Response of Some Watermelon Accessions to a Puerto Rico Strain of *Zucchini yellow mosaic virus*

Giseiry Rosa-Valentín and Linda Wessel-Beaver

Department of Agroenvironmental Sciences, University of Puerto Rico, Mayagüez Campus, Box 9000, Mayagüez, PR 00681-9000

Jose Carlos V. Rodrigues

Puerto Rico Agricultural Experiment Station, University of Puerto Rico, South Botanical Garden, 1193 Guayacan Street, San Juan, PR 00926

Additional index words. *Citrullus*, cucurbits, disease resistance, germplasm, potyvirus

Abstract. One of the most important members of the Potyviridae is *Zucchini yellow mosaic virus* (ZYMV). It affects watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai] as well as other cucurbits in most parts of the world. Although several genotypes have been reported as having resistance to ZYMV, differential responses to ZYMV strains are known to occur. Using a Puerto Rico strain of ZYMV (ZYMV-PR, GenBank accession number MN422959), we tested the response of 11 genotypes [PIs from the U.S. Department of Agriculture (USDA) National Genetic Resources Program] previously reported as having resistance to this virus. In two greenhouse trials, the first three leaves of seedlings of each genotype were mechanically inoculated with ZYMV-PR. An enzyme-linked immunosorbent assay (ELISA) was done on each seedling's fourth leaf and symptom severity was rated on the first, third, fifth, and seventh leaves. There were significant genotype $\times$ trial interactions for most variables, but some genotypes performed consistently in both trials. All seedlings of PI 537277 tested negative for ELISA (absorbance < 0.200) across both trials. PI 537277, PI 595200, PI 595201, and PI 595203 were generally among the accessions with the lowest symptom severity scores. Overall, symptom severity correlated poorly with ELISA readings. But all plants of PI 537277, and most plants of PI 595201 and PI 595203, had low ELISA readings and low severity scores. Despite having low severity scores, PI 595200 was among the genotypes with the highest ELISA readings in trial 2. For the plant breeder, the most useful genotypes are those that exhibit reduced severity as well as low ELISA. PI 537277, PI 595201, and PI 595203 met those criteria in this study. Of these three accessions, PI 595203 would be the most useful in a breeding program because it has shown resistance to the Puerto Rico, Florida, and China strains of ZYMV.

ZYMV impacts cucurbit production around the world. Desbiez and Lecoq (1997) list more than 62 countries in all regions of the world where ZYMV has been reported. This member of the Potyviridae family has been known since

1973, when it was first observed in northern Italy in *Cucurbita pepo* (Lisa et al., 1981). It is now known to affect watermelon [*C. lanatus* (Thunb.) Matsum. & Nakai] in many parts of the world (Desbiez and Lecoq, 1997), including North America (Ali et al., 2012; Juarez et al., 2013; Nameth et al., 1985; Provvidenti et al., 1984), South America (Rabelo Filho et al., 2010), Asia (Chang et al., 1987), Europe (Vučković et al., 2012), and the Middle East (Bananej and Vahdat, 2008; Topkaya and Ertunç, 2012). Because ZYMV is transmitted in a nonpersistent manner by a variety of aphid species (Castle et al., 1992), chemical control methods are not effective. Genetic resistance would provide the most cost-efficient control method.

Provvidenti (1986) observed that the resistance reaction to ZYMV in watermelon PI 494528 and PI 494532 varied with environmental conditions; these genotypes were susceptible when evaluated under the low light and cool temperature conditions of a winter greenhouse, but were completely resistant during the warm summer months. Provvidenti (1991) later noted that these sources of resistance to ZYMV were viral strain nonspecific. Other sources of resis-

tance were observed to be strain-specific but not temperature dependent. Provvidenti (1991) tested PI 482261, PI 482299, PI 482308, and PI 482322 and found that resistance in these PIs was specific to the Florida strain of ZYMV (ZYMV-FL); these same accessions were susceptible to Connecticut and Egyptian strains of ZYMV (ZYMV-CT and ZYMV-E, respectively). Provvidenti (1991) conducted an inheritance study using PI 482261 with ZYMV-FL as the inoculum source and concluded that resistance was controlled by the single recessive gene *zym*.

As expected of a widely distributed virus affecting a number of cucurbit species, ZYMV is biologically variable (Antignus et al., 1989; Davis and Mizuki, 1987; Desbiez [sic] and Lecoq, 1999; Desbiez and Lecoq, 1997; Desbiez et al., 1996, 2002; Juarez et al., 2013). Differential response of genotypes to different ZYMV strains is known to occur. Provvidenti (1991) screened 57 watermelon PIs and 11 cultivars and found all genotypes to be susceptible to ZYMV-CT and ZYMV-E strains of ZYMV. Four accessions, PI 482322, PI 482299, PI 482261, and PI 482308, had a least some plants with resistance to ZYMV-FL. Progeny derived from these plants were also resistant. He concluded that resistance to ZYMV-FL was controlled by a single recessive gene in resistant plants derived from PI 482261. Boyhan et al. (1992) showed that 'Egun' (apparently PI 595203) had strong resistance to ZYMV-FL, and demonstrated that PI 386025, PI 386026, PI 482261, PI 494528, and PI 494529 also have some degree of resistance. Xu et al. (2004) determined that PI 595203 is resistant to the China strain of ZYMV (ZYMV-CH) and described that resistance as a single recessive gene. Ling et al. (2006) confirmed resistance in PI 595203 to ZYMV-FL and concluded that resistance to that strain is also controlled by a single recessive gene. Guner (2004) tested 1644 cultigens of watermelon using ZYMV-FL. The accessions with the highest resistance to ZYMV were PI 386016, PI 386019, PI 485580, PI 494529, PI 537277, PI 560016, PI 595200, and PI 595203. Guner and Wehner (2008) also mentioned PI 482276, PI 595201, and PI 596662 as having ZYMV resistance.

