Inheritance of Resistance to *Watermelon mosaic virus* in *Cucumis melo* that Impairs Virus Accumulation, Symptom Expression, and Aphid Transmission

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ABSTRACT

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The *Cucumis melo* accession TGR-1551 was found to be resistant to *Watermelon mosaic virus* (WMV, genus *Potyvirus*, family *Potyviridae*). The resistance resulted in a drastic and significant reduction of virus titer and infected plants were asymptomatic or exhibited mild disease symptoms. The same gene or closely linked genes restricted virus accumula-

tion and ameliorated symptom expression. No effect was observed on virus accumulation in inoculated leaves, which suggested that the initial phases of infection were not affected. The resistance was effective against a range of WMV isolates from Spanish melon production areas. Using aphid inoculations, resistant plants showed a lower propensity for infection by WMV and for serving as virus sources for secondary spread once infected. Resistance was determined to be under recessive genetic control.

Additional keywords: potyvirus resistance, recessive resistance.

Most melon (*Cucumis melo* L.) cultivars are susceptible to a number of mosaic diseases caused by aphid-borne viruses. These viruses are a worldwide problem for field-grown melons and include the cucumovirus (genus *Cucumovirus*, family *Bromoviridae*) *Cucumber mosaic virus* (CMV) and the potyviruses (genus *Potyvirus*, family *Potyviridae*) *Papaya ringspot virus* strain W (PRSV-W), *Watermelon mosaic virus* (WMV), and *Zucchini yellow mosaic virus* (ZYMV) (2,12,24,32,38,45). Although the relative importance of mosaic-inducing viruses in field-grown melons varies between regions and years, CMV and WMV are common in many production areas such as California (24,45), Hawaii (63), Italy (35), Spain (2,38), and Turkey (58).

Mosaic-inducing viruses are of great concern for melon growers because, in addition to mosaic symptoms, serious yield and quality loss may occur (1,7). Transmission in nature occurs in a nonpersistent manner by different aphid (*Hemiptera*, *Aphididae*) species, with *Aphis gossypii* Glover and *Myzus persicae* Sulz. being efficient vectors in the field (10,13). Aphids can acquire and inoculate these viruses during superficial probes that take only a few seconds (33), making prevention of spread through the use of insecticides impractical (49,53).

Mosaic-inducing viruses seriously limit melon production, and much effort has been directed toward identifying sources of resistance. Commercial melons with resistance to CMV and PRSV-W have been produced (32), but melon cultivars with resistance to WMV or ZYMV are not available even though resistance to these viruses has been reported (3,14,22,25). We recently screened a collection of *C. melo* and wild species accessions for useful sources of resistance to mosaic-inducing viruses and found that the *C. melo* accession TGR-1551 exhibited resistance to WMV (16). This study describes the phenotype and inheritance of WMV resistance derived from TGR-1551.

MATERIALS AND METHODS

Plant materials, virus isolates, and aphid colonies. Seed of the parental genotypes used in this study were obtained from the CSIC-La Mayora germ plasm bank. The melon accession TGR-1551, originally collected in Zimbabwe and maintained by self-pollination, previously was suggested as a possible source of WMV resistance (16). *C. melo* 'Bola de Oro' and PMR 45 were used as susceptible controls. Inheritance of WMV resistance in TGR-1551 was studied using the two parents (TGR-1551 and 'Bola de Oro'), their F₁ hybrid and F₂ generations, a first backcross to the resistant parent (F₁ × TGR-1551, BC_{1R}), and a first backcross to the susceptible parent (F₁ × 'Bola de Oro', BC_{1S}).

Twenty-one isolates of WMV (Table 1) were used to evaluate resistance of TGR-1551. 'Bola de Oro' also was inoculated with each isolate as a control. Four plants were tested for each virus isolate-plant genotype combination. The WMV isolates were selected randomly from a collection obtained during a survey of major areas of melon field production in Spain. Prior to inoculation, pure stocks of each virus isolate, not containing other viruses or other WMV isolates, were obtained by single-local-lesion transfers as described previously (42). CMV isolate M730 and ZYMV isolate C16 were described by Díaz et al. (16). Infectious virus stocks were maintained at 4°C as lyophilized infected leaves.

