



Characterization and Identification of Chili Pepper Accessions with Tolerance to Biotic Stress for Sustainable Agriculture

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ABSTRACT

Chili peppers worldwide are severely affected by both biotic and abiotic stresses, leading to substantial yield losses. The heavy reliance on chemical pesticides poses environmental risks and has proven ineffective, particularly in controlling vectors that transmit viral diseases. Therefore, sustainable chili pepper production necessitates urgent efforts to develop biotic stress-resistant cultivars. This study aimed to identify valuable genetic material and introduce resistance genes against insects, fungal pathogens, and viral diseases into commercial cultivars. From July 2022 to February 2023, five accessions and four varieties of chili pepper were screened for broad-spectrum resistance under field conditions at the Indonesian Vegetables Research Institute. The results indicated that accessions 5x1, 5x2, 2x5, and 4x5, as well as the varieties 'Carla Agrihorti', 'Hot Beauty', 'Inata Agrihorti', and 'Pilar', exhibited resistance to various stresses. Conversely, accession 2x1 was classified as moderately resistant, displaying reduced feeding damage. Accession 2x5 was identified as resistant to oriental fruit flies. Anatomical traits such as fruit weight, pedicel length, fruit diameter, fruit locus count, and pericarp thickness were crucial components of the resistance mechanism against fruit flies. Additionally, accessions 5x1, 2x1, 2x5, and 4x5 demonstrated resistance to anthracnose. The highest fruit yields were recorded in varieties 'Carla Agrihorti', 'Inata Agrihorti', and 'Hot Beauty', while accessions 2x5 and 5x1 represent valuable genetic resources for breeding programs aimed at combining high yield with durable resistance, thereby enhancing the sustainability of chili pepper production in the face of ongoing biotic challenges.

Introduction

Chili peppers (*Capsicum annuum* L.) are widely cultivated worldwide and are believed to be one of the earliest cash crops. Originally from South and Central America (Saxena et al., 2016; Karim et al., 2021), chili peppers are widely used for medicinal purposes, as well as in culinary applications as a vegetable, spice, and natural colorant. Recent estimates report its global production at approximately 3.1 million t across 2.06 million ha. China is the leading producer, contributing 16.7 million t, which accounts for around 35% of the global cultivated area. Other major producers are

Turkey (3.0 million t), Mexico (2.3 million t), and Indonesia (2.7 million t) (Penella and Calatayud, 2019).

Plant performance and fruit quality are significantly affected by biotic and abiotic stress conditions, particularly when most chili peppers are cultivated in open fields (Khaitov et al., 2019). Chili pepper production is severely impacted by pests and diseases, which reduce productivity and export potential, affecting many commercially grown *Capsicum* accessions and varieties (Khaitov et al., 2019; Parisi et al., 2020). Aphids, thrips, mites,

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whiteflies, oriental fruit flies, fruit borers, and microbial pathogens such as *Colletotrichum*, *Phytophthora*, *Fusarium*, *Xanthomonas*, and various viruses are among the most common biotic stress agents (Parisi et al., 2020; Jeevenandham et al., 2018; Syamsudin et al., 2022; Shivalingaswamy et al., 2022; Islam et al., 2020; Bashir et al., 2018; Utami et al., 2022; Ashwathappa et al., 2022).

Begomoviruses, i.e., the whitefly-borne Pepper Yellowing Leaf Curl Virus (*PepYLCV*), pose a significant threat to chili pepper cultivation. According to Wahyono et al. (2023), in lowland regions, the prevalence of Begomovirus infection reached 93%, while in highland areas it was 88.78%. Moreover, disease severity was higher in lowlands (54.50%) than in highlands (38.11%). Setiawati et al. (2022) reported that insect infestations are a major cause of yield losses in chili peppers, ranging from 25% to 100% globally. Insect pests such as aphids, thrips, and whiteflies also serve as important vectors of plant viruses.

Furthermore, climate change has intensified the frequency of extreme weather events, which not only reduce chili pepper yields but also facilitate the rapid spread of viral diseases. Subedi et al. (2023) emphasized that climate change will likely make the distribution and impact of pests and diseases more unpredictable, potentially affecting broader geographic regions. Chemical pesticides remain the primary method for managing pests and diseases in chili pepper cultivation. However, their indiscriminate and prolonged use has resulted in several problems, including the development of pest resistance, environmental contamination, increased production costs, labor intensiveness, and food safety concerns (Kanaan, 2021).

To address these challenges, it is essential to enhance the profitability, sustainability, and productivity of chili pepper farming. One promising approach is the development of improved cultivars with greater durability and reduced dependence on chemical inputs. Unlike strategies targeting specific pests or pathogens, breeding for broad-spectrum resistance offers a more environmentally friendly and sustainable solution. As highlighted by Lozada et al. (2022), developing chili pepper varieties with resistance to a wide array of pests and diseases represents the most eco-efficient and cost-effective strategy for long-term pest and disease management. Future breeding programs targeting multi-resistant chili pepper varieties should prioritize the development of broad-spectrum resistance, achievable through the integration of physical, biochemical, and genetic defense mechanisms. Leveraging these rare genetic resources enables the creation of cultivars with enhanced resistance to a wide range of pests and diseases. Notable resistant genotypes include 'Criollo de Moralos-334', 'PI 201232', and 'PI 201234' from Mexico; 'AC2258'

from Central America; and 'Perennial' from India. These genotypes exhibit resistance to bacterial wilt, anthracnose, viral pathogens, and insect pests (Corozo-Quiñónez et al., 2024).