In Puerto Rico, ZYMV was first identified as affecting watermelon plantings in 1982 (Escudero, 1992). A 2001–2002 survey of the island found ZYMV to be the most common virus in cucurbit crops (Paz-Carrasco and Wessel-Beaver, 2002). The objective of this study was to use the Puerto Rico strain of ZYMV (ZYMV-PR) to test a group of watermelon accessions previously shown to have resistance to other strains of this virus.

Materials and Methods

Two trials were conducted in a greenhouse in Mayagüez, PR, during February to April of 2012. These months were generally sunny and greenhouse temperatures varied from a low of ≈ 23 °C to a high of ≈ 35 °C. In trial 1, watermelon accessions (Plant Genetic Resources Conservation Unit, USDA-ARS, Griffin, GA) listed in Table 1 were seeded in

Received for publication 20 May 2020. Accepted for publication 10 July 2020.

Published online 13 August 2020.

This work was supported by the U.S. Department of Agriculture (USDA) National Institute of Food and Agriculture (NIFA), Special Grants in Tropical/Subtropical Agriculture Research Program project 2010-34135-21022, Specialty Crop Research Initiative project 2015-51181-24285, and the Puerto Rico Agricultural Experiment Station. The contents are solely the responsibility of the authors and do not necessarily represent the official views of the USDA or NIFA. This paper is a portion of a thesis submitted by the first author in partial fulfillment of the requirements in a master's degree program.

G.R.-V. is a Former Graduate Student.

L.W.-B. is the corresponding author. E-mail: lindawessel-beaver@upr.edu.

This is an open access article distributed under the CC BY-NC-ND license (<https://creativecommons.org/licenses/by-nc-nd/4.0/>).

Table 1. Watermelon accessions tested for resistance to a Puerto Rico isolate of *Zucchini yellow mosaic virus* (ZYMV). Accessions in the table were previously reported as resistant to ZYMV as cited in the last column of the table.

Accession	<i>Citrullus</i> group ²	Country of origin	Reference where accession is reported as resistant to ZYMV ³
PI 482261	<i>Citrullus amarus</i> Schrad.	Zimbabwe	Provvidenti, 1991; Boyhan et al., 1992; Guner 2004
PI 482276	<i>C. amarus</i> Schrad.	Zimbabwe	Guner 2004; Guner and Wehner, 2008
PI 485580	<i>C. amarus</i> Schrad.	Botswana	Guner, 2004; Guner and Wehner, 2008; Guner et al., 2019
PI 494528	<i>Citrullus mucospermus</i> (Fursa) Fursa	Nigeria	Provvidenti, 1986; Provvidenti, 1991; Boyhan et al., 1992; Guner 2004; Guner and Wehner, 2008; Guner et al., 2019
PI 494529	<i>C. mucospermus</i> (Fursa) Fursa	Nigeria	Boyhan et al., 1992; Guner, 2004; Guner et al., 2019
PI 537277	<i>Citrullus colocynthis</i> (L.) Schrad.	Pakistan	Guner, 2004; Guner et al., 2019
PI 560016	<i>C. mucospermus</i> (Fursa) Fursa	Nigeria	Guner, 2004; Guner and Wehner, 2008; Guner et al., 2019
PI 595200	<i>Citrullus lanatus</i> (Thunb.) Matsum. & Nakai	Selection from PI 189316 - Zaire	Guner, 2004; Guner et al., 2019
PI 595201	<i>C. lanatus</i> (Thunb.) Matsum. & Nakai	Selection from PI 189316 - Zaire	Guner, 2004; Guner and Wehner, 2008; Guner et al., 2019
PI 595203	<i>C. mucospermus</i> (Fursa) Fursa	Nigeria	Boyhan et al., 1992; Xu et al., 2004; Guner, 2004; Ling et al., 2006; Guner and Wehner, 2008; Guner et al., 2018, 2019
PI 596662	<i>C. amarus</i> Schrad.	South Africa	Guner, 2004; Guner and Wehner, 2008; Guner et al., 2019

²Taxonomy according to U.S. Department of Agriculture National Plant Germplasm System.

³Except for Xu et al., 2004 (China strain) and Provvidenti, 1991 (Connecticut, Egypt and Florida strains), all reported resistances are to the Florida strain of ZYMV.

