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ORIGINAL PAPER

Dissection of the oligogenic resistance to *Cucumber mosaic virus* in the melon accession PI 161375

Ali Essafi · Juan A. Díaz-Pendón · Enrique Moriones · Antonio J. Monforte · Jordi Garcia-Mas · Ana M. Martín-Hernández

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Abstract Resistance to *Cucumber mosaic virus* (CMV) in the exotic melon accession PI 161375, cultivar "Sonwang Charmi" (SC) had previously been described as oligogenic, recessive and quantitative, with a major QTL residing in linkage group XII (LGXII). We have used a collection of near isogenic lines (NILs) with introgressions of SC into the genome of the susceptible accession Piel de Sapo (PS) to further characterise this resistance. Infection of NILs carrying introgressions on LGXII showed that only NIL SC12-1 was resistant to CMV strains P9 and P104.82, but not to strains M6 and TL. Further mapping of this region showed that the resistance, named *cmv1* maps in an area of 2.2 cM, between markers CMN61_44 and CMN21_55. Moreover, *cmv1* confers total resistance to strains P9 and P104.82, indicating that in these cases it is not quantitative and that *cmv1* is sufficient to confer full resistance to these CMV strains. Candidate gene mapping of ten translation initiation factors in the melon genome failed to find any of them in the interval between markers CMN61_44 and CMN21_55. All these results suggest that the resistance to CMV present in SC is oligogenic, where different loci confer resistance to different CMV strains, but not necessarily quantitative, since at least one of these genes (*cmv1*) confers total resistance, similar to that of the parental SC, and does not need the contribution of other loci.

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A. Essafi · A. J. Monforte · J. Garcia-Mas ·
A. M. Martín-Hernández (✉)
IRTA, Centre de Recerca en Agrigenòmica CSIC-IRTA-UAB,
Carretera de Cabrils, Km 2, 08348 Cabrils, Barcelona, Spain
e-mail: montse.martin@irta.es

J. A. Díaz-Pendón · E. Moriones
Estación Experimental "La Mayora" CSIC,
29759 Algarrobo-Costa, Málaga, Spain

Present Address:
A. J. Monforte
Instituto de Biología Molecular y Celular de Plantas
(IBMCP)(CSIC-UPV) Ingeniero Fausto Elio,
s/n CPI "Ciudad Politécnica de la Innovación
DE LA INNOVACIÓN"- Edificio 8E, 46022 Valencia, Spain

Introduction

Cucumber mosaic virus (CMV) has the largest host range of any plant virus, infecting over 800 plant species and causing severe damage worldwide in some of the most economically important species, such as members of the *Solanaceae* and *Cucurbitaceae* families. CMV isolates are placed into two main subgroups, I and II, on the basis of biological, serological and molecular properties (Palukaitis et al. 1992). Genetic resistance against CMV is scarce and partial and polygenic in the few cases reported (Caranta et al. 2002). There are few examples reported of dominant resistance against CMV, such as the *RCY1* gene in *Arabidopsis* ecotype C24 (Takahashi et al. 2001) or the *RT4-4* gene in common bean (Seo et al. 2006). These genes belong to the NBS-LRR type of dominant genes producing total resistance through hypersensitive response reaction. In *Arabidopsis*, *cum1* and *cum2* have been reported as two single, independent, recessive mutations that confer resistance to CMV, affecting cell-to-cell and long-distance movement (Yoshii et al. 1998). Resistance to CMV, however, is usually described as partial and polygenic. In pepper, Caranta et al. 2002 described a dominant but partial resistance that showed restriction to CMV long-distance movement. This resistance was governed by one major-