Developing cultivars or hybrids resistant to various pests and diseases has been a major focus of research worldwide in recent decades. However, identifying chili pepper accessions (such as landraces, wild relatives, pre-breeding materials, and closely related species) with biotic stress resistance remains challenging despite these efforts. The urgent need to create new resistant cultivars suited to different pedoclimatic conditions is further emphasized by the effects of climate change and the risk of resistance breakdown, both of which threaten the durability of disease resistance (Parisi et al., 2020).

Despite extensive research into pest and disease management in chili peppers, a critical gap persists in the identification and development of accessions with broad-spectrum, durable resistance to multiple biotic stressors, particularly under changing climatic conditions. Moreover, the limited exploration of genetic diversity, coupled with an overreliance on chemical control and insufficient yield-performance correlation studies, underscores the need for integrated resistance screening, characterization, and evaluation of accessions to support sustainable chili pepper production.

Therefore, the primary objective of this study was to identify chili pepper accessions with resistance to a broad range of biotic stress factors. The potential benefits of these resistant accessions will be assessed by evaluating their impact on yield improvement and the long-term sustainability of chili pepper production. Ultimately, the development of broad-spectrum resistant varieties is expected to enhance both the resilience and sustainability of the chili pepper industry.

Materials and Methods

This research was conducted in an experimental site of the Indonesian Vegetables Research Institute, Lembang, West Java, Indonesia (107° 30' EL, 60° 30' SA; 1,250 m asl), from July 2022 to February 2023. All climatological factors in the experiment were considered. The temperatures ranged from 24-26 °C, humidity levels ranged from 84-88%, and the average annual rainfall was 724 mm. The pH of the soil at the location of the experiment was 5.0, classifying it as Andosol.

Five chili pepper accessions (5x1, 5x2, 2x1, 2x5, 4x5, and 5x5) and four improved varieties from Indonesia ('Carla Agrihorti', 'Hot Beauty', 'Pilar', and 'Inata Agrihorti') were used as materials in this study. After spending one month in the nursery, the nine accessions and varieties were transplanted to the field. A randomized complete block design (RCBD) was employed for the field experiment, consisting of

nine treatments and four replications. Each accession and variety was transplanted into a 10 × 1 m plot with a spacing of 70 cm × 50 cm, and forty chili plants were planted per plot.

To record insect pests, fruit number, and yield, ten plants were randomly selected from each U-shaped plot. The severity of pest and disease infestation was recorded weekly starting three weeks after transplanting, while the fresh weight of chili pepper fruits was measured after harvest. The average pest population was determined by counting sucking pests—aphids, mites, whiteflies, and thrips—on three leaves per plant using a 10× magnifying hand lens. Between 30 and 93 days after transplanting (DAT), at 7-day intervals, ten plants were randomly selected and assessed individually for curling symptoms using a 0–4 scale (Niles, 1980) (Table 1).

Leaf curl index (LCI) was determined using the following formula:

$$LCI = \frac{\text{The sum of the scores for all plant}}{\text{total number of all plants} \times \text{number of score category}} \times 100\% \quad (1)$$

The intensity of fruit bored due to damage was calculated using the formula:

$$\text{Fruit damage intensity (\%)} = \frac{\text{Number of infected fruits per plot}}{\text{Total number of fruits per plot}} \times 100\% \quad (2)$$

Table 1. Standard procedure for scoring leaf curl index (LCI).

LCI/Grade (0-4)	Category	Symptoms
0	Immune (I)	No symptoms (No curling, completely healthy plant)
1	Resistant (R)	1-25 percent of leaves/plants show curling, less damage
2	Moderately Resistant (MR)	26-50 percent of leaves/plants show curling, moderately damaged
3	Susceptible (S)	51-75 percent of leaves/plants show curling, heavily damaged, malformation of growing points, and reduction in plant height
4	Highly Susceptible (HS)	>76 percent of leaves/plants show curling, severe and complete destruction of growing points, and a drastic reduction in plant height, defoliation, and severe malformation

Based on the resistance classification system established by Kumar and Omkar (2021), chili pepper accessions were categorized according to the percentage of fruit infestation observed under field conditions. Accessions were considered immune if no fruit infestation occurred, moderately resistant if infestation ranged from 1–20%, tolerant if infestation was between 21–30%, susceptible if infestation reached 31–40%, and highly susceptible if infestation exceeded 40%.

Beginning at the first fruit harvest, data on fruit morphological parameters were recorded. To assess their function in providing resistance to the fruit borer (*Bactrocera dorsalis*), phenotypic parameters such as fruit weight (g), fruit pedicel (cm), length (cm), diameter (cm), fruit locus (cm), and pericarp fruit thickness (cm) were recorded from fruits of each accession that were harvested independently.

The intensity of anthracnose attack on chili fruit was measured using the following formula:

$$\text{Intensity} = \frac{\sum \text{infected fruits}}{\sum \text{observed fruits}} \times 100\% \quad (3)$$

For each harvest, the number of fruits per plant and the total yield per plot were recorded. The average values were then used to estimate yield per ha.

A one-way analysis of variance (ANOVA) was conducted to evaluate differences across all parameters among the seven treatments. The means were separated using a post hoc test known as Tukey's honestly significant difference (HSD), which allowed for a 5% level of significance comparison of the variances among the seven treatments.

Observation of symptoms of pepper yellow leaf curl virus (PepYLCV) in the field

Observations on the incidence and intensity of PepYLCV symptoms in all plant populations per treatment were made once a week, starting from 30 d after transplanting (DAT).

The observed variable for virus incidence was calculated using the following formula:

$$Incidence (\%) = \left(\frac{a}{A}\right) \times 100 \quad (4)$$

where: a = number of symptomatic plants, A = number of plants observed.

