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Field Resistance Status of Hot Pepper to the Pepper Yellow Leaf Curl Indonesian Virus in Relation to Volatile Compounds

(Status Rintangan Ladang Lada Pedas terhadap Virus Daun Lada Kuning Keriting Indonesia Berkaitan Sebatian Meruap)

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ABSTRACT

The Pepper Yellow Leaf Curl Indonesian (PYLCI) virus, transmitted by the whitefly, has a significantly detrimental impacts on the productivity of hot peppers. Despite the implementation of extensive breeding programs, the development of varieties resistant to PYLCI remains a challenging endeavor. This study proposes a novel strategy that considers vector-host interactions with the objective of enhancing resistance. A total of 37 genotypes of hot pepper, collected by IPB University and the National Research and Innovation Agency (NRIA), along with two registered varieties of hot pepper (Bonita and Sigantung), were utilized in this study. The results were classified into five categories based on the disease reaction observed in the tested genotypes. The IPB RF 41 genotype exhibited high resistance, with no instances of diseases and minimal symptom intensity. In contrast, the IPB RF 20 genotype demonstrated high susceptibility, with a 64% incidence of disease and a symptom intensity of 41.85%. The distribution of the whitefly was consistent across the area, with an average of 29–39 imagos per week in each trap. The flowers and leaves of the CR-2022-46 (resistant) and IPB RF 29 (susceptible) genotypes were found to contain volatile compounds. The resistant genotypes exhibited a reduced number of volatile floral compounds. Leaf analysis showed the presence of D-limonene, indole, naphthalene, 1.4.5-trimethyl-, whereas these compounds were not detected in the flowers. Conversely, α-fenchene and naphthalene, 2-methyl-, were found exclusively in the flowers. Beta-ocimene plays a role in the whitefly-hot pepper interaction, with varying patterns of increasing from the vegetative to generative phases between resistant and susceptible genotypes. Resistant plants released higher levels of (E)-4,8-Dimethylnona-1,3,7-triene (DMNT) in both phases. This information could prove beneficial for the further development of alternative breeding programs focused on PYLCI resistance, and potentially revolutionize the agricultural practices in Indonesia.

Keywords: Hot pepper (*Capsicum frutescens*); pepper yellow leaf curl Indonesian virus; resistance; volatile compounds

ABSTRAK

Virus Daun Lada Kuning Keriting Indonesia (PYLCI) yang disebarkan oleh lalat putih, secara signifikan menghalang produktiviti lada pedas. Walaupun pelaksanaan program pembiakan yang meluas, pembangunan varieti rintang PYLCI masih sukar diperoleh. Penyelidikan ini mencadangkan strategi baharu yang mengambil kira interaksi vektor-perumah sebagai objektif untuk meningkatkan ketahanan. Sebanyak 37 genotip lada pedas yang dikumpulkan oleh Universitas IPB dan Badan Penelitian dan Inovasi Nasional (NRIA), serta dua varieti lada pedas berdaftar (Bonita dan Sigantung) digunakan dalam kajian ini. Hasil telah dikelaskan kepada lima kategori berdasarkan reaksi penyakit yang diperhatikan dalam genotip yang diuji. Genotip IPB RF 41 menunjukkan ketahanan tinggi dengan tiada kejadian penyakit dan keamatan gejala yang minima, manakala genotip IPB RF 20 sangat mudah terjejas dengan 64% kejadian penyakit dan keamatan

gejala 41.85%. Taburan lalat putih adalah tekal di seluruh kawasan dengan purata 29 - 39 imago setiap minggu dalam setiap perangkap. Sebatian volatil dikenal pasti dalam bunga dan daun genotip CR-2022-46 (rintang) dan IPB RF 29 (mudah terjejas). Genotip rintang menunjukkan pengurangan sebatian meruap bunga. Analisis daun menunjukkan kehadiran D-limonena, indola, naftalena, 145-trimetil-, tetapi sebatian ini tidak terdapat pada bunga. Sebaliknya, α-fensena dan naftalena, 2-metil- dijumpai eksklusif pada bunga. Beta-ocimene memainkan peranan dalam interaksi lalat putih-lada pedas yang berbeza antara genotip tahanan dan genotip rentan dengan pola yang berubah daripada fasa vegetatif kepada fasa generatif. Tanaman rentan melepaskan tahap (E)-4,8-Dimetilnona-1,3,7-trien (DMNT) yang lebih tinggi dalam kedua-dua fasa. Maklumat ini boleh memberi manfaat untuk pembangunan program pembiakan alternatif yang memberi tumpuan kepada rintang PYLCI dan berpotensi merevolusi amalan pertanian di Indonesia.

Kata kunci: Lada merah (*Capsicum frutescens*); rintang; sebatian volatil; virus daun lada kuning keriting Indonesia

INTRODUCTION

Chili peppers are a strategic vegetable commodity in Indonesia, and their impact on economic inflation makes them a national priority. In 2021, global production of fresh chili peppers reached 53 million tons (FAOSTAT 2023), with a global export turnover of 550 thousand tons. China is the primary exporter of fresh chili peppers, accounting for 31.5% of global exports (Trademap 2023). Although Indonesia accounts for 5.18% of global fresh chili pepper production and is the third-largest producer after China and Turkey (FAOSTAT 2023), it contributes a mere 0.33% to world fresh chili pepper exports (Trademap 2023). The chili pepper cultivated in Indonesia is typically of two species: the red chili pepper (*Capsicum annuum* L.) and the hot pepper (*C. frutescens* L.). The hot pepper contributes only 53.1% to national chili pepper production, with a national productivity of 8.23 tons/ha, which is lower than the productivity of the red chili pepper, which is 9.1 tons/ha (Kementerian Pertanian Republik Indonesia 2023).