10-cm plastic pots filled with Promix PGX (Premier Tech Horticulture, Quakertown, PA) and arranged in a completely randomized design (CRD). The experimental unit was a pot with a single seedling. Each genotype was replicated six times. In trial 2, the same accessions were tested along with susceptible watermelon cultivars Crimson Sweet and Sugar Baby (Willhite Seed Company, Poolville, TX). Genotypes were again arranged in a CRD but replicated 10 times. PI 485580 did not germinate adequately in trial 1; only data from trial 2 are presented for this PI. For both trials, 14-d-old seedlings were mechanically inoculated with a Puerto Rico isolate of ZYMV (ZYMV-PR, GenBank accession number MN422959, Rodrigues Virology Collection No.161). In each trial, buffer-inoculated plants were included as controls. ZYMV-PR was originally collected from a single leaf of a single plant of *Cucurbita moschata* Duchesne in a 2008 survey in Puerto Rico and produced severe disease symptoms when inoculated to various cucurbit species. ZYMV-PR was multiplied on seedlings of *C. moschata* 'Waltham' [True Leaf Market (previously Mountain Valley Seed Company), Salt Lake City, UT] and the infected tissue was lyophilized and preserved for further use. As needed for this and other studies, 'Waltham' seedlings were inoculated with the original lyophilized material and fresh tissue from those plants were used as inoculum. Reverse-transcriptase polymerase chain reaction and sequencing of a coat protein gene fragment were conducted to monitor the stability of the isolate (GenBank accession MN422959). No meaningful variations were observed. Mechanical inoculation was done as follows: The first three true leaves of watermelon genotypes were inoculated 14 d after seeding; in a few plants, the third leaf was not sufficiently expanded to allow for inoculation, in which case only the first two leaves were inoculated. Leaves were lightly dusted with 350 mesh carborundum (Thermo Fisher Scientific, Waltham, MA). Fresh leaves of ZYMV-infected 'Waltham' were macerated in a cold mortar and pestle

with 0.02 M phosphate buffer at pH 7.0. A tissue-to-buffer ratio of 1:10 (wt/vol) was used. At 20 d post inoculation, the first, third, fifth, and seventh leaves were visually evaluated for symptom severity using a 0 to 3 scale where 0 = no symptoms, 1 = mild chlorosis or mottling, 2 = strong chlorosis or mottling, and 3 = severe virus symptoms including blistering and leaf deformity. Ratings of 0 or 1 would be considered resistant. At 28 d post inoculation, a 0.1-g sample of tissue from the fourth leaf of each plant was sampled and tested for ZYMV using a commercial ELISA kit (Agdia, Elkhart, IN). The kit uses a double antibody sandwich assay and alkaline phosphatase label. The ZYMV ELISA from Agdia "detects isolates... from all over the world, including CT, USDA, SJBCA, CA, IT, NY, FL, Z18" (<https://orders.agdia.com/pathoscreen-zymv-alkphos-psa-77700>). Positive, negative, and extraction buffer controls were included on each ELISA plate. All controls were supplied by Agdia. Within a plate, controls and individual plant samples were replicated two times (two subsamples). Absorbance at 405 nm was measured with a Dias Microplate Reader (Dynex Technologies, Chantilly, VA). Individual absorbance values were adjusted by subtracting the average reading of the negative controls within a plate. Negative adjusted readings were changed to zero.

The absorbance reading of the fourth leaf (mean of two subsamples per plant); the symptom severity of first, third, fifth, and seventh leaves; the average severity of the first, third, and fifth leaves; and the average severity of the first, third, fifth, and seventh leaves were analyzed using InfoStat (Di Rienzo et al., 2019). Data from the buffer-inoculated control plants and, in the case of ELISA, from the positive, negative, and buffer controls were not included in the analysis of variance (ANOVA) to comply with the assumption of a common variance. Because there was a significant trial $\times$ genotype interaction, trials were analyzed separately using a one-way ANOVA. Means were separated with a Fisher's protected test of

least significant difference using $\alpha = 0.05$. In addition, for each trial a Pearson's correlation coefficient was calculated for each pair of variables used to assess resistance. Correlations were calculated using individual plant data (the mean of two subsamples per plant for ELISA readings, and a single observation per leaf for symptom severity at each leaf position).

Results

Although there was a significant trial $\times$ genotype interaction for the various measures of symptom severity, PI 537277 and PI 595201 were almost always among the accessions with the lowest severity, no matter which leaf was scored (Tables 2 and 3). Two other accessions, PI 595200 and PI 595203, were also often among the genotypes with the lowest severity, although less consistently so than the previously mentioned accessions.

Trial $\times$ genotype interaction was even greater for absorbance readings (ELISA) (Tables 2 and 3). The relative ranking of ELISA readings for genotypes varied between the two trials, except for PI 537277 and PI 595203. These two accessions were among the genotypes with the lowest ELISA readings in both trials. In trial 1, PI 595200 was among the genotypes with the lowest ELISA reading, but this same accession was among the genotypes with the highest ELISA reading in trial 2.

Correlations between assessment methods (symptom severity and ELISA) were always positive and generally significant, but some correlations were noticeably stronger than others (Table 4). Among measurements of symptom severity, correlations were much stronger between the fifth vs. the seventh leaf ($r = 0.886$ in trial 1 and $r = 0.867$ in trial 2) compared with the first vs. the seventh leaf ($r = 0.442$ in trial 1 and $r = 0.607$ in trial 2). When the severity scores of either the first to the fifth leaf or the first to the seventh leaf were averaged, this value was reasonably well correlated even with scores from the first leaf ($r \geq 0.542$). Correlations between

Table 2. Trial 1: Means of enzyme-linked immunosorbent assay (ELISA) absorbance readings (405 nm) in the fourth leaf and symptom severity of leaves at various positions in watermelon accessions mechanically inoculated (first three leaves, 14 d after seeding) with a Puerto Rico isolate of *Zucchini yellow mosaic virus* (ZYMV).