The determination of the severity attack of viral symptoms was done following the formula of Willocquet et al. (2008) as follows:

$$S = \frac{\sum(n \times v)}{N \times V} \times 100\% \quad (5)$$

where: S = severity of symptoms; n = number of plants that fall into a given symptom scale; v = scoring value of a particular symptom; N = number

of plants observed; V = highest symptom severity scoring value.

The symptom severity score is classified as follows (Fig. 1): 0 = plants showing no symptoms of the virus (healthy) (0 %); 1 = plants show mild mosaic symptoms (1 % - 25 %); 2 = the plant shows mosaic symptoms, yellow grooves are visible (> 25% - 50%); 3 = the plant shows mosaic symptoms; yellow grooves are visible and there is a change in the shape of the growth (> 50% - 75%); 4 = plants show symptoms of heavy mosaic, yellow grooves are visible (contrast), changes in growth shape occur, and dwarf plants (> 75% - 100%).

The infection rate (r) was calculated using the formula:

$$r = \frac{e}{t} \left(\frac{1}{1 - X_{i+1}} - \log \frac{1}{1 - X_i} \right) \quad (6)$$



Fig. 1. Illustrative scale used for evaluating symptom severity of Pepper Yellow Leaf Curl Virus (*PepYLCV*) in chili plants.

where e is the natural logarithm base, t is the time interval between observations, and X is disease severity.

The total area under the disease progression curve (AUDPC) was determined with the formula of Simko and Piepho (2021):

$$AUDPC = \sum_i^{n-1} \left| \frac{Y_i + Y_{i+1}}{2} \right| (t_{i+1} - t_i) \quad (7)$$

where: AUDPC = area under the disease progression curve; Y_{i+1} = observational data to $i+1$; Y_i = first observation data; t_{i+1} = time of observation to $i+1$; t_i = first observation time.

To compute the percentage of inhibition of the spread (P) of mosaic virus severity as a result of the treatment, the following formula was used:

$$P = 1 - \left| \frac{AUDPC_{Treatment}}{AUDPC_{Control}} \right| \times 100\% \quad (8)$$

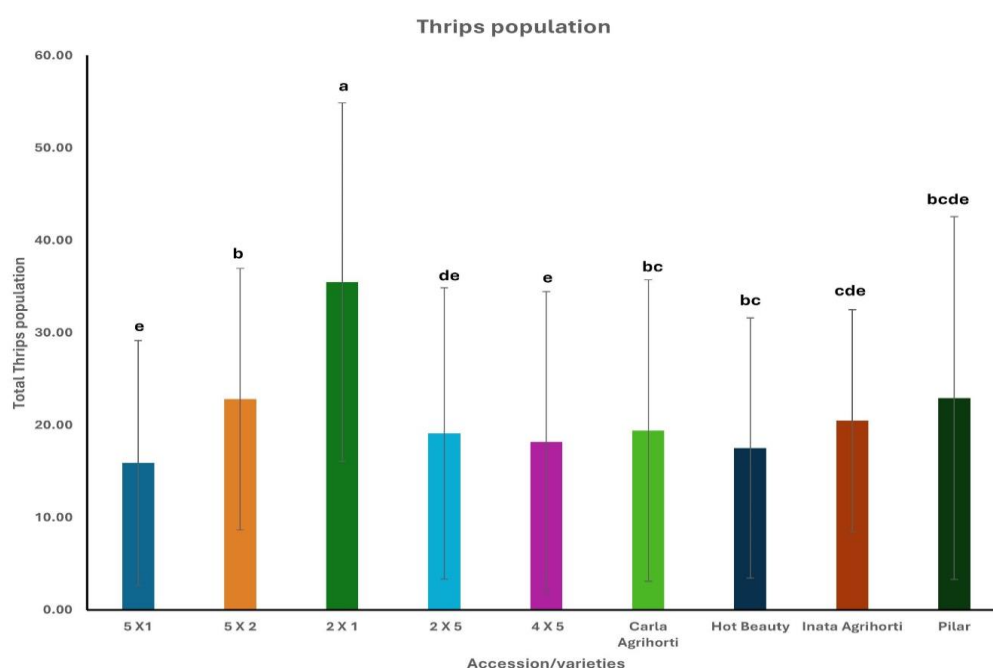
Results

Reaction of chilli pepper accessions to insect pest incidence

The results of the experiment indicated significant variations in thrips populations among different accessions and varieties, as recorded at 7 d intervals (Table 2). The total thrips population ranged from 4.48 thrips per leaf to 12.42 thrips per leaf (Fig. 2). Among the various accessions and varieties, smaller thrips populations were observed in accession 5x1 (4.48 thrips/leaf), accession 4x5 (4.60 thrips per leaf), and accession 2x5 (5.06 thrips per leaf) with no significant difference compared to 'Inata Agrihorti' (5.48 thrips per leaf) and 'Pilar' (6.98 thrips per leaf). Conversely, a higher population was recorded in 4x5 (4.60 thrips per leaf), 'Hot Beauty' (8.24 thrips per leaf), and 2x1 (12.42 thrips per leaf).

Table 2. Classified degree of resistance to the pepper yellow leaf curl virus (*PepYLCV*).

Disease Attack intensity (%)	Durability rating
0	Immun (I)
$x \leq 10$	Resistance (R)
$10 < x \leq 20$	Moderately Resistant (MR)
$20 < x \leq 30$	Moderately Susceptible (MS)
$30 < x \leq 40$	Susceptible (S)
$x \geq 50$	Highly Susceptible (HS)

**Fig. 2.** Total numbers of thrips *T. parvis pinus* populations on different chili pepper accessions/varieties.

Many studies have explored sources of thrips resistance within the *Capsicum* genus, highlighting the importance of identifying resistant varieties to enhance sustainable pest management strategies. The thrips preference, feeding rate, mortality, and reproduction of resistant accessions were all negatively affected.