One of the primary factors contributing to the low national productivity of hot peppers is the presence of Pepper Yellow Leaf Curl Indonesia (PYLCI) viral disease (Kustanto 2022; Selangga et al. 2021), which is transmitted by the whitefly (*Bemisia tabaci* Genn.) as the disease vector. The whitefly is a significant pest of chili peppers in Indonesia and other Asian countries, as well as Australia, sub-Saharan Africa, Mediterranean countries, and the Middle East (De Barro et al. 2011, 2008). Whiteflies are polyphagous insects that inflict damage on various plant species, including weeds and ornamental plants (Jones 2003). The piercing and sucking of plant fluids by whiteflies causes damage to leaf cells and tissues (Abubakar et al. 2022; Ghelani et al. 2020; Kamaliah et al. 2022; Narendra, Phabiola & Yuliadhi 2017; Soumia et al. 2020). Additionally, whiteflies act as vectors for various viruses, especially Begomovirus (Abdillah, 2021; Fiallo-Olive & Navas-Castillo 2023; Fiallo-Olive et al. 2020; Naveed et al. 2023). In cases of Gemini virus attack, yield losses can reach 100%. In 2018, whiteflies attacks carrying yellow virus disease reached 30% of the total area affected by pests, and in 2020, this figure increased to 40% (Direktorat Perlindungan Hortikultura Kementerian Pertanian 2023).

The PYLCI virus is a pathogen that infects hot pepper plants, causing the younger leaves or shoots to develop a wrinkled appearance and a light mosaic coloration. Eventually, the coloration will evolve to a bright yellow hue, and the concave and wrinkled leaves will diminish in size and increase in thickness. Begomovirus is an unstable virus, therefore, it is unable to survive for an extended period outside of its host. In order for natural transmission to occur, the assistance of a vector is required. The whitefly is an insect that is capable of acting as a vector for Begomovirus. Begomovirus pathogens are transmitted persistently by whitefly due to the AV1 gene in the Begomovirus genome, which is capable of producing a protein that protects against the degradation of virus particles when entering the digestive system of the whitefly.

The most common method of pest and disease control employed by farmers is the use of insecticides. However, the extensive application of insecticides has the potential to result in the development of resistance among whitefly populations and the subsequent proliferation of pests (Horowitz et al. 2020). One factor that determines the efficacy of an integrated pest management (IPM) program is the resistance of the host plant to infestation by the whitefly (Czosnek et al. 2017; Srivastava et al. 2017; Sulistyo 2016). Despite the intensive breeding of hot peppers to develop varieties resistant to the PYLCI virus, the disease-transmitted whitefly pests have not been effectively controlled. It is anticipated that losses in production will be decreased by developing a hot pepper variety resistant to the PYLCI virus through the incorporation of the interaction between insect vectors and host plants, specifically the synthesis of volatile compounds.

The tolerance mechanism of hot pepper genotypes against the PYLCI virus has yet to be extensively documented. Whiteflies act as vectors for the spread of PYLCI virus, indicating the tolerance mechanism of hot pepers against PYLCI virus can be elucidated through the interaction of hot peppers with whiteflies. Several studies have documented that the interaction between insects and chili pepper plants is mediated by the release of volatile compounds by chili pepper plants in response to herbivores. The interaction between whiteflies and hot pepper plants

facilitated by volatile compounds has received scant attention. The objective of this study was to ascertain the resistance status of the hot pepper breeding genotypes and the profile of volatile compounds in hot pepper as a host plant against the whitefly as a PYLCI virus vector. The present study's contributions are expected to include explaining the mechanism of hot pepper resistance to yellow virus and obtaining a profile of volatile compounds that can be used as selection criteria in hot pepper breeding programs.

MATERIALS AND METHODS

GENETIC MATERIAL AND EXPERIMENT DESIGN

The experimental research was conducted in Cikole Village, Lembang District, West Bandung Regency, West Java Province, Indonesia, from July to November 2022. The experimental treatment comprised 37 lines of hot chili pepper (*Capsicum frutescens*), which were working collections from IPB (IPB University) and NRIA (National Research and Innovation Agency), and two commercial varieties (*Bonita* and *Sigantung*) (Table 1). The experimental design employed a randomized block design, with two replications and 12 plants in each replication.

CULTIVATION TECHNIQUE

The seeds of all chili genotypes were sown in trays at a screen house and planted in a double-row system with a spacing of 50 cm × 80 cm. The base fertilizer consisted of 20 tonnes/ha of manure, 220 kg/ha of N fertilizer, 110 kg/ha of P_2O_5, and 180 kg/ha of K_2O (Moekasan, Basuki & Prabaningrum 2012). Pest control measures were implemented when the population density or attack intensity of *Spodoptera litura* and *Thrips parvispinus* reached the established economic threshold. Specifically, insecticide application was conducted when the attack intensity for *S. litura* exceeded 12.5%, and for *T. parvispinus*, when it surpassed 15%, as outlined by Prabaningrum and Moekasan (2014). Furthermore, disease management was performed through the application of fungicides, a decision that was informed by the prevailing conditions of the plants in the field. Irrigation was performed manually on daily basis since the planting of the crops.

OBSERVATION

The population of *B. tabaci* was observed, as were the symptoms of the pepper yellow leaf curl Indonesia virus in plants and volatile compound profiles. The population of *B. tabaci* was observed using a double-strap yellow rope. The installation was performed using a bamboo stake set at a height of 20 cm above the plant canopy surface, adjusted according to the increasing height of the additional plants. Four traps (T1–T4) were placed in a vertical and random configuration (Figure 1). Yellow sticky traps were replaced once a week after observations were conducted. Observations were conducted once a week at intervals by counting *B. tabaci* trapped in yellow sticky traps.