Nonviruliferous *M. persicae* individuals were obtained from colonies reared on pepper (*Capsicum annuum* L.) 'California Wonder' F₁ (Ramiro Arnedo S.A., La Rioja, Spain) plants. Nonviruliferous *A. gossypii* individuals were obtained from colonies reared on melon (accession ANC-42, CSIC-La Mayora germ plasm bank) plants.

Inoculation and symptom evaluation. Mechanical and aphid inoculations were performed as described previously (61). Mechanical inoculations were performed on cotyledons of plants at expanded cotyledons and emerging-first-leaf growth stage. Aphid inoculations were performed onto the first leaf of plants at expanded-cotyledons and one-leaf growth stages. For transmission studies, two levels of infestation were examined, 4 aphids (six

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experiments) or 10 aphids (four experiments) per plant. In every replicate, transmission to 15 healthy plants of each TGR-1551 and 'Bola de Oro' genotypes was performed. TGR-1551 was not inoculated using *A. gossypii* because previous studies (61) have indicated that it is resistant to virus transmission by this aphid species. Virus sources for mechanical inoculations were young leaves of infected zucchini squash ('Diamante' F₁; Seminis Vegetable Seeds Iberica, Almería, Spain) harvested 15 days postinoculation. Except when indicated, virus sources for aphid inoculations were young leaves of infected PMR 45 melon plants. Mock-inoculated (mechanically with inoculation buffer or by using virus-free aphids) plants served as negative controls. Experiments were conducted in an insect-proof glasshouse at ≈ 25 and 18°C (day and night, respectively) and a day length of 16 h, with light supplementation when needed (inoculation of the collection of WMV isolates); or in a growth-chamber at 25 and 22°C (day and night, respectively), 70% relative humidity, and a 16-h photoperiod (radiation level at 400 to 700 nm, $120 \mu\text{mol m}^{-2} \text{s}^{-1}$) (aphid inoculation and genetic analysis). Inoculated plants were assessed up to 6 weeks postinoculation (wpi) (6- to 10-leaf growth stage). Symptoms were rated according to an integer scale of 0 to 5, where 0 = no symptoms; 1 = apical leaf with dispersed vein clearing or mottle, and no mosaic; 2 = mild mosaic only in the apical leaf; 3 = mild mosaic and mild leaf distortion in the three youngest leaves; 4 = mosaic and leaf distortion in the three or four youngest leaves; and 5 = severe mosaic and leaf distortion in the five or six youngest leaves. For the examination of resistance in F₂ and BC_{1R} populations, plants with a symptom score ≤ 2 were considered resistant. Curves of symptom progress were constructed, and the area under the symptom progress curve (AUSPC) was determined using the following formula: $\text{AUSPC} = \sum_i [(x_i + x_{i+1})/2]t_i$, where x_i = mean value of disease score at date i , and t_i = time (in days) between scoring date i and scoring date $i + 1$ (8). The AUSPC formula integrates disease severity between several assessment times using the trapezoidal integration method, dividing the symptom progress curve into a series of rectangles and summing the area of each rectangle to approximate the total area under the curve (8). AUSPC can be used as a descriptor of the disease progression (9).

Virus detection and quantification. Double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) (11) was used to detect the presence of virus in plants as described previously (16), using commercial polyclonal antisera for WMV, CMV, or ZYMV (Loewe Biochemica GmbH, Otterfing, Germany). When needed, the amount of WMV present in inoculated plants was determined based on DAS-ELISA from extracts obtained as described previously (16). To avoid sampling bias due to nonuniform virus distribution in plant tissues, whole cotyledons or leaves were analyzed. For quantitative DAS-ELISA, a preparation of M116-WMV virus particles was obtained as described previously (42). Known amounts (25, 50, 100, 150, and 250 ng) of M116-WMV preparation were included as controls in each ELISA plate to obtain the standard curve that related absorbance at 405 nm value to virus concentration. Serial dilutions (wt/vol) of sample extracts included 1:10, 1:100, and 1:500 for cotyledons and young leaves of TGR-1551 or of asymptomatic plants of any genotype; and dilutions of 1:500, 1:1,000, 1:5,000, 1:10,000, and 1:20,000 for young leaves of symptomatic plants. Three replicate wells were included for each sample, from which mean absorbance value was calculated. Absorbance values used for quantification in every ELISA plate were only those from dilutions that placed within the linear range of the relationship between sample dilution and absorbance values.