effect and six minor-effect QTLs (Caranta et al. 2002). A similar partial resistance to long-distance CMV movement governed by QTLs with different effect has also been reported in potato (Valkonen and Watanabe 1999) and tomato (Stamova and Chatelat 2000). In cucurbits, however, resistance against CMV has been described mostly as recessive (Risser et al. 1977). During a survey of 253 melon (*Cucumis melo* L.) accessions of different geographical origins, Diaz et al. (2003) reported that resistances to CMV were almost absent. However, there was heterogeneity in the responses of some accessions to some strains. For example, resistance in the Korean accession 'Sonwang charmi, PI 161375' (from now on, SC) and Freeman's Cucumber was isolate-specific for three strains of the common type. Freeman's Cucumber exhibited partial resistance to isolate B20.2 and total resistance to M6, whereas SC showed partial resistance to B20.2 and total resistance to isolate M373. Dogimont et al. (2000) used a collection of recombinant inbred lines (RILs) between the CMV-resistant parental SC and the susceptible Védrantais to investigate the genetic control of the resistance present in SC when challenged with three different CMV isolates, P9, TL, both of subgroup I, and To 72, belonging to subgroup II. They reported that the trait was governed by several QTLs, one of them with major effect, located in linkage group XII of the map of Perin et al. (2002).

Since viruses depend on host factors to complete their infectious cycle, it is likely that mutations in these host factors will result in resistance to such viruses (Fraser 1990). In fact, all recessive resistance genes characterised to date, either identified in natural mutations or mutagenized populations, have been found to correspond to mutations in components of the eukaryotic translation initiation complex (for a review, see Robaglia and Caranta 2006; Maule et al. 2007). Mutations in eIF4E, eIF4G and their paralogs eIF(iso)4E and eIF4(iso)G are involved in resistance mainly against potyviruses, but also against the potyvirus-related *Barley yellow mosaic bymovirus* (Stein et al. 2005), and potyvirus non-related *Melon necrotic spot carmovirus* (MNSV) (Nieto et al. 2006) and *Rice yellow mottle sobemovirus* (Albar et al. 2006). This indicates that the resistance mechanism might be related to inability of the virus to use the translation machinery to multiply and/or accumulate in the resistant cell. In some cases, a combination of mutations in more than one eIF is required to achieve complete resistance. For example, simultaneous mutations in both eIF4E and eIF(iso)4E are required for complete resistance to *Pepper veinal mottled potyvirus* in pepper (Ruffel et al. 2006). However, in other cases, viral movement is compromised rather than viral translation. For example, eIF4E, apart from its role in translation of the viral RNA, has been implicated in cell-to-cell movement of *Pea seed borne mosaic potyvirus* (PSbMV) in pea (Gao et al. 2004). Likewise, eIF(iso)4E has been suggested to act in *Turnip mosaic potyvirus* (TuMV) transport in *Arabidopsis*, as it interacts with the viral encoded protein VPg and eIF(iso)4G, and this complex interacts with microtubules (Lellis et al. 2002). Instead, eIF4E and eIF4G (*cum 1* and *cum2*) have a role in CMV movement in *Arabidopsis* by participating in translation of the MP, but not in translation of other viral proteins (Yoshii et al. 2004). In this case, resistance to CMV accounts for the lack of movement of the virus, rather than for the lack of translation.

Here we have used a collection of NILs containing introgressions of SC in the background of the cultivar "Piel de Sapo" (PS) (Eduardo et al. 2005) to genetically dissect the resistance to CMV. We report that the recessive resistance to CMV is strain-specific and that the major QTL previously reported in LGXII was, in fact, enough to confer total resistance to two strains of CMV. We have also mapped ten eukaryotic translation initiation factors in the melon genetic map to test them as candidates for the resistance.

Materials and methods

Plants and viruses

Genotypes used for the characterisation of the resistance to CMV present in *C. melo* were: cultivar PS and the Korean accession PI 161375 cultivar Sonwang charmi (syn. PI161375) (SC) as susceptible and resistant controls, respectively, near isogenic lines (NILs) SC12-1, SC12-2, SC12-3 and SC12-4, with a single, independent introgression from SC on linkage group XII (Fig. 1) (Eduardo et al. 2005), and an F1 from the cross SC12-1 × PS. Double haploid lines (DHLs) from the cross SC × PS (Gonzalo et al. 2005) were used for mapping of candidate genes. NILs (or subNILs) with shorter introgressions to increase mapping resolution were obtained by selecting recombinant F_2 plants generated from the cross between SC12-1 and PS using markers flanking the introgression (see below). Recombinant plants were selected and selfed. F3 plants were scored with appropriate markers to fix the recombinant chromosomes and to determine the exact point of recombination (see below).

