Evaluation of *Citrullus* sp. Germ Plasm for Resistance to Watermelon Mosaic Virus 2

A. G. GILLASPIE, JR., and J. M. WRIGHT, U.S. Department of Agriculture, Agricultural Research Service, Southern Regional Plant Introduction Station, Griffin, GA 30223-1797

ABSTRACT

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A total of 670 *Citrullus* species accessions were evaluated for resistance to watermelon mosaic virus 2. In greenhouse tests seedlings to be evaluated were mechanically inoculated, and in field tests plants in spreader rows were mechanically inoculated. Plants were considered virus free by the absence of disease symptoms and by negative results in enzyme-linked immunosorbent assay. Our working definition of resistance was the ability of a plant to withstand, oppose, lessen, or overcome the attack of a pathogen. Plants were considered resistant if virus free 10–14 days after final inoculation in the greenhouse, or virus free 4–6 wk after inoculation of the spreader-row plants in the field, even though many were subsequently infected. Selections from 10 *C. lanatus* accessions (PI 189316, PI 189317, and PI 189318 from Zaire; PI 244018, PI 244019, and PI 255137 from South Africa; PI 164708 from India; and PI 494529 and Egun, which are Egusi-types, and PI 306782 from Nigeria) were resistant in both field and greenhouse tests. Five *C. colocynthis* accessions (PI 386016, PI 386024, PI 386025, and PI 386026 from Iran and PI 388770 from Morocco) possessed some resistance in both field and greenhouse tests.

Watermelon mosaic virus 2 (WMV-2) is a potyvirus (6,11) that causes losses in cantaloupe, cucumber, pumpkin, squash, and watermelon (7). It also infects leguminous, malvaceous, and chenopodiaceous plants (7). Several strains of WMV-2 have been described from various areas of the world (7). WMV-2 has been confused with watermelon mosaic virus 1, but the latter is now considered a strain of papaya ringspot virus (7).

Resistance to WMV-2 in watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai) and in colocynth (*C. colocynthis* (L.) Schrader) has been reported, but the degree of resistance varies with the virus strain tested. Bhargava and Bhargava (2) found 59 commercial cultivars of watermelon to be resistant to an Indian strain of WMV-2, but Sowell and Demski (8) reported that all commercial watermelon cultivars tested (including some of the same cultivars tested in India) were susceptible to WMV-2. Webb (10) found resistance in cultivar Egun from Nigeria, and Provvidenti (5) found that Nigerian Egusi types (PI 494528 and PI 494532) are resistant to WMV-2 and to zucchini yellow mosaic virus (ZYMV). The term Egun is probably Egusi miswritten or misspelled. The Egusi types discussed here are accessions of *C. lanatus* with a particularly bitter fruit and large seeds. Resistance in Egun is thought to be controlled by a single dominant gene (R. E. Webb, *personal communication*). Demski and Sowell (4) reported that all *C. lanatus* accessions in the collection at Griffin, Georgia are susceptible. Adlerz and Crall (1) found that *C. lanatus* accessions PI 248178, PI 249010, and PI 255137 remain free of symptoms in the field in Florida.

Our research was undertaken to locate useful resistance to WMV-2 in *C. lanatus* accessions and to identify sources of resistance in *C. colocynthis*. For the purposes of our tests, resistance was defined as the ability of a plant to withstand, oppose, lessen, or overcome the attack of a pathogen (13). We knew that most accessions in the collection had been increased by open pollination; so if genes for resistance were present, some populations were probably still segregating for resistance.

MATERIALS AND METHODS

General procedures. All *Citrullus* accessions tested were derived from the collection at the Southern Regional Plant Introduction Station at Griffin, Georgia, except for Egun and PI 482261-1, which were supplied by G. Boyhan at Auburn University. A Florida isolate (FC-1656) of WMV-2 supplied by D. Purcifull, University of Florida, Gainesville, was used throughout these tests. The host plant used for virus multiplication as a source of inoculum was *Cucurbita pepo* L. 'Small Sugar' (Sun Seeds Genetics Inc., Hollister, CA). Inoculum was produced by grinding leaves of the infected Small Sugar pumpkin in 0.025 M phosphate buffer, pH 7.2, and adding a small amount of 600-grit Carborundum. Early greenhouse tests and the 1990 field tests used a rabbit polyclonal antiserum to WMV-2 (supplied by D. Purcifull) in an indirect enzyme-linked immunosorbent assay (ELISA). Subsequent greenhouse and field testing was accomplished with the potyvirus monoclonal antibody (Agdia Inc., Elhart, IN), also used in an indirect ELISA. A WMV-2 antiserum kit from Agdia (peroxidase-IgG in a double-sandwich ELISA) was used to test plants in the greenhouse in 1991–92 and in the field in 1992.