Genotype ^y	ELISA-ZYMV ^x	Symptom severity ^z					
		First leaf	Third leaf	Fifth leaf	Seventh leaf	Mean of first to fifth leaf	Mean of first to seventh leaf
482261	0.79 b ^w	1.17 b	1.33 b	2.33 d	2.33 c	1.61 c	1.79 d
482276	0.50 ab	0.00 a	1.00 ab	2.00 cd	2.67 c	1.00 bc	1.42 cd
494528	0.43 ab	1.17 b	1.50 b	1.67 cd	1.17 b	1.44 c	1.38 cd
494529	0.55 b	0.50 a	0.33 a	0.67 ab	0.67 ab	0.50 ab	0.54 bc
537277	0.18 a	0.40 a	0.00 a	0.00 a	0.00 a	0.13 a	0.10 ab
560016	0.86 b	1.00 ab	1.00 ab	0.00 ab	0.00 ab	0.67 abc	0.50 abc
595200	0.42 ab	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a
595201	0.28 ab	0.40 a	0.00 a	0.00 a	0.00 a	0.13 a	0.10 ab
595203	0.28 ab	0.80 ab	0.20 a	0.00 a	0.00 a	0.33 a	0.25 ab
596662	0.52 ab	0.80 ab	1.20 b	1.40 bc	1.40 a	1.13 c	1.20 cd
LSD	0.399	0.596	0.939	0.868	0.928	0.600	0.617

^zSymptom severity measured 20 d postinoculation on a 0 to 3 scale where 0 = no symptoms, 1 = mild chlorosis or mottling, 2 = strong chlorosis or mottling, and 3 = severe virus symptoms including blistering and leaf deformity. Leaves numbered from youngest to oldest (first leaf is the first true leaf produced by the plant).

^yNumbers refer to PI numbers of the U.S. Department of Agriculture Plant Genetic Resources System.

^xFourth leaf sampled 28 d postinoculation.

^wWithin a column, means followed by a common letter are not different at the 0.05 *P* level according to Fisher's protected least significant difference (LSD) test.

Table 3. Trial 2: Means of enzyme-linked immunosorbent assay (ELISA) absorbance readings (405 nm) in the fourth leaf and symptom severity of leaves at various positions in watermelon accessions mechanically inoculated (first three leaves, 14 d after seeding) with a Puerto Rico isolate of *Zucchini yellow mosaic virus* (ZYMV).

Genotype ^y	ELISA-ZYMV ^x	Symptom severity ^z					
		First leaf	Third leaf	Fifth leaf	Seventh leaf	Mean of first to fifth leaf	Mean of first to seventh leaf
482261	0.65 bcde ^w	0.8 bc	1.4 cd	1.9 cd	2.0 cde	1.4 cd	1.5 d
482276	0.47 abcd	0.7 abc	1.3 cd	1.7 bcd	1.3 abc	1.2 cd	1.3 cd
485580	0.42 abc	1.0 cd	1.3 cd	2.5 d	2.6 de	1.6 de	1.8 def
494528	1.00 e	1.1 cd	1.8 de	1.1 abc	0.6 a	1.4 cd	1.2 cd
494529	0.93 de	0.9 c	2.0 def	1.9 cd	1.8 bcd	1.6 de	1.6 de
537277	0.10 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a
560016	0.84 cde	0.9 c	1.7 cde	2.0 cd	2.2 cde	1.5 cde	1.6 de
595200	0.71 cde	0.8 bc	1.6 cd	1.4 abc	0.9 ab	1.2 cd	1.2 cd
595201	0.47 abc	0.3 ab	0.6 ab	0.6 a	0.2 a	0.5 ab	0.4 ab
595203	0.25 ab	0.8 bc	1.1 bc	0.9 ab	0.7 a	0.9 bc	0.9 bc
596662	0.41 abc	0.9 c	1.7 cd	1.9 cd	2.0 cde	1.5 cd	1.6 de
CS	0.46 abc	1.5 de	2.4 ef	2.7 d	2.6 de	2.1 ef	2.2 ef
SB	0.39 abc	2.0 e	2.5 f	2.8 d	3.0 e	2.3 f	2.3 f
LSD	0.467	0.57	0.71	1.05	1.00	0.61	0.63

^zSymptom severity measured 20 d postinoculation on a 0 to 3 scale where 0 = no symptoms, 1 = mild chlorosis or mottling, 2 = strong chlorosis or mottling, and 3 = severe virus symptoms including blistering and leaf deformity. Leaves numbered from youngest to oldest (first leaf is the first true leaf produced by the plant).

^yNumbers refer to PI numbers of the U.S. Department of Agriculture Plant Genetic Resources System. CS = Crimson Sweet, SB = Sugar Baby.

^xFourth leaf sampled 28 d postinoculation.

^wWithin a column, means followed by a common letter are not different at the 0.05 *P* level according to Fisher's protected least significant difference (LSD) test.

symptom severity measured in different leaves and absorbance in the ZYMV assay using the fourth leaf were much smaller and sometimes not significant. The strongest correlation was only $r = 0.452$.

To determine if the weak correlation between symptom severity and ELISA was a general phenomenon or specific to certain genotypes, we graphed absorbance against symptom severity for each genotype across both trials. Because results were similar for each leaf scored, and some genotypes had not expanded to the seventh leaf, we present only the graph for ELISA (sampled in the fourth leaf) vs. symptom severity in the fifth leaf (Fig. 1). Most watermelon genotypes showed a range of absorbance readings, from low (clearly negative for ZYMV) to high (strongly positive for ZYMV). The only genotype with all plants testing negative (absorbance < 0.200) was PI 537277. In addition, all plants of this genotype had a severity score of 0 in the fifth leaf (Fig. 1) and in all other leaves as well (data not shown). Most plants of PI 595201 and PI 595203 had both low ELISA readings and low symptom

severity. However, there were exceptions. Some plants of these accessions had ELISA readings strongly positive for ZYMV, but almost all these cases were of plants with mild disease symptoms (severity of 0 or 1). Most plants of PI 595200 had a score of 0 or 1 for symptom severity, but some of these plants were strongly positive for ZYMV. The results for PI 494529 were similar to PI 595200, but more plants had severity scores of 2 or 3. The remaining accessions, as well as 'Crimson Sweet' and 'Sugar Baby', had highly variable ELISA readings and severity ratings, with little association between the two assessment methods. There were multiple cases of genotypes with plants with high absorbance readings (positive for ZYMV) but low severity (0 or 1). The reverse was also observed: 15 plants over the two trials had severity ratings of 2 or 3, but tested negative for ZYMV in the ELISA test.