For virus inoculation experiments, seeds were pre-germinated by soaking them in water overnight and then kept for 2 days in light at 28°C. Seedlings were grown in growth chambers SANYO MLR-350H in long-day conditions consisting of 25°C for 16 h with 20,000 lux of light and 23°C for 8 h in dark for all infections except for P104.82 infections, which required 22°C for 16 h with 20,000 lux light, and 18°C for 8 h in dark. CMV strains P9, TL, M730, and M6 belonging to subgroup I isolates were described previously (Diaz et al. 2003; Dogimont et al. 2000). M730 is a

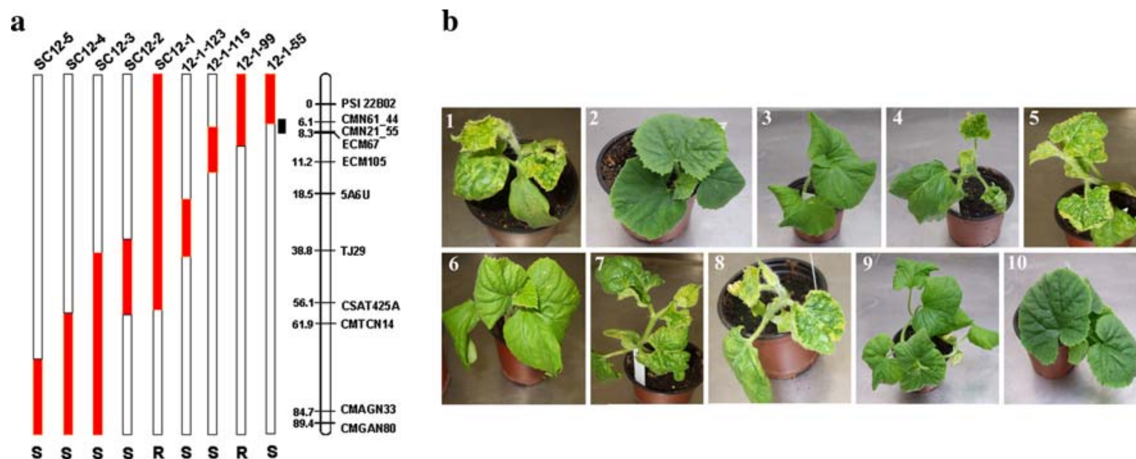

Fig. 1 **a** Near isogenic lines (*NILs*) of linkage group XII and subNILs developed from NIL SC12-1. In *red*, the introgression from the resistant parental SC. In *white*, the genome of the susceptible parental PS. The *bar on the right* shows the LGXII map with some molecular markers and their relative genetic distances recalculated after the addition of new molecular markers to those reported, after Gonzalo et al. (2005), in Fig. 3. *R* resistant to CMV strain P104.82. *S* susceptible to CMV P104.82. The *black rectangle* indicates the region where *cmv1* is located. **b** Plants from relevant accessions showing symptoms 30 days after inoculation with CMV P104.82. *1* PS, *2* SC, *3* SC12-1, *4* SC12-3, *5* SC12-1-55, *6* SC12-1-99, *7* SC12-1-115, *8* SC12-1-123, *9* PS noninoculated, *10* SC noninoculated

song-like pathotype (Leroux et al. 1979), able to overcome SC resistance. P104.82 belonging to subgroup II was kindly provided by Marisol Luis-Arteaga (Plant Protection Dept., CITA, Zaragoza, Spain).

