Iran Agricultural Research 19:129-144 (2000)

STUDY OF RESISTANCE TO CUCUMBER MOSAIC VIRUS, WATERMELON MOSAIC VIRUS AND ZUCCHINI YELLOW MOSAIC VIRUS IN MELON CULTIVARS

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(Received: November 4, 1998)

ABSTRACT

Sources of resistance to *Watermelon mosaic virus* (WMV), *Zucchini yellow mosaic virus* (ZYMV), and two local isolates of *Cucumber mosaic virus* (CMV) as the most destructive viruses to melon (*Cucumis melo* L.) fields in Iran, were investigated. This study evaluated the resistance of melon germplasm under high disease pressure achieved with natural inoculations by indigenous aphid vector in the field, mechanical inoculations in the greenhouse, and then field comparison of resulting resistant and tolerant cultivars (cvs.) by natural and mechanical inoculations. The majority of genotypes used in this study were open-pollinated (OP). Some selfed (S_1) seeds and F_1 hybrids were also included. The results indicated that most of the melon cvs. were susceptible to both natural infection of cucurbit viruses in the field and mechanical inoculation in the greenhouse. However, melon cvs. 'Magolalena Vertbrod', 'Soski' and

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'Bahramabadi' (OPs) were immune to ZYMV. Melon cvs. 'Galicum' (S1), 'Latifah-1' (S1), 'Tashkandi', (OP) and 'Khorasgani' (OP) were resistant to CMV and WMV under both greenhouse and field conditions. Melon cvs. 'Baghkomeh-Lenjan', 'Oshtorjan', 'Lenjan', 'Firozan', 'Shahd-Shiraz', 'Ardian' and 'Latifah' (OPs) were tolerant considering both infection type and fruit yield reduction traits. Virulence of CMV#1, CMV#2, WMV and ZYMV differed significantly, where CMV#1 was the most virulent type having the highest mean of disease severity (3.59). The reassessment of the progenies of resistant and tolerant cvs. with doubly infected plants in the field, provided evidence that the sources of resistance or tolerance found in the earlier field and greenhouse germplasm evaluations are heritable.

Key words: CMV, Melon, Resistance, WMV, ZYMV.

تحقیقات کشاورزی ایران

(۱۳۷۹) ۱۴۴-۱۲۹: ۱۹

بررسی مقاومت به ویروس موزائیک خیار، ویروس موزائیک هندوانه و ویروس موزائیک کدو در ارقام طالبی و خربزه

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چکیده

منابع مقاومت به ویروس موزائیک هندوانه (WMV)، ویروس موزائیک زرد کدو(ZYMV) و دو جدایه محلی ویروس موزائیک خیار(CMV) به عنوان مخرب ترین ویروس های بیماری زای طالبی و

خربزه (*Cucumis melo* L.) در مزارع ایران، مورد مطالعه قرار گرفت. در این بررسی مقاومت ژرم پلاسم طالبی و خربزه تحت فشار زیاد بیماری با مایه کوبی های طبیعی بوسیله شته محلی، مایه زنی مکانیکی در گلخانه و سپس ارزیابی مزرعه ای ارقام مقاوم و متحمل حاصل از طریق مایه کوبی های طبیعی و مکانیکی انجام گرفت. نتایج نشان داد که بیشتر ارقام طالبی و خربزه به هر دو شرایط آلودگی طبیعی ویروس های جالیزدر مزرعه و آلودگی مکانیکی در گلخانه حساس بودند. با این وجود، رقم طالبی مگولالینا ورتبرود و ارقام خربزه سوسکی و بهرام آبادی نسبت به ZYMV مصون بودند. ارقام طالبی گالیکوم و خوراسگانی در برابرCMV وWMV در هر دو شرایط مزرعه و گلخانه مقاوم بودند. به همین ترتیب ارقام تاشکندی و لطیفه-۱ مقاوم بودند. ارقام طالبی باغ کومه لنجان، اشترجان، لنجان، فیروزان، شهد شیراز و ارقام خربزه آردیان و لطیفه با توجه به صفات تیپ آلودگی و میزان کاهش عملکرد میوه متحمل شناخته شدند. بیماری زایی 'CMV#1' ,'CMV#2', WMV و ZYMV بطور معنی داری متفاوت بود، به نحوی که CMV#1 بیماری زا ترین نوع با بالاترین شدت (۳/۵۹) بود. ارزیابی مجدد نتاج ارقام مقاوم و متحمل از طریق آلودگی مضاعف بوته ها درمزرعه، شواهدی دال بر ارثی بودن منابع مقاومت یا تحمل موجود در ارزیابی های قبلی مزرعه ای و گلخانه ای ژرم پلاسم بود.

