, Mishra RS. 2018. Environmental factors influencing the population of whitefly and leaf curl disease incidence in chilli. J Environ Biol 39 (2): 216-220. DOI: 10.22438/jeb/39/2/ms-278.

Choudhary DK, Nirala YS, Pradhan A, Koshale KN. 2025. Emerging threats of whitefly-transmitted plant viruses: Insights into global spread, management, and vector-host interactions. J Adv Biol Biotechnol 28 (5): 26-41. DOI: 10.9734/jabb/2025/v28i52266.

Deguine J-P, Aubertot J-N, Flor RJ, Lescourret F, Wyckhuys KAG, Ratnadass A. 2021. Integrated pest management: Good intentions, hard realities. A review. Agron Sustain Dev 41: 38. DOI: 10.1007/s13593-021-00689-w.

Devendra R, Kumar M, Ghosh D, Yogindran S, Karim MJ, Chakraborty S. 2022. Capsicum-infecting begomoviruses as global pathogens: Host-virus interplay, pathogenesis, and management. Trends Microbiol 30 (2): 170-184. DOI: 10.1016/j.tim.2021.05.007.

Dos Santos TLB, Baldin ELL, do Prado Ribeiro L, de Souza CM, Bueno NM, da Silva IF. 2020. Silverleaf whitefly-resistant common beans: An investigation of antibiosis and/or antixenosis. Bragantia 79 (s1): 1-12. DOI: 10.1590/1678-4499.20190309.

Douglas AE. 2018. Strategies for enhanced crop resistance to insect pests. Ann Rev Plant Biol 69: 637-660. DOI: 10.1146/annurev-arplant-042817-040248.

Firdaus S, Van Heusden A, Harpenas A, Supena EDJ, Visser RGF, Vosman B. 2012. Identification of silverleaf whitefly resistance in pepper. Plant Breed 130 (6): 708-714. DOI: 10.1111/j.1439-0523.2011.01894.x.

Gothe RM, Karrem A, Gowda RSR, Onkarappa D, Jaba J, Ahn S-J, Pathour S, Yogendra K, Bheemanahalli R. 2024. Decoding plant defense: Accelerating insect pest resistance with omics and high-throughput phenotyping. Plant Physiol Rep 29: 793-807. DOI: 10.1007/s40502-024-00835-y.

Ingale AS, Mutkule DS, Deshmukh KV, Kumbhar SC, Bharadwaj GS. 2019. Screening of sunflower germplasm lines for resistance/tolerance to leafhopper and whitefly. J Entomol Zool Stud 7 (5): 803-807.

Kamaliah TL, Hidayat P, Maharijaya A, Syukur M. 2022. Preference *Bemisia tabaci* Genn. and its relation to leaf anatomical and morphological characters of chili (*Capsicum annuum* L.). J Agron Indonesia 50 (3): 291-298. DOI: 10.24831/jai.v50i3.40312.

Kaviya E, Ambethgar V, Chandraleka R, Kalyanasundaram A, Santhi VP, Geethanjali S. 2024. Impact of major homopteran pests on biochemical changes in guava (*Psidium guajava* L.) fruits and foliage. Phytoparasitica 52: 99. DOI: 10.1007/s12600-024-01220-z.

Li D, Li H-Y, Zhang J-R, Wu Y-J, Zhao S-X, Liu S-S, Pan L-L. 2023. Plant resistance against whitefly and its engineering. Front Plant Sci 14: 1232735. DOI: 10.3389/fpls.2023.1232735.

Millán-Chaidez R, Garzón-Tiznado JA, Linares-Flores PJ, Velarde-Félix S, Lugo-García GA, Retes-Manjarrez JE. 2021. Resistance to *Bemisia tabaci* (Hemiptera: Aleyrodidae) Mediterranean (Q biotype) in landrace and wild tomato populations from Mexico. Florida Entomol 103 (4): 472-478. DOI: 10.1653/024.103.00409.

Mwila N, Rubaihayo S, Kyamanywa S, Odong TL, Nuwamanya E, Mwala M, Agbahoungba S, Badji A. 2017. Biochemical factors associated with cassava resistance to whitefly infestation. Afr Crop Sci J 25 (3): 365-385. DOI: 10.4314/acsj.v25i3.9.

Naveed H, Islam W, Jafir M, Andoh V, Chen L, Chen K. 2023. A review of interactions between plants and whitefly-transmitted begomoviruses. Plants 12 (21): 3677. DOI: 10.3390/plants12213677.