Inoculations and virus detection

Viral inocula were freshly prepared from infected zucchini squash ('Diamant F1', Seminis Vegetable Seeds Iberica, Almería, Spain or 'Chapin F1', Semillas Fito SA, Barcelona, Spain) and rub-inoculated onto the cotyledons of 7- to 10-day-old melon plants. Symptoms were scored visually 30 days after inoculation. Then, viral detection was performed either by DAS-ELISA or by RT-PCR from young newly developed leaves. DAS-ELISA was performed with CMV-specific polyclonal antisera from Loewe Biochemica GmbH (Otterfing, Germany) according to manufacturer's instructions. ELISA reactions were measured spectrophotometrically at 405 nm using the plate reader Anthos2001 (Anthos Labtec Instruments GmbH, Salzburg, Austria). Negative controls consisted of leaf tissue extracts from mock-inoculated plants of each of the genotypes tested. Positive controls consisted of leaf tissue extracts from PS plants infected with the different CMV strains tested. A sample was considered positive if the *A*405 nm value was greater than twice that of the negative control. For RT-PCR, RNA was isolated from young newly developed leaves using TriReagent (SIGMA-ALDRICH, St Louis, MO, USA) according to manufacturer's instructions. RT-PCR was made using Superscript RT (Invitrogen Life Technologies, Carlsbad, CA, USA) and Taq polymerase (Promega Corporation, Madison, WI) according to manufacturer's instructions. The primers used were CMVCP (5′TTTAGAGTCTTGTCGCAGCAGCAGC) as forward primer and PCVMC (5′AGGAGGCAGGAACTTTACGGAC) as reverse primer, amplifying a 300 bp fragment of the coat protein.

Development of molecular markers from candidate genes

DNA extraction from melon leaves was carried out using the Doyle method (Doyle and Doyle 1990) as modified by Garcia-Mas et al. (2000). For PCR, primers were designed using Primer 3 programme (http://frodo.wi.mit.edu/cgibin/primer3/primer3_www.cgi) (Rozen and Skaletsky 2000). Amplified fragments from both PS and SC were purified with sepharose columns and sequenced using the ABI PRISM Bigdye Terminator Cycle Sequencing kit (Applied Biosystems, Foster City, CA, USA) in an ABI PRISM 3130 sequencer (Applied Biosystems, Foster City, CA, USA). For SNP discovery, sequences obtained from both parental lines were aligned using BioEdit (Hall 1999). SNPs were detected either by enzyme restriction digestion (CAPS) or SNaPshot (Applied Biosystems, Foster City, CA, USA) (Morales et al. 2004). Simple-sequence repeat markers (SSRs) were amplified with primers flanking the repetitive sequence. One of the primers was labelled with IRD-800 (MWG Biotech AG, Ebersberg, Germany) and fragments were visualized with a LICOR IR2 sequencer (Li-cor Inc, Lincoln, New England, USA) as described in Gonzalo et al. (2005).

Mapping

Molecular markers used to saturate the region in LGXII were PSI_22-B02 (with forward primer GCAGGCTGAAC

AAATGTGG and reverse primer CCAAGAACTTTGCCT CCAAC amplifying a 800 bp fragment polymorphic after digestion with *Hinf*I, Deleu and Garcia-Mas, personal communication), 5A6U (with forward primer TCCATTGGTT AAAAAGAAAGACG and reverse primer TTCATTTTT GTATTCACTGCATTT amplifying a 213 bp fragment), CMN61_44 and CMN21_55 (Fukino et al. 2007), ECM67 (with forward primer CTCAACGAGTCGCCCAAG and reverse primer TTGGTCGACGCTGTAATGTC amplifying a 128 bp fragment) and ECM105 (with forward primer TTCTTGCAAATTGTCCAGACC and reverse primer AGAGATTGGCCATCATCGAC amplifying a 87 bp fragment). Markers were mapped in the SC × PS melon genetic map using the DHL mapping population (Gonzalo et al. 2005). All of them were SSRs, except PSI_22-B02, which was mapped as a CAPS. A linkage map was constructed independently with MAPMAKER, version 3.0 (Lander et al. 1987). Markers were ordered using the order command with LOD > 3.0 and distances were calculated with the Kosambi function (Kosambi 1944). Markers for candidate genes were mapped by selective genotyping using the bin-mapping strategy (Howad et al. 2005) as in Moreno et al. (2007).