INTRODUCTION

Melon and muskmelon (*Cucumis melo* L.) varieties are subjected to severe losses due to an array of aphid-transmitted viruses including *Cucumber mosaic Cucumovirus* (CMV), *Watermelon mosaic Potyvirus* (WMV) (formerly WMV 2), Watermelon strain of *Papaya ringspot virus* (PRSV-W) (formerly watermelon mosaic virus-1) and *Zucchini yellow mosaic Potyvirus* (ZYMV) (2, 5, 8, 16, 17). CMV and WMV are the most prevalent viruses in Iran, causing severe losses to the melon, muskmelon and other cucurbit varieties (4, 23). However, ZYMV is probably one of the most damaging, and since its first description in 1981 (11), it has been found world-wide (2, 12, 13, 19). ZYMV

was identified in Iran, and the results of surveys in different growing regions in Markazi province indicated that it was one of the major components of the viral pathosystem of cucurbit crops in this country (6).

CMV, WMV and ZYMV are transmitted in a non-persistent manner by aphids, and aphicides are generally ineffective in reducing the spread of the viruses in the field since transmission occurs before the aphids obtain a lethal dose of aphicides (15, 24). Breeding for resistance, therefore, is considered as the main strategy for controlling these viruses. Wasuwat and Walker (25) attributed CMV resistance to a single dominant gene, designated *Cmv*. Other workers, however, found the inheritance to be more complex (8, 9). Karchi *et al.* (8) reported that 3 recessive genes influence resistance to CMV. Resistance to PRSV-W in the muskmelon line 'PI 180280' or in its derivate cv. 'WMR 29' was attributed to a single dominant gene designated as *Wmv* (new nomenclature *Prv*) (3, 26). Pitrat and Lecoq (16) reported another source of resistance to PRSV with two alleles at the same locus including *Prv*1 from PI 180280 and *Prv*2 from PI 180283. Resistance to WMV in *C. melo* L. has not been reported although cultivars appear to vary in field tolerance to WMV. ZYMV causes symptoms resembling those incited by PRSV-W (21) and is related serologically to WMV (11, 22). Resistance to ZYMV has been found in a muskmelon line 'PI 414723' from India (17). This resistance was effective against two strains of ZYMV and is governed by two dominant genes (*Zym* and *Fn*). Recently, Fuchs *et al.* (5) reported a genetically engineered melon containing coat protein genes of CMV, WMV and ZYMV. The homozygous plants of this line were highly resistant, and showed only few symptomatic leaves confined close to the vine tips.

Studying the genetic variability of resistance to cucurbit viruses among *C. melo* germplasm, as a prerequiste for effective breeding program, is of critical importance. The results will be used for understanding mechanisms and factors affecting the variability, and in turn for planning a durable control strategy in the breeding programs via employing the resistant sources of *C. melo*. The aim of this study was to evaluate the resistance of melon cvs.

originating from native and exotic sources against CMV (two local isolates), WMV and ZYMV.

MATERIALS AND METHODS

Field Evaluation of Germplasm

Ninety-nine genotypes of melon (*C. melo*) as listed in Table 1 were grown in the Research Farm of College of Agriculture, Isfahan University of Technology in 1986. The majority of genotypes used in this investigation were open-pollinated (OP), and the remainder were either F1 hybrid or self-pollinated (S1) population. Seeds of each genotype were dusted with Benlate T (30% benomyl+30% thiram), germinated in a moist cloth bag at room temperature, and sown in two rows of twenty eight plants (20 m row length) with 75-cm spacing between plants and 3 m spacing between rows.

Leaves infested with melon aphid, *Aphis gossypii* Glover, the green peach aphid, *Myzus persicae*, and the faba-bean aphid, *Aphis fabae*, were collected from other fields and placed randomly on melon plants. No insecticide was used in the field.