Distribution of WMV in entire leaves of infected plants was studied by immunoblot analysis after leaf-press blotting. Leaves were frozen at -80°C and then blotted onto a nitrocellulose membrane as described previously (62). The detection procedure, based on the method described by Hawkes et al. (29), included blocking the membranes with 4% no-fat milk powder in $1\times$ AFT (136 mM NaCl, 250 mM $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$, 8 mM $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$, pH 7.2 to 7.4) containing 0.5% (vol/vol) Tween 20. Virus was localized by using WMV alkaline phosphatase (AP)-conjugated immunoglobulin Gs (Loewe Biochemica GmbH). The membrane was developed by incubating with luminescent substrate (CDP-Star, Roche Diagnostics GmbH, Mannheim, Germany), and exposed to X-ray films (Kodak, X-Omat AR).

Statistical and inheritance analyses. Statistical analyses were performed using the statistical software SPSS for Windows

TABLE 1. Geographic origin, year of collection, and host species from which the *Watermelon mosaic virus* (WMV) isolates used in this study originally were collected

Geographic origin	WMV isolate ^a	Host species	Year of collection
Western Spain			
Badajoz	M95/14 (BAD95.1)	<i>Cucumis melo</i>	1995
	M95/47 (BAD95.3)	<i>C. melo</i>	1995
Northeastern Spain			
Barcelona	B9.2 (BAR95.1)	<i>C. melo</i>	1995
	B10.1 (BAR95.2)	<i>C. melo</i>	1995
	M719 (BAR99.3)	<i>C. melo</i>	1999
	M721 (BAR99.5)	<i>C. melo</i>	1999
Zaragoza	C3W/99 (ZAR99.2)	<i>Cucurbita pepo</i>	1999
	M23/95 (ZAR95.1)	<i>Cucumis melo</i>	1995
	M780	<i>C. melo</i>	1999
Central Spain			
Madrid	M95/158.8 (MAD95.1)	<i>C. melo</i>	1995
	M95/162.8 (MAD95.4)	<i>C. melo</i>	1995
	M95/303.8 (MAD95.8)	<i>C. melo</i>	1995
	M95/323.8 (MAD95.7)	<i>C. melo</i>	1995
	M730W277 (MAD99.2)	<i>C. melo</i>	1999
Segovia	S748 (SG99.2)	<i>Citrullus lanatus</i>	1999
Southern Spain			
Málaga	M656 (MAL99.1)	<i>Cucumis melo</i>	1999
	M692 (MAL99.5)	<i>C. melo</i>	1999
Eastern Spain			
Murcia	M239	<i>C. melo</i>	1995
	M236 (MUR95.2)	<i>C. melo</i>	1995
	M261 (MUR95.4)	<i>C. melo</i>	1995
Valencia	M116 (VAL95.1)	<i>C. melo</i>	1995

^a The code name used in Moreno et al. (42) is shown in parentheses.

(release 11.0.1; SPSS Inc., Chicago). To stabilize variance and normalize data distributions, values for virus quantities were $\log_{10}$ transformed and percentages of aphid transmission were arcsine-transformed prior to analysis. Correlations between AUSPCs, symptom scores, and virus accumulation data were calculated using Pearson's r product-moment coefficient. Separations of means were made using the Games-Howell test ($P \leq 0.05$), an analysis of variance-based post hoc procedure suitable for cases in which treatment variances are nonhomogeneous. Determination of the number of loci involved in the inheritance of resistance in the TGR-1551 $\times$ 'Bola de Oro' genetic family were made upon χ^2 testing of departure from expected resistant:susceptible ratios for different genetic models.