Results

Localization of a major QTL for resistance to CMV strains P9 and P104.82

To characterise the major QTL for resistance to three CMV strains described by Dogimont et al. (2000), we used a collection of NILs with introgressions of SC into the genome of PS (Eduardo et al. 2005). This collection contains five NILs representing LGXII (SC12-1 to SC12-5) (Fig. 1). We have used in this study NILs SC12-1 and SC12-3, since they cover the whole LGXII (Fig. 1). To identify the NIL harbouring the major QTL described by Dogimont et al. (2000), we performed a series of inoculations in plants from both NILs using several strains of CMV. As shown in Table 1, out of all of them, NIL SC12-1 was resistant to strains P9 and P104.82, whereas NIL SC12-3 was susceptible to these virus strains. Conversely, CMV strains TL and M6 were able to infect both SC12-1 and SC12-3, implying that the locus for resistance to these two strains was located in a different linkage group. Control SC was resistant to all strains except when inoculated with the song strain M730, which is reported to overcome the resistance present in SC. Control PS was susceptible to all strains, although strain P9 was less aggressive than P104.82, infecting typically only 30–40% of inoculated PS and SC12-3 plants. Therefore, to more precisely characterise the resistance present in NIL SC12-1, strain P104.82 was used, since this strain was able to infect more than 90% of inoculated plants of the susceptible control PS and SC12-3. In complementary experiments we observed that NILs SC12-2 and SC12-4 (Fig. 1) were also susceptible to strains P9 and P104.82 (data not shown). Therefore, we concluded that the major QTL for resistance to CMV described by Dogimont et al. (2000) was effective for only some strains, like P9 and P104.82 and resided in the introgression of SC present in NIL SC12-1 in the area flanked by markers TJ29 (Gonzalo et al. 2005) and PSI_22-B02 (Deleu and Garcia-Mas, personal communication) (see Fig. 1). Furthermore, this major QTL was not effective against strain TL, contrary to the results reported by Dogimont et al. (2000). We have named the resistance to CMV present in SC12-1 as *cmv1*.

In order to fine map the *cmv1* locus, five molecular markers (CMN61_44, CMN21_55, ECM67, ECM105, and 5A6U) located in the interval between PSI_22-B02 and TJ29 were phenotyped in the whole mapping population (Fig. 1). To delimit the region containing *cmv1*, 171 plants of the F2 between NIL SC12-1 and the susceptible parental PS, were genotyped using the flanking markers PSI_22-B02 and TJ29. Sixty-six plants were found to contain recombinations between the flanking markers, and the markers in the interval were used to define the introgression present in each recombinant. Four subNILs, spanning the introgression of SC12-1 associated with the major QTL for resistance to CMV, were chosen to test susceptibility to CMV strain P104.82. As shown in Fig. 1, whereas subNIL SC12-1-99 was resistant, subNILs SC12-1-55, SC12-1-115

Table 1 Infection of melon accessions with different CMV strains

CMV strains	PS	SC	SC12-1	SC12-3	F1 (SC12-1× PS)	Planters Jumbo	Gulfstream
P9	4/11[a]	0/11	0/24	6/14	8/20	ND	ND
P104.82	12/13	0/9	0/14	13/13	7/7*	7/8*	7/9*
M6	6/6	0/20	10/13	13/13	5/5*	5/5*	5/5*
TL	5/10	0/10	10/16	8/12	ND	ND	ND
M730	6/6*	6/6*	6/6*	6/6*	ND	ND	ND

Numbers are the result of at least two independent experiments except in the cases indicated with an asterisk

[a] Number of plants showing symptoms/number of inoculated plants

and SC12-1-123 were susceptible indicating that *cmv1* mapped in the 2.2 cM interval between CMN61_14 and CMN21_55.