The percentage of damaged plants varied significantly among the tested accessions. According to Niles' (1980) criteria, eight accessions/varieties were classified as resistant (score 1), while one was categorized as highly resistant (score 2). The resistant accessions/varieties were 5x1, 5x2, 2x5, 4x5, 'Carla Agrihorti', 'Hot Beauty', 'Inata Agrihorti', and 'Pilar'. Notably, accession 2x1 was classified as highly resistant (Table 3).

All treatments showed resistance against fruit borer, *H. armigera*, to varying degrees, as shown in Table

4. There were no discernible changes in fruit damage severity across treatments throughout the investigation. The amount of fruit damage varied between 0.34 and 1.18% depending on the experimental settings. Damage to chili fruits by the oriental fruit fly varied from 22.35 to 49.06%, with accession 4x5 suffering the most (49.06%).

Fruit diameter across all accessions ranged from 1.34 to 2.04 cm, while fruit weight varied from 8.57 to 18.00 g (Table 5). The largest fruit diameter (2.04 cm) and weight (18.00 g) were observed in accession 4x5, whereas the smallest values were recorded in the 'Hot Beauty' variety. A strong positive correlation was observed between infection rates and several morphological traits, including pedicel length ($r = 0.96$), fruit diameter ($r = 0.93$), and pericarp thickness ($r = 0.96$) (Fig. 3).

Table 3. Percentage of leaf curling index on thrips damage.

Accessions	Leaf curling Index (%) at 7 d intervals										Mean	Score	Resistant Category*
	-----DAT-----												
	30	37	44	51	58	65	72	79	86	93			
5x1	0	0.28	1.1	1.39	20	22.5	20.83	24.72	33.61	34.72	15.915	1	R
5x2	5.56	7.78	7.8	7.78	20.28	25.56	33.89	39.16	43.61	36.67	22.809	1	R
2x1	5.56	5.56	20	21.94	37.78	49.16	51.38	50.27	55.28	57.78	35.471	2	MR
2x5	0.28	1.11	0.83	3.88	13.51	28.89	35.83	31.39	38.05	37.2	19.097	1	R
4x5	0.28	0.83	0.56	4.44	10.56	25	23.38	32.22	37.17	47.2	18.164	1	R
Carla Agrihorti	0.28	0.83	0.83	3.89	11.94	30.83	35.83	32.78	39.72	37.22	19.415	1	R
Hot Beauty	0	1.11	3.3	4.72	10.56	26.16	29.67	30.17	31.67	37.78	17.514	1	R
Inata Agrihorti	5.56	5.56	7.78	15.56	15.56	20	25.83	33.61	36.94	38.33	20.473	1	R
Pilar	0.28	0.83	1.67	4.72	12.78	32.5	36.94	43.89	46.77	48.88	22.926	1	R

DAT - Days After transplanting; * = Resistance category; R = Resistant; MR = Moderately resistant; S = Susceptible; HS = Highly Susceptible.

Table 4. Infestation of fruit damage due to fruit fly, fruit borer, and anthracnose on different chili accessions/ varieties.

Accessions/variety	Mean infestation of fruit damage due to pest/disease		
	-----percent-----		
	<i>Fruit fly</i>	<i>Fruit borer</i>	<i>Anthracnose</i>
5x1	36.92 ^{bc*}	0.91 ^a	4.43 ^{ab}
5x2	46.42 ^{ab}	1.12 ^a	15.45 ^a
2x1	41.05 ^{abc}	0.45 ^a	5.60 ^{ab}
2x5	33.31 ^{cd}	0.89 ^a	6.27 ^{ab}
4x5	49.06 ^a	1.08 ^a	5.52 ^{ab}
Carla Agrihorti	39.53 ^{abc}	0.40 ^a	1.63 ^b
Hot Beauty	22.35 ^d	0.34 ^a	12.33 ^{ab}
Inata Agrihorti	32.88 ^{cd}	1.18 ^a	2.33 ^b
Pilar	34.56 ^c	0.54 ^a	9.02 ^{ab}
CV	11.05	1.50	12.7

* = means followed by the same letter are not significant ($P < 0.05$). CV=coefficient variation.

Table 5. Morphological characters of chili pepper fruit accession/varieties.

Accessions /variety	Fruit weight (g)	Fruit pedicel length (mm)	Fruit length (cm)	Fruit diameter (cm)	Fruit locus	Pericarp fruit thickness(mm)
5x1	14.16 ^{ab*}	3.95 ^{ab}	12.20 ^a	16.82 ^{bc}	2.23 ^a	1.41 ^a
5x2	16.82 ^a	4.50 ^{ab}	13.43 ^a	18.12 ^{ab}	2.34 ^a	1.73 ^a
2x1	16.14 ^{ab}	4.41 ^{ab}	13.23 ^a	17.42 ^b	2.20 ^a	1.53 ^a
2x5	11.43 ^{bc}	3.45 ^{ab}	12.02 ^a	15.37 ^{cd}	2.11 ^a	1.38 ^a
4x5	18.00 ^a	4.75 ^a	11.89 ^a	20.35 ^a	2.16 ^a	1.76 ^a
Carla Agrihorti	15.73 ^{ab}	3.98 ^{ab}	11.98 ^a	17.42 ^b	3.11 ^a	1.50 ^a
Hot Beauty	8.57 ^c	3.07 ^b	12.00 ^a	13.44 ^d	2.36 ^a	1.13 ^a
Inata Agrihorti	11.34 ^{bc}	3.14 ^{ab}	13.39 ^a	15.07 ^c	2.20 ^a	1.16 ^a
Pilar	11.50 ^{bc}	3.82 ^{ab}	12.93 ^a	15.40 ^c	2.27 ^a	1.40 ^a
CV	5.22	1.65	2.83	2.62	1.30	5.56

* = means followed by the same letter are not significant ($P < 0.05$). CV = coefficient variation.

