Journal of Heredity 2004:95(6):498–502
doi:10.1093/jhered/esh076
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Inheritance of Resistance to Zucchini Yellow Mosaic Virus and Watermelon Mosaic Virus in Watermelon

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Abstract

High resistance to zucchini yellow mosaic virus–China strain (ZYMV-CH) and moderate resistance to watermelon mosaic virus (WMV) were found in a selection of PI 595203 (*Citrullus lanatus* var. *lanatus*), an Egusi type originally collected in Nigeria. Mixed inoculations showed primarily that these two viruses have no cross-protection. This fact may explain the high frequency of mixed infection often observed in commercial fields. When plants were inoculated with a mixture of the two viruses, the frequency of plants resistant to ZYMV was lower than expected, indicating that WMV infection may reduce the ability of a plant to resist ZYMV. We studied inheritance of resistance to ZYMV-CH and WMV, using crosses between a single-plant selection of PI 595203 and the ZYMV-susceptible watermelon inbreds 9811 and 98R. According to virus ratings of the susceptible parents, the resistant parent, and the F_1, F_2, and BC_1 generations, resistance to ZYMV-CH was conferred by a single recessive gene, for which the symbol *zym-CH* is suggested. The high tolerance to WMV was controlled by at least two recessive genes.

Watermelon (*Citrullus lanatus* [Thunb.] Matsum. & Nakai) is a major crop in China. The area and production in China is the largest in the world. In 2001, 1.2 million ha of watermelon were grown. The area and production is about 60% of the world total (FAOSTAT 2003). Virus diseases are destructive to watermelon and are difficult to control (Sherf and Macnab 1986). Recently, frequent occurrences of viruses have caused serious reduction in watermelon production in some areas of China.

Major control strategies for diseases caused by virus include the use of insecticides to eliminate virus vectors, herbicides to remove alternate hosts for virus, and genetic resistance (Provvidenti, 1993). Of those controls, the most economical method is breeding resistant cultivars. Zucchini yellow mosaic virus (ZYMV) and watermelon mosaic virus (WMV, formerly watermelon mosaic virus-2) are the most common virus diseases of watermelon in China (Gu 2001). The U.S. Department of Agriculture watermelon germplasm collection has been screened for resistance to several virus diseases. Boyhan et al. (1992) and Lecoq et al. (1998) identified PI accessions resistant to ZYMV, and Gillaspie and Wright (1993) identified PI accessions resistant to WMV. Provvidenti and Gonsalves (1982) found in *Cucumis metuliferus* that accessions resistant to WMV were also resistant to PRSV and that the double virus resistance was controlled by a single dominant gene. Provvidenti (1991) identified a single recessive gene in watermelon PI 482261 conferring resistance to the Florida isolate of ZYMV (ZYMV-FL). Other than a report by McCuistion (1998) that there was intermediate resistance to WMV in the F_1 of resistant × susceptible diploid inbred parents, there have been no reports on the inheritance of resistance to WMV in watermelon.

Provvidenti collected and purified viruses associated with watermelon disease in China on a trip in the 1990s. He identified PI 595203 (*Citrullus lanatus* var. *lanatus*), an Egusi type sometimes referred to as Egun, as being highly resistant to ZYMV, WMV, and cucumber mosaic virus. The objective of this study was to measure the inheritance of resistance to ZYMV and WMV in watermelon PI 595203, and to study the interaction of the two viruses when they are inoculated in a mixture.

Materials and Methods

The genetic material used in this investigation was derived from crosses and backcrosses of virus-resistant PI 595203

with virus-susceptible inbred lines 9811 and 98R. Seeds of PI 595203 were obtained from Provvidenti, Cornell University, New York. PI 595203 is an Egusi type watermelon from Africa, having many branches, dark green leaves with deep lobing, late maturity, gray rind pattern, small round fruit, white flesh with bitter flavor, and large tan seeds. Seeds of 9811 and 98R were obtained from the China National Engineering Research Center for Vegetables, Beijing. Lines 9811 and 98R are elite inbred lines having few branches, light green leaves with slight lobing, early maturity, narrow striped rind, large round fruit, red flesh with sweet flavor, and medium-sized black seeds.

