horticulturae

MDPI

Article

Evaluation of Squash (*Cucurbita pepo* L.) Genotypes for Resistance to Cucurbit Chlorotic Yellows Virus

Saritha Raman Kavalappara [1,*], Sudeep Bag [1,*], Alexander Luckew [2], Cecilia E. McGregor [2], Albert K. Culbreath [1] and Alvin M. Simmons [3]

[1] Department of Plant Pathology, University of Georgia, Tifton, GA 31793, USA
[2] Department of Horticulture, University of Georgia, Athens, GA 30602, USA
[3] U.S. Vegetable Research, USDA-ARS, 2700 Savannah Highway, Charleston, SC 29414, USA
* Correspondence: sarirk@uga.edu (S.R.K.); sudeepbag@uga.edu (S.B.)

Abstract: Cucurbit chlorotic yellows virus (CCYV), a Crinivirus transmitted by whiteflies, poses a significant threat to cucurbit crops globally. Summer squash (*Cucurbita pepo* L.), an important vegetable crop in the Southeastern United States, is particularly affected. The absence of commercially available resistant summer squash cultivars necessitates the exploration of resistant sources. Cucurbita germplasms with potential resistance to CCYV were previously identified through field screening. In this study, we describe the controlled greenhouse screening of these germplasm lines aimed at validating resistance to CCYV infection. The susceptible cultivar Gentry used as control exhibited early and severe symptoms in response to CCYV infection. In contrast, all the PI accessions tested, including PI 512749, PI 615141, PI 136448, PI 442312, PI 458731, and PI 420328, displayed delayed and less severe symptoms. Nevertheless, CCYV RNA accumulated in all the PI accessions. Lower symptom severity while harboring a considerable amount of CCYV indicates their inherent tolerance to the yellowing disease induced by CCYV. When comparing CCYV RNA accumulation in PI accessions with the commercial cultivar 'Gentry', lower virus titers were observed across all tested accessions. Specifically, PI 420328 and PI 458731 exhibited significantly reduced CCYV titers compared to the susceptible cultivar in both mass exposure and clip cage experiments. These accessions, displaying reduced symptoms and lower virus titers, hold promise as sources of resistance to CCYV in breeding programs. This study also highlights the importance of utilizing a reliable method to assay the resistance or tolerance of selected germplasm to infection by CCYV.

Keywords: cucurbit chlorotic yellows virus; host resistance; *Cucurbita pepo* L.

Citation: Kavalappara, S.R.; Bag, S.; Luckew, A.; McGregor, C.E.; Culbreath, A.K.; Simmons, A.M. Evaluation of Squash (*Cucurbita pepo* L.) Genotypes for Resistance to Cucurbit Chlorotic Yellows Virus. *Horticulturae* **2024**, *10*, 264. https://doi.org/10.3390/horticulturae10030264

Academic Editors: Rafael José Carvalho Mendes, Leandro Pereira Dias, Renato Lopes Gil and Fernando Tavares

Received: 16 February 2024
Revised: 7 March 2024
Accepted: 8 March 2024
Published: 10 March 2024

1. Introduction

Cucurbit chlorotic yellows virus (CCYV), classified under the genus *Crinivirus* in the family *Closteroviridae*, is part of an emerging complex of whitefly-transmitted viruses associated with cucurbit yellows disease [1,2]. CCYV is transmitted in a semi-persistent manner by *Bemisia tabaci* MEAM1 and MED [2]. Originally identified in Japan in 2004 [3], the virus is now present not only in Asia [3–5], but also in Africa [6], the Mediterranean regions of Europe [7,8], and North America [9]. It was first identified in the USA in the Imperial Valley of California [9] and subsequently spread to other production areas where the whitefly vector is present [10]. CCYV infects important cucurbits, including squash (*Cucurbita pepo* L.), melon (*Cucumis melo* L.), watermelon (*Citrullus lanatus* (Thunb.) Matsum. and Nakai), and cucumber (*Cucumis sativus* L.) [2]. In addition, the experimental host range extends to species within the families Asteraceae, Chenopodiaceae, Convolvulaceae, Solanaceae, and Fabaceae, including weeds and alfalfa (*Medicago sativa*) [2]. CCYV infection induces chlorosis on leaves, which is more prominent on the lower leaves of the plant [2]. Early infection of cucumber, melon, and watermelon plants by CCYV is associated with reduced yields. Infection may also adversely affect the quality of melons as evidenced

by a reduction in Brix, a measure of sugar content, in the fruits of infected melon and watermelon plants [11].

Cucurbita pepo is of significant economic importance, particularly as a summer squash. In the state of Georgia, summer squash is a major vegetable crop that is predominantly cultivated in the fall season [12]. Extensive surveys on fall cucurbits in Georgia have revealed heavy incidence of CCYV along with other whitefly-transmitted viruses (WTVs), cucurbit leaf crumple virus (CuLCrV), and cucurbit yellow stunting disorder virus (CYSDV) and in mixed infections of all three viruses [13]. The current management practices of CCYV heavily rely on the use of insecticides to control the vector whiteflies, yet their efficiency is limited when whitefly populations are high. Utilizing resistant genotypes stands out as the most effective and environmentally safe way of managing viral diseases [14,15]. However, there are no commercially available summer squash cultivars with resistance to any of the WTVs [16]. While research on host resistance has not been conducted extensively for CCYV, resistant sources were reported in melon (*C. melo*) accessions from India, Pakistan, and Bangladesh by a group in Japan [17]. The resistance in the Indian accession JP 138,332 is recessive and the major locus for resistance is located on chromosome 1 [18]. Field evaluation of Cucurbita germplasm in the Southeastern United States identified potential resistance sources to CuLCrV, CYSDV [19], and CCYV (Luckew et al., unpublished).