RESULTS

Effective resistance of TGR-1551 to Spanish isolates of WMV. An initial screening of TGR-1551 resistance was performed by mechanical inoculation of this breeding line with 21 isolates representative of the Spanish WMV population (Table 1), including WMV isolate M116 used for preliminary screening (16). All plants of TGR-1551 exhibited uniform resistance to all 21 isolates of WMV tested. No appreciable difference was observed between virus-inoculated TGR-1551 and mock-inoculated TGR-1551 plants even at 6 wpi, except for a few plants for which scattered chlorotic mottle, vein clearing, or mosaic was barely visible in young leaves at that time (Fig. 1B). In contrast, all inoculated 'Bola de Oro' plants exhibited severe symptoms (severe mosaic, leaf distortion, and stunting) with all 21 WMV isolates after 1 wpi (Fig. 1D). DAS-ELISA analysis of young, noninoculated leaves at 5 wpi indicated that WMV was present in all the 'Bola de Oro' and TGR-1551 plants inoculated except for isolates S748 and M95/47, which were not detected in any of the TGR-1551 plants tested. WMV was not detected nor were symptoms observed in mock-inoculated plants (Fig. 1A and C). No appreciable differences were observed in symptoms or virus titer between

Fig. 1. Systemic symptoms observed in plants of **B**, TGR-1551 or **D**, 'Bola de Oro' 6 weeks after mechanical inoculation with *Watermelon mosaic virus* isolate M-116 compared with **A** and **C**, mock-inoculated plants of each genotype, respectively. In TGR-1551 plants, no symptoms were observed in most of the inoculated plants; a few exhibited the mild symptoms shown in **B**.

the WMV isolates that infected TGR-1551. Therefore, more detailed analysis were performed using M116-WMV as a representative isolate.

WMV accumulation and symptom expression are impaired in TGR-1551. The amount of virus present in TGR-1551 and 'Bola de Oro' plants was studied after mechanical inoculation with M116-WMV. The inoculated cotyledons were analyzed at 1 wpi, and the youngest, newly developed leaf was analyzed at 1, 2, 3, and 4 wpi. Because a destructive sampling method was used, only one cotyledon per plant was analyzed for all plants, and quantification in young leaves was from groups of five plants per sampling date that then were discarded. Symptoms were evaluated twice per week. No symptoms were observed in the cotyledons at any time for either TGR-1551 or 'Bola de Oro' plants. DAS-ELISA analysis indicated that WMV was present in all the inoculated cotyledons, and that a significantly greater (Games-Howell test, $P \leq 0.05$) mean amount of virus was present in TGR-1551 than in 'Bola de Oro' plants (Fig. 2, open and black boxes, respectively). The initial infection phases, therefore, were not impaired in TGR-1551. In contrast, significantly greater levels of virus were detected consistently through 6 wpi in young non-inoculated leaves of 'Bola de Oro' compared with TGR-1551. Virus was not detected in young leaves of TGR-1551 plants until 3 wpi, and subsequent virus titers were always $<1\%$ of those detected in 'Bola de Oro' plants (Fig. 2, dashed and spotted boxes, respectively). The greatest virus titers in TGR-1551 plants were detected at 4 wpi. Systemic accumulation of WMV obviously was impaired in TGR-1551 relative to accumulation in 'Bola de Oro'. Moreover, symptom development differed significantly between plants of the two genotypes. 'Bola de Oro' plants exhibited severe symptoms 1 wpi, with the highest symptom scores obtained 3 wpi. In contrast, symptoms were not observed in any of the TGR-1551 plants until 4 wpi, with only very mild mosaic or scattered vein clearing symptoms observed in a few plants, always with a rating <2 (Fig. 2, open circles curve). Infection was visually evident only in those plants in which symptoms rated ≥ 3 . Differences in symptom development between TGR-1551 and 'Bola de Oro' plants (Fig. 2) resulted in large differences in disease severity suffered by each genotype during the evaluation period as estimated by AUSPCs ($\text{AUSPC}_{\text{TGR-1551}} = 6$; $\text{AUSPC}_{\text{'Bola de Oro'}} = 127$). A similar result also was obtained after aphid inoculation using M116-WMV and *M. persicae* (20 aphids per plant). *A. gossypii* was not used as vector because resistance to virus inoculation by this vector is present in TGR-1551 (16,59). In the case of aphid inoculation, differences with 'Bola de Oro' were even more pronounced because no symptoms at all were exhibited by any of the inoculated TGR-1551 plants although, as before, low amounts of virus could be detected in young, noninoculated leaves (data not shown).