A series of observations were conducted on all treated plant populations. The first observation was recorded at the 30-day mark, and the final observation was documented 90 days following the planting procedure. The observation interval was established as once per week. The data obtained from the observation of each of the four sticky yellow traps installed were not subjected to statistical testing; rather, they were summed and averaged. The intensity of attack of virus symptoms was observed according to the criteria established by Dolores (1997), with modifications as outlined by Gunaeni, Wulandari and Hudayya (2015). The classification of symptom severity scores was conducted as follows: 0 = Plant not showing virus symptoms (healthy plant) (0%); 1 = Plant showing mild mosaic symptoms (1% - 25%); 2 = Plant showing mosaic symptoms, with yellow streaks clearly visible (contrast); (> 25 % - 50 %); 3 = Plant showing mosaic symptoms, with yellow streaks clearly visible (contrast) and a change in growth form (> 50% - 75%); 4 = Plant showing severe mosaic symptoms, with yellow streaks clearly visible (contrast) (Figure 2).

The volatile compounds were profiled at the Flavor Laboratory of the Rice Research Center in Subang Regency, following the methods outlined by Junior et al. (2012) and Kirana et al. (2019), with several modifications. The extraction and identification of chili flower volatiles were conducted by solid phase microextraction-headspace (SPME-HS/GC-MS). Four grams of chili leaves and flowers were placed into a vial (Sigma-Aldrich) and extracted by heating at 30 °C for 30 min. The solid phase microextraction (SPME) fiber utilized was a 50/30 µm DVB/CAR/PDMS (divinylbenzene/carboxen/polydimethylsiloxane) blend, supplied by Supelco (Sigma-Aldrich, Bellefonte, PA, USA). The fibers were then injected directly into a Hewlett Packard 6890 device (Agilent Technologies Inc., Santa Clara, USA), a Gas Chromatograph-Mass Spectrometer (GC-MS), where they remained for a period of 15 min to ensure maximal distribution of volatile compounds. The device was programmed in split-less mode, a method that allows for the analysis of all volatile compounds present in the sample. Subsequently, the metabolites were separated using an Innowax capillary column (film thickness: 60 m × 0.25 mm I.D. × 0.25 µm) with helium as the carrier gas, at a flow rate of 0.7 mL min^{-1}. The oven temperature was programmed from 40 to 200 °C, and the total GC running time was 25 min. The MS transfer temperature was set at 260 °C, the ionization energy was maintained at 70 eV, and the mass range was approximately 50-550 m.

TABLE 1. The experimental treatment of 37 genotypes of hot chilli pepper (*Capsicum frutescens*)

Lines number	Genotype	Sources
1	IPB RF 7	IPB
2	IPB RF 8	IPB
3	IPB RF 9	IPB
4	IPB RF 12	IPB
5	IPB RF 15	IPB
6	IPB RF 16	IPB
7	IPB RF 17	IPB
8	IPB RF 18	IPB
9	IPB RF 20	IPB
10	IPB RF 21	IPB
11	IPB RF23	IPB
12	IPB RF 24	IPB
13	IPB RF 26	IPB
14	IPB RF 27	IPB
15	IPB RF 28	IPB
16	IPB RF 29	IPB
17	IPB RF 30	IPB
18	IPB RF 33	IPB
19	IPB RF 34	IPB
20	IPB RF 35	IPB
21	IPB RF 38	IPB
22	IPB RF 39	IPB
23	IPB RF 40	IPB
24	IPB RF 41	IPB
25	IPB RF 42	IPB
26	IPB RF 43	IPB
27	IPB RF 44	IPB
28	Bonita	Commercial variety
29	CR-2022-46	NRIA
30	Sigantung	Commercial variety
31	CR-2022-48	NRIA
32	CR-2022-49	NRIA
33	CR-2022-52	NRIA
34	CR-2022-53	NRIA
35	CR-2022-54	NRIA
36	CR-2022-55	NRIA
37	CR-2022-56	NRIA
38	CR-2022-57	NRIA
39	CR-2022-58	NRIA