The resistance present in SC12-1 is recessive, but not quantitative

The evaluation of infections was performed by scoring symptoms and confirmed by DAS-ELISA in extracts from young newly developed leaves. DAS-ELISA did not show any positive reaction in extracts of P104.82-inoculated SC12-1 plants (data not shown). However, since the resistance in SC had been described as quantitative, we expected to find some level of viral accumulation in the young newly developed leaves that perhaps was under the level of detection of the technique. However, as shown in Fig. 2, RT-PCR analysis was unable to detect any CMV presence in our SC12-1 inoculated plants, whereas the viral nucleic acids were readily detected in young newly developed leaves of the susceptible NIL SC12-3 and parental PS (Fig. 2). Therefore, we concluded that the QTL present in SC 12-1 was necessary and sufficient to confer total resistance to CMV strains P9 and P104.82, similarly to the resistance present in the parental SC. Furthermore, it suggests that the other QTLs described by Dogimont et al. (2000) either had no role in the resistance of SC to these CMV strains or had a redundant role, indicating that the resistance conferred by *cmv1* is not quantitative, but qualitative. To further characterise the nature of the resistance present in SC12-1 as dominant or recessive, the hybrid between SC12-1 and PS was inoculated with strain P104.82. As shown in Table 1, all hybrid plants were susceptible, confirming that the resistance to CMV present in LGXII was recessive.

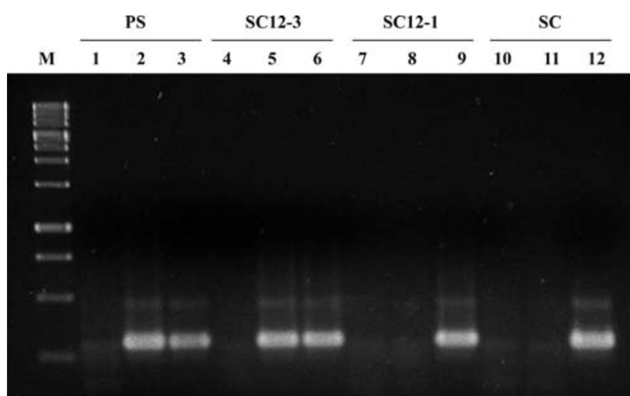

Fig. 2 RT-PCR analysis from the upper systemically infected leaves of PS, SC12-3, SC12-1 and SC plants. Plants were either mock-inoculated (*lanes 1*, *4*, *7* and *10*) or infected with CMV P104.82 (*lanes 2*, *5*, *8* and *11*) and CMV M730 (*lanes 3*, *6*, *9* and *12*). M lane is the 1 kb DNA ladder size marker

Mapping of candidate genes

As an approach to identify the resistance gene present in the SC introgression of SC12-1, we carried out a strategy of mapping candidate genes involved in recessive resistance in order to see if any of them mapped in the interval of the introgression. The Melogen database (Gonzalez-Ibeas et al. 2007; http://www.melogen.upv.es/) was screened to search for ESTs corresponding to melon orthologues of translation initiation factors from *Arabidopsis thaliana*. We retrieved from the database sequences for one eIF(iso)4E, one eIF4G, two eIF(iso)4G, four eIF4A, one novel Cap Binding protein (nCBP) and three polyA binding proteins (PABP) (Table 2). All of them were mapped in the SC × PS genetic map (Gonzalo et al. 2005) after SNP discovery and bin-mapping (Fig. 3), except two sequences encoding PABP and one sequence with homology to eIF4A that were monomorphic between the parental lines (data not shown). The position of eIF4E was previously known, in LGXII near marker 5A6U (Morales et al. 2005), as it corresponded to the recessive resistance *nsv* against MNSV present in SC (Nieto et al. 2006). Mapping of all these translation factors is summarised in Fig. 3 and showed that none of them mapped in linkage group XII, in the area of the introgression present in SC12-1, except eIF4E. Nevertheless, we ruled out eIF4E as a candidate for the CMV resistance, since it co-segregates with marker 5A6U, which in our mapping experiments with the subNILs is located outside the region delimited by markers CMN61_44 and CMN21_55 (Fig. 1). Furthermore, melon accessions Gulfstream and Planters Jumbo, which harbour the single mutation found in eIF4E that confers resistance against MNSV, are susceptible to CMV strains P9 and p104.82 (Table 1). Taken together, these results show that none of the candidate genes mapped to date in the melon genetic map correspond to *cmv1*.