Uniform germination of seeds was achieved by placing seeds that had been sterilized in 0.01% $HgCl_2$ on moist blotter paper in glass dishes that were incubated at 30°C. All experiments were performed in insect-free greenhouses at the National Engineering Research Center for Vegetables, Beijing, China. The greenhouse temperature was 23–35°C at day and 16–20°C at night.

The ZYMV-CH isolate was collected in Beijing, China, and provided by R. Provvidenti, Cornell University, New York, who tested it and confirmed it as a China strain. The WMV isolate was provided by Gu Qingsheng, Zhengzhou Institute of Pomology, China Agricultural Academy. We prepared inoculum by grinding leaves of ZYMV and WMV-infected squash hybrid Zaoqinq No.1 (*Cucurbita pepo* L.) in 0.02 M phosphate buffer at pH 7.0, using a mortar and pestle. Leaf-to-buffer ratio was 1:5 (1 g infected leaf to 5 ml buffer). The inoculation procedure was the same, whether we were using a squash for inoculum increase, or testing watermelon for resistance.

Plants of the parental inbreds and their progeny were mechanically inoculated at the cotyledonary stage and reinoculated when the first true leaf was fully expanded. Inoculation consisted of dusting one leaf with an 800-mesh carborundum and then applying the inoculum to the leaf with the thumb rotating in a circular motion 8–10 times, as if painting the leaf with inoculum. After inoculation, carborundum was rinsed off the leaves to improve light interception.

Symptoms of virus infection on plants were rated weekly for 4 weeks. The rating was on a scale of 0–5 (0 = no symptoms; 1 = slightly mosaic on leaves; 2 = mosaic patches and/or necrotic spots on leaves; 3 = leaves near apical meristem deformed slightly, yellow, and reduced in size; 4 = apical meristem with mosaic and deformation; and 5 = extensive mosaic and serious deformation of leaves, or plant dead). Virus tests were run with 20–31 plants of each parent and F_1 generation, 95–124 plants of the F_2, and 40–82 of each BC_1 generation. Segregation ratios were compared to the expected ratios with the Chi-square test.

Variance components and heritability and effective factors were analyzed in this experiment. Additive (s^2_A) and dominance (s^2_D or nonadditive) genetic variances and narrow-sense heritability h^2 were estimated by the method of Warner (1952). Environmental variance was estimated as $s^2_E = (s^2_{P1} + s^2_{P2} + 2s^2_{F1})/4$ (Wright 1968). Broad-sense heritability was estimated as $H = (s^2_{F2} - s^2_E)/s^2_{F2}$. Gene effects based on a six-parameter model were estimated with the nonweighted method and notation described by Gamble (1962) and are defined as follows:

$$m = F_2;$$
$$a = +B_1 - B_2;$$
$$d = -0.5P_1 - 0.5P_2 + F_1 - 4F_2 + 2B_1 + 2B_2;$$
$$aa = -4F_2 + 2B_1 + 2B_2;$$
$$ad = -0.5P_1 + 0.5P_2 + B_1 - B_2;$$
$$dd = P_1 + P_2 + 2F_1 + 4F_2 - 4B_1 - 4B_2.$$

The number of effective factors controlling resistance was estimated by five methods. Method 1 was proposed by Wright (1968), method 2 was proposed by Mather and Jinks (1982), and methods 3 through 5 were proposed by Lande (1981). All of the effective factor formulas assumed that segregating genes for resistance were all located in one parent, resistance genes were not linked, all resistance genes had equal effects on resistance, epistatic effects were absent, dominance effects were absent, and genotype × environment effects were absent (Wright 1968). The assumption of no dominance effects appears to be a problem in this study, because they were found to be large in PI 595203 × 9811 and PI 595203 × 98R.