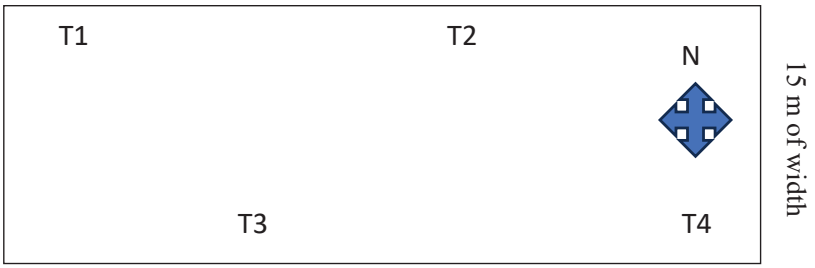

FIGURE 1. The configuration for the disposition of yellow traps

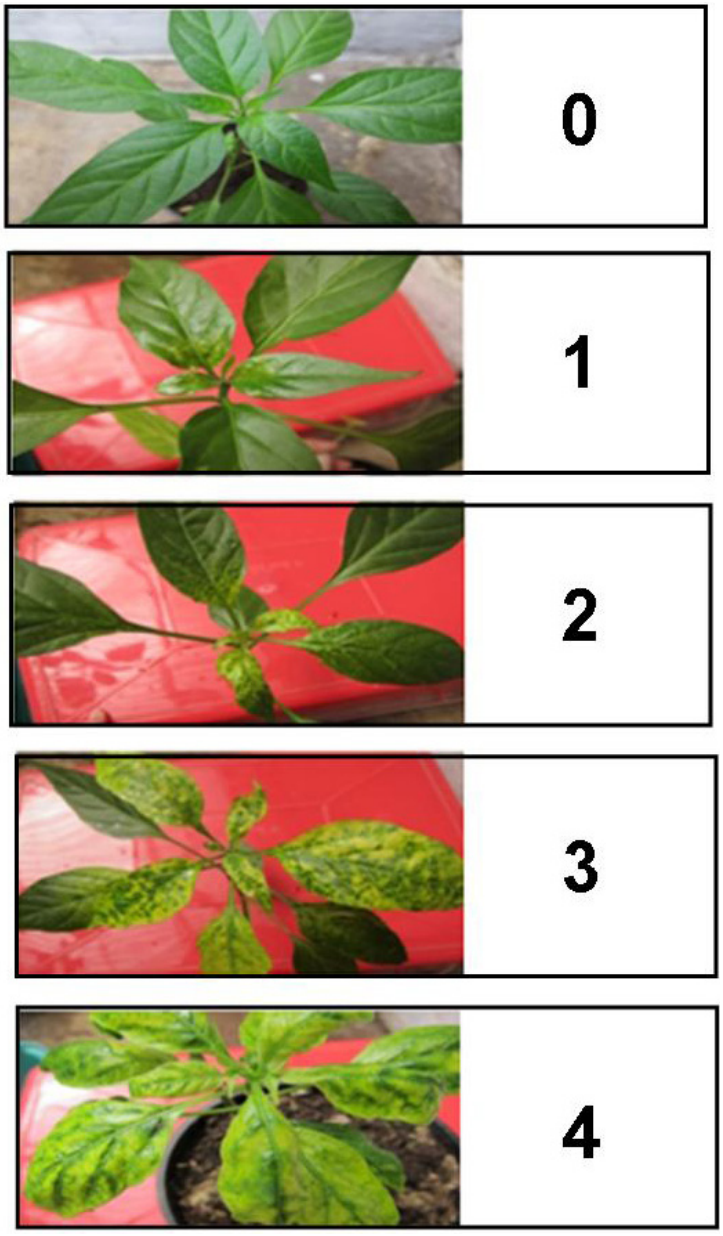

FIGURE 2. Scoring of the symptoms of the curly yellow virus (Gunaeni, Wulandari & Hudayya 2015)

DATA ANALYSIS

The data on incidence observed, disease intensity (Table 2) were analyzed using simple statistical analysis (mean value and standard deviation), and the *B. tabaci* population was analyzed using Tukey test, both employing PKBT-STAT. The analysis was performed for data on incidence and intensity, Area Under the Disease Progress Curve (AUDPC) value calculation from yellow virus intensity data, and strain mapping using Principal Component Analysis (PCA) using PAST programs.

The incidence of virus symptom attack was determined by the following formula:

$$Incidence\ (\%) = \left(\frac{a}{A}\right) \times 100$$

where a is the number of symptomatic plants; and A is the number of plants observed.
The intensity of the virus symptom attack was determined by the following formula:

$$I = \frac{\sum(n \times v) \times 100}{N \times V}$$

where I is the intensity of attack symptoms; n is the number of plants exhibiting symptoms on a certain scale; v is the scoring value of certain symptoms; N is the number of plants observed; and V is the highest symptom severity scoring value.

The total area under the disease progression curve (AUDPC) of the disease was calculated by formula 2 (Louws et al. 1996). The formula is expressed as follows:

$$AUDPC = \sum_{i}^{n-1} \left|\frac{Yi + Yi + 1}{2}\right| ti + 1 - ti$$

where AUDPC is the disease progression curve; Yi+1 is the observational data 1+1; Yi is the 1st observation data; ti+1 is the time of the i-th observation + 1; and ti is the time of the 1st observation.

The relative volatile compound content data were determined using the NIST-14 library. The data were then tabulated and the presence index of volatile compounds was calculated. The presence index of volatile compounds is the ratio of the presence of volatile compounds to the total detected volatile compounds. The detected volatile compounds were selected based on the area and the degree of similarity with the literature (Faizal et al. 2017; Kirana et al. 2019; Luning et al. 1994).

RESULTS AND DISCUSSIONS

B. tabaci POPULATION

The experimental plot was situated in region where the Pepper Yellow Leaf Curl Indonesia virus is endemic. The results of capturing whiteflies from the four traps indicated the presence of the insect population in the experimental area since the vegetative phase. The initial observation was recorded 85 days after planting the plants. A total of 691 *B. tabaci* imagos were trapped, with a range of 145-194 imagos in a single trap. The average number of *B. tabaci* imagos captured per week in each trap ranged from 29 to 39, exhibiting no significant variations acrosss the four traps (Figure 3). These findings suggest that the whitefly population was evenly distributed within the designated test area.

A number of studies have indicated that the presence of a single *B. tabaci* carrying the virus within a population is sufficient to facilitate its transmission (Brown & Czosnek 2002; Devendran et al. 2022; Duriat 2008; Hasyim, Setiawati & Liferdi 2016; Sulandari 2006). According to Brown and Czosnek (2002), Devendran et al. (2022), Roy, Chakraborty and Ghosh (2021), and Sulandari (2004), the presence of a low epidemiological population of *B. tabaci* can transmit the virus to hot peppers when using varieties that are susceptible to PYLCI viral disease. In certain types of plants, the process of transferring the begomovirus carried by whitefly insects will take place in a very short time, even in just a matter of seconds or minutes, resulting on the immediate movement or inoculation of parts of the plant cells (persistent transmission) (Thakur et al. 2018).

The data on whitefly capture demonstrated that the whiteflies present in the experimental fields carried the virus, subsequently infecting the healthy plants and thriving on the tested plants. This was evidenced by the symptoms of the PYLCI virus infection in the hot pepper plants, which included the following: Leaf edges curling upwards (cupping), thickening of the leaf veins, yellowing of the leaves, and curling. The symptoms persisted, resulting in the majority of the leaves turning bright yellow or light green due to chlorosis. This was followed by the emergence of leaf abnormalities, such as sunken and wrinkled leaves that are smaller and thicker in sizes, and stunted plant growth. This observation aligns with the findings of research conducted on chilies (Akhter et al. 2014; Septariani et al. 2020; Singh et al. 2021; Tuhumury & Amanupunyo 2018; Windarningsih et al. 2018) that were infected with the PYLCI virus. The initial symptoms manifested as yellowing of the foliage, beginning at the uppermost leaves or in the young leaf tissue. The virus then disseminated, leading to the thickening of the veins within the chili plant leaves. The advanced stage of the PYLCI viral disease is characterized by the emergence of smaller young leaves.

