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

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Abstract. One-hundred ninety U.S. PIs of bottle gourd [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 7 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.

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 symptoms 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 symptoms and ELISA.

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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.
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 535455), 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 accessions (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, Roineh, 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 resistance to ZYMV was used as the ZYMV-FL infection.

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

<table>
<thead>
<tr>
<th>Resistant PI (36/190)</th>
<th>Partially Resistant PI (64/190)</th>
<th>Susceptible PI (90/190)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PI 271351, PI 271352, PI 271354, PI 271356, PI 271357</td>
<td>PI 170928, 269507, 269508, 271353, 273662</td>
<td>PI 170463, 181948, 269505, 270456, 280636</td>
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<td>PI 287534, 358065, 358099, 368638, 368639</td>
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<td>PI 381824, PI 381825, PI 381826, PI 381828, PI 381829</td>
<td>PI 368640, 379365, 381821, 381822, 381827</td>
<td>PI 370474, 370477, 370478, 379367, 381850</td>
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<td>PI 381831, PI 381832, PI 381834, PI 381835, PI 381836</td>
<td>PI 381830, PI 381854, 406857, 491089, 492125</td>
<td>PI 419090, 423240, 432341, 432342, 435291</td>
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<td>PI 381837, PI 381838, PI 381839, PI 381840, PI 381842</td>
<td>PI 458736, 491274, 491280, 491281, 491283</td>
<td>PI 438844, 438846, 438847, 442368, 442369</td>
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<td>PI 491304, 491305, 491306, 491307</td>
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<tr>
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<td>PI 491308, 491309, 491310, 491311</td>
<td>PI 491312, 491313, 491314, 491315</td>
</tr>
</tbody>
</table>

These PI were divided into three groups based on their reactions to *Zucchini* yellow mosaic virus infection.

1Resistant: all the tested plants in an accession were resistant (partially resistant/total accessions tested).

2Partially resistant: one or several but not all the tested plants in an accession were resistant (partially resistant/total accessions tested).

3Susceptible: all the tested plants in an accession were infected (susceptible/total accessions tested).

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 386635, which was in partial resistance in the primary test, was also in partial resistance in the repeated test. All of the susceptible PIs 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 1) 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 information.
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 previously 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-F. 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.

**Literature Cited**