In order to evaluate the presence of cross-protection, mixed inoculations with WMV and ZYMV in F_2 populations of PI 595203 × 98R were performed in two ways: (1) one cotyledon was inoculated as outlined above with ZYMV and the other with WMV; or (2) the two viruses were mixed, and the mixture was used to inoculate two cotyledons.

Enzyme-linked immunosorbent assay (ELISA) was used to determine (1) the kind of virus in each plant receiving mixed inoculation, and (2) whether there was virus present in plants that remained symptomless. Tissue used for testing was taken from a sample of the top leaves of the plant. Plants without virus that also had a low rating were considered resistant. The antibodies of the two viruses were kindly provided by Piero Roggero, Italy Science Academy.

Results

Plants of PI 595203 inoculated with ZYMV did not show any symptoms, and no virus was detected by ELISA, whereas those of 9811 and 98R had severe systemic mosaic symptoms and distorted leaves. All F_1 plants had severe systemic symptoms (Table 1). The F_2 segregated 3:1 (resistant:susceptible). The BC_1 to the susceptible parent were all susceptible, whereas the BC_1 to the resistant parent segregated in a 1:1 ratio. Thus, the high level of resistance to ZYMV in PI 595203 was controlled by a single recessive gene. The gene is named *ZYMV-China strain resistance*, with the symbol *zym-CH*. These results are similar to those obtained by Provvidenti (1991), who reported a single recessive gene for resistance to the Florida strain of ZYMV (ZYMV-FL) in PI 482261. ZYMV isolates have been reported to differ in pathogenicity, aphid transmissibility, and serological or molecular properties (Desbiez et al. 2002; Lecoq and Purcifull 1992). Thus, it is important to determine

Table 1. Inheritance of resistance to zucchini yellow mosaic virus–China strain (ZYMV-CH) in crosses of watermelon PI 595203 with lines 9811 and 98R

Parent or cross	Total no. of plants	Resistant plants	Susceptible plants	Expected ratio	χ^2	Probability
PI 595203 P_1	26	26	0	1:0	—	—
9811 P_2	25	0	25	0:1	—	—
PI 595203 × 9811 F_1	24	0	24	0:1	—	—
PI 595203 × 9811 F_2	95	27	68	1:3	0.424	.50–.75
PI595203 × (PI595203 × 9811) BC1	40	17	23	1:1	0.626	.30–.50
9811 × (PI 595203 × 981) BC2	77	0	77	0:1	—	—
PI 595203 P_1	26	26	0	1:0	—	—
98R P_2	22	0	22	0:1	—	—
PI 595203 × 98R F_1	26	0	26	0:1	—	—
PI 595203 × 98R F_2	102	22	80	1:3	0.471	.40–.50
PI595203 × (PI595203 × 98R) BC1	40	18	22	1:1	0.226	.50–.75
98R × (PI 595203×98R) BC2	79	0	79	0:1	—	—

whether the two ZYMV strains are the same, and whether the two resistance genes are the same.

Plants of PI 595203 inoculated with WMV were slightly stunted, with leaves reduced in size but normal in color and shape. ELISA results indicated that the virus was present in the plants. Thus, PI 595203 is moderately resistant to WMV. No obvious pattern of single-gene inheritance was produced when plants were grouped into categories of resistant (rating 0, 1, 2) and susceptible (rating 3, 4, 5), even when an intermediate category was used to account for heterozygotes (Table 2). Plants of the F_1 and BC_1 to the susceptible parent all had systemic infections by the virus. In the F_2 and the BC_1 to the resistant parent, the segregation patterns indicated that resistance was not controlled by a single gene, so we analyzed it as a quantitative trait.