Symptom score and yield assessment. Disease severity was visually scored on a 0 (symptomless) to 5 (severe symptoms) basis as described by Walkey and Pink (24) with some modifications: 0: no leaf symptom (Immune), 1: no stunting, very mild mosaic (or mottle) symptoms or chlorotic local lesions on at least one leaf (resistant), 2: no stunting, distinct mosaic symptoms on one or more leaves together with no significant fruit yield reduction (ranked as tolerant), 3: stunting to approximately ¾ normal size, leaf deformity and moderately severe mosaic (moderately susceptible), 4: plants stunted to between ½ and ¼ normal size, leaf deformity and severe mosaic (susceptible), and 5: plants severely stunted, little or no growth, severe mosaic, necrosis or early death. Growth rate reduction of infected plants within each genotype was determined visually in comparison with the non-infected plants.

Mature fruits were harvested in two subsequent harvests and assessed for each genotype in infected and healthy plants according to their fruit size,

weight, and number. The fruit yield losses of genotypes were sorted into six classes 0 (no fruit yield reduction) to 5 (100% fruit yield reduction).

Table 1. Response of melon cultivars to the natural infection of cucurbit viruses in the field.

Cultivars	Origin	Seed type†	Infection type	Fruit yield reduction (%)
Galicum	Exotic	S_1	1	0
Khorasgani	Iran	OP	1	0
Baghkomeh-Lenjan; Oshtorjan; Lenjan; Firozan; Shahd-Shiraz	Iran	OP	2	8
Samsouri Varamin; Larjan; Laki; Laki 731; Bahramabadi; Dastanbouh; Talaei1; Barada; Shamam; Samsouri; Talebi Isfahan; Borazjan	Iran	OP	3	10-20
Chilton; Cavaillion-roseflesh; Hales Best 936; SMS; Star H; Magolalena-vertbrod; Balanco; King Henry; Knightsenrly; Selfstrile; Hales-Jumbo; PMR45;Irogois; Delicious51; HDGF	Exotic	OP	3	10-20
Dixie Jumbo; MHB45; N45; TaniaA; Wdd230	Exotic	F_1	3	10-20
Amarloo; Talaei; Bami; Majidi; Yazdani; Ghaleh-Sorch; Abarkhohi; Databbouh; Varamin; Miandoab	Iran	OP	4	20-50
Perlita; Marked Pride; Hales Best; D.J.H.; HQFM; Harvest Queen; Ghaled; KasturaH.B.J.6827; Queen of Colorado; Charentais;	Exotic	OP	4	20-50
CGB; HPX-922; Tania	Exotic	F_1	4	20-40
Pinto Fusarium-2	Exotic	S_1	4	30
Donjuon	Exotic	F_1	5	100
Honey Dew	Exotic	OP	5	100
Tashkandi	Iran	OP	1	0
Latifah-1	Iran	S_1	1	0
Ardian; Latifah	Iran	OP	2	8
Abas-shori; Khaghani 1; Khaghani 2; Marini; Arya; Hamadani; Khaki-Marini; Mashhadi; Shahreza; Izadi; Zard-Karaj; Postab; Ebrameh; Firouzi	Iran	OP	4	20-50
Souski; Hajabas Teh; Sehrlenjan; Evaneki;	Iran	OP	3	10-20
Ardian 1; Ardian 2	Iran	S_1	3	10-20
Mirpanjami; Mahmoudabadi; Arya-1; Arya-2; Garmsari-1; Garmsari-2	Iran	S_1	4	20-50
Mila; Notiro	Exotic	F_1	4	20-30
Bokar	Iran	OP	5	100

† OP= open pollinated; S_1 =1st generation of selfed OP plant; F_1= F_1 hybrid.

Greenhouse Evaluation of Germplasm

The same genotypes as in the field (99 genotypes) were investigated using two local isolates of CMV, WMV (provided by Dr. M. Bahar, Isfahan University

of Technology, Isfahan, Iran) and ZYMV (provided by Dr. V. Lisa, Instituto di Fitovirologia del Consiglio Nazionale delle Ricerche, Torino, Italy). Seeds were pregerminated as described in the previous section and sown in 20cm- diameter claypots filled with a potting soil. Plants were maintained at 20-25°C in an insect-protected greenhouse that was regularly sprayed with aphicides. Two pots with inoculated plants and one pot with uninoculated plants (control), with four plants in each pot were used, for each of viruses applied for individual plant genotype.