INCIDENCE, INTENSITY, AND AUDPC OF PEPPER YELLOW LEAF CURL INDONESIAN

The incidence of symptoms associated with PYLCI viral disease exhibited an increasing trend with the advancement of plant age. In this study, the average incidence of the PYLCI among all genotypes tested was 24.25%, with

TABLE 2. The degree of resistance to the pepper yellow leaf curl Indonesia virus

Disease intensity (%)	Resistance rating
0	Highly resistant
$x \leq 10$	Resistant
$10 < x \leq 20$	Moderately resistant
$20 < x \leq 30$	Moderately susceptible
$30 < x \leq 40$	Susceptible
$x > 40$	Highly Susceptible

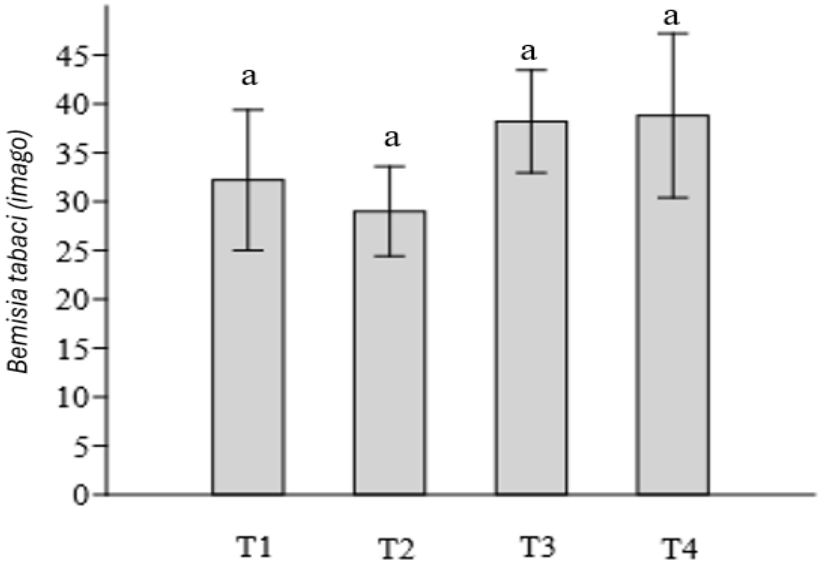

FIGURE 3. The population of *B. tabaci* in the experimental field determined based on the catch of four traps (T1-T4) (unit of imago). Error bars represent standard deviation, and the number followed by the same letter does not differ significantly based on the Tukey test level of 5%

a range from 0% (IPB RF 41) to 64.55% (IPB RF 20) (Table 3). The average number of *B. tabaci* captured per week in each trap ranged from 29 to 39 imagos. An increase in vector insect populations invariably leads to enhanced opportunities for virus transmission to plants, as the greater the whitefly population, the more effective the transmission and the shorter the incubation period (Sulandari 2004). This virus multiplication mechanism occurs rapidly in the nucleus of infected plant cells through recombination-mediated replication (Thakur et al. 2018). The prevalence of PYLCI virus symptoms exhibited a range of 0% to 64.60%, with an average intensity of 17.77%, ranging from 0% to 41.90%. The increase in symptoms was observed to be concomitant with an increase in the whitefly population, which ranged from 29 to 39 imagos.

In regard to the degree of resistance to the PYLCI virus, IPB RF 41 was identified as a highly resistant genotype, exhibiting no symptoms of infection for up to 90 days post-planting. Conversely, IPB RF 20 was classified as a highly susceptible genotype, displaying a high intensity of symptoms that exceeded 40% (Table 3). The intensity of PYLC1 disease on hot peppers ranges from 50% to 100%, while on large chilies, it ranges from 20% to 100%.

In terms of the level of resistance to the PYLCI virus, the IPB RF 41 genotype exhibited high resistance, demonstrating no symptoms of PYLCI virus infection for up to 90 days post-planting. This suggests that the plant can effectively survive the virus attack over an extended period, which is a critical factor in determining crop yields. Conversely, the IPB RF 20 genotype was identified as exhibiting a high level of susceptibility to the PYLCI virus, a trait that can result in significant economic losses.

Within the context of PYLCI disease, the infection intensity in cayenne pepper ranges from 50% to 100%, while in large chilies, the intensity ranges from 20% to 100%. This range suggests that cayenne pepper is more

susceptible to viral infection compared to large chili peppers. This is an important consideration in choosing the variety of chili pepper to be planted, especially in areas that are prone to virus attacks. A comprehensive understanding of these differences in resistance facilitates more informed decisions regarding variety selection, which can enhance crop yields and mitigate losses due to disease. Furthermore, continued research on the resistance of this genotype to the virus could contribute to the development of new, more resistant varieties, thereby ensuring the sustainability of agricultural practices in the future.

The intensity of the PYLCI viral disease escalated with the advancement of the plant's age, influenced by the presence of inoculums in the field, which can affect the response of the chili strains to the disease. The increased sensitivity of chili genotypes to the PYLCI virus was influenced by the presence of inoculums that infect plants at a young stage (Ganefianti et al. 2008).

The intensity of disease symptoms is influenced by genetic, environmental, plant age, and insect vector factors (Marianah 2020). From a genetic perspecitve, a plant genome contains receptors that recognize viruses entering its cells and induce resistance responses (Subekti et al. 2006). Furthermore, Suganda et al. (2002) posited that the intensity of the disease attack is manifested by the scoring of symptoms, as well as the number and size of the leaves.