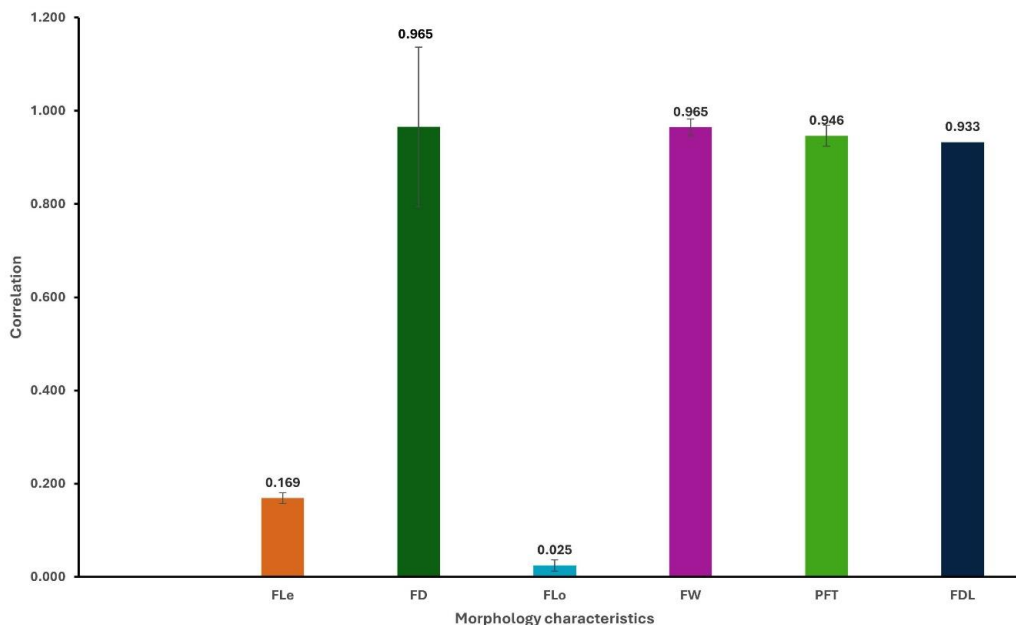


Fig. 3. Correlation of morphological characteristics of chili fruit and infestations of oriental fruit fly: FLe—fruit length, FD—fruit diameter, FLo—fruit locus, FW—fruit weight, PFT—pericarp fruit thickness, FDL—fruit pedicel length.

Reaction of chili pepper accessions against pathogen incidence

Research has shown varying responses among chili pepper accessions and varieties to this disease. Table 4 indicates that ‘Inata Agrihorti’ exhibited resistance to anthracnose with 2.23% fruit damage, while seven accessions/varieties (5x1, 2x1, 2x5, 4x5, ‘Carla Agrihorti’, ‘Hot Beauty’, and ‘Pilar’) demonstrated tolerance. Accession 5x2 was classified as susceptible.

Incidence and severity of PepYLCV symptoms

The progression of *PepYLCV* infection, measured in terms of incidence and symptom severity over an eight-week observation period, was illustrated in Tables 6 and 7. Disease onset began at 44 d after transplanting (DAT) across all accessions. The severity of infection increased over time, with notable differences among chili accessions. The most susceptible accessions were 5x2 (20.2%), 5x1 (17.93%), and ‘Carla Agrihorti’ (17.12%), which showed rapid disease progression and higher final severity at 79 DAT. Moderately susceptible were ‘Hot Beauty’ (14.61%), ‘Inata Agrihorti’ (12.32%), and ‘Pilar’ (12.01%), which showed gradual symptom development and moderate disease impact. Most tolerant accessions, i.e., 2x1 (8.61%), and 4x5 (9.20%), exhibited minimal disease symptoms throughout the observation period. In addition, infection rates ranged between 0.002 and 0.006, which suggested that the infection has a low prevalence across all varieties. This indicated slow disease spread or effective resistance. Both incidence

and severity increased parallel to plant age, showing fluctuating patterns throughout the observation period. Accessions 5x1, 5x2, and 2x5 exhibited the shortest incubation periods, while 4x5, ‘Hot Beauty’, and ‘Inata Agrihorti’ demonstrated longer incubation periods.

AUDPC and viral disease resistance

Table 8 presents the severity of symptoms associated with *PepYLCV*, as measured by the Area Under the Disease Progress Curve (AUDPC) over the observation period from 30 to 79 days after planting. The results showed that the resistant accessions 4x5 and 2x1 performed significantly better than the control. Moderately resistant varieties included ‘Carla Agrihorti’, ‘Hot Beauty’, ‘Pilar’, and ‘Inata Agrihorti’, while the susceptible accessions 5x1, 5x2, and 2x5 performed significantly worse than the control. These findings suggest that accessions 4x5 and 2x1 are the most promising candidates for future research and development focused on disease resistance in chili pepper breeding programs.