Discussion

In agreement with Guner (2004) and Guner et al. (2019), we found PI 535277 to

be highly resistant to ZYMV (Tables 2 and 3; Fig. 1). This accession is classified as *Citrullus colocynthis* (L.) Schrad. a perennial species known as the desert watermelon (Levi et al., 2012). Among the tested plants of this accession, none exhibited disease symptoms. ZYMV was not detected in serological tests, suggesting the failure of virus infection and/or virus replication. In phylogenetic studies, both Levi et al. (2001) and Chomicki and Renner (2015) grouped accessions of *C. colocynthis* as quite genetically distinct from the other phylogenetic groups tested in our study. *C. colocynthis* can be crossed with watermelon only with difficulty (Levi et al., 2002). Therefore, deploying PI 533277 in a watermelon breeding program for ZYMV resistance will present challenges.

All other PIs showing good levels of ZYMV resistance in our screening belonged to either *C. lanatus* (PI595200, PI 595201) or the sister species [according to Chomicki and Renner (2015)] *Citrullus mucososperma* (PI 595203). PI 595203 should be of interest to watermelon breeders because it has been

Table 4. Correlation coefficients between enzyme-linked immunosorbent assay (ELISA) absorbance readings (405 nm) in the fourth leaf and symptom severity of leaves at various positions in watermelon plants mechanically inoculated (first three leaves, 14 d after seeding) with a Puerto Rico isolate of *Zucchini yellow mosaic virus* (ZYMV).

		Symptom severity				
Symptom severity	ELISA-ZYMV ^z	First leaf	Third leaf	Fifth leaf	Seventh leaf	Mean of first, third, and fifth leaf
Trial 1						
First leaf	0.209					
Third leaf	0.249	0.442**				
Fifth leaf	0.367**	0.362*	0.788**			
Seventh leaf	0.452**	0.301*	0.638**	0.886**		
Mean of first, third, and fifth leaf	0.341*	0.627**	0.919**	0.918**	0.783**	
Mean of first, third, fifth and seventh leaf	0.399**	0.542**	0.866**	0.954**	0.903**	0.974**
Trial 2						
First leaf	0.242					
Third leaf	0.424**	0.607**				
Fifth leaf	0.397**	0.466**	0.693**			
Seventh leaf	0.201	0.370**	0.562**	0.867**		
Mean of first, third, and fifth leaf	0.445**	0.763**	0.898**	0.890**	0.746**	
Mean of first, third, fifth, and seventh leaf	0.409**	0.686**	0.844**	0.933**	0.883**	0.976**

²Fourth leaf sampled 28 d postinoculation.

*, **Significant at $\alpha = 0.05$ and 0.01 , respectively.

Fig. 1. Enzyme-linked immunosorbent assay (ELISA) absorbance readings (405 nm) in the fourth leaf and symptom severity in the fifth leaf of individual plants of watermelon accessions inoculated with a Puerto Rico isolate of *Zucchini yellow mosaic virus* (ZYMV). Genotype numbers refer to PI numbers of the U.S. Department of Agriculture Plant Genetic Resources System. CS = Crimson Sweet, SB = Sugar Baby. ELISA readings below the lower horizontal line are considered negative for ZYMV; readings above the upper horizontal line are considered positive for ZYMV. Some points on the graph cover other points with a similar or equal absorbance reading.

shown to also be resistant to both ZYMV-CH (Xu et al., 2004) and ZYMV-FL (Guner 2004; Guner et al., 2019; Ling et al., 2006). Guner (2004) found this PI to be the most resistant of 1644 cultigens tested. PI 595203

has been shown to have a single nucleotide polymorphism mutation in the eIF4E genomic region of watermelon closely associated with resistance to ZYMV (Harris et al., 2009; Ling et al., 2009). USVL-370, a breeding line

with PI 595203 as the source of resistance to ZYMV-FL, was recently released (Levi et al., 2016).

The remaining PIs tested showed strong disease symptoms and/or high ELISA readings

even though previous studies had shown them to be resistant. Provvidenti (1991) found a single recessive gene for resistance to ZYMV-FL in PI 483361, and Boyhan et al. (1992) confirmed its resistance. But like Guner and Wehner (2008), our study did not confirm this resistance. In fact, we found this accession to be among the most susceptible (Tables 2 and 3; Fig. 1). Likewise, we found PI 482276, PI 485580, PI 494528, PI 560016, and PI 595662 to be susceptible to ZYMV-PR, whereas previous studies had found these accessions to be resistant (Boyhan et al., 1992; Guner, 2004; Guner and Wehner, 2008; Guner et al., 2019). Possible explanations include a differential response to ZYMV isolates, with these PIs being resistant to ZYMV-FL but not to ZYMV-PR. Another possible explanation is that, for a specific accession, the genetic composition of plants tested in our study differs from those in previous studies. Watermelon is assumed to be a highly cross-pollinated crop (Kumar et al., 2013). Thus, many accessions in the USDA collection are likely to be a composite of multiple genotypes.