Challenges in field screening include uncertainties surrounding whitefly pressure, intensity of inoculation, viral inoculum levels, interactions during mixed infections, and the age of the plant at the time of infection [20,21]. Consequently, the effectiveness and reproducibility of field screens remain uncertain, demanding a more rigorous method using controlled greenhouse inoculations with a pure culture, delivering higher virus titers of each virus to validate results from field evaluations. Greenhouse screening benefits from a controlled environment and ensures the reliability of the identified resistance sources before their integration into breeding programs. The objective of this study was to assess in the greenhouse the potential of resistance to a pure culture of CCYV by six accessions of summer squash (*Cucurbita pepo*) which had previously performed well under field conditions (Luckew et al., unpublished).

2. Materials and Methods

2.1. Source of Seeds, Whiteflies, and Virus Culture

Seeds of summer squash plant introduction (PI) accessions were obtained from the germplasm collection of the USDA-Agricultural Research Service (North Central Regional Plant Introduction Station, Ames, IA, USA) (Table 1). The CCYV susceptible commercial cultivar Gentry was sourced from Seedway (Hall, New York, NY, USA). Whitefly (*Bemisia tabaci* MED) populations were reared on upland cotton (*Gossypium hirsutum*, DG3615B3XF), a non-host of CCYV [22]. The isolate of CCYV used in this study was collected in the fall of 2020 from squash grown in research plots at the University of Georgia (UGA), Tifton, GA, USA. The CCYV isolates from Georgia (MW629381; MW629379; MW685455 and MW629380, OM489401, MW685461) share more than 99% of nucleotide sequence identity with isolates of CCYV reported from the USA and Europe [13]. Because the virus is not mechanically transmitted [23], the CCYV culture was maintained by periodic whitefly transmissions on squash cultivar Gold Star (Seedway, Hall, New York, NY, USA). The virus culture was maintained inside insect-proof cages (BugDorm, 160 µm aperture, MegaView Science Co., Ltd., Taichung, Taiwan).

Table 1. Characteristics of plant introduction (PI) accessions and their phenotypic responses to cucurbit chlorotic yellows virus (CCYV) in comparison with the susceptible cultivar 'Gentry'. Symptom severity scores at 15 and 30 days post inoculation during mass exposure and clip cage inoculations are provided.

Accession/Cultivar	Origin	Name	Symptom Severity Scores			
			Mass Exposure		Clip Cage Inoculations	
			15 DPI	30 DPI	15 DPIDPI	30 DPI
Gentry	Seedway, USA	NA	2	5	2	5
PI 512749	Castilla y León, Spain	AS-CU-1	0	3	0	3
PI 615141	Alma-Ata, Kazakhstan	Ames 19040	0	3	0	2
PI 136448	Manchuria, China	NA	0	1	0	1
PI 442312	Guanajuato, Mexico	Calabaza de India	0	1	0	1
PI 458731	Buenos Aires, Argentina	VAV 3738	0	1	0	1
PI 420328	Sakarya, Turkey	NA	0	1	0	1

NA indicates information is not available. Symptom severity scores are average values from six plants.

2.2. CCYV Transmissions

Six squash accessions (Table 1) potentially resistant to CCYV, previously identified in a field screen [19], were evaluated. Commercial squash variety Gentry was used as CCYV susceptible control. The experiment comprised three treatments: (1) inoculations with viruliferous whiteflies, (2) mock inoculations with aviruliferous whiteflies to eliminate the possibility of feeding damage being mistaken for viral symptoms, and (3) non-inoculated plants. The experiment was performed twice with inoculations being carried out by mass inoculations in the first round and using clip cages in the second round. In all three treatments, six plants of each genotype were included both in mass inoculations and clip cage inoculations. Inoculation was performed using approximately two-week-old squash seedlings with at least one fully developed true leaf.

CCYV infection on Gold Star source plants was confirmed by RT-PCR before using them in transmissions, following previous protocols [13]. Adult whiteflies raised on cotton were released onto CCYV-infected squash plants, allowing an acquisition access period (AAP) of 48 h. Subsequently, the whiteflies were provided an inoculation access period (IAP) of 48 h on test plants. Mock inoculations were performed by allowing whiteflies, directly collected from cotton, to feed on squash seedlings. In the initial screening round, mass inoculations were carried out by placing test plants of each PI line and Gentry in a single cage. Thereafter, adult whiteflies from the CCYV source plants were released on top of the test plants by gently shaking the source plant with approximately 50 whiteflies per plant (Figure 1A,B). Similarly, for mock inoculations, a similar number of whiteflies grown on cotton were released onto plants kept in a single cage. In the second round of screenings, inoculations were conducted using clip cages, prepared by cutting a circle from pool noodles and sealing one end with a fine mesh. Approximately 50 whiteflies were aspirated from the CCYV source plant or cotton and clipped on to the lower side of leaves with a clip cage (Figure 1C). Clip cage experiments were conducted in two batches with the initial batch comparing PI 420328 and PI 458731. The remaining PI accessions were subsequently compared with Gentry, as their seeds were unavailable at the time.