The AUDPC value, representing the total area under the disease development curve for each genotype, was determined at observation 30–90 days after planting. These values are presented in Table 3 and Figure 4. The AUDPC values of the different genotypes varied significantly, with the lowest value recorded for the IPB RF 41 genotype (0.0 ± 0.0) and the highest for the IPB RF 20 genotype (1700.4 ± 979.6) (Figure 4). The observed differences in symptom development suggest that the resistance of hot pepper genotypes to the PYLCI virus may be contingent on the interaction between the genotype and the virus. The AUDPC values of the genotypes were indicative of their capacity to impede viral infection, with lower AUDPC values corresponding to greater inhibitory effects. Conversely, higher AUDPC values indicated diminished plant resistance. As yellow virus disease progresses, the symptoms that manifest will intensify and accelerate.

MAPPING OF RESISTANCE EXHIBITED BY CHILI STRAINS AGAINST THE PEPPER YELLOW LEAF CURL INDONESIAN VIRUS

The results of the multivariate analysis showed that the tested genotypes could be grouped based on their eigenvalues into five categories: highly tolerant, tolerant, moderately tolerant, susceptible, and highly susceptible. The IPB RF 41 strain exhibited the highest tolerance level, while the IPB RF 20 strain displayed the lowest tolerance level. A total of 18 strains showed moderate tolerance to yellow virus, 9 strains exhibited high tolerance, and 10 strains displayed low tolerance levels (Figure 5).

The results of mapping the resistance of the strains to the PYCLI virus can be used to understand the mechanism of interaction between *B. tabaci* as a PYCLI virus vector because 80% of plant viruses depend on vectors for dissemination (Legarrea et al. 2015). Insect interactions with chili plants that can be mediated by volatile compounds are known as herbivore-induces plant volatiles (HIPVs). Zhang et al. (2019) have provided a comprehensive overview of the functions of HIPVs, as reported in several studies. These functions include direct defense, characterized by a toxic effect and repulsion of herbivores, and as a form of indirect defense, marked by attraction of natural enemies and the production of signal or early warnings that alert neighboring plants to impending insect attack. It is hypothesized that resistant and susceptible strains will exhibit different volatile compound profiles, thereby facilitating a more nuanced understanding of the defense mechanisms employed by chili peppers against *B. tabaci* infestations.

PROFILE OF VOLATILE COMPOUNDS OF STRAINS RESISTANT AND SUSCEPTIBLE TO PEPPER YELLOW CURL LEAF INDONESIAN VIRUS

The volatile compounds were extracted from two parts of the plant, namely flowers and leaves, of resistant (46) and susceptible (29) strains (Table 4). The total number of volatile compounds identified by the GCMS tool from the two strains was 97 compounds. The total number of volatile compounds extracted from the leaves of the susceptible and resistant strains was both 95 compounds, while the total number of volatile compounds extracted from the flowers of the resistant and susceptible strains differed. The resistant strains had fewer volatile compounds than the susceptible strains. Several volatile compounds were detected in the leaves but not in the flowers, namely Phenylethyl alcohol, D-Limonene, Indole, Amorphadiene, 1R-α-Pinene, 1,3,8-p-Menthatriene, Hexyl isobutyrate, Naphthalene, 1,6-dimethyl-, trans-Farnesol, Azulene, 4,6,8-trimethyl-, and Isomyocorene. Conversely, two volatile compounds, β-Myrcene and trans-β-Ocimene, were exclusively released by the flowers and not detected in the leaves.

The potential role of volatile compounds in vector-plant interactions may be that these compounds are generated in response to stress, including viral infections, and may affect interactions with vectors. Resistant strains have been observed to produce more diverse and higher amounts of volatile compounds (Furdikova et al. 2019). In addition to the production of volatile compounds, resistant strains were shown to have better defense mechanisms and accumulation of phenolic compounds that can inhibit viral replication. Conversely, the susceptible strains exhibited limited production of volatile receptors and an inadequate defense system to fight viral infections.

The present study compared volatile compounds that exhibited the largest area based on the average and standard

TABLE 3. Average incidence, intensity, and AUDPC value of yellow virus symptoms

No. Massage	No. Genotype	Incidence (%) at 90 dap	Intensity (%) at 90 dap	AUDPC Value (30-90 dap)
1	IPB RF 7	35.0±21.2	26.3±15.9	515.4±336.2
2	IPB RF 8	33.3±0.0	25.0±0.0	682.2±5.7
3	IPB RF 9	35.0±21.2	26.3±15.9	667.7±19.1
4	IPB RF 12	46.0±12.1	34.1±8.4	870.6±156.4
5	IPB RF 15	41.7±11.8	31.3±8.8	833.5±151.8
6	IPB RF 16	27.9±0.9	19.7±2.4	418.7±218.5
7	IPB RF 17	19.2±15.3	14.3±11.7	273.8±360.6
8	IPB RF 18	19.1±1.3	15.8±3.1	363.6±387.4
9	IPB RF 20	64.6±32.5	41.9±23.8	1700.4±979.6
10	IPB RF 21	43.2±9.6	32.1±6.9	814.1±240.7
11	IPB RF23	16.7±0.0	12.3±0.4	100.0±30.9
12	IPB RF 24	29.2±29.5	21.8±22.3	492.2±240.9
13	IPB RF 26	29.2±5.9	21.5±4.9	257.8±165.7
14	IPB RF 27	45.8±5.9	33.1±2.7	775.6±672.6
15	IPB RF 28	38.6±16.1	28.3±14.6	388.6±131.4
16	IPB RF 29	46.7±18.9	35.0±14.1	798.3±909.7
17	IPB RF 30	13.2±7.0	9.8±5.4	212.5±154.7
18	IPB RF 33	21.6±4.8	15.8±3.1	94.6±54.3
19	IPB RF 34	13.2±7.0	9.8±5.4	187.9±119.9
20	IPB RF 35	8.3±0.0	5.1±1.3	57.8±64.1
21	IPB RF 38	13.5±7.3	9.6±4.8	140.0±9.9
22	IPB RF 39	4.2±5.9	3.1±4.4	80.7±114.1
23	IPB RF 40	8.3±11.8	6.3±8.8	286.6±171.1
24	IPB RF 41	0.0±0.0	0.0±0.0	0.0±0.0
25	IPB RF 42	4.2±5.9	3.0±4.2	51.6±72.9
26	IPB RF 43	17.4±1.1	15.9±4.0	387.4±147.2
27	IPB RF 44	37.9±40.7	28.0±31.1	714.6±665.9
28	Bonita	17.4±1.1	12.8±0.4	334.0±5.0
29	CR-2022-46	12.5±5.8	9.3±4.6	200.0±256.3
30	Sigantung	20.8±5.9	15.4±4.7	323.4±103.9
31	CR-2022-48	8.0±0.0	6.1±0.2	201.6±139.2
32	CR-2022-49	26.1±1.6	19.4±0.9	431.9±70.1
33	CR-2022-52	9.2±1.2	6.1±0.2	202.9±141.1
34	CR-2022-53	4.2±5.9	3.0±4.2	139.1±72.9
35	CR-2022-54	16.7±0.0	11.3±1.8	203.7±24.3
36	CR-2022-55	25.0±11.8	19.5±4.9	368.9±118.3
37	CR-2022-56	29.2±5.9	21.5±4.9	370.3±99.4
38	CR-2022-57	34.5±36.0	25.5±27.6	635.7±469.6
39	CR-2022-58	29.1±15.4	18.8±8.8	386.4±254.8

