HortScience 42(5):1124–1126. 2007.

Sources of Resistance to *Zucchini Yellow Mosaic Virus* in *Lagenaria siceraria* Germplasm

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Additional index words. bottlegourd, *Citrullus lanatus*, cucurbits, rootstock, watermelon

***Abstract*. One-hundred ninety U.S. PIs of bottlegourd [*Lagenaria siceraria* (Mol.) Standl.] were evaluated for their resistance to the Florida strain of *Zucchini yellow mosaic virus* (ZYMV-FL). Seedlings in the first leaf stage were mechanically inoculated with freshly prepared ZYMV-FL tissue extract in a greenhouse. Four weeks postinoculation, plants were visually evaluated for symptom expression and tissue samples from upper noninoculated leaves were collected for serological analysis with enzyme-linked immunosorbent analysis (ELISA). A combination of symptom expression and ELISA value was considered in determining the resistance or susceptibility for each accession. Of the 190 *L. siceraria* PIs screened, 36 accessions were in complete resistance (no disease symptom with negative ELISA on all tested plants), 64 PIs showed partial resistance (some of the tested plants were resistant, whereas others were susceptible), and 90 PIs were susceptible (severe symptom and positive ELISA on all tested plants). The ZYMV-FL resistance exists mostly among *L. siceraria* PIs collected in India. Thirty-three of the 36 *L. siceraria* PIs showing ZYMV-FL resistance were collected in India, one in Indonesia, one in South Africa, and one in Zimbabwe. To rule out any potential escapes in the primary screening, a repeated test using representative accessions, including 10 susceptible, three partially resistant, and three completely resistant PIs, was done to confirm the ZYMV-FL resistance. Furthermore, the resistance to ZYMV-FL was shown to be heritable in progenies generated through self-pollination of single plants in each of five resistant PIs as well as in three F1 hybrids.**

Zucchini yellow mosaic virus (ZYMV), a member of genus *potyvirus* in the family *Potyviridae,* is one of the major viruses on cucurbits. Cultivars of *Cucurbita pepo*, *Cucumis melo*, and *Citrullus lanatus* are particularly affected. However, all other cucurbits, including bottlegourd [*Lagenaria siceraria* (Mol.) Standl.], are vulnerable to ZYMV infection. Natural infection of bottlegourd by ZYMV has been reported in Hawaii (Ullman et al., 1991), India (Verma et al., 2004), and Serbia (Dukić et al., 2006). Provvidenti et al. (1984) identified two major ZYMV strains in the United States (ZYMV-FL and ZYMV-CT) and showed that although ZYMV-CT incites more severe disease symptoms, its distribution is limited to the northeast. ZYMV-FL is the most prevalent strain in cucurbit crops in North America.

In many cases, planting a disease-resistant cultivar is the best solution for controlling virus diseases in vegetable crops. Resistance to ZYMV in bottlegourd exists in germplasm collected throughout the world (Gerber, 1978; Provvidenti, 1977, 1995). However, the only major test for virus resistance in *L. siceraria* was carried out with 18 accessions (Provvidenti, 1981). It is therefore necessary to screen U.S. *L. siceraria* germplasm to identify potential sources of disease resistance that may be useful in developing different bottlegourd lines as vegetables or as rootstocks for watermelon grafting. The objective of this study was to evaluate the U.S. PI collection (190 PIs) of *L. siceraria* against ZYMV-FL infection. We report the identification of *L. siceraria* PIs resistant to ZYMV-FL.

Materials and Methods

Virus isolate and inoculation. The ZYMV-FL culture (provided by Dr. Todd Wehner, North Carolina State University) was derived from the original ZYMV-FL strain isolated by Provvidenti et al. (1984). The virus was propagated and maintained on Gray zucchini squash (*Cucurbita pepo* L.). Virus inoculum was prepared by macerating virus-infected leaves (1:5 w/v) in 0.02 M phosphate-buffered saline, pH 7.4, with a mortar and pestle. Seedlings were inoculated by lightly dusting the leaves with carborundum. Then, they were mechanically rubbed with a cotton swab soaked in the virus inoculum. Application involved several circular motions until the entire leaf was covered with the inoculum. Excess carborundum was rinsed with water and the inoculated seedlings were placed under the shade for a few hours to minimize direct sunlight damage to the newly inoculated leaves. A repeated inoculation was performed within 2 weeks. Four weeks after the initial inoculation, plants were evaluated for symptom expression (Fig. 1) and the presence of ZYMV was analyzed using enzyme-linked immunosorbent assay (ELISA). Both results were considered in determining the resistance or susceptibility. Resistance was designated as all the tested plants in an accession that remained free of symptom expression and negative ELISA for ZYMV (apparently immune). Partial resistance was designated when only a portion of seedlings tested in an accession remained free from ZYMV infection as indicated by symptom and ELISA. When all the tested plants in an accession were infected, it was then considered susceptible.