Figure 1. Screening of genotypes by (**A**) mass inoculation of squash plant introduction (PI) accessions. (**B**) After landing on top of the leaves, whiteflies move to the abaxial side of the leaves. (**C**) Clip cage inoculation involved aspirating whiteflies from cotton plants and directly clipping them onto the abaxial side of leaves.

Following the IAP, the whiteflies were killed by applying neonicotinoid insecticide ASSAIL® 30SG (UPL NA Inc., King of Prussia, PA, USA) containing the active ingredient acetamiprid at a rate of 0.025 g (a.i.)/100 mL water, on the recipient plants. This ensured that the symptoms of CCYV would not be confused with squash silverleaf disorder, a condition caused by persistent feeding by immature whiteflies [22,24]. The plants received weekly fertilization with Miracle-Gro® Water-Soluble All-Purpose Plant Food. Both inoculated plants and controls were maintained within the cages (BugDorm, 160 µm aperture, MegaView Science Co., Ltd., Taichung, Taiwan) in a greenhouse facility at the University of Georgia, Tifton, GA, USA. The greenhouse was maintained at a temperature of 28 ± 3 °C and 50 ± 20% relative humidity throughout the duration of the experiment. Plants were monitored daily for symptom development.

2.3. Disease Phenotyping

Symptoms of CCYV-induced chlorosis were assessed using a visual scale of 0 to 5, where '0' indicated the absence of symptoms and '5' denoted highly severe symptoms. The scores 1, 2, 3, 4, and 5 corresponded to increasing percentages of symptom coverage: up to 20%, 40%, 60%, 80%, and 100% of the plant, respectively. Each genotype was scored according to this scale. A similar scale has been used previously to evaluate symptoms induced by cucurbit yellow stunting disorder virus (CYSDV), a closely related Crinivirus that produce virtually identical symptoms [25,26]. Symptom development was monitored, and the symptom scores were recorded at 15 and 30 days post inoculation (DPI) using the visual scale.

2.4. Quantification of CCYV

Leaf samples were collected from the fifth leaf from the apical meristem of each plant at 30 days post inoculation (DPI) for quantification of CCYV. RNA extractions were performed with Spectrum™ Plant Total RNA Kit (Sigma Aldrich, St. Louis, MO, USA), following the manufacturer's protocol. cDNA was synthesized from 100 ng of total RNA using CCYV RdRp-specific primers [27], following the protocol described earlier [13]. A volume of 5 µL of cDNA served as a template in the qPCR reaction of 25 µL, which also included 12.5 µL of SSOAdvanced Universal SYBR Green Supermix and 1 µL each (10 µM) of forward and reverse primers and 6.5 µL H2O in a CFX96 Touch Deep Well Real-Time PCR System (Bio-Rad, Hercules, CA, USA). The primers and PCR profiles previously published for quantification of CCYV were used in qRT-PCR [28]. An initial denaturation step (3 min at 95 °C) was followed by 40 cycles of denaturation (10 s at 95 °C) and a combined step of annealing and extension at 62 °C for 30 s. The cycle threshold values were calculated, and melting curve analysis was performed to ensure the specificity of amplifications by CFX

Maestro Software (Bio-Rad, Hercules, CA, USA). There were six biological replicates of each genotype and each biological sample was tested in triplicate.

Cycle threshold (Ct) values from samples were compared with an external standard to estimate the copy number of CCYV in each sample, following protocols described earlier [29]. The standard consisted of a series of six ten-fold dilutions of plasmid containing the fragment of the CCYV RdRp gene amplified by the primer. The concentration of plasmid in the initial dilution was measured in ng/µL using a Nanodrop spectrophotometer (Thermo Scientific, Wilmington, DC, USA). The number of copies of the gene fragment was estimated based on the formula: the number of copies = (amount in ng * 6.022×10^{23})/(length of a vector in bp * 1×10^{9} * 650), where the weight of a base pair (bp) is assumed to be 650 Da [30].

2.5. Statistical Analyses

Data analyses were performed in JMP®, Version 16 (SAS Institute Inc., Cary, NC, USA, 1989–2021). Differences between log-transformed CCYV titers of different genotypes were assessed using one-way ANOVA. Treatment means were separated with a post hoc Student *t*-test and were considered significant at $p < 0.05$.

3. Results

3.1. Symptom Severity

The observations regarding virus symptomatology were consistent in both mass inoculations and clip cage experiments across the susceptible cultivar Gentry and tested accessions (Table 1). In the susceptible cultivar Gentry, chlorosis was restricted between the interveinal region emerged as early as 10 DPI on the lowermost leaf (Figure 2A). Over time, the chlorosis expanded and merged to cover the entire leaf (Figure 2B). The symptoms gradually progressed onto upper leaves, first appearing as chlorotic spots (Figure 2C) that eventually coalesced to cover entire leaves. By 30 DPI, more than 80% of the leaves exhibited symptoms induced by CCYV, with the lowest leaves displaying severe chlorosis and the upper leaves showing chlorotic spots (Figure 3A,B). Gentry was rated a symptom severity score of '2' at 15 DPI and the maximum symptom severity score of '5' at 30 DPI.