How ZYMV-PR compares with ZYMV isolates used in previous screening studies is difficult to determine because tracing back previous isolates is problematic. ZYMV-PR produces severe symptoms in susceptible cucurbits. Guner (2004) described the isolate ZYMV-FL he used for screening as one producing severe symptoms and deriving from a subculture of isolate 2088 received from E. Hiebert at the University of Florida. Therefore, ZYMV-FL used in Guner (2004), Guner and Wehner (2008), and Guner et al. (2019) was likely the same as the isolate designated “SV” (for “severe”) in the University of Florida study by Wisler et al. (1995). The screening studies of Provvidenti (1986, 1991) do not specify where he obtained his isolate of ZYMV-CT and ZYMV-FL, but it is likely these isolates were collected in Connecticut in 1982 and Florida in 1983, respectively, and reported in Provvidenti et al. (1984). The screening by Boyhan et al. (1992) used ZYMV-FL obtained from R. Provvidenti. The inheritance study by Xu et al. (2004) used an isolate from R. Provvidenti who confirmed it to be ZYMV-CH. Confirmation was likely done using serology, line differentials, and/or biological reaction, not sequencing. Wisler et al. (1995) described the P1 protein of SV (=ZYMV-FL) and deposited the sequence as GenBank: L35589.1 (“Florida severe”). No genomic comparison can be made between ZYMV-FL and ZYMV-PR because the latter was characterized using the coat protein (CP) and not the P1 protein. The CP region of the genome is the most widely used for *Potyvirus* (Adams et al., 2005; Shukla and Ward, 1988). The ZYMV-PR CP gene fragment showed a high percentage of identity to ZYMV isolates recently reported in squash in Iran (98.17%), Turkey, Venezuela, and Egypt, and in bees in Australia (97.72%) (Roberts et al., 2018). Our work appears to be the only reported

watermelon screening study in which the ZYMV isolate used has had its CP sequence clearly documented. Considering the advances in sequencing and the importance of this virus to cucurbits in general, it would be useful to obtain the full genomes of ZYMV-FL, ZYMV-CT, and ZYMV-PR to better compare these isolates.

The response of plants exposed to virus can be expressed in terms of the behavior of the virus within the plant and in terms of the disease response of the plant (Bos and Parlevliet, 1995; Cooper and Jones, 1983). The ideal assessment method from the plant breeder’s perspective is one that is easy to carry out, reliably indicates the virus’s ability to infect and/or replicate within the plant, consistently measures the disease response of the plant, and can be obtained at the earliest moment possible in the life cycle of the plant. In this study, symptom severity assessed on either the first or third leaf of a seedling was often not indicative of degree of severity on later leaves. This was especially the case when the first few leaves showed few or no symptoms. However, once a leaf exhibited strong disease symptoms in any leaf, the following leaves generally expressed the same or greater symptom severity (data not shown).

ELISA readings were not reported in previous studies assessing resistance among watermelon accessions (Boyhan et al., 1992; Guner, 2004; Guner and Wehner, 2008; Provvidenti, 1986, 1991), although they were used in studies of inheritance of ZYMV resistance in PI 595203 (Ling et al., 2006; Xu et al., 2004) and for assessing presence or absence of virus in individual accessions in Guner et al. (2019). We believe that ELISA absorbance readings offer an additional tool for assessing virus resistance in watermelon accessions. The simultaneous use of more than one technique to assess a plant’s response to a virus should be encouraged among plant breeders. In some cases, we observed plants with severity ratings of 0 or 1, but strongly positive absorbance readings. This suggests that virus replication was occurring within these plants without the usual development of symptoms and damage to plant tissue. The reverse situation was also observed in those plants with strong symptoms but with ELISA readings negative for ZYMV. To rule out the possibility of accidental contamination with *Papaya ringspot virus* (PRSV), these plants were tested by ELISA for PRSV. Together with ZYMV, PRSV is the other most common cucurbit virus in Puerto Rico (Paz-Carrasco and Wessel-Beaver, 2002). All readings for PRSV were negative in these symptomatic plants. Astier et al. (2007) points out that virus titer within a plant varies over time and in different parts of the plant. Serological tests sometimes fail to detect the virus in highly symptomatic leaves. Nevertheless, serological assays potentially offer the breeder an easy means of quantifying the degree to which a virus has replicated in a plant. The ideal ZYMV resistant genotype would be one

that limits the replication of a virus within a plant and minimizes the intensity of disease symptoms when subjected to a variety of strains of the virus. Genotypes showing resistance to multiple virus strains, such as PI 595203, should rank high as sources of resistance.