Fig. 1. Symptom expression on the susceptible (three leaves on the left) or resistant (last leaf on the right) *Lagenaria siceraria* to *Zucchini yellow mosaic virus* infection.

Received for publication 18 Dec. 2006. Accepted for publication 25 Feb. 2007.

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Evaluation of L. siceraria *accessions*. The 190 accessions representing the majority of the available U.S. PI collection of *L. siceraria* germplasm originated from 17 countries (including Argentina, China, Cyprus, Ethiopia, Greece, Honduras, India, Indonesia, Iraq, Israel, Mexico, Guatemala, South Africa, Syria, United States, Yugoslavia, and Zimbabwe) were supplied by the USDA Southern Plant Introduction Station in Griffin, GA. Five seeds of each of the 190 *L. siceraria* PI accessions were planted in an insect-free greenhouse with temperature 20 to 30 °C and natural lighting period of 14 to 16 h at the U.S. Vegetable Laboratory in Charleston, SC. Depending on the genotype, not all the seeds planted were able to germinate. Plants were evaluated for reaction to ZYMV-FL in an unreplicated test using symptom expression and ELISA.

Repeated test. Selected accessions representing the susceptible, partially resistant, or resistant groups were reevaluated in a repeated test in a greenhouse. This test included 10 susceptible (PI 181948, PI 280632, PI 368636, PI 370474, PI 379367, PI 406857, PI 451857, PI 458736, PI 491354, and PI 534555), three partially resistant (PI 270456, PI 491346, and PI 368635), and three resistant accessions (PI 381825, PI 381831, and PI 381834) in the initial test. Fifteen seeds of each accession were planted and the plants were evaluated for reactions to ZYMV-FL with symptom expression and ELISA. Watermelon cultivars, 'New Hampshire Midget' and 'Calhoun Grey' (*Citrullus lanatus var. lanatus*), were included as susceptible reference checks and PI 595203 (*C. lanatus var. lanatus*) was used as the ZYMV-resistant control.

Progenies from single plant selection and F1 hybrids. To test whether the identified resistance to ZYMV is inheritable to the progenies, two selected resistant plants from each of the five resistant or partially resistant PIs (PI 271360, PI 381825, PI 381831, PI 381834, and PI 368635) were saved and used for self-pollination and for making F1 hybrids (PI 381825 × PI 368635, PI 381834 × PI 381825). F1 progenies generated from a cross made between the resistant (PI 381831) and the susceptible accession (PI 181948) were also used to test for ZYMV resistance.

Enzyme-linked immunosorbent assay. ELISA was performed according to the manufacturer's instructions (BioReba, Reinach, Switzerland). Microtiter plates were first coated with 1 μg/ml of ZYMV antibody, and virus particles were trapped after incubating the prepared tissue extract on the coated plates. Leaf extract was prepared by processing the tissue samples collected from the upper noninoculated leaves in tissue extraction buffer (1:20 w/v) with a homogenizer, Homex-6 (BioReba). The alkaline phosphatase conjugated antibody to ZYMV was then added to the plate. Finally, the yellow color (from enzyme-substrate hydrolysis), which developed in positive samples, was measured with an ELISA reader, SpectraMax Plus 384 (Molecular Devices, Sunnyvale, CA). A sample with absorbance value (OD405 nm) of at least twice the mean health plant controls was regarded as positive.

Results and Discussion

Primary screening. The results generated from the primary screening of the 190 accessions for ZYMV resistance could be classified into three distinct groups: 1) complete resistance (36 accessions); 2) partial resistance (64 accessions); and 3) susceptible (90 accessions) (Table 1). The control watermelon cultivars ('New Hampshire Midget' and 'Calhoun Grey') were highly susceptible. As expected, PI 595203 was resistant. The high percentage (19%) of accessions with complete resistance to ZYMV-FL infection was surprising. Among these 36 completely resistant accessions, 33 were collected in India (Table 1). The other three (PI 280633, PI 470260, and PI 491352) originated in different regions, South Africa, Indonesia, and Zimbabwe, respectively.