Gene effects for resistance to WMV were estimated by means of the nonweighted method and notation of Gamble (1962). The midpoints were 4.62 and 4.52 in the two crosses PI 595203 × 9811 and PI 595203 × 98R (Table 3). Additive (a), additive × additive (aa), and dominance × dominance (dd) effects were negative (toward the resistant parents) for both crosses. Dominance (d) and additive × dominance (ad) were positive (toward the susceptible parents), and additive × dominance (ad) had the greatest magnitude of any single effect. The environmental component of variance was small in the two crosses. Dominance variance was larger than additive variance in PI 595203 × 9811, and smaller than additive variance in PI 595203 × 98R. Broad-sense heritabilities were high (0.84 to 0.85) for both crosses, indicating a large genetic component for WMV resistance. Narrow-sense heritability in PI 595203 × 9811 was low (0.14), due to the small additive and large dominance variance in that cross, but was moderate (0.58) in PI 595203 × 98R. Selection for WMV resistance should be effective, but may require testing of progeny rows rather than single plants.

Estimates of the minimum number of effective factors (gene loci) controlling resistance to WMV ranged in the two crosses from 0.8 to 10.4 (Table 3). Mean estimates were 4.7 and 2.7 for PI 595203 × 9811 and PI 595203 × 98R, respectively. Calculation methods depending mainly on

Table 2. Rating for resistance to watermelon mosaic virus (WMV) in crosses of watermelon PI 595203 with lines 9811 and 98R

Parent or cross	No. of plants	Disease rating (no. of plants in category)						Average resistance rating	Total variance
		0	1	2	3	4	5		
PI 595203 P_1	27	0	11	16	0	0	0	1.59	0.25
9811 P_2	29	0	0	0	0	2	27	4.93	0.07
PI 595203 × 9811 F_1	26	0	0	0	0	6	20	4.77	0.18
PI 595203 × 9811 F_2	124	4	1	0	8	7	104	4.62	1.12
PI595203 × (595203 × 9811) BC1	72	0	5	15	9	9	34	3.72	2.01
9811 × (PI595203 × 9811) BC2	72	0	0	0	0	6	66	4.92	0.08
PI 595203 P_1	27	0	11	16	0	0	0	1.59	0.25
98R P_2	31	0	0	0	0	0	31	5.00	0.00
PI 595203 × 98R F_1	20	0	0	0	0	4	16	4.80	0.17
PI 595203 × 98R F_2	119	3	1	0	5	28	82	4.52	0.95
PI595203 × (PI595203 × 98R) BC1	67	0	2	6	8	15	36	4.15	1.28
98R × (PI 595203×98R) BC2	82	0	0	0	0	7	75	4.91	0.07

Disease rating was 0 to 5 (0 = no disease symptoms; 1 = slight mosaic on leaves; 2 = mosaic patches and/or necrotic spots on leaves; 3 = leaves near apical meristem deformed slightly, yellow, and reduced in size; 4 = apical meristem with mosaic and deformation; 5 = extensive mosaic and serious deformation of leaves, or plant dead).

Table 3. Estimates of gene effects; additive (s^2_A), dominance (s^2_D), and environmental variances (s^2_E); broad-sense (H) and narrow-sense (h^2) heritabilities; genetic gain (G_S); and minimum number of genes (or effective factors, EF) for resistance to watermelon mosaic virus in crosses of watermelon PI 595203 with lines 9811 and 98R

Effect	PI 595203 × 9811	PI 595203 × 98R
Gene effects[a]		
m	4.62	4.52
a	−1.2	−0.76
d	0.31	1.55
aa	−1.2	−0.04
ad	0.47	0.94
dd	−0.02	−1.57
Variance components[b]		
s^2_A	0.15	0.55
s^2_D	0.83	0.25
s^2_E	0.17	0.15
H	0.85	0.84
h^2	0.14	0.58
G_S	0.27	0.95
Effective factors[c]		
EF1	2.1	2.5
EF2	10.4	5.3
EF3	1.5	1.8
EF4	8.7	2.6
EF5	0.8	1.5
Mean	4.7	2.7
Mean (no EF2, EF4)	1.5	1.9

Heritabilities calculated as $H = (s^2_{F2} - s^2_E)/s^2_{F2}$ and $h^2 = s^2_A/s^2_{F2}$; genetic gain calculated as $G_s = (1.76)\ (h^2)\ (s_{F2})$.