Literature Cited

- Adams, M.J., J.F. Antoniwi, and C.M. Fauquet. 2005. Molecular criteria for genus and species discrimination within the family *Potyviridae*. *Arch. Virol.* 150:459–479.
- Ali, A., O. Abdalla, B. Bruton, W. Fish, E. Sikora, S. Zhang, and M. Taylor. 2012. Occurrence of viruses infecting watermelon, other cucurbits and weeds in the parts of the Southern United States. *Plant Health Prog.* 13(1). <<https://aps-journals.apsnet.org/doi/pdfplus/10.1094/PHP-2012-0824-01-RS>>.
- Antignus, Y., B. Raccach, A. Gal-on, and S. Cohen. 1989. Biological and serological characterization of *Zucchini yellow mosaic* and *Watermelon mosaic virus-2* isolates in Israel. *Phytoparasitica* 17(4):289–298.
- Astier, S., J. Albouy, Y. Maury, C. Robaglia, and H. Lecoq. 2007. Principles of plant virology: Genome, pathogenicity, virus ecology. Science Publishers, Enfield, NH.
- Bananej, K. and A. Vahdat. 2008. Identification, distribution and incidence of viruses in field-grown cucurbit crops of Iran. *Phytopathol. Mediterr.* 47:247–257.
- Bos, L. and J.E. Parlevliet. 1995. Concepts and terminology in plant/pest relationships: Toward consensus in plant pathology and crop protection. *Annu. Rev. Phytopathol.* 33:69–102.
- Boyhan, G., J.D. Norton, B.J. Jacobsen, and B.R. Abrahams. 1992. Evaluation of watermelon and related germ plasm for resistance to zucchini yellow mosaic virus. *Plant Dis.* 76:251–252.
- Castle, S.J., T.M. Perring, C.A. Farrar, and A.N. Kishaba. 1992. Field and laboratory transmission of watermelon mosaic virus 2 and zucchini yellow mosaic virus by various aphid species. *Phytopathology* 82:235–240.
- Chang, Y.M., C.H. Hsiao, W.Z. Yang, S.H. Hseu, Y.J. Chao, and C.H. Huang. 1987. The occurrence and distribution of five cucurbit viruses on melon and watermelon in Taiwan. *J. Agr. Res. China* 36(4):389–397.
- Chomici, G. and S.S. Renner. 2015. Watermelon origin solved with molecular phylogenetics including Linnaean material: Another example of museomics. *New Phytol.* 205:526–532.
- Cooper, J.I. and A.T. Jones. 1983. Responses of plants to viruses: Proposals for the use of terms. *Phytopathology* 73(2):127–128.
- Davis, R.F. and M.K. Mizuki. 1987. Detection of cucurbit viruses in New Jersey. *Plant Dis.* 71:40–44.
- Desbie [sic], C. and H. Lecoq. 1999. Evolution of zucchini yellow mosaic potyvirus (ZYMV) variability on Martinique Island since its first detection. Noble Foundation, Virus Evolution Workshop, Ardmore, OK, 21–24 Oct. 1999.
- Desbiez, C. and H. Lecoq. 1997. Zucchini yellow mosaic virus. *Plant Pathol.* 46:809–829.
- Desbiez, C., C. Wipf-Scheibel, and H. Lecoq. 2002. Biological and serological variability, evolution and molecular epidemiology of *Zucchini yellow mosaic virus* (ZYMV, *Potyvirus*) with special reference to Caribbean islands. *Virus Res.* 85:5–16.
- Desbiez, C., C. Wipf-Scheibel, C. Robaglia, T. Delaunay, and H. Lecoq. 1996. Biological and molecular variability of zucchini yellow