Repeated test. Sixteen accessions representing the susceptible (10 PIs), partially resistant (3 PIs), and resistant groups (3 PIs) in the primary screening were reevaluated for their resistance to ZYMV-FL in a greenhouse. The data generated from the repeated test were in general agreement with the primary screening (Table 2). The three resistant accessions (PI 381825, PI 381831, and PI 381834) were still in complete resistance. PI 368635, which was in partially resistance in the primary test, was also in partially resistance in the repeated test. All of the susceptible lines were in susceptible or in partially resistant in the repeated test. These repeatable results indicated that the resistance screening was effective.

Zucchini yellow mosaic virus *resistance in the selected lines was inheritable*. All the progenies generated from three single plant selected lines (3, 4, and 5), as well as two F1 hybrids (6 and 7) showed complete resistance to ZYMV infection (Table 3). Two other lines (1 and 2) were still segregating for the resistance, which would require additional single plant selection to obtain stable resistance to ZYMV. The result in line 8 showed that resistance to ZYMV in PI 381831 was transferable to the susceptible plants in PI 181948 (line 10) in the F1 population. Although the inheritance of ZYMV resistance in *L. siceraria* is still unknown, resistant in PI 381831 may be dominant, because many F1 plants (nine of 16) were not infected or the virus titer in the infected plants (mean absorbance value, 0.230) was much lower when compared with the susceptible parent (mean, 2.196) (Table 3). Additional experiments are underway to generate F2 and backcross populations for more definite

Table 1. Evaluation of *Lagenaria siceraria* accessions for their resistance against *Zucchini yellow mosaic virus*.[z]

Resistant PI (36/190)[y]	Partially Resistant PI (64/190)[x]	Susceptible PI (90/190)[w]
271351, 271352, 271354, 271356, 271357, 271359, 271360, 271477, 280633, 381823, 381824, 381825, 381826, 381828, 381829, 381831, 381832, 381834, 381835, 381836, 381837, 381838, 381839, 381840, 381842, 381843, 381844, 381845, 381846, 381847, 381848, 381849, 381851, 470260, 491352, 636137	179298, 269507, 269508, 271353, 273662, 273663, 280631, 280632, 368635, 368636, 368640, 379365, 381821, 381822, 381827, 381830, 381854, 406857, 419089, 419215, 458736, 491274, 491280, 491281, 491283, 491286, 491287, 491288, 491289, 491295, 491297, 491298, 491300, 491302, 491303, 491306, 491314, 491315, 491316, 491318, 491319, 491320, 491321, 491322, 491323, 491325, 491328, 491329, 491332, 491337, 491338, 491339, 491340, 491341, 491345, 491346, 491349, 491350, 491351, 491353, 500818, G-11933, G-11938,G-11942	170463, 181948, 269505, 270456, 280636, 287534, 358056, 358059, 368638, 368639, 370474, 370477, 370478, 379367, 381850, 419090, 432340, 432341, 432342, 435291, 438844, 438846, 438847, 442368, 442369, 451856, 451857, 487482, 491252, 491266, 491267, 491268, 491269, 491270, 491271, 491272, 491273, 491275, 491276, 491277, 491278, 491279, 491282, 491290, 491291, 491292, 491293, 491294, 491296, 491299, 491304, 491305, 491307, 491308, 491309, 491310, 491311, 491312, 491313, 491317, 491324, 491326, 491327, 491330, 491331, 491333, 491334, 491335, 491336, 491342, 491343, 491344, 491347, 491348, 491354, 534552, 534553, 534554, 534555, 534556, 639723, 641946, 642039, 642040, 642041, 642042, 642044, 642045, G-11931, G-11936

[z]These PIs were divided into three groups based on their responses to *Zucchini yellow mosaic virus* infection.
[y]Resistant: all the tested plants in an accession were resistant (resistant/total accessions tested).
[x]Partially resistant: one or several but not all the tested plants in an accession were resistant (partially resistant/total accessions tested).
[w]Susceptible: all the tested plants in an accession were infected (susceptible/total accessions tested).

Table 2. A repeated test with selected accessions confirmed the resistant potential in *Lagenaria siceraria* to *Zucchini yellow mosaic virus*.