[a] Rating is from 0 (no disease symptoms) to 5 (extensive mosaic and serious deformation of leaves, or plant dead).

[b] Calculated as $s^2_A = 2s^2_{F2} - (s^2_{B1} + s^2_{B2})$, $s^2_E = (s^2_{P1} + s^2_{P2} + 2s^2_{F1})/4$, and $s^2_D = s^2_{B1} + s^2_{B2} - s^2_{F2} - s^2_E$.

[c] Calculated as follows: $EF_1 = (P_2 - P_1)^2[1.5 - 2h(1 - h)]/8[s^2_{F2} - 0.25(s^2_{P1} + s^2_{P2} + 2s^2_{F1})]$, where $h = F_1 - P_1/P_2 - P_1$; $EF_2 = [0.5(P_2 - P_1)]^2/[2s^2_{F2} - (s^2_{B1} + s^2_{B2})]$; $EF_3 = (P_2 - P_1)^2/8[s^2_{F2} - 0.25(s^2_{P1} + s^2_{P2} + 2s^2_{F1})]$; $EF_4 = (P_2 - P_1)^2/8[2s^2_{F2} - (s^2_{B1} + s^2_{B2})]$; $EF_5 = (P_2 - P_1)^2/8[s^2_{B1} + s^2_{B2} - (s^2_{F1} + 0.5s^2_{P1} + 0.5s^2_{P2})]$.

backcross variances (EF2 and EF4) produced large estimates of the number of effective factors. If these methods of calculation were excluded from the mean, the estimates were 1.5 and 1.9 for PI 595203 × 9811 and PI 595203 × 98R, respectively. Thus, resistance to WMV was controlled by at least two genes.

There was no difference in time of symptom expression and in the incidence of symptoms for the two mixed inoculation treatments. These results support the idea that there is no cross-protection. These results are also consistent with the observation that mixed infections are often observed in the field. The ELISA results for the F_2 populations inoculated with both viruses indicated a frequency of 3.5% WMV-infected plants, the same as that obtained by inoculating with WMV alone. The frequency of ZYMV-infected plants was about 6.0%, less than that obtained by inoculating with ZYMV alone. Infection by WMV may reduce the ability of the plant to resist ZYMV. Thus, the two viruses appear to have cooperative interaction. This area requires further investigation.

Discussion

At present, there are two sources of resistance for the control of ZYMV in cucurbit species, both inherited recessively. The first is not viral-strain-specific and is available in some accessions of the Nigerian Egusi (e.g., PI 494528 and PI 494532; Provvidenti 1993). However, this resistance is temperature dependent and is best expressed in warm or hot climates (Provvidenti 1993). The second source confers a high level of resistance, is not temperature dependent, but is specific for ZYMV-FL (Provvidenti, 1991).

There are few sources of resistance to WMV, mainly for some wild accessions from Africa that are moderately resistant (PI 494528, PI 494532, PI 295848, and PI 381740; Lecoq et al.1998). Our research indicates that PI 595203 is resistant to ZYMV and moderately resistant to WMV. Recently, Strange et al. (2002) reported PI 595203 is also resistant to papaya ringspot virus watermelon strain. This is the first time that the inheritance of resistance to two different viruses has been studied in this accession.

PI 595203 may be useful in the development of multivirus-resistant cultivars. However, according to the results obtained with a mixture of viruses, the expected ratio of plants resistant to two viruses in the F_2 is about 1:600, so a large population would be necessary to obtain multivirus-resistant plants.

According to virus ratings of the susceptible parents, the resistant parent, and the F_1, F_2, and BC_1 generations, resistance to ZYMV-China strain was conferred by a single recessive gene, for which the symbol *zym-CH* is suggested. The high tolerance to WMV was controlled by at least two recessive genes. Broad-sense heritability was high (0.84–0.85, depending on the cross), and narrow-sense heritability was low to high (0.14–0.58, depending on the cross).

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Received May 23, 2003
Accepted June 4, 2004

Corresponding Editor: William F. Tracy