Viral inocula and inoculation procedures. Plants were inoculated 15-20 days after sowing, when the first true leaf had begun to expand. The CMV isolates and WMV were multiplied on a local cv. of zucchini (*Cucurbita pepo* L.) and ZYMV was multiplied on zucchini cv. 'Superzeni'. Inocula were obtained by grinding infected zucchini leaves with 0.02 M phosphate buffer, pH 7.2, using a mortar and pestle. The infective sap was inoculated to the Celite-dusted cotyledons of seedlings before their first true leaf appeared or expanded. Virus symptoms were visually assessed according to the same scale described for the field plants. All non-biological materials were sterilized prior to use.

Virus indexing. The presence of virus in greenhouse plants was tested by serological tests and differential species. Double diffusion tests as described by Ball (1) were conducted using antisera M, W (provided by Dr. J.A. Tomlinson, National Vegetable Research Station, Wellesbourne, UK), and C (provided by Professor H.A. Scott, University of Arkansas, Department of Plant Pathology, Fayetteville, USA) of CMV. M and W antisera were diluted by phosphate-buffer saline at a ratio of 5:1, but antiserum C was used undiluted. Double-diffusion tests were conducted in sodium dodecyl sulfate (SDS) as reported by Purcifull and Batchelor (20) for ZYMV indexing. ZYMV atiserum was provided by Dr. V. Lisa, Instituto di Fitovirologia del Consiglio Nazionale delle Ricerche, Torino, Italy. Serological tests for WMV indexing were not conducted because the antiserum could not be prepared.

Differential species from Chenopodiaceae, Cucurbitaceae, Leguminosae and Solanaceae were also used for virus indexing.

Field Evaluation of Resistant and Tolerant Cultivars

The melon cvs. showing either resistant or tolerant reaction in the field/greenhouse, were grown with a susceptible cv. in the field (Table 2). Self-pollinated seeds (S1 or S2) harvested from the resistant or tolerant plants of the first trial (field evaluation germplasm) were used in this study. Each cultivar was sown in two rows of twenty eight plants as described earlier, and only one of the rows was inoculated. Resistance was assessed under high disease pressure achieved by mechanical inoculations of mixed inocula of CMW#1, CMV#2 and WMV as the most prevalent viruses in the region. Inoculation procedure, disease assessment, and fruit yield losses were conducted similar to that described in the previous sections.

Table 2. Response of resistant and tolerant *Cucumis melo* L. cvs. inoculated with a mixture of two local isolates of CMV and WMV in the field.

Cultivars		Mean[1] symptom scores	Growth rate[2]		Fruit yield[3]		Fruit yield loss[4] (%)
			Healthy plants	Infected plants	healthy plants	Infected plants	
Galicum	R[5]	1.0	4	4	8	8	0
Khorasgani	R	1.0	5	5	10	10	0
Baghkomeh	T	1.8	5	5	9	9	0
Shahd-Shiraz	T	2.0	4	4	9	9	0
Abarkohi	S	4.3	3	5	6	9	26
Tashkandi	R	1.0	4	4	9	9	0
Latifah-1	R	1.2	5	5	10	10	0
Ardian	T	2.0	5	5	9	9	0
Honey Dew	S	4.0	4	2	8	5	25

1. Symptom severity based on a 0 (no symptoms) to 5 (severe symptoms) scale. 2. Growth rate based on 0 (no growth) to 5 (maximum growth) scale.
3. Fruit yield based on conversion of fruit yield weights to a 0 (no fruit production) to 10 (maximum fruit production) scale.
4. Fruit yield losses based on the mean weights and scores of fruit yield reduction and growth yield reduction.
5. R, T, and S refer to resistant, tolerant and susceptible, respectively.

Data Analysis

In all experiments, scores and percentages were arcsine-transformed before being submitted to a *t* test for comparing the virulency of the tested viruses as well as estimation of genetic variation within the germplasm for their reactions to the diseases. Average of fruit reduction rate (score) and growth reduction rate of individual genotype as calculated by comparing the infected and non-infected plants was used to compute fruit yield losses.

Spearman's rank correlation coefficients were calculated for ranked cvs. based on symptom score and ranked cvs. based on fruit yield, and between field and greenhouse symptom scores.