Limited distribution of WMV in TGR-1551-infected leaves. The location and distribution of WMV in young, noninoculated leaves of TGR-1551 or 'Bola de Oro' plants systemically infected with M116-WMV, by mechanical or aphid inoculation, was examined 3 wpi by leaf-press blotting assays. Clear differences were observed between TGR-1551 and 'Bola de Oro' plants. WMV was present in a limited number of infection foci with reduced size in leaves of TGR-1551 plants whereas, in 'Bola de Oro', the virus was distributed uniformly throughout the leaf (Fig. 3). This result was consistent with the lower amount of virus detected in equivalent leaves after DAS-ELISA analysis of TGR-1551 or 'Bola de Oro' plants (Fig. 2).

Resistance to aphid transmission of WMV in TGR-1551. The possible epidemiological consequences derived from the use of a resistant genotype like TGR-1551, which supports low virus titers, was studied in experiments using *M. persicae* and *A. gossypii* as vectors and either 'Bola de Oro' or TGR-1551 as virus sources. Results showed that TGR-1551 had a significantly lower rate of infection using virus acquired from 'Bola de Oro'

(4 and 10 aphids per plant) (Table 2). Previous work indicated that TGR-1551 has a resistance mechanism that prevents *A. gossypii* from initiating ingestion from the phloem, but superficial probes are performed, aphid stylets reach the phloem, and salivation occurs (21). Therefore, acquisition and transmission of a nonpersistent virus like WMV was expected for *A. gossypii* from TGR-1551 plants. In fact, our results showed that both *A. gossypii* and *M. persicae* were able to acquire and transmit WMV from TGR-1551, with no significant difference in rates of infection of TGR-1551 and 'Bola de Oro' (Table 2). However, compared with 'Bola de Oro', TGR-1551 was a significantly less efficient virus source with either aphid species at both levels of infestation (Table 2).

Recessive inheritance of resistance to WMV in TGR-1551. The mode of inheritance of the resistance to WMV found in TGR-1551 was determined using mechanical inoculation with M116-WMV. Symptom expression was monitored for 5 wpi, AUSPC was calculated, and the amount of virus in the youngest noninoculated leaf 4 wpi was analyzed in a number of plants of TGR-1551, 'Bola de Oro', F_1 (7 plants of each genotype), and F_2 , BC_{1R} , and BC_{1S} plants (61, 27, and 33 plants, respectively). TGR-1551 and 'Bola de Oro' plants behaved as described above. The F_1 and BC_{1S} plants showed the phenotype of the susceptible parent. Plants of the F_2 and the BC_{1R} populations exhibited segregation for the three variables described above. In these segregating populations, significant positive correlations were obtained between AUSPCs and symptom scores 5 wpi ($r = 0.681$, $P \leq 0.001$ for the F_2 ; $r = 0.831$, $P \leq 0.001$ for the BC_{1R}). Also, the amount of virus detected in young leaves positively correlated with symptom scores ($r = 0.665$, $P \leq 0.001$ for the F_2 ; $r = 0.826$, $P \leq 0.001$ for the BC_{1R}) and AUSPCs ($r = 0.842$, $P \leq 0.001$ for the F_2 ; $r = 0.846$, $P \leq 0.001$ for the BC_{1R}). All 'Bola de Oro' plants were susceptible, whereas all TGR-1551 plants were resistant (Table 3). All F_1

Fig. 3. Watermelon mosaic virus (WMV) location and distribution in entire young noninoculated leaves of **B**, TGR-1551 and **D**, 'Bola de Oro' plants inoculated mechanically with WMV isolate M-116 or **A** and **C**, mock-inoculated, respectively 3 weeks earlier at expanded cotyledons growth stage. Leaf-press blots were obtained and analyzed using an anti-WMV antibody.