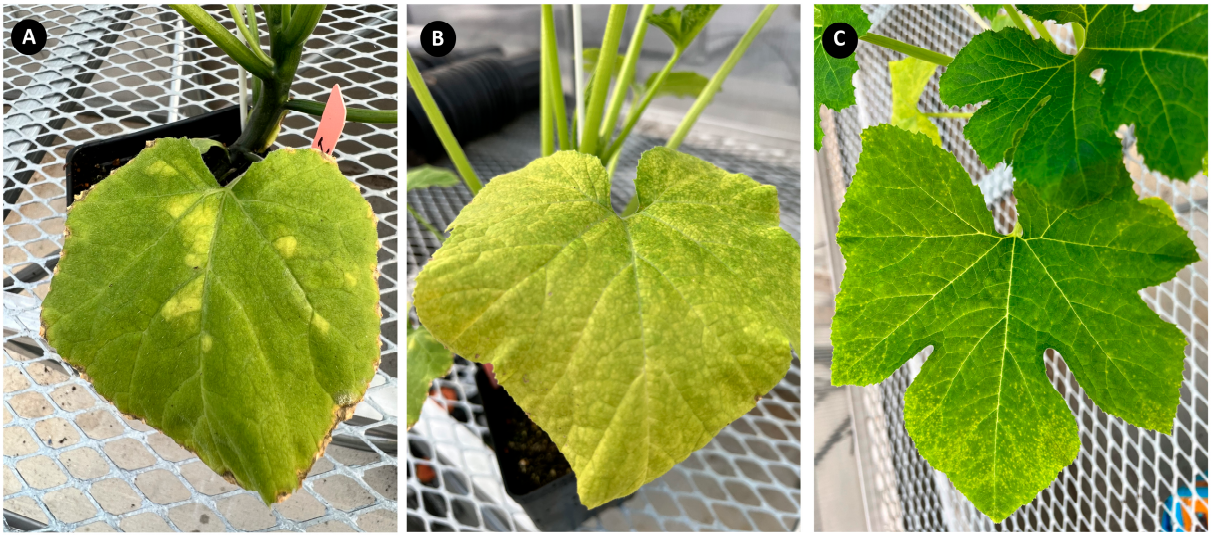

Figure 2. Symptoms induced by cucurbit chlorotic yellows virus on Gentry: (**A**) Earliest symptoms appear on lower leaves as yellow mottle. (**B**) Chlorosis covering the entire area of same leaf at later stages. (**C**) Early symptoms on systemically infected upper leaves appear as chlorotic spots, initially concentrated at the margin.

In contrast, in all the PI accessions assessed, the appearance of symptoms was notably delayed and less severe compared to Gentry. No symptoms were observed on any of the PI accessions up to three weeks post inoculation. All plants of each PI accession were given a symptom severity score of '0' at 15 DPI. On the PI lines, PI 512749 and PI 615141, mild chlorosis became apparent on the lower leaves around three weeks after inoculation, in comparison to mock inoculated plants of same accession. At 30 DPI, pronounced chlorosis on lower leaves and chlorotic spots on upper leaves covered around 60% of the CCYV inoculated plants of PI 512749. This accession was given a symptom severity score of '3'. For PI 615141, mild chlorosis on lower leaves and chlorotic spots extending to cover around 40–60% of the plants was observed. PI 136448 and PI 442312, as well as PI 458731 and PI 420328 exhibited mild chlorosis on the lower leaves at 30 DPI in comparison to mock inoculated plants. However, chlorotic spots on the newest leaf as observed on Gentry PI 512749 and PI 615141 were not observed. Symptoms covered less than 20% of the plants and, hence, these four accessions were given a symptom severity score of '1' at 30 DPI. PI 420328 (Figure 3C) and PI 458731 (Figure 3D) stood out among the rest by exhibiting very mild chlorosis on the lower leaves and no chlorotic spots. The distinction between CCYV inoculated and mock inoculated plants were barely visible (Figure 3C,D).