RESULTS

Field Evaluation of Germplasm

Most of the melon cvs. were susceptible to the natural infection of cucurbit viruses in the field (Table 1). Melon cvs. 'Galicum' (S1) and 'Khorasgani' (OP), originated respectively from an exotic and a native source were resistant to the natural infection in the field. Similarly, melon native cvs. 'Tashkandi', (OP), and 'Latifah-1' (S1) were resistant. Melon cvs. 'Baghkomeh-Lenjan', 'Oshtorjan', 'Lenjan', 'Firozan', 'Shahd-Shiraz', 'Ardian' and 'Latifah' (OPs) were tolerant in comparison with the susceptible cvs. for both infection type (IT 2) and fruit yield reduction (8%). Melon cvs. 'Donjuon' (F1), 'Honey Dew', and 'Bokar' (OPs) were highly susceptible with IT 5, and fruit yield reduction of 100% (fruit yield loss 5). The majority of cvs. as presented in Table 1 were ranked as susceptible (IT 4). Melon cvs. 'Samsouri Varamin', 'Bahramabadi', 'Talaei', 'Shamam', 'Samsour', 'Barada', 'Cavaillion roseflesh', 'Magolalena vertbord', 'Balanco', 'Talebi Isfahan', 'Chilton', 'Hales Best 936', 'S.M.S.', 'Hales Best Jumbo', 'Knightsenrly', 'PMR45', 'Star H', 'Iroguois', 'King Henry', 'Delicious51', 'Self strile', 'HDGF', 'Dastanbouh', 'Borazjan', 'Laki', 'Laki 731', 'Largan', 'Souski', 'HajabasTeh', 'Sehrlenjan', 'Evaneki',

'Ghengah' (OPs); 'Dixie jumbo', 'MHB 45', 'N.45', 'Tania Wdd230' (F1s), 'Ardian1', and 'Ardian 2', (S1s) were moderately susceptible.

Spearman's rank correlation coefficient ($r=0.12$, $P>0.05$) calculated between ranked cvs. for symptom score and ranked cvs. for the fruit yield loss was not statistically significant.

Greenhouse Evaluation of Germplasm

Ninety-nine cvs. were screened simultaneously for resistance to two local isolates of CMV, WMV and ZYMV. The majority of the germplasm tested was susceptible to these three viruses. However, the melon cvs. 'Galicum', 'Baghkomeh-lenjan' and 'Tashkandi' and 'Latifah-1' showed to be resistant to CMV isolates and WMV. The melon cv. 'Galicum' was moderately susceptible to ZYMV, whereas, 'Tashkandi' and 'Latifah-1' were immune to ZYMV. In addition, melon cvs. 'Magolalena Vertbrod', 'Soski' and 'Bahramabadi' were immune to ZYMV.

The ranking of cultivars for their mean of CMV+WMV symptom scores in the greenhouse and in the field showed that the two environmental conditions were highly correlated ($r=0.47$, $P<0.01$), while these environments for the mean of CMV+WMV+ZYMV were correlated statistically at $P<0.05$ ($r=0.23$). Hence, a complete list of the reactions of cultivars to the tested viruses was not presented.

In the double-diffusion serological tests, the local CMV isolates failed to react with WMV-M and WMV-W antisera, however, they reacted with prominent precipitin lines with the antiserum C of CMV. All antigens of ZYMV reacted with the ZYMV antiserum both prepared from Italy. Serology tests on inoculated plants of two cvs. from each group of reactions to viruses (IT 1 to 5) also confirmed that all had the tested virus.

Virulency of CMV#1, CMV#2, WMV and ZYMV differed significantly (Table 3). CMV#1 was the most virulent type having the highest mean of disease severity (3.59). This isolate also identified as CMV (Y), and showed more disease severity as causing necrosis on cowpea (*Vigna sinensis* L.) than CMV#2

that showed chlorotic and mosaic symptoms on this species. No significant differences were observed between CMV#2, WMV and ZYMV for virulency.

Table 3. Comparison of virulency between CMV#1, CMV#2, WMV and ZYMV in *Cucumis melo* L. cultivars in the greenhouse.

Virus type	Mean symptom score	Compared with	*t* value
CMV#1	3.59	CMV#2	2.99†
CMV#1	3.59	WMV	3.05†
CMV#1	3.59	ZYMV	3.20†
CMV#2	3.20	WMV	0.43ns
CMV#2	3.20	ZYMV	0.53ns
WMV	3.08	ZYMV	0.04ns
ZYMV	2.98		

† Significant at P<0.01 , ns = Non -significant.