Fig. 2. Mean Watermelon mosaic virus (WMV) accumulation (micrograms of virus per gram of tissue) values in inoculated cotyledons or in noninoculated young leaves of *Cucumis melo* TGR-1551 (open and dashed boxes, respectively) or 'Bola de Oro' (black and spotted boxes, respectively) plants inoculated mechanically with WMV isolate M-116 at expanded cotyledons growth stage, and mean severity of symptoms (from 0 = absence to 5 = maximum severity) observed in TGR-1551 (open circles) or 'Bola de Oro' (black circles) plants at different times postinoculation. Different letters above bars at each time postinoculation indicate statistically significant differences (uppercase letters for inoculated cotyledons, lowercase letters for noninoculated young leaves).

plants were susceptible, suggesting a recessive control of resistance. All BC_{1S} plants were susceptible, and the F₂ and BC_{1R} plants showed segregation for resistance. Based on the segregation ratio observed in these two generations, we estimated the number of genes involved in the inheritance of the resistance. For the simplest model studied, one recessive gene (Table 3), the F₂ segregation did not fit the expected 1R:3S ratio, but two more resistant plants would have made the segregation acceptable. The BC_{1R} segregated in an acceptable fit to the ratio 1R:1S expected for this model (Table 3). Neither the F₂ nor the BC_{1R} data fit the two-recessive-gene model. The observed F₂ departure from the expected ratio for the single-gene model could be explained by the existence of an additional dominant gene, epistatic to the recessive resistance gene (Table 3).

DISCUSSION

TGR-1551 was consistently resistant to WMV when inoculated mechanically or via aphid transmission. The resistance was found to be effective against a collection of WMV isolates from Spain collected in melon production areas. This resistance likely will be useful to control WMV infection throughout the melon-growing

regions of Spain. A recent detailed analysis of the WMV population present in Spain indicated that high biological and genetic homogeneity existed among isolates (42). Also, limited data are available concerning WMV variation elsewhere (15,42). Therefore, usefulness of the resistance found in TGR-1551 for WMV control in other geographical regions needs to be evaluated.

The WMV resistance described here is significant, because few sources of resistance to WMV are available in *C. melo* (32) and have not been transferred to commercial cultivars despite frequent damage caused by this virus to melon crops worldwide (12,24, 36,45,63). The most promising resistance to WMV reported to date in *C. melo* was found several years ago in the breeding line 91213, a line derived from PI 371795 (25,43). However, in contrast to TGR-1551, unambiguous WMV symptoms were reported in line 91213. During early infection stages, plants of line 91213 inoculated with WMV exhibited severe mosaic or veinal chlorosis symptoms, which later became less severe as the leaves matured (43). Additionally, in line 91213, mosaic and distortion occasionally appeared in the young leaves of older plants (25). A similar resistant response to WMV was reported for breeding line PI 414723-4S₃ which is closely related to line 91213 and also is derived from PI 371795 (22,41). Inoculated plants of PI 414723-4S₃ initially exhibited severe systemic symptoms in the first one to four leaves above the site of inoculation, but later recovered (3,22). Resistance to WMV in PI 414723-4S₃ was demonstrated to be controlled by a single dominant gene (3,22), in contrast to the recessive control suggested for the resistance described here. Therefore, the inheritance of resistance to WMV in TGR-1551 differs from inheritance patterns previously reported in *C. melo*.