		Primary screening			Repeated test		
PI No.[z]	Origin	n[y]	S[x]	R[x]	n[y]	S[x]	R[x]
181948*	Syria	6	6	0	3	3	0
280632	S. Africa	1	1	0	1	0	1
368636	Yugoslavia	6	6	0	5	4	1
370474	Yugoslavia	9	9	0	5	4	1
379367	Yugoslavia	12	12	0	4	4	0
406857	Honduras	5	5	0	5	3	2
451857	Guatemala	6	6	0	2	2	0
458736	Argentina	4	4	0	5	4	1
491354	Zimbabwe	9	9	0	4	4	0
534555	Syria	3	3	0	5	5	0
270456	Mexico	10	8	2	5	5	0
491346	Zimbabwe	12	7	5	1	0	1
368635*	Yugoslavia	6	2	4	4	1	3
381825*	India	12	0	12	5	0	5
381831*	India	9	0	9	2	0	2
381834*	India	13	0	13	5	0	5

[z]USDA P.I. accessions number. [y]Number of plants tested under this accession.
[x]Number of ZYMV susceptible (S) or resistant (R) plants determined by symptom expression and confirmed by enzyme-linked immunosorbent assay.
*Plants in these accessions were saved for single plant selection and for making crosses.

Table 3. Analysis of resistance to *Zucchini yellow mosaic virus* in selected *Lagenaria siceraria* accessions using progenies generated from single plant selection or their F1 hybrids.

			Susceptible		Resistance	
Line[z]	Pedigree	n[y]	S[x]	(mean ELISA)[w]	R[x]	(mean ELISA)[w]
		Lagenaria siceraria				
1	271360	18	7	(0.749)	11	(0.054)
2	368635	4	1	(1.101)	3	(0.024)
3	381825	4	0	(NA)	4	(0.072)
4	381831	3	0	(NA)	3	(0.030)
5	381834	18	0	(NA)	18	(0.031)
6 (F1)	381825 × 368635	4	0	(NA)	4	(0.038)
7 (F1)	381834 × 381825	9	0	(NA)	9	(0.044)
8 (F1)	181948 × 381831	16	7	(0.230)	9	(0.047)
9	271353	5	1	(1.270)	4	(0.019)
10	181948	9	8	(2.196)	1	(0.032)
		Citrullus lanatus var. citroides				
	482261	10	5	(1.755)	5	(0.047)
		C. lanatus var. lanatus controls				
	595203	4	0	(NA)	4	(0.064)
	N.H. Midget	7	7	(1.473)	0	(NA)
	Calhoun Grey	7	7	(1.073)	0	(NA)

[z]Breeding lines designation, progenies generated from single plant selection or F1 hybrids.
[y]Number of plants tested under each line.
[x]Number of susceptible (S) or resistant (R) plants was determined by their ELISA absorbance readings. A reading with at least twice of that of the health control and above 0.100 was considered susceptible (S). Resistant (R) plants were apparently immune, neither with symptom expression nor with detectable level of virus accumulation in ELISA.
[w]The value presented here was an average of the ELISA absorbance readings collected on these plants assigned in that particular R or S group.
ELISA = enzyme-linked immunosorbent assay; NA = no available plant assigned to that particular group.

determination. Our test also confirmed the ZYMV resistance in the previously identified accession, PI 271353 (Provvidenti et al., 1984). However, under our testing conditions, this line (9) was defined as partially resistant because only four of the five tested plants were actually free from systemic ZYMV infection (Table 3). The partial resistance was also confirmed in PI 482261, a *C. lanatus var. citroides* genotype that was previous identified (Provvidenti, 1991). The test also confirmed ZYMV resistance in PI 595203, a *C. lanatus var. lanatus* (Boyhan et al., 1992; Guner, 2004). The total infection in the susceptible controls, including plants in the cultivars 'New Hampshire Midget' and 'Calhoun Grey', indicated that our inoculation technique was thorough and sufficient.

Rootstock grafting has become a common practice, vital in overcoming soilborne diseases in fruit-bearing vegetables. In Asia, rootstock grafting is commonly used in the cultivation of *Cucurbitaceae* crops, including watermelon. In recent years, there has been an increasing interest in the United States to use grafted watermelon for production. Grafting watermelon on different cucurbits proved effective in controlling soilborne diseases and in enhancing fruit production and quality (Roberts et al., 2005; Roberts et al., 2006). Bottlegourd is proven to be a valuable rootstock for watermelon grafting. The PIs identified in this study might be useful in genetic programs aiming to enhance disease and pest resistance of bottlegourd lines used as rootstocks for watermelon grafting.

Conclusions

The result in the present study demonstrates that there is significant genetic resistance to ZYMV in U.S. *L. siceraria* germplasm collections. Numerous *L. siceraria* accessions were identified as potential sources of resistance to ZYMV-FL. As watermelon grafting becomes more popular in the United States, demands for disease-resistant rootstocks will increase. Thus, future germplasm evaluation for resistance to cucurbit viruses should focus on *L. siceraria* accessions.

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