Since total immunity of watermelon to WMV-2 has not been reported, our approach was to look for resistance as expressed in plants which were virus free early in their development after being exposed to infection by WMV-2. The plants infected late in their development will still produce normal fruit.

Greenhouse screening. Greenhouse tests were conducted during the fall-to-spring period to avoid the high temperatures that favor the development of other watermelon disease problems, such as gummy stem blight. In the 1989–90 greenhouse tests, 25 seeds of each plant introduction to be screened were planted in flats in Metro Mix 220 potting medium. Cotyledons of the seedlings were inoculated by rubbing with fresh inoculum. Two weeks after the first inoculation, all plants with virus symptoms were rogued. The symptomless plants were tested by ELISA. Plants testing negative were reinoculated and retested by ELISA after 10 days. Plants showing no disease symptoms or negative in ELISA at this stage were transplanted into individual 30.5-cm plastic pots containing Metro Mix 220, grown to the flowering stage, self-pollinated, and the resulting seeds saved for field evaluation.

In the 1990–91 and 1991–92 greenhouse tests, inoculations of seedlings with virus (10:1 buffer/tissue by weight) were accomplished with a Binks artist's airbrush at 276 kPa. When the cotyledons were fully expanded, the abaxial surface was inoculated on Monday, Wednesday, and Friday of successive weeks until 14 total inoculations had been achieved. This technique gave a maximum number of infected plants.

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The remaining procedures were the same as those used in 1989–90.

Field screening. Field-plot studies were performed at the U.S. Fruit and Tree Nut Research Facility, Byron, Georgia. The experimental fields were fertilized according to the recommendations of the Georgia Cooperative Extension Service. The herbicide, bensulide (Prefar 4E), was applied 3 days prior to planting at a rate of 14 L/ha. Seeds were treated with a slurry of thiram and captan at a ratio of 4:1 prior to planting. Spreader rows of host plants were planted in early June, 1 wk before the test plants, and were inoculated at the cotyledon stage with 40:1 inoculum (buffer/tissue) with an airbrush at 620 kPa. In 1990, 16 cultivars from the family Cucurbitaceae were used as host plants. Host plants and test plants were alternated in the row in a randomized block design. One hundred plants each of 17 accessions were planted. Plots were spaced with 2.4- × 2.4-m centers with 2.4-m alleyways between blocks. Host plants were placed midway between these centers. In 1991 and 1992, host plants (*C. lanatus* 'Baby Bush II' hybrid [Park Seed, Greenwood, SC) and test plants were planted in separate rows in a 2:1 ratio of test-plant rows to host-plant rows in a randomized block design. Plots were spaced with 3- × 3-m centers and 6-m alleyways. Testing by ELISA began 2 wk after germination of the test plants and continued on alternating weeks throughout the growing season.

Plants that tested negative for WMV-2 6 wk after germination were selfed. In 1990, selfs were made and the blossoms taped closed with masking tape. In 1991 and 1992, male and female flowers were covered with cloth bags on wire frames as described by Walker (9). The bags were removed for selfing; then the flowers were re-covered for 1 day to prevent cross-pollination.

RESULTS AND DISCUSSION

A total of 670 *Citrullus* accessions were screened for resistance to WMV-2. In these tests, 19 *C. lanatus* accessions had individual plants with resistance (Table 1). Ten of these (PI 189316, PI 189317, and PI 189318 from Zaire; PI 244018, PI 244019, and PI 255137 from South Africa; PI 164708 from India; and Egusi-types PI 494529 and Egun, and PI 306782 from Nigeria) had individual plants with resistance in the field as well as in the greenhouse tests.