The ± symbol indicates the mean accompanied by the standard deviation values. The abbreviation 'DAP' refers to 'days after planting'

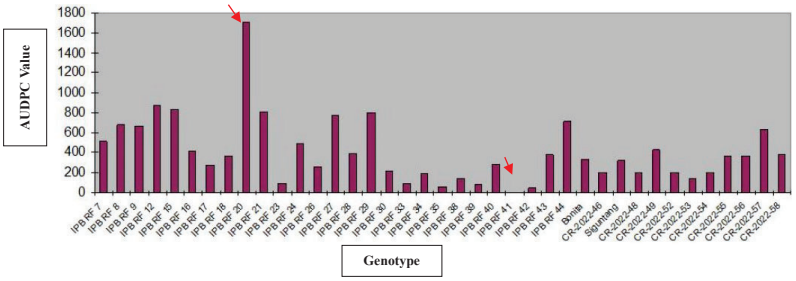

FIGURE 4. Graphical representation of the disease development of various genotypes (30-90 DAP)

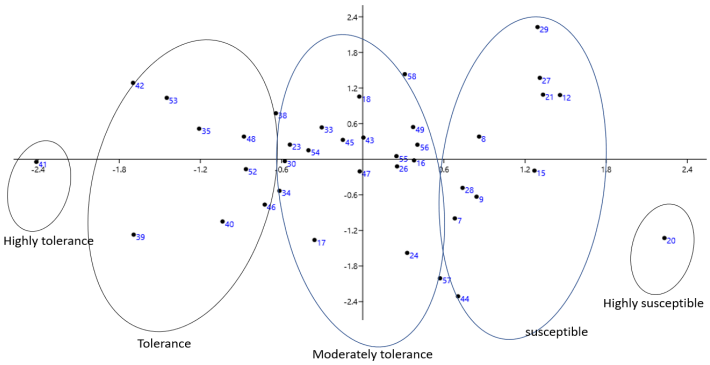

FIGURE 5. The results of resistance mapping of 50 genotypes of cayenne pepper against the PYCLI viral disease, based on eigen values

TABLE 4. Area of volatile compounds in leaves and flowers of resistant and susceptible genotype (1 million times)

Dominant volatile compounds	Leaf		Flower	
	Resistant	Susceptible	Resistant	Susceptible
β-Ocimene (check pattern, position as common compound)	123.0	26.1	1,405.0	2,291.5
Oxime-, methoxy-phenyl-	49.3	43.9	79.7	60.1
(E)-4,8-Dimethylnona-1,3,7-triene	50.7	26.0	124.0	46.4
Phenylethyl alcohol	12.4	0.5	-	-
Indole	8.5	4.4	-	-
D-Limonene	8.3	3.8	-	-
Amorphadiene	10.5	0.8	-	-
1R-α-Pinene	8.2	1.4	-	-
1,3,8-p-Menthatriene	5.4	3.3	-	-
Neo-allo-ocimene	6.3	2.0	43.7	59.9
Hexyl isobutyrate	4.4	3.9	-	-
Naphthalene, 1,6-dimethyl-	2.5	4.7	-	-
trans-Farnesol	0.7	6.5	-	-
Azulene, 4,6,8-trimethyl-	2.4	4.7	-	-
Isomyocorene	8.7	140.8	-	-
β-Myrcene	-	-	34.5	37.3
trans-β-Ocimene	-	-	28.1	38.0

deviation of all volatile compounds detected by the GC-MS tool (dominant volatile compounds). A comparison of the dominant volatile compounds released by the leaves and flowers of resistant and susceptible strains is presented in Table 4. The analysis showed that of the 17 volatile compounds detected in the leaves, 14 were more prevalent in resistant leaves, with the exception of Naphthalene, 1,6-dimethyl-,trans-Farnesol, Azulene, 4,6,8-trimethyl-, and Isomyocorene. In contrast, flowers exhibited a wider range of volatile compounds, with a maximum of six dominant volatile compounds. The flowers of the susceptible strains exhibited a higher release of β-Ocimene, while the resistant strains demonstrated elevated release of (E)-4,8-Dimethylnona-1,3,7-triene. These observations suggest a potentidal role for these two volatile compounds in the chili defense mechanism against PYLCI.