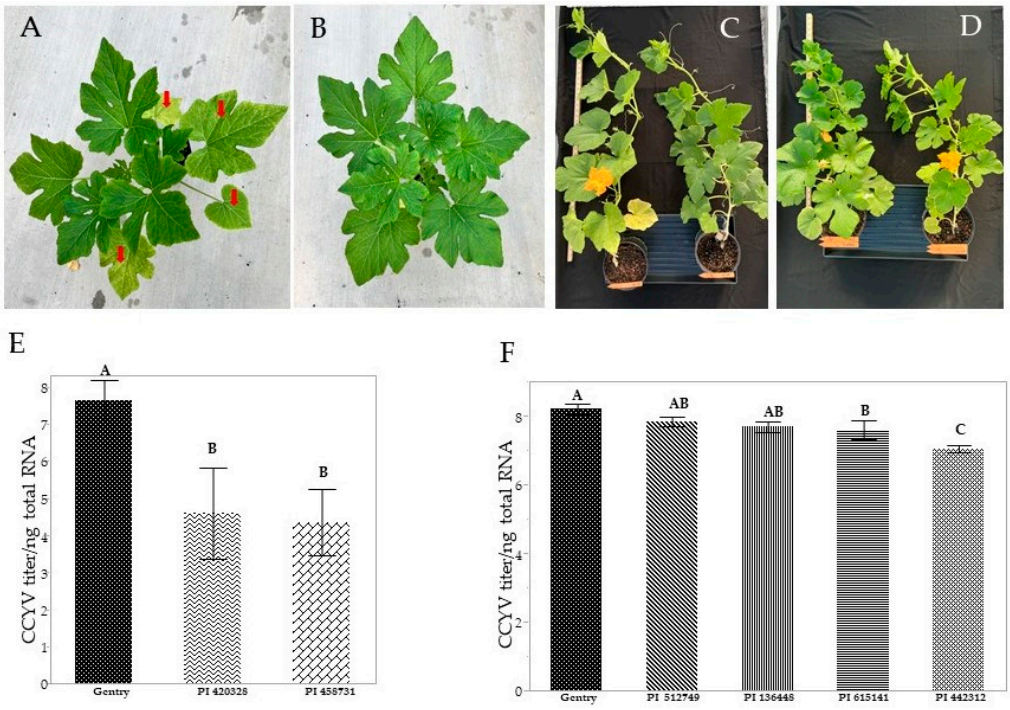

Figure 3. Response of the genotypes tested to CCYV infections. Virus-induced chlorosis covered most of the lower leaves of the inoculated Gentry plants (**A**) with the lowest leaves displaying severe chlorosis (Red arrow) and the upper leaves showing chlorotic spots (Red arrow) by 30 DPI in comparison to (**B**) mock inoculated plants which remains asymptomatic. CCYV inoculated plants of PI 420328 (**C**) and PI 458731 (**D**) (plants on the left) exhibited very mild chlorosis on the lower leaves and no chlorotic spots compared to mock inoculated plants (plants on the right). Significantly lower CCYV virus titers were observed in PI 420328 and PI 458731 (**E**) as well as PI 615141 and PI 442312 (**F**) compared to Gentry in clip cage inoculations.

3.2. Virus Titer

The CCYV titer in the PI accessions varied slightly in both mass exposure (Supplementary Figure S1) and in clip cage experiments (Figure 3E,F). In mass inoculation experiments, CCYV titers were significantly lower in all the PI accessions compared to Gentry ($p < 0.001$). Clip cage experiments were conducted in two batches with the first batch comparing PI 420328 and PI 458731 against the susceptible line Gentry. In this comparison, the CCYV

titers in PI 420328 ($p < 0.0001$) and PI 458731 ($p < 0.0001$) were several times lower than in Gentry (Figure 3E). In the second batch of clip cage experiments, the remaining four PI accessions (PI 512749, PI 136448, PI 615141, and PI 442312) were compared with Gentry. CCYV titers in PI 615141 ($p < 0.022$) and PI 442312 ($p < 0.001$) were significantly lower than in Gentry, but comparatively higher than those observed in PI 420328 and PI 458731 (Figure 3F). There was no significant difference in CCYV titers among PI 512749, PI 136448, and Gentry. Among the six PI accessions tested, PI 512749 displayed relatively higher CCYV titers in both mass exposure and in clip cage experiments.

4. Discussion

Cucurbit chlorotic yellows virus (CCYV), a recently emerged virus that infects most cucurbits has become prevalent in many parts of the globe where the vector whiteflies are present [1,2]. The impact of CCYV on squash (*C. pepo*), a significant cucurbit species cultivated in the Southeastern United States, has been particularly severe, with high incidence reported during the fall season, often in mixed infections with CYSDV and CuLCrV [10]. In addition to losses due to CCYV infections alone on cucurbits [3,11], CYSDV, a closely related Crinivirus, was shown to exacerbate the symptoms on squash in mixed infections with CuLCrV [29].

Given the absence of commercially available resistant cultivars, there is a need for developing resistance against whitefly-transmitted viruses [16]. Although resistance sources have been identified in melon [17], these are not accessible in the United States [31]. Potential resistance sources for squash have been identified in mixed-infected fields in the Southeast [19]. However, the challenge with spontaneous field inoculation is that CCYV resistant plants may be infected by an unrelated virus, or any other pathogen, and erroneously considered susceptible [32]. Consequently, the resistance identified by field evaluations needs to be further validated by controlled greenhouse inoculations of pure culture of each virus to validate the results from field evaluations.

Criniviruses, including CCYV [33], exhibit low genetic variability in the coding regions among different isolates [23,34–36] and the CCYV isolates from Georgia are 99% identical to other isolates of CCYV available in the GenBank [13]. Consequently, the resistance identified in this study is anticipated to confer protection against a broad spectrum of CCYV isolates.

Two methods of whitefly inoculation were employed: mass inoculation (Figure 1A,B) and the use of clip cages (Figure 1C). While mass inoculation provides an initial assessment of resistance and susceptibility, clip cage experiments were also conducted to account for challenges in uniformity regarding the number of whiteflies on each plant, when co-inoculating different genotypes. Individual inoculation through clip cages ensures uniform whitefly distribution on each plant, facilitating precise virus transmission by placing a single plant with viruliferous whiteflies.

The investigation revealed consistent and distinctive patterns of CCYV symptom development in the susceptible cultivar Gentry and the different PI accessions tested. Gentry exhibited early and severe symptoms, with over 80% of leaves displaying chlorosis and/or chlorotic spots by day 30 post inoculation (DPI). In contrast, all the tested accessions demonstrated delayed and less severe symptomatology. PI 420328 and PI 458731 exhibited minimal differences in symptoms between CCYV inoculated and mock inoculated plants. In these two accessions, even at the end of 30 DPI, the yellowing on the lower leaves was not distinguishable from age-related yellowing on the lowest leaves (Figure 3).

The titers of CCYV in all tested PI accessions, except PI 512729 and PI 136448, at 30 DPI were consistently lower than those in the susceptible cultivar 'Gentry', regardless of differences in some PI accessions between both types of inoculation methods. PI 512729 and PI 136448 had lower CCYV titers in the mass inoculation experiment, but not in the clip cage experiment. The variations in virus titers in mass inoculations and clip cage inoculations may be attributed to whitefly preferences for certain genotypes over others during mass inoculations as reported earlier in wild tomato screening by whitefly inoculations [32].