Accessions PI 189316, PI 189317, PI 189318, and Egun have the most promise, because their degree of resistance and their potential ease of handling were good. PI 244018, PI 244019, and PI 255137 were late bloomers that produced few mature fruit after selfing and before the end of the growing season. These last three accessions produced male and perfect flowers. PI 494529 produced very mild symptoms of WMV-2; the infection

Table 1. Accessions of *Citrullus* (of 670 evaluated in greenhouse and field tests) demonstrating resistance to watermelon mosaic virus 2

			Resistant plants/total plants tested								
			Greenhouse[c]			Field[d]					
						1990		1991		1992	
PI[a]	Country of origin	Species[b]	1989–90	1990–91	1991–92	4–6 wk	12–14 wk	4–6 wk	12–14 wk	4–6 wk	12–14 wk
164708	India	C.l.	1/19	...[e]	...	21/96	0/96	...	...	...	...
164804	India	C.l.	0/26	...	...	5/97	0/97	...	...	...	...
164977	Turkey	C.l.	0/31	...	...	2/97	0/97	...	...	...	...
167126	Turkey	C.l.	1/23	...	...	0/97	...	...	...	...	...
185635	Ghana	C.l.	...	2/25	...	...	...	...	...	...	...
189316	Zaire	C.l.	...	2/25	...	...	...	46/87	22/87	37/37	32/37
189317	Zaire	C.l.	...	1/23	1/25	...	...	...	...	33/39	34/40
189318	Zaire	C.l.	...	3/24	...	...	...	58/92	15/92	...	...
235118	India	C.c.	1/16	...	...	...	...	...	...	...	...
244018	S. Africa	C.l.	...	1/21	...	...	...	33/88[f]	22/88	24/34	25/36
244019	S. Africa	C.l.	...	5/24	...	...	...	...	...	29/38	28/39
248178	Zaire	C.l.	...	1/25	...	...	...	...	...	...	...
255137	S. Africa	C.l.	...	2/19	...	...	...	79/90[g]	22/90	...	...
306364	Zaire	C.l.	...	...	...	...	...	22/69	0/69	...	...
306782	Nigeria	C.l.	...	1/15	...	...	...	31/80	2/77	...	...
346082	Afghanistan	C.l.	...	1/15	...	...	...	...	...	...	...
386016	Iran	C.c.	2/19	0/20	...	25/83	11/83	...	...	...	...
386018	Iran	C.c.	1/28	...	...	...	...	...	...	...	...
386024	Iran	C.c.	1/10	1/18[h]	...	23/98	14/98	...	...	7/34	1/35
386025	Iran	C.c.	...	9/26[h]	...	17/90	10/90	...	...	7/30	2/31
386026	Iran	C.c.	2/15	1/11[h]	...	36/103	10/103	...	...	8/29	3/29
388770	Morocco	C.c.	3/19	...	...	9/84	0/84	...	...	...	...
432337	Cyprus	C.c.	...	...	...	5/57	0/57	...	...	...	...
482261-1	Zimbabwe	C.l.	...	1/25	...	...	...	...	...	...	...
494529	Nigeria	C.l.[i]	1/21	1/24	10/25	75/100	41/100	47/85[j]	7/85[j]	11/36	11/36
494530	Nigeria	C.l.	3/28	...	...	...	...	...	...	...	...
Egun[k]	Nigeria	C.l.	...	6/15	107/164[l]	...	...	78/82[m]	47/82	31/32	31/33

[a] Plant inventory number.
[b] *Citrullus lanatus* (C.l.) and *C. colocynthis* (C.c.).
[c] ELISA results 10–14 days after final mechanical inoculation (14 total inoculations at 2–3 day intervals).
[d] ELISA results 4–6 wk and 12–14 wk after plants in spreader rows inoculated.
[e] Not tested.
[f] 23 S-1 seedlings among the 88 total seedlings. S-1 data = 7/31 (15/23) and 9/26 (2/23).
[g] 15 S-1 seedlings among the 90 total seedlings. S-1 data = 7/31 (13/15) and 9/26 (6/15).
[h] S-1 seedlings from 1990 field screening.
[i] Egusi-type of *C. lanatus*.
[j] All S-1 seedlings.
[k] Egun and 482261-1 seeds from Auburn University zucchini yellow mosaic virus screening tests.
[l] S-2 seedlings from 1991 field tests.
[m] S-1 seedlings from 1990–91 greenhouse tests.

could only be detected by serological testing of many plants. The Egusi-type melons have hard fruit with bitter flesh and large seeds, but they do cross readily with other watermelons.