The β-Ocimene is a volatile compound that has been widely documented as an interaction medium between insects and their host plants. Ocimenes, volatile compounds utilized by insects as host site markers (Farre-Armengol et al. 2017; Jayanthi et al. 2012; Syamsudin et al. 2022), have been shown in this study to play a role in the interaction between *B. tabaci* and chili plants, although the underlying mechanisms remain to be fully explained. The study showed that the pattern of β-Ocimene increase differed between resistant and susceptible genotypes during the transition from the vegetative to the generative phase. Furthermore, the synthesis of β-Ocimene was more pronounced in the flowers of susceptible plants, suggesting a potential role in the early warning system of chili's defense against *B. tabaci*. This defense mechanism may also affect the defense of other plants or different strains, as reported by Zhang et al. (2019). Additionally, compounds such as trans-Farnesol and Isomyocorene were synthesized in greater abundance in susceptible plants. Isomyocorene, a terpenoid compound, belongs to a group that includes volatile organic compounds (VOCs), which are responsible for the primary aroma of the resulting fruits (Yadav et al. 2022).

The next volatile compound that exhibited a different pattern between resistant and susceptible strains was (E)-4,8-dimethylnona-1,3,7-triene (DMNT). Resistant strains demonstrated a greater release of DMNT volatile compounds than susceptible genotypes during the vegetative (leaf) and generative (flower) phases. Consequently, it can be inferred that the employed defensive strategy is direct defense by means of releasing volatile compounds as repellents. The findings are consistent with previous studies that have reported the presence of DMNT in plant-insect interactions, particularly in cases of herbivory (Li et al. 2021; Meents et al. 2019; Richter et al. 2016; Schlaeger, Picket & Birkett 2018). The findings of the present study are in allignment with those of previous study by Li et al. (2021), which reported that DMNT functions as a repellent that can impede the oviposition of *B. tabaci* in plants.

In the present study, the volatile compounds D-limonene and α-pinene were also found to be released in greater quantities by resistant strains compared to susceptible strains in response to yellowing disease caused by the virus. In addition, Johnston et al. (2022) reported a new finding that kaolin with D-limonene aroma effectively repels aphids during the dry season. D-limonene has been identified as a repellent for *B. tabaci*, which may lead to a reduction in the number of adult whiteflies settling on host plants, a decrease in oviposition rates, nymph populations, and ultimately, a decrease in yellowing disease symptoms. These findings are consistent with the studies by Guarino et al. (2021) and Saraiva et al. (2022), which also reported that α-pinene is released in smaller amounts by susceptible strains compared to resistant strains in cashew and citrus plants.

There are also volatile compounds that have been found to be synthesized in greater quantities in resistant plants, including phenylethyl alcohol, amorphadiene, and neo-allo-ocimene. Phenylethyl alcohol, also known as 2-phenylethanol, is a colorless, aromatic, viscous liquid with a floral odor. In a study by Herera et al. (2020), 2-phenylethanol was mixed with acetic acid (AA) as a food lure to catch both male and female Lepidoptera and Neuroptera insects. This compound is also an aggregate of the pheromone of the beetle *Megacyllene caryae* (Lacey et al. 2008). Amorphadiene, a sesquiterpene, is a precursor in the biosynthesis of secondary metabolites in plants (Pyne, Narcross & Martin 2019). In *Artemisia annua*, amorphadiene plays a crucial role in the production of artemisinin, a secondary metabolite that is part of the plant's defense mechanisms. Furthermore, a study has shown that the activity of the amorphadiene synthase (pADS) gene in *Artemisia annua* is inducible by salicylic acid (SA) and methyl jasmonate (MJ), which are well-known signals in plant defense responses (Guo et al. 2010). In addition, neo-allo-ocimene, a terpenoid, contributes to the primary aroma in fruits (Yadav et al. 2022). The relationship between these three types of volatile compounds and their role as repellents against *B. tabaci* remains to be elucidated; further studies are necessary to test the validity of these volatile compounds as repellents in resistant chili plants.

CONCLUSION

The highly resistant genotype of hot pepper against the PYLCI viral disease is IPB RF 41, exhibiting 0% incidence, 0% intensity, and AUPDC value of 0, while IPB RF 20 is a susceptible genotype with 64% incidence, 41.85% intensity and AUPDC value of 1700,4. The PYLCI virus vector, whitefly, exhibited even population distribution across the study area, with the average number of *B. tabaci* captured per week per trap ranging from 29 to 39 imagos. The volatile compounds present in the flowers and leaves of the resistant (46) and susceptible (29) genotypes were identified. The

resistant genotypes exhibited a reduced level of floral volatile compounds compared to the susceptible genotypes. Volatile compounds detected in the leaves but not in the flowers included Phenylethyl alcohol, D-Limonene, Indole, Amorphadiene, 1R-α-Pinene, 1,3,8-p-Menthatriene, Hexyl isobutyrate, Naphthalene, 1,6-dimethyl-, trans-Farnesol, Azulene, 4,6,8-trimethyl-, and Isomyocorene. Conversely, volatile compounds that are released in the flowers but not in the leaves included β-Myrcene and trans-β-Ocimene. The role of β-ocimene in the interaction between *B. tabaci* and chili plants is noteworthy. Differences in the pattern of increase of β-ocimene from the vegetative phase to the generative phase were observed in resistant and susceptible genotypes. Additionally, a different pattern was observed between resistant and susceptible genotypes for (E)-4,8-Dimethylnona-1,3,7-triene (DMNT). The resistant genotype exhibited higher levels of DMNT release compared to the susceptible genotype, both in the vegetative (leaf) and generative (flower) phases.

RECOMMENDATION

The utilization of plant volatile compounds has the potential to facilitate the formulation of strategies for the control of pests and diseases, thereby contributing to the development of more effective and environmentally friendly approaches within the agricultural sector. Further research is required to conduct controlled experiments in greenhouse settings, wherein resistant and susceptible lines will be examined. This will serve to validate the hypothesis that the compounds identified in this study are, in fact, confirmed as resistant genotype markers.

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