Notably, the CCYV titers in PI 420328 and PI 458731 were significantly lower than in Gentry, in the more efficient albeit time-consuming clip cage inoculations, underscoring their ability to suppress CCYV infections.

The term resistance is employed to characterize the impact of host on the virus, whereas tolerance is used to denote the disease reaction of the plant in response to virus infection [37]. Plant resistance to a particular virus is characterized by reduced virus infection, multiplication, and invasion [37], that ultimately translates into lower virus titers, whereas a plant is deemed tolerant when it exhibits mild or no symptoms and shows no or reduced yield loss in response to virus infection [37–40]. All PI accessions tested in this study exhibited reduced symptoms regardless of supporting a substantial amount of CCYV, indicating tolerance to the yellowing disease caused by CCYV in all these lines. In PI 420328 and PI 458731, the CCYV titers were also significantly lower than the susceptible check, indicating resistance to CCYV, and thus, these two accessions are particularly promising. It is worth mentioning that this resistance may be partial or dose-dependent, as the level of reduction in virus titers observed in clip cage experiments using a limited number of whiteflies on each plant was larger than in mass inoculations, where the introduction of a higher initial inoculum is possible.

Furthermore, PI 420328 has previously been reported to be resistant to CYSDV, another closely related Crinivirus, [41]. Lower virus titers and elevated levels of 21- and 22-nucleotide (nt)-sized class CYSDV RNAs, the hallmarks of RNA silencing, were observed in this line, indicating more robust and efficient RNA silencing [41]. These lines hold promise as potential sources of resistance to CCYV in breeding programs. Considering the resistance of PI 420328 to another closely related Crinivirus, it is plausible that the resistance mechanism in this line, and possibly PI 458731, could have a broad-spectrum effect against Criniviruses. Future research should focus on identifying host loci associated with resistance and tolerance in these lines and incorporating these loci into summer squash varieties to enhance resistance against CCYV.

5. Conclusions

CCYV presents a significant threat to the sustainable production of cucurbit crops globally, particularly impacting summer squash in the Southeastern United States. The absence of commercially available resistant cultivars necessitates the exploration of resistance sources. Previous field screenings identified specific Cucurbita germplasm lines as potential sources of resistance against whitefly-transmitted virus infections. Our controlled greenhouse screenings confirmed the potential resistance of Cucurbita germplasm lines, particularly PI 420328 and PI 458731, which displayed delayed and milder symptoms compared to the susceptible cultivar Gentry. Quantification of CCYV RNA accumulation further supported their resistance, indicating significantly lower virus titers in these accessions compared to the susceptible cultivar. These findings suggest PI 420328 and PI 458731 as promising candidates for breeding resistant summer squash cultivars. This study also establishes a reliable method for assessing resistance to CCYV. Future efforts should focus on exploiting the resistance mechanisms of these germplasm lines to enhance the resilience of cucurbit crops against CCYV, ensuring their sustainability and productivity.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae10030264/s1, Figure S1: The mean virus titer in all the PI accessions was significantly lower than that in Gentry at 30 DPI in mass exposure experiments.

Author Contributions: Conceptualization, S.R.K., S.B., C.E.M. and A.K.C.; methodology, S.R.K.; validation, S.R.K.; formal analysis, S.R.K., S.B. and C.E.M.; investigation, S.R.K. and S.B.; resources, S.B. and A.M.S.; data curation, S.R.K.; writing—original draft preparation, S.R.K.; writing—review and editing, S.R.K., S.B., C.E.M., A.L., A.K.C. and A.M.S.; supervision, S.R.K., A.K.C. and S.B.; project administration, S.B.; funding acquisition, S.B. and A.M.S. All authors have read and agreed to the published version of the manuscript.

Funding: This study was partially supported by the USDA-HATCH grant awarded to S.B., number 1020319, and the USDA, ARS-UGA Cooperative Agreement, project number 58-6080-9-006. The funders had no role in the study design, data collection and analyses, the decision to publish or the manuscript preparation.

Data Availability Statement: Data are contained within the article.

Acknowledgments: The authors appreciate the anonymous reviewers for their constructive criticism and suggestions on the manuscript. The mention of a proprietary product does not constitute an endorsement or a recommendation for its use by the USDA or the University of Georgia.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Wintermantel, W.M. Cucurbit Chlorotic Yellows Virus. 2022. Available online: https://ecucurbitviruses.org/ (accessed on 7 March 2024).
2. Okuda, M.; Okazaki, S.; Yamasaki, S.; Okuda, S.; Sugiyama, M. Host range and complete genome sequence of Cucurbit chlorotic yellows virus, a new member of the genus Crinivirus. *Phytopathology* **2010**, *100*, 560–566. [CrossRef] [PubMed]
3. Gyoutoku, Y.; Okazaki, S.; Furuta, A.; Etoh, T.; Mizobe, M.; Kuno, K.; Hayashida, S.; Okuda, M. Chlorotic yellows disease of melon caused by cucurbit chlorotic yellows virus, a new crinivirus. *Jpn. J. Phytopathol.* **2009**, *75*, 109–111. [CrossRef]
4. Bananej, K.; Menzel, W.; Kianfar, N.; Vahdat, A.; Winter, S. First report of cucurbit chlorotic yellows virus infecting cucumber, melon, and squash in Iran. *Plant Dis.* **2013**, *97*, 1005. [CrossRef] [PubMed]
5. Kumar, A.; Rout, B.M.; Choudhary, S.; Sureja, A.K.; Baranwal, V.K.; Pant, R.P.; Kaur, B.; Jain, R.K.; Basavaraj, Y.B. First report of cucurbit chlorotic yellows virus infecting pumpkin in India. *Plant Dis.* **2021**, *106*, 1767. [CrossRef]
6. Hamed, K.; Menzel, W.; Dafalla, G.; Gadelseed, A.M.A.; Winter, S. First report of cucurbit chlorotic yellows virus infecting muskmelon and cucumber in Sudan. *Plant Dis.* **2011**, *95*, 1321. [CrossRef] [PubMed]
7. Abrahamian, P.E.; Sobh, H.; Abou-Jawdah, Y. First report of cucurbit chlorotic yellows virus on cucumber in Lebanon. *Plant Dis.* **2012**, *96*, 1704. [CrossRef] [PubMed]
8. Orfanidou, C.; Maliogka, V.; Katis, N. First report of cucurbit chlorotic yellows virus in cucumber, melon, and watermelon in Greece. *Plant Dis.* **2014**, *98*, 1446. [CrossRef] [PubMed]
9. Wintermantel, W.M.; Hladky, L.L.J.; Fashing, P.; Ando, K.; McCreight, J.D. First report of cucurbit chlorotic yellows virus infecting melon in the New World. *Plant Dis.* **2019**, *103*, 778. [CrossRef]
10. Devendran, R.; Kavalappara, S.R.; Simmons, A.M.; Bag, S. Whitefly-transmitted viruses of cucurbits in the Southern United States. *Viruses* **2023**, *15*, 2278. [CrossRef]
11. Peng, J.; Huang, Y. The occurrence of cucurbit chlorotic yellows virus disease in Taiwan and evaluation of the virus infected fruit quality and yield. *Phytopathology* **2011**, *101*, S139–S140. [CrossRef]
12. UGA Farm Gate Value 2022. Available online: https://caed.uga.edu/publications/farm-gate-value.html (accessed on 7 March 2024).
13. Kavalappara, S.R.; Milner, H.; Konakalla, N.C.; Morgan, K.; Sparks, A.N.; McGregor, C.; Culbreath, A.K.; Wintermantel, W.M.; Bag, S. High throughput sequencing-aided survey reveals widespread mixed infections of whitefly-transmitted viruses in cucurbits in Georgia, USA. *Viruses* **2021**, *13*, 988. [CrossRef] [PubMed]
14. Lapidot, M.; Friedman, M. Breeding for resistance to whitefly-transmitted geminiviruses. *Ann. Appl. Biol.* **2002**, *140*, 109–127. [CrossRef]
15. Morales, F.J. Conventional breeding for resistance to *Bemisia tabaci*-transmitted geminiviruses. *Crop Prot.* **2001**, *20*, 825–834. [CrossRef]
16. Candian, J.S.; Coolong, T.; Dutta, B.; Srinivasan, R.; Sparks, A.; Barman, A.; Ribeiro da Silva, A.L.B. Yellow squash and zucchini cultivar selection for resistance to cucurbit leaf crumple virus in the Southeastern United States. *HortTechnology* **2021**, *31*, 504–513. [CrossRef]
17. Okuda, S.; Okuda, M.; Sugiyama, M.; Sakata, Y.; Takeshita, M.; Iwai, H. Resistance in melon to cucurbit chlorotic yellows virus, a whitefly-transmitted crinivirus. *Eur. J. Plant Pathol.* **2013**, *135*, 313–321. [CrossRef]
18. Kawazu, Y.; Shimomura, K.; Maeda, S.; Yamato, Y.; Ueda, S.; Okuda, S.; Okuda, M.; Sugiyama, M. QTL mapping for resistance to cucurbit chlorotic yellows virus in melon (*Cucumis melo* L.). *Euphytica* **2018**, *214*, 239. [CrossRef]
19. Luckew, A.; Meru, G.; Wang, Y.-Y.; Mwatuwa, R.; Paret, M.; Carvalho, R.; Kalischuk, M.; da Silva, A.L.B.R.; Candian, J.; Dutta, B. Field evaluation of cucurbita germplasm for resistance to whiteflies and whitefly-transmitted viruses. *HortScience* **2022**, *57*, 337–344. [CrossRef]
20. Cohen, S.; Kern, J.; Harpaz, I.; Ben-Joseph, R. Epidemiological studies of the tomato yellow leaf curl virus (TYLCV) in the Jordan Valley, Israel. *Phytoparasitica* **1988**, *16*, 259–270. [CrossRef]
21. Lapidot, M.; Ben-Joseph, R.; Cohen, L.; Machbash, Z.; Levy, D. Development of a scale for evaluation of tomato yellow leaf curl virus resistance level in tomato plants. *Phytopathology* **2006**, *96*, 1404–1408. [CrossRef]

22. Costa, H.; Ullman, D.; Johnson, M.; Tabashnik, B. Squash silverleaf symptoms induced by immature, but not adult, *Bemisia tabaci*. *Phytopathology* **1993**, *83*, 763. Available online: https://www.apsnet.org/publications/phytopathology/backissues/Documents/1993Abstracts/Phyto_83_763.htm (accessed on 7 March 2024). [CrossRef]
23. Tzanetakis, I.E.; Martin, R.R.; Wintermantel, W.M. Epidemiology of criniviruses: An emerging problem in world agriculture. *Front. Microbiol.* **2013**, *4*, 119. [CrossRef]
24. Young, K.; Kabelka, E.A. Characterization of resistance to squash silverleaf disorder in summer squash. *HortScience* **2009**, *44*, 1213–1214. [CrossRef]
25. Tamang, P.; Ando, K.; Wintermantel, W.M.; McCreight, J.D. QTL mapping of cucurbit yellow stunting disorder virus resistance in melon Accession PI 313970. *HortScience* **2021**, *56*, 424–430. [CrossRef]
26. McCreight, J.D.; Wintermantel, W.M. Genetic resistance in melon PI 313970 to cucurbit yellow stunting disorder virus. *HortScience* **2011**, *46*, 1582–1587. [CrossRef]
27. Orfanidou, C.; Katsiani, A.; Papayiannis, L.; Katis, N.I.; Maliogka, V.I. Interplay of cucurbit yellow stunting disorder virus with cucurbit chlorotic yellows virus and transmission dynamics by *Bemisia tabaci* MED. *Plant Dis.* **2021**, *105*, 416–424. [CrossRef]
28. Kavalappara, S.R.; Riley, D.G.; Cremonez, P.S.G.; Perier, J.D.; Bag, S. Wild Radish (*Raphanus raphanistrum* L.) is a potential reservoir host of cucurbit chlorotic yellows virus. *Viruses* **2022**, *14*, 593. [CrossRef] [PubMed]
29. Gautam, S.; Gadhave, K.R.; Buck, J.W.; Dutta, B.; Coolong, T.; Adkins, S.; Srinivasan, R. Virus-virus interactions in a plant host and in a hemipteran vector: Implications for vector fitness and virus epidemics. *Virus Res.* **2020**, *286*, 198069. [CrossRef] [PubMed]
30. Rotenberg, D.; Krishna Kumar, N.K.; Ullman, D.E.; Montero-Astúa, M.; Willis, D.K.; German, T.L.; Whitfield, A.E. Variation in tomato spotted wilt virus titer in *Frankliniella occidentalis* and its association with frequency of transmission. *Phytopathology* **2009**, *99*, 404–410. [CrossRef] [PubMed]
31. McCreight, J.D.; Davis, A.A.; Reitsma, K. Melon (*Cucumis melo*) Crop Vulnerability Statement. 2020. Available online: https://www.ars-grin.gov/documents/cgc/cvs/2020-Melon%20Crop%20Vulnerability%20Statement.pdf (accessed on 7 March 2024).
32. Pico, B.; Díez, M.; Nuez, F. Evaluation of whitefly-mediated inoculation techniques to screen *Lycopersicon esculentum* and wild relatives for resistance to tomato yellow leaf curl virus. *Euphytica* **1998**, *101*, 259–271. [CrossRef]
33. Orfanidou, C.; Baltzi, A.; Dimou, N.; Katis, N.; Maliogka, V. Cucurbit chlorotic yellows virus: Insights into its natural host range, genetic variability, and transmission parameters. *Plant Dis.* **2017**, *101*, 2053–2058. [CrossRef]
34. Akhter, M.S.; Bhor, S.A.; Hlalele, N.; Nao, M.; Sekine, K.-T.; Yaeno, T.; Yamaoka, N.; Nishiguchi, M.; Gubba, A.; Kobayashi, K. Review of beet pseudoyellows virus genome structure built the consensus genome organization of cucumber strains and highlighted the unique feature of strawberry strain. *Virus Genes* **2016**, *52*, 828–834. [CrossRef]
35. Orílio, A.F.; Navas-Castillo, J. The complete nucleotide sequence of the RNA2 of the crinivirus tomato infectious chlorosis virus: Isolates from North America and Europe are essentially identical. *Arch. Virol.* **2009**, *154*, 683–687. [CrossRef]
36. Rubio, L.; Galipienso, L.; Ferriol, I. Detection of plant viruses and disease management: Relevance of genetic diversity and evolution. *Front. Plant Sci.* **2020**, *11*, 1092. [CrossRef]
37. Cooper, J.; Jones, A. Responses of plants to viruses: Proposals for the use of terms. *Phytopathology* **1983**, *73*, 127–128. Available online: https://www.apsnet.org/publications/phytopathology/backissues/Documents/1983Articles/Phyto73n02_127.PDF (accessed on 7 March 2024). [CrossRef]
38. Fraser, R. The genetics of resistance to plant viruses. *Annu. Rev. Phytopathol.* **1990**, *28*, 179–200. [CrossRef]
39. Kang, B.C.; Yeam, I.; Jahn, M.M. Genetics of plant virus resistance. *Annu. Rev Phytopathol* **2005**, *43*, 581–621. [CrossRef] [PubMed]
40. Pagán, I.; García-Arenal, F. Tolerance to plant pathogens: Theory and experimental evidence. *Int. J. Mol. Sci.* **2018**, *19*, 810. [CrossRef] [PubMed]
41. Kavalappara, S.R.; Bag, S.; Luckew, A.; McGregor, C.E. Small RNA profiling of cucurbit yellow stunting disorder virus from susceptible and tolerant squash (*Cucurbita pepo*) lines. *Viruses* **2023**, *15*, 788. [CrossRef] [PubMed]