Seven *C. colocynthis* accessions showed resistance in greenhouse tests, and five of these (PI 386016, PI 386024, PI 386025, and PI 386026 from Iran and PI 388770 from Morocco) also exhibited some resistance in field tests (Table 1). Unfortunately, *C. colocynthis* is somewhat difficult to cross with *C. lanatus*. The five *C. colocynthis* accessions listed for the 1989–90 greenhouse tests and for the 1990 field tests were all from untested, open-pollinated seeds. The *C. colocynthis* accessions tested exhibited few virus symptoms, but by late season a large number of the plants became infected.

There were probably no selfs in the 1990 field test, because we attempted (unsuccessfully) to make these selfs early in the morning before the bees had visited the pollen. In 1991, this approach was replaced by the covering method. Seed from the 1990 field test was included in the 1990–91 greenhouse test.

The low number of resistant seedlings in greenhouse tests of the progeny of some selfs and of the seedlings from some previously tested accessions was a concern. Some of the plants chosen for selfing may have been escapes, rather than truly resistant plants. Another possible explanation is that the method of inoculation employed in the greenhouse tests may have utilized a virus titer that was too severe compared to that of insect transmission from spreader rows in the field tests. However, selfs of those resistant plants from the greenhouse tests produced resistant progeny more often, as determined by field-test results.

The inconsistent results in the initial 1991–92 greenhouse tests led to more extensive tests in which we observed plants that tested positive for WMV-2, later tested negative, and a third time tested positive again. Leaf samples taken from main vines and secondary branches of known infected plants were tested by ELISA. The main vines were found to contain virus more often than did the secondary branches. A recent report indicates that WMV-2 is unevenly distributed in plants infected by viruliferous aphids (12). Unequal distribution of the virus in infected watermelon was attributed to the virus occurring only in tissue produced after inoculation by the aphid. The uneven distribution of virus in mechanically inoculated plants requires further explanation. In 1992 field tests, main vines and their secondary branches were tested for the presence of WMV-2. In the aphid-inoculated plants, only some of the main vines were infected; and in some cases secondary branches were infected without main vine infection. These results agree with the findings from Florida (12). Most of the vines and branches of colocynth were infected, probably indicating a difference in susceptibility and/or virus movement within these plants. Explaining the distribution will require further studies, but in the meantime we have altered our sampling protocol to include the youngest fully developed leaves of at least three main vines.

Generally, plants of accessions which did not have WMV-2 10–14 days after final inoculation in the greenhouse tests or 4–6 wk after inoculation of the spreader-row plants in the field tests were resistant enough to produce unblemished fruit with viable seeds. Some of these plants, which were virus free in their early development, became infected as the season progressed.

Egun and PI 482261-1 were reported to be resistant to ZYMV (3), and Egun was reported resistant to WMV-2 (10). PI 494528 and PI 494532 from Nigeria were reported to be resistant to an isolate of WMV-2 (5), but we found no resistant seedlings in our greenhouse tests of these accessions. However, we did find resistance in other Egusi-type melons that were collected in Nigeria at the same time as the ones tested previously (5). Differences in virus-strain pathogenicity or testing procedures may explain the lack of resistance in some reported accessions. If resistance to this virus is strain specific, it will have a significant impact on the breeding and development of new watermelon cultivars resistant to WMV-2. The sources of resistance to the WMV-2 strain we tested must now be tested against other strains of the virus. Most resistant accessions of *C. lanatus* originated in Africa; the resistant accessions of *C. colocynthis* came from Iran.

We plan to proceed to the S_5 stage with the sources of resistance we have selected, to reduce the amount of segregation in the progenies. We will then attempt to determine how the resistance is inherited. This germ plasm should then be of greater use to breeders.

Those desiring a list of all of the accessions tested in these experiments should contact the authors.

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