- mosaic virus on the island of Martinique. *Plant Dis.* 80:203–207.
- Di Rienzo, J.A., F. Casanoves, M.G. Balzarini, L. Gonzalez, M. Tablada, and C.W. Robledo. 2019. InfoStat versión 2019. Centro de Transferencia InfoStat, FCA, Universidad Nacional de Córdoba, Argentina. 8 July 2020. <<http://www.infostat.com.ar>>.
- Escudero, J. 1992. Situación de los virus que afectan a las cucurbitáceas en la costa sur de Puerto Rico, p. 44–51. In: *El cultivo de cucurbitáceas: Memorias del foro técnico*, June 26, 1992, Lajas, Puerto Rico, Universidad de Puerto Rico, Recinto Universitario de Mayagüez, Estación Experimental Agrícola.
- Guner, N. 2004. Papaya ringspot virus watermelon strain and zucchini yellow mosaic virus resistance in watermelon. North Carolina State University, Raleigh, Ph.D. Diss. 8 July 2020. <<http://repository.lib.ncsu.edu/ir/bitstream/1840.16/3271/1/etd.pdf>>.
- Guner, N. and T. Wehner. 2008. Overview of Potyvirus resistance in watermelon, p. 445–451. In: M. Pitrat (ed.). *Proceedings of the IXth EUCARPIA Mtg. on Genetics and Breeding of Cucurbitaceae*, Institut National de la Recherche Agronomique (INRA), Avignon (France), 21–24 May 2008.
- Guner, N., Z. Pesic-VanEsbroeck, L.A. Rivera-Burgos, and T.C. Wehner. 2019. Screening for resistance to *Zucchini yellow mosaic virus* in watermelon germplasm. *HortScience* 54:206–211.
- Guner, N., L.A. Rivera-Burgos, and T.C. Wehner. 2018. Inheritance of resistance to *Zucchini yellow mosaic virus* in watermelon. *HortScience* 53:1115–1118.
- Harris, K.R., K.S. Ling, W.P. Wechter, and A. Levi. 2009. Identification and utility of markers linked to the zucchini yellow mosaic virus resistance gene in watermelon. *J. Amer. Soc. Hort. Sci.* 134:529–534.
- Juarez, M., P. Legua, C.M. Mengual, M.A. Kassem, R.N. Sempere, P. Gómez, V. Truniger, and M.A. Aranda. 2013. Relative incidence, spatial distribution and genetic diversity of cucurbit viruses in eastern Spain. *Ann. Appl. Biol.* 162(3):362–370.
- Kumar, R., M. Dia, and T.C. Wehner. 2013. Implications of mating behavior in watermelon breeding. *HortScience* 48:960–964.
- Levi, A., C.E. Thomas, A.P. Keinath, and T.C. Wehner. 2001. Genetic diversity among watermelon (*Citrullus lanatus* and *Citrullus colocynthis*) accessions. *Genet. Resources Crop Evol.* 48:559–566.
- Levi, A., C.E. Thomas, T. Joobeur, X. Zhang, and A. Davis. 2002. A genetic linkage map for watermelon derived from a testcross population: (*Citrullus* var. *citroides* x *C. lanatus* var. *lanatus*) x *Citrullus colocynthis*. *Theor. Appl. Genet.* 105:555–563.
- Levi, A., J.A. Thies, W.P. Wechter, C. Kousik, K. Ling, H. Harrison, A. Simmons, U.K. Reddy, P. Nimmakayala, Z. Fei, S. Mitchel, Y. Xu, K. Tadmor, and K. Katzir. 2012. Exploiting genetic diversity in *Citrullus* spp. to enhance watermelon cultivars, p. 41–48. In: S. Sari, I. Solmaz, and V. Aras (eds.). *Proceedings of the Xth EUCARPIA Meeting on Genetics and Breeding of Cucurbitaceae*. 15–18 Oct. 2012, Antalya, Turkey. Çukurova University, Adana, Turkey.
- Levi, A., K.R. Harris-Shultz, and K.-S. Ling. 2016. USVL-370, a *Zucchini yellow mosaic virus*-resistant watermelon breeding line. *HortScience* 51:107–109.
- Ling, K.S., A. Levi, N. Guner, and T. Wehner. 2006. Evaluating *Zucchini yellow mosaic virus* resistance in watermelon, p. 468–472. In: G.J. Holmes (ed.). *Cucurbitaceae 2006*, Asheville, NC, 17–21 Sept. 2006. Universal Press, Raleigh, NC.
- Ling, K.S., K.R. Harris, J.D.F. Meyer, A. Levi, N. Guner, T.C. Wehner, A. Bendahmane, and M.J. Havey. 2009. Non-synonymous single nucleotide polymorphisms in the watermelon eIF4E gene are closely associated with resistance to zucchini yellow mosaic virus. *Theor. Appl. Genet.* 120:191–200.
- Lisa, V., G. Boccardo, G. D'Agostino, G. Della-valle, and M. d'Aquilio. 1981. Characterization of a potyvirus that causes Zucchini Yellow Mosaic. *Phytopathology* 71:667–672.
- Nameth, S.T., J.A. Dodds, A.O. Paulus, and A.K. Kishaba. 1985. Zucchini Yellow Mosaic Virus associated with severe diseases of melon and watermelon in Southern California desert valleys. *Plant Dis.* 69:785–788.
- Paz-Carrasco, L. and L. Wessel-Beaver. 2002. Survey of *Cucurbita* viruses found in Puerto Rico, p. 256–264. In: D.N. Maynard (ed.). *Cucurbitaceae 2002*. ASHS Press, Alexandria, VA.
- Provvidenti, R., D. Gonsalves, and H.S. Humaydan. 1984. Occurrence of *Zucchini yellow mosaic virus* from Connecticut, New York, Florida and California. *Plant Dis.* 68(5):443–446.
- Provvidenti, R. 1986. Reaction of accessions of *Citrullus colocynthis* from Nigeria to Zucchini yellow mosaic virus. *Cucurbit Genet. Coop. Rpt.* 9:82–83.
- Provvidenti, R. 1991. Inheritance of resistance to the Florida strain of Zucchini yellow mosaic virus in watermelon. *HortScience* 26:407–408.
- Rabelo Filho, F. de A.C., K.F. Carvalho, J.A. de A. Lima, M.A. de Queiroz, W.O. de Paiv, and A.K.Q. do Nascimento. 2010. Fontes de resistência em melancia e meloeiro a vírus do gênero *Potyvirus*. *Revista Brasileira de Ciências Agrárias (Agrária)* 5(2):187–191.
- Roberts, J.M.K., K.B. Ireland, W.T. Tay, and D. Paini. 2018. Honey-assisted surveillance for early plant virus detection. *Ann. Appl. Biol.* 173(3):285–293.
- Shukla, D.D. and C.W. Ward. 1988. Amino acid sequence homology of coat proteins as a basis for identification and classification of the potyvirus group. *J. Gen. Virol.* 69:2703–2710.
- Topkaya, Ş. and F. Ertunç. 2012. Cucurbitaceae 2012. Current status of virus infections in cucurbit plantations in Ankara and Antalya provinces, p. 759–762. In: S. Sari, I. Solmaz, and V. Aras (eds.). *Proceedings of the Xth EUCARPIA Meeting on Genetics and Breeding of Cucurbitaceae*, Antalya, Turkey, 15–18 Oct. 2012. Çukurova University, Adana, Turkey.
- Vučurović, A., A. Bulajić, I. Stanković, D. Ristić, D. Nikolić, J. Berenji, and B. Krstić. 2012. First report of *Zucchini yellow mosaic virus* in watermelon in Serbia. *Plant Dis.* 96(1):149.
- Wisler, G.C., D.E. Purcifull, and E. Hiebert. 1995. Characterization of the P1 protein and coding region of zucchini yellow mosaic virus. *J. Gen. Virol.* 76:37–45.
- Xu, Y., Z. Kang, Z. Shi, H. Shen, and T. Wehner. 2004. Inheritance of resistance to zucchini yellow mosaic virus and watermelon mosaic virus in watermelon. *J. Hered.* 95(6):498–502.