Yield of chili pepper

Figure 4 illustrates statistically significant differences in fruit yield among the cultivars ($P < 0.05$). ‘Inata Agrihorti’, ‘Carla Agrihorti’, and ‘Hot Beauty’ produced higher fruit yields overall. Although the total yields of accessions 2x5 and 5x1 were relatively low, they were notable for their high commercial-grade fruit production. In contrast, accessions 4x5, 2x1, and 5x2 exhibited lower yields compared to the other cultivars.

Table 6. Incidence of pepper yellow leaf curl virus (*PepYLCV*) on chili accessions/varieties at weekly intervals of observation.

Accession/ Variety	Incidence (%)								Infection rate
	-----Observation (DAT)-----								
	30	37	44	51	58	65	72	79	
5x1	0	0	1.04	1.85	3.48	7.01	17.7	24.80	0.007
5x2	0	0	0.93	1.39	3.48	11.57	15.69	26.77	0.008
2x1	0	0	0	1.62	3.48	8.33	7.43	13.00	0.004
2x5	0	0	2.09	3.47	1.39	15.18	17.28	25.99	0.007
4x5	0	0	0	0	1.39	6.25	7.29	15.26	0.004
Carla Agrihorti	0	0	0	1.62	2.78	7.19	15.53	21.52	0.006
Hot Beauty	0	0	0	0	2.78	4.92	7.77	19.40	0.006
Inata Agrihorti	0	0	0	0	2.78	10.54	9.52	16.65	0.005
Pilar	0	0	0	0.7	2.78	7.13	9.36	15.81	0.004

DAT = days after transplanting.

Table 7. Severity of pepper yellow leaf curl virus (*PepYLCV*) on chili accession/varieties at weekly intervals of observation.

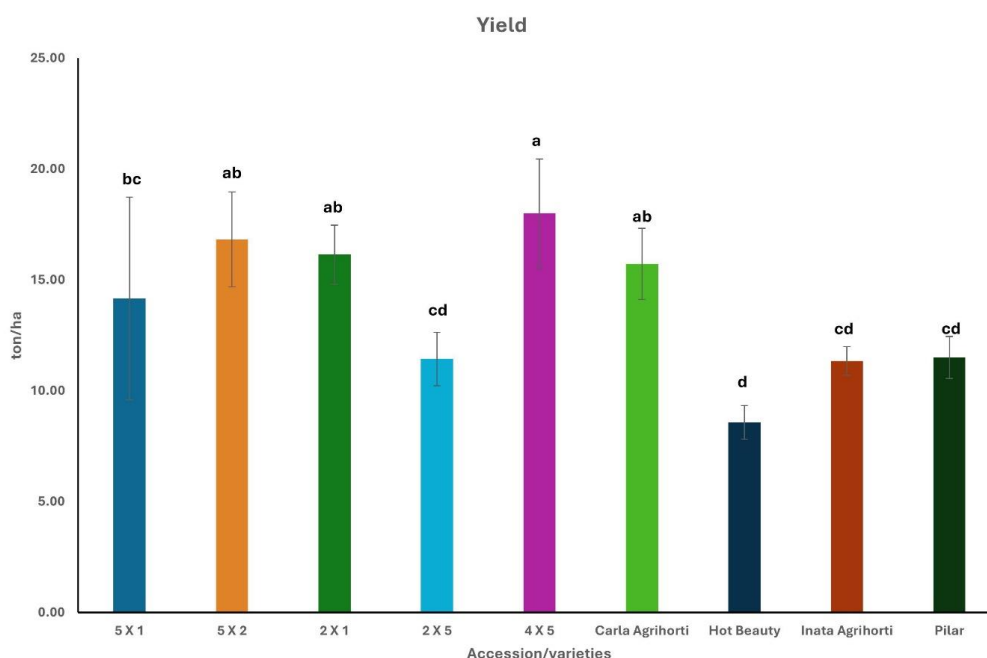
Accession/ Variety	Severity (%)								Infection rate
	-----observation (DAT)-----								
	30	37	44	51	58	65	72	79	
5x1	0	0	0.35	1.6	2.08	4.78	13.55	17.93	0.005
5x2	0	0	0.7	1.04	3.74	4.78	11.48	20.2	0.006
2x1	0	0	0	0.52	2.6	6.95	5.38	8.61	0.002
2x5	0	0	1.39	2.08	1.04	8.34	12.16	11.10	0.003
4x5	0	0	0	0	1.04	9.20	4.73	9.20	0.002
Carla Agrihorti	0	0	0	0.52	2.81	5.5	11.25	17.12	0.005
Hot Beauty	0	0	0	0	2.08	3.69	8.29	14.61	0.004
Inata Agrihorti	0	0	0	0	3.65	5.14	7.19	12.32	0.003
Pilar	0	0	0	0.35	2.6	4.99	6.06	12.01	0.003

DAT = days after transplanting.

Table 8. Effect of accession treatment on AUDPC value at observation 30 to 79 d after planting.

Accessions/varieties	AUDPC	Different from the control means
5x1	219.29	- 51
5x2	222.89	- 54
2x1	138.30*	4
2x5	213.93	- 48
4x5	137.0*	6
Carla Agrihorti	141.87	0
Hot Beauty	143.01	0
Inata Agrihorti	155.0	0
Pilar	140.12	0
Average	167.93	- 143

Remarks: * = best treatment. The average value of 4 control commercial chili varieties, Carla Agrihorti, Hot Beauty, Inata Agrihorti, and Pilar, is 145.

**Fig. 4.** Effect of accessions/varieties on chili pepper yield.

Discussion

Thrips were observed in the field a few weeks after transplanting, with higher incidence recorded during the dry season. These pests primarily attack the upper parts of young leaves, buds, and flowers, causing upward leaf curling and, in severe cases, complete defoliation and significant crop loss. Effective management of insect pests requires the identification of host plant resistance; however, information on resistant sources against thrips remains limited, necessitating further research. To enhance chili pepper resistance to thrips, breeding programs should prioritize accessions 4x5 and 2x5.