A significant reduction in WMV titer and disease symptom expression occurred in TGR-1551 plants (Fig. 2). Thus, TGR-1551 is resistant, not immune to infection by WMV (5,6). Virus accumulation and symptoms expression appeared to be under the same or closely linked genetic control.

Genetic analysis indicated that the resistance to WMV found in TGR-1551 was recessive. It is interesting to note that recessive control of resistance is frequent in potyvirus resistance systems (17,19,20,52). We analyzed the possible number of genes involved in the inheritance of the resistance trait. Based on a single gene model, the BC_{1R} segregated in a close fit to the expected 1:1, whereas the F₂ segregated in a close but not acceptable fit to a 1:3 resistant:susceptible ratio (Table 3). Thus, the single-gene model appears to be valid for use in a backcross breeding scheme to transfer resistance to different horticultural plant and fruit types. However, lack of fit for this single-gene model in F₂ segregants suggests that epistatic or genotype-environment effects may influence the expression of resistance. Environmental conditions (18,51), or the susceptible parents employed (14) are known to influence the expression of resistance traits against potyviruses. Further analysis will be needed, therefore, to elucidate the genetic basis of this resistance.

TABLE 2. Efficiency of *Myzus persicae* and *Aphis gossypii* in the transmission of *Watermelon mosaic virus* isolate M116 from infected plants of *Cucumis melo* TGR-1551 and 'Bola de Oro' to healthy plants of the two same genotypes

		Average percentage of plants infected when using for inoculation ^z	
Transmitter, source ^y	Recipient	4 aphids/plant	10 aphids/plant
<i>M. persicae</i>			
Bola de Oro	Bola de Oro	81.33 ± 7.22 ab	98.33 ± 1.67 a
	TGR-1551	30.00 ± 4.94 cd	58.33 ± 11.01 bc
	Bola de Oro	0.00 e	8.34 ± 4.19 de
	TGR-1551	4.00 ± 1.63 e	8.33 ± 3.19 de
<i>A. gossypii</i>			
Bola de Oro	Bola de Oro	53.57 ± 5.84 bc	81.33 ± 10.20 ab
	TGR-1551	ND	ND
	Bola de Oro	0.00 e	1.33 ± 1.33 e
	TGR-1551	ND	ND

^y Transmitter aphid species and virus acquisition source. Virus sources were young leaves of TGR-1551 or 'Bola de Oro' plants systemically infected after mechanical inoculation with M116-WMV (obtained from an aphid-inoculated plant) three weeks prior to inoculation of the test materials.

^z Six and four replicates were done when using 4 or 10 aphids/plant inoculation pressures, respectively. In every replicate, transmission to 15 healthy plants of each TGR-1551 and Bola de Oro genotype were performed. The number of plants that became infected was determined 30 days after inoculation by visual observation and double-antibody sandwich enzyme-linked immunosorbent assay testing. Mean ± standard error; mean percentages with the same letter are not significantly different (Games-Howell test procedure, $P \leq 0.05$); ND = not included because TGR-1551 is resistant to virus transmission by *A. gossypii* (61).

TABLE 3. Goodness of fit (χ^2 and P) for genetic models of resistance to *Watermelon mosaic virus* (WMV) isolate M116 in populations derived from a cross between the resistant TGR-1551 and the susceptible 'Bola de Oro' melons

Generation ^x	No. of plants ^y			One recessive gene ^z			Two recessive genes ^z			Double recessive × dominant epistasis ^z		
	Total	R	S	R:S	χ^2	P	R:S	χ^2	P	R:S	χ^2	P
Bola Oro	22	0	22	0:1	...	...	0:1	...	...	0:1	...	...
TGR-1551	22	22	0	1:0	...	...	1:0	...	...	1:0	...	...
F ₁	22	0	22	0:1	...	...	0:1	...	...	0:1	...	...
BC _{1S}	70	0	70	0:1	...	...	0:1	...	...	0:1	...	...
BC _{1R}	63	28	35	1:1	0.778	0.378	1:3	12.704	<0.001	1:1	0.778	0.378
F ₂	131	22	109	1:3	4.705	0.030	1:15	24.855	<0.001	3:13	0.329	0.566

^x F₁ = TGR-1551 × Bola Oro, BC_{1S} = F₁ × Bola Oro, and BC_{1R} = F₁ × TRG-1551.