Field Evaluation of Resistant and Tolerant Cultivars

The results of field reassessment of the cultivars with either resistant or tolerant reaction to prevalent cucurbit viruses (CMV#1, CMV#2 and WMV) are shown in Table 2. These cultivars differed considerably from susceptible cultivar (control) for not being affected by the virus diseases, neither in disease score form nor fruit yield loss. The progeny test for the sources of resistance was conducted, and the results obtained from field and greenhouse experiments were consistent. The exotic cvs. like 'Galicum' (S2) and 'HoneyDew' (OP) were less adapted and hence produced less fruit yield than the native cvs.

DISCUSSION

The results of these studies confirm those of other workers (9, 10, 26) that cultivars of *C. melo* are generally susceptible to CMV, WMV and ZYMV; however, 'Galicum', 'Baghkomeh-lenjan', 'Tashkandi' and 'Latifah-1' cvs. were resistant to CMV as well as WMV, and the two latter were immune to ZYMV. In

addition, melon cvs. 'Magolalena Vertbrod', 'Soski' and 'Bahramabadi' were immune to ZYMV. The reduction in the fruit yield of the infected plants compared with non-infected plants, ranged from 0% (resistant cvs.) to 100% (highly susceptible cvs.). The French melon cv. 'Charentais' was highly susceptible to CMV, which agrees with Lecoq *et al.* (10). In the present study, immunity was not found in the *C. melo* germplasm tested for CMV and WMV. Likewise, no symptomless *C. melo* has been reported yet for CMV and WMV, which was prevalent in our field. A melon breeding line 'WMR 29' derived from PI 180280 was reported to be tolerant to WMV (3). Similarly, in a search for resistant lettuce germplasm, Provvidenti *et al.* (18) screened over 500 accessions of lettuce (*Lactuca sativa* L.) against a local CMV isolate in greenhouse and field experiments and found that all were susceptible. It has been shown in an inbred line of cucumber (*Cucumis sativus* L.) derived from a Chinese cv. 'Taichung Mou Gua' (TMG) a multiple allelic system differing in effectiveness and dominance relationships that works at the *Zym* locus (7). Resistance to ZYMV has been found in a muskmelon line 'PI 414723' from India (17). This resistance was effective against two strains of ZYMV and is governed by two dominant genes (*Zym* and *Fn*). In the present study although, no significant differences were observed between CMV#2, WMV and ZYMV for virulencey in the greenhouse, ZYMV was less virulent than the others, in which three cvs. showed exclusively immune reaction to it.

The study of relationship between field and greenhouse results for disease severity showed that CMV and WMV are the main prevalent cucurbit viruses in the field. The poor adaptability of some cultivars may be the cause of lack of relationship between the disease reactions and the fruit yield reduction in the field. This suggests that adapted cvs. may be affected less than introduced cvs. by the virus infections. Previous studies by Yamamoto *et al.* (27) indicated that the mixture of CMV and WMV inoculum was more virulent than each of these viruses *per se*. In the present study, therefore, the reassessment of the progenies of resistant and tolerant cvs. with doubly infected plants in the field, provides evidences that the founding of the sources of resistance or tolerance

found in the earlier field and greenhouse germplasm evaluations are heritable. Absence of fruit yield reduction of infected plants in tolerant cvs. in the second field trial may be attributed to the selective seeds from superior plants, which were used in the trial (Tables 1 and 2). Although *C. melo* response to virus disease may be influenced by the stage of plant development and environmental conditions, the impact of level of resistance seems to be more pronounced in the present study.

In conclusion, these trials showed that disease and yield assessments are the most appropriate measurement for determining resistance to cucurbit viruses naturally occurring in the field of *C. melo*, and that useful sources of resistance to the viruses does occur in some commercial cultivars. These resistant cultivars may be used by plant breeders in future melon and muskmelon breeding programs, either alone, or in combination with the reported CMV, WMV and ZYMV resistance genes of the wild or domestic relatives.

ACKNOWLEDGMENTS

We thank Dr. V. Lisa (Insituto di Fitovirologia applicata del Consiglio Nazionale delle Ricerche, Italy) for providing zucchini yellow mosaic virus and its antiserum, Dr. J.A. Tomlinson (National Vegetable Research Station, UK) for providing M and W antisera of CMV, Professor H.A. Scott (University of Arkansas, U.S.A.) for providing C antiserum of CMV, Dr. M. Bahar (Isfahan University of Technology) for providing CMV#1 and CMV#2 isolates and technical advice, and Dr. D. Danesh (University of Minnesota, U.S.A.) for valuable suggestions.

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