As shown in Table 3 and Figure 2, there was a positive correlation between thrips population density and the proportion of plants damaged. Previous research has demonstrated that foliar morphological and chemical traits, such as glandular trichomes and secondary metabolites, play a significant role in host plant resistance to thrips. Resistant accessions are often characterized by lower chlorophyll content, higher trichome density, and the presence of specific secondary metabolites that deter feeding. Studies have also emphasized the roles of epicuticular papillae and acyl sugars in reducing thrips infestation.

Extensive investigations into *Capsicum* species have identified accessions with traits that reduce thrips preference, feeding activity, survival, and reproductive capacity. Notably, several thrips species exhibit highly specific resistance mechanisms during their larval stages. Plants that produce certain secondary metabolites, such as methyl ketones and sesquiterpene carboxylic acid derivatives, have been shown to adversely affect thrips populations (Pavani et al., 2024).

Both the physical and chemical properties of leaves may influence a host plant's resistance to insects, as reported by Kumar and Omkar (2021). Plants with thin leaves, low chlorophyll levels, or other traits may be more attractive to highly susceptible thrips populations. Glandular trichomes are the main defense mechanism against thrips in host plants, as stated by Bac-Molenaar et al. (2019). These trichomes can limit the ability of pests to adhere to or move across plant surfaces. In tomato, trichome density, chlorophyll content, and phenol concentration were all inversely correlated with thrips population. According to Broughton et al. (2015), a greater number of thrips were captured when type VI glandular trichomes were induced in response to methyl jasmonate treatment. Shorter mesophyll and epidermal cells, along with higher epicuticular papillae density, were also observed in genotypes showing reduced thrips damage (Wahyuni et al., 2021). Thrips incidence and oviposition were both reduced by various combinations of acyl sugars with differing fatty acid profiles (Mouden and Leiss, 2021). Any leaf characteristic that disrupts the pest's life cycle may serve as a potential defense mechanism against thrips (Visschers et al., 2019; Sadafale et al., 2025; Usman et al., 2014; Chaudhary and Pandya, 2019).

Without proper management, the oriental fruit fly can cause up to 100% production losses during the dry season (Vargas et al., 2015). Compounds such as β -ocimene may have contributed to the greater fruit fly oviposition observed in certain accessions. Physical barriers against pests are provided by morphological characteristics of chili pepper fruits, including fruit length, diameter, weight, pericarp thickness, and pedicel length. Consistent with this study, Syamsudin et al. (2022) found that larger fruits were more resistant to fruit fly infestation. These traits may deter pests due to their associated nutritional and chemical properties, including water content, carbohydrates, fiber, and volatile compounds.

Anthrachnose, caused by *Colletotrichum* spp., significantly impacts chili pepper production worldwide, leading to substantial yield losses. In recent years, anthrachnose incidence has increased in Indonesia, primarily due to *C. acutatum* (Hasyim et al., 2014). Research has revealed considerable variation in the responses of chili pepper accessions

and varieties to this disease, suggesting distinct resistance mechanisms inherent to each genotype (Budi et al., 2023). Studies aimed at introgressing anthrachnose resistance into chili peppers have identified promising lines exhibiting moderate resistance coupled with high yield potential (Hasyim et al., 2014). Moreover, Ridzuan et al. (2018) and Usman et al. (2014) reported a positive association between capsaicin concentration and anthrachnose resistance.

Genetic resistance to *Colletotrichum* spp. is influenced by several factors, including pathogen species, resistance sources, inoculation methods, and fruit developmental stages (Sun et al., 2015; Suwor et al., 2017). The complexity of resistance is demonstrated by the differential reactions between ripe and unripe fruits (Sun et al., 2015; Hasyim et al., 2014). *C. gloeosporioides* tends to infect ripe fruits, whereas *C. acutatum* is more prevalent in unripe fruits. Both stages of resistance are governed by quantitative trait loci (QTLs), and as fruits mature, antioxidant levels increase, thereby enhancing the plant's defense system (Baba et al., 2019; Sun et al., 2015). Despite extensive research, the involvement of multiple *Colletotrichum* species complicates the development and commercialization of anthrachnose-resistant chili cultivars (Saxena et al., 2016). Therefore, further studies are essential to fully elucidate the underlying resistance mechanisms.

Young, sunken, wrinkled, and light-yellow mosaic-colored leaves or shoots were the first symptoms of PepYLCV in pepper plants. As the infection advanced, the majority of the young leaves became a vibrant yellow color, while a small number displayed a combination of yellow and green shades. Infected leaves shrank, thickened, became concave, and developed wrinkles. Severe infections caused growth retardation and decreased flower output, which in turn caused farmers to lose a significant amount of money and produce less. These symptoms were consistent with what had been found in other research (Sayekti, 2023; Selangga and Listihani, 2021; Selangga et al., 2023). The 2x1, 4x5, and Pilar accessions were the most promising with respect to disease resistance, showing slow progression and low overall severity. In contrast, 5x2 and 5x1 were highly susceptible, with early symptom development and rapid disease progression. The disease rate values corroborated these observations, offering a quantitative summary of disease dynamics. Infected plants exhibited phenotypic symptoms due to virus–host–environment interactions (Bennett and Agbandje-McKenna, 2017). Symptom severity indicated viral infection intensity, with viral DNA-induced chloroplast damage inhibiting photosynthesis, as evidenced by yellow mosaic patterns and leaf vein clearing. This disruption of photosynthesis hindered plant growth and reduced yields. These findings aligned with previous research

(Bhattacharyya et al., 2015; Anikina and Seitzhanova, 2015; Anikina et al., 2023), suggesting suboptimal defense gene function in chili peppers. The severity of a disease was greatly affected by its incidence (Ayu et al., 2021). According to Katherine et al. (2022) and García et al. (2024), the rates of resistance and symptom manifestation were affected by variations in incubation times, which in turn were determined by genetic composition. Varieties with strong resistance genes tended to exhibit longer incubation periods and lower disease intensity compared to susceptible ones. In resistant varieties, virus replication and spread were limited, resulting in an extended incubation period and reduced disease intensity (Sheat and Winter, 2023; García et al., 2024).