Ni X, Quisenberry SS. 1997. Distribution of Russian wheat aphid (Homoptera: Aphididae) salivary sheaths in resistant and susceptible wheat leaves. J Econ Entomol 90: 848-853. DOI: 10.1093/jee/90.3.848.

Osuna-Caballero S, Olivoto T, Jiménez-Vaquero MA, Rubiales D, Rispail N. 2023. RGB image-based method for phenotyping rust disease progress in pea leaves using R. Plant Methods 19: 86. DOI: 10.1186/s13007-023-01069-z.

Padilha G, Pozebon H, Patias LS, Ferreira DR, Castilhos LB, Forgiarini SE, Donatti A, Bevilaqua JG, Marques RP, Moro D, Rohrig A, Bones SAS, Filho AC, Pes LZ, Arnemann JA. 2021. Damage assessment of *Bemisia tabaci* and economic injury level on soybean. Crop Prot 143: 105542. DOI: 10.1016/j.cropro.2021.105542.

Painter RH. 1951. Insect Resistance in Crop Plants. The Macmillan Company , New York.

Panwar N, Thirumurugan S, Kumar S. 2023. Host plant resistance in Brassicaceae against aphids. In: Kumar S (eds). Brassica-Recent Advances. IntechOpen, London. DOI: 10.5772/intechopen.110204.

Paweer MMZ, Namikoye ES, Nchore SB, Akutse KS. 2025. Can fungal endophytes suppress *Trialeurodes vaporariorum* and the transmission of tomato infectious chlorosis and chlorosis viruses in field conditions? Front Cell Infect Microbiol 15: 1470821. DOI: 10.3389/fcimb.2025.1470821.

Peñalver-Cruz A, Garzo E, Prieto-Ruiz I, Díaz-Carro M, Winters A, Moreno A, Fereres A. 2020. Feeding behavior, life history, and virus transmission ability of *Bemisia tabaci* Mediterranean species (Hemiptera: Aleyrodidae) under elevated CO_2. Insect Sci 27 (3): 558-570. DOI: 10.1111/1744-7917.12661.

Prithiva JN, Ganapathy N, Muthukrishnan N, Mohankumar S, Chandrasekhar CN. 2019. Preliminary screening of okra genotypes for leafhopper resistance *Amrasca biguttula* (Ishida) (Homoptera: Cicadellidae). J Pharmacogn Phytochem 8 (3): 2537-2541.

Sandra YMA, Maharijaya A, Sobir. 2022. Screening of resistance to geminivirus and whitefly in pepper. Euphytica 218: 155. DOI: 10.1007/s10681-022-03109-6.

Sayekti TWDA, Syukur M, Hidayat SH, Maharijaya A. a. 2021. Diversity and genetic parameter of chili pepper (*Capsicum annuum*) based on yield component in three location. Biodiversitas 22 (2): 823-829. DOI: 10.13057/biodiv/d220236.

Sayekti TWDA, Syukur M, Hidayat SH, Maharijaya A. b. 2021. Morphological response and genetic variability of four species of chili pepper (*Capsicum* spp.) under infection of pepper yellow leaf curl virus. Biodiversitas 22: 4758-4765. DOI: 10.13057/biodiv/d221107.

Selangga DGW, Listihani L, Temaja IGRM, Wirya GNAS, Sudiarta IP, Yuliadhi KA. 2023. Determinants of symptom variation of Pepper yellow leaf curl Indonesia virus in bell pepper and its spread by *Bemisia tabaci*. Biodiversitas 24 (2): 869-877. DOI: 10.13057/biodiv/d240224.

War AR, Taggar GK, Hussain B, Taggar MS, Nair RM, Sharma HC. 2018. Plant defence against herbivory and insect adaptations. AoB Plants 10 (4): ply037. DOI: 10.1093/aobpla/ply037.

Yadav RK, Jayanthi K, Kumar S, Kumar M, Ponnam N, Reddy M. 2022. Evaluation of chilli genotypes and understanding biochemical basis of whitefly (*Bemisia tabaci* Genn.) resistance. S Afr J Bot 151 (Part A): 433-444. DOI: 10.1016/j.sajb.2022.10.024.

Yorozuya H, Sudo M, Sato Y. 2021. Field evaluation of resistance to tea green leafhopper, *Empoasca onukii*, in tea plant. Entomol Exp Appl 169 (11): 1049-1056. DOI: 10.1111/eea.13096.