Discussion

We have described here the locus *cmv1* for resistance to CMV, present in the exotic accession SC and in the NIL SC12-1. This locus confers recessive and qualitative resistance to several strains of CMV. Resistance to CMV had previously been reported as oligogenic and recessive (Karchi et al. 1975) and quantitative, with most of the phenotypic variation explained by a QTL present in LGXII for resistance to three strains (Dogimont et al. 2000). These authors performed a QTL analysis using a RIL collection between Védrantais and SC. Our analysis has been carried out using a NIL collection with introgressions from the same resistant genotype. NIL collections are the best tool to dissect complex characters, since they permit to mendelize

Table 2 Melon candidate genes encoding translation initiation factors

Sequence[a]	GenBank accession	Gene name	Best *Arabidopsis* Hit (BlastX)	*E* value	Primer sequences (forward and reverse, 5′ → 3′)	SNP/indel (PI → PS)	SNP detection method	Linkage group
15d_04-C12	AM738186	*CmeIF(iso)4E*	Eukaryotic initiation factor 4E isozyme form At5g35620	1E-69	CAGGTGAAAATCTGCATTCG CGTCGAAGAATTTTGGTGGT	G → C	CAPS (*Pfl*MI)	V
PS_25-G02	AM716939	*CmeIF4G*	Eukaryotic initiation factor 4G At3g60240	9E-64	GAGAAAGGCGAAGGGTTCTT TAAATCGTGACAGCTCATGG	A → C	CAPS (*Nla*III)	V
PSI_38-G06	AM723260	*CmeIF(iso)4G-1*	Eukaryotic initiation factor 4G isozyme form At5g57870	3E-71	TATGCTCCATGACGCAGGTA CCTGTTCTTTGTAGCCACCTT	A → G	CAPS (*Hha*I)	II
A_31-C07	AM728729	*CmeIF(iso)4G-2*	Eukaryotic initiation factor 4G isozyme form At2g24050	4E-54	GTCGCTCTCAACCCTAAACCT TCAACATCCCGAGAATCTCAC	C → T	CAPS (*Pst*I)	VII
PS_18-G09	AM719341	*CmnCBP*	Novel cap binding protein At5g18110	1E-96	GCATTAGTGGGCGATCAACT TCAGATGCATTACGGTTCCA	T → C	CAPS (*Nde*I)	IV
A_24-E01	AM727204	*CmPABP*	Poly (A) binding protein At1g49760	2E-66	GTACGTTGGAAAAGCCCAGA CGCGTCCTCTAGGTAGCATC CTGTATTTTCAATTGTGGATGGAGTT[b]	T → G	SNaPshot	I
CI_36-D02	AM731697	*CmeIF4A-1*	Eukaryotic initiation factor 4A At1g54270	1E-25	TGCCGCTGCTACACTTAACTT GACTGGGCTTGTTGGATTACA	A → T	CAPS (*Nla*III)	XI
PS_33-A09	AM718612	*CmeIF4A-2*	Eukaryotic initiation factor 4A At1g54270	0	CTGCTACAATGCCTCCTGAAG TGCTGGCAGCTCTTCAATTAT	T → C	CAPS (*Fok*I)	IV
HS_15-F12	AM724572	*CmeIF4A-3*	Eukaryotic initiation factor 4A At1g54270	0	GGCAAACCCTAGCTTCCTCT GTCGGTGCCAAAACTAATGC TAATTCCTAGAGTTTTCCCCATT[b]	G → C	SNaPshot	I
–	DQ393830	*CmeIF4E*[c]	Eukaryotic initiation factor 4E At4G18040	2E-84	CCGATCTATACCTTCTCTACC TACAAATCTGCCCTCATCGCC	–	CAPS (*Rsa*I)	XII

The best BlastX hit for each sequence is indicated. Primer sequences, SNPs identified, SNP detection method and melon linkage group are indicated. For CAPS markers the restriction enzyme is also shown

[a] ESTs from MELOGEN database. Some of them are singletons. For ESTs contained in contigs, a representative EST from the contig is given

[b] Primers for SNaPshot polymorphism detection

[c] Published in Nieto et al. (2006)