^y Plants (expanded cotyledons growth stage) were inoculated mechanically with WMV M116, and scored for disease symptoms at the end of the fifth week postinoculation, according to an integer scale from 0 (no symptoms) to 5 (severe symptoms). Resistant (R) plants were those with symptom score ≤2; susceptible (S) plants were those with symptom score >2.

^z R:S = expected ratio.

Recessive resistance genes may interfere with the viral life cycle either at the single-cell level (44,55), during cell-to-cell movement (4,40), or during long-distance transport through the plant (28,30). The mechanism of resistance to WMV of TGR-1551 plants was not associated with early infection stages, because the virus accumulated efficiently in inoculated leaves. Resistant plants supported virus spread into young, noninoculated leaves, although systemic accumulation was greatly reduced. This was observed clearly with the leaf-press blotting analysis of young leaves of WMV-infected plants (Fig. 3). Therefore, some movement-based form of resistance may be operating in TGR-1551. Extensive data are available about the processes associated with virus movement within plants (23,37,47,48,54,57), showing that impaired virus spread is one of the major resistance mechanism of plants to virus infection (23). In fact, many resistance traits have been associated with alteration of virus movement in plants at different levels (cell-to-cell movement or long-distance translocation) (27,31,56). Our data do not distinguish at which stage virus transport may be impaired in TGR-1551 plants, nor can we discern the mechanism responsible for reduced virus titer. Further studies will be needed to more precisely establish the mechanism that is limiting WMV accumulation in TGR-1551 plants.

Restriction of systemic accumulation results in partial resistance and is an efficient means to reduce virus incidence (46). In fact, restricted accumulation of virus in plants may limit availability for vectors, thus reducing subsequent viral spread. It is apparent from our data that the resistance in TGR-1551 significantly limits the ability of WMV to accumulate in infected plants. Localization of WMV within the leaves of TGR-1551-infected plants indicated an uneven distribution with fewer infection foci of smaller size compared with that observed in susceptible plants (Fig. 3). Therefore, reduced amounts of virus are predicted to be available for aphid acquisition in this genotype, which may reduce subsequent virus transmission. This was confirmed during aphid-transmission studies (Table 3). In contrast, with other non-persistently transmitted viruses (ZYMV and CMV) that readily infect TGR-1551 (16), equivalent transmission rates were obtained for both TGR-1551 and 'Bola de Oro', as demonstrated for *M. persicae* (data not shown). Therefore, the differences observed between TGR-1551 and 'Bola de Oro' were due to different virus availability and not to resistance of TGR-1551 to the vector (21,59). Accordingly, a significantly lower epidemiological risk of WMV spread is expected for TGR-1551 than for a susceptible melon like 'Bola de Oro' because (i) TGR-1551 plants showed a lower propensity to be infected by WMV and (ii) TGR-1551 plants infected with WMV were less efficient virus sources for secondary spread. Therefore, our results suggest that TGR-1551 could be a valuable resistance source to reduce production losses caused by WMV.

Interestingly, TGR-1551 plants also exhibit resistance to the vector *A. gossypii* (21), similar to plants of the breeding line 91213 (26). Moreover, virus inoculation by *A. gossypii* is impeded in this genotype (61), similar to previous observations for *C. melo* accession PI 161375 (50). In addition, TGR-1551 plants are resistant to *Cucurbit yellowing stunting disorder virus* (genus *Crinivirus*, family *Closteroviridae*) (34,39), and to the biotype B of its natural vector, the sweet-potato whitefly *Bemisia tabaci* (Hemiptera: *Aleyrodidae*) (60). The results obtained here emphasize the potential importance of the line TGR-1551 as a donor of multiple disease and pest resistance for melon breeding programs.

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