However, viruses may mutate to overcome plant defenses, which requires time and energy. Not all resistant varieties show long incubation periods and low disease intensity; some may have short incubation periods with low intensity or vice versa. The incubation period is the time needed for the virus to replicate and induce symptoms, and plant defense mechanisms can extend this period by inhibiting viral replication. Enzymes such as peroxidases and proteases, along with Pathogenesis-Related (PR) proteins, play crucial roles in combating viruses and inhibiting their replication. PR proteins also attract immune cells and induce the death of infected cells (Musidlak et al., 2017; Dos Santos and Franco, 2023). Disease progression refers to symptom severity and virus-induced damage, which can be mitigated by plant defense mechanisms (Min Huang et al., 2023). As shown in Table 8, the progression of *PepYLCV* symptoms for each treatment was analyzed using the Area Under the Disease Progress Curve (AUDPC) from 37 to 79 days after transplanting (DAT). Although the responses varied among treatments, a general trend of increasing disease severity with plant age was observed. Higher AUDPC values were associated with lower disease inhibition percentages in the control group. Notably, accessions 4x5 and 2x1 recorded lower AUDPC values and higher disease inhibition rates compared to the control, suggesting effective suppression of *PepYLCV* progression. Among the five treatments evaluated, these two accessions demonstrated significantly greater efficacy, with accession 4x5 showing an AUDPC of 137 and 4% inhibition, and accession 2x1 an AUDPC of 138.30 and 6% inhibition. These findings indicate that both accessions were capable of reducing *PepYLCV* incidence and maintaining disease severity at relatively low levels.

Improving fruit quality, increasing tolerance to abiotic stresses, building resistance to pests and diseases, and raising production are the main goals of chili pepper genetic breeding (Manzur et al., 2014). Marketable yield was primarily attributed to

resistance to pests and diseases, including thrips, fruit borers, anthracnose, and geminivirus. 'Carla Agrihorti', 'Inata Agrihorti', and 'Hot Beauty' exhibited significantly higher marketable yields, while accessions 2x5 and 5x1 also showed high marketable yields compared to the other accessions. Based on the overall data mentioned above, the study highlights accessions 4x5 and 2x5 as promising candidates that exhibit both reduced thrips infestation and lower *PepYLCV* severity, as supported by field data on pest population, AUDPC values, and disease inhibition. The integration of pest and virus resistance in a single genotype is rarely reported and offers significant value for breeding programs. The correlation of morphological traits with thrips resistance provides detailed evidence linking leaf morphological features to thrips resistance in chili peppers, advancing the current understanding of host plant resistance mechanisms. The study applies disease progression analysis (AUDPC) to distinguish between accessions based on their incubation period and symptom intensity, providing insight into quantitative resistance against *PepYLCV* concerning host genotype. Unlike most studies that examine pest or disease resistance in isolation, this research links biotic stress resistance directly with yield performance, identifying high-yielding accessions (e.g., 2x5, 5x1) that maintain productivity under pest and pathogen pressure, demonstrating their suitability for sustainable production. By combining phenotypic and field performance data, this work supports the breeding of resilient cultivars with stacked resistance traits for tropical environments, where simultaneous biotic stressors are common.

Conclusions

The following accessions and cultivars were shown to be resistant to thrips: 'Carla Agrihorti', 'Hot Beauty', 'Inata Agrihorti', 'Pilar', 2x5, 4x5, 5x2, and 5x1. Feeding damage was lower in 2x1, which was fairly resistant. Resistance to oriental fruit flies was observed in accession 2x5. This resistance was influenced by anatomical factors such as high fruit weight, pedicel length, fruit diameter, fruit locus number, and pericarp thickness. Anthracnose was not present in accessions 5x1, 2x1, and 4x5. Accession 4x5 had a longer incubation time, mild disease symptoms, and was resistant to *PepYLCV*. 'Carla Agrihorti', 'Inata Agrihorti', and 'Hot Beauty' produced more fruits, followed by 2x5 and 5x1, which produced moderate yields. Chili pepper cultivars with long-lasting, broad-spectrum resistance and high productivity may be developed with the help of accessions 2x5, 5x1, and 4x5.

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Author contributions

Conceptualization, NG, WS and RK; methodology, NG, WS, WK, EK, TKM, IS, BKU, AH, RM; software, WS, TKM, YS; resources, IRS, RK, EK, TKM, ED, IS, BKU, AH, RM, and AKK; investigation, IRS, RK, and AKK; validation, IRS, RK, EK, TKM, ED, IS, BKU, AH, RM, AKK, YS, W; writing—original draft preparation, NG, WS, YS, W; all authors are involved in writing, review and editing; supervision, NG, WS; project administration, NG and IRS; funding acquisition, RK and NG. All authors have read and agreed to the published version of the manuscript.

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Conflict of Interest

The authors indicate no conflict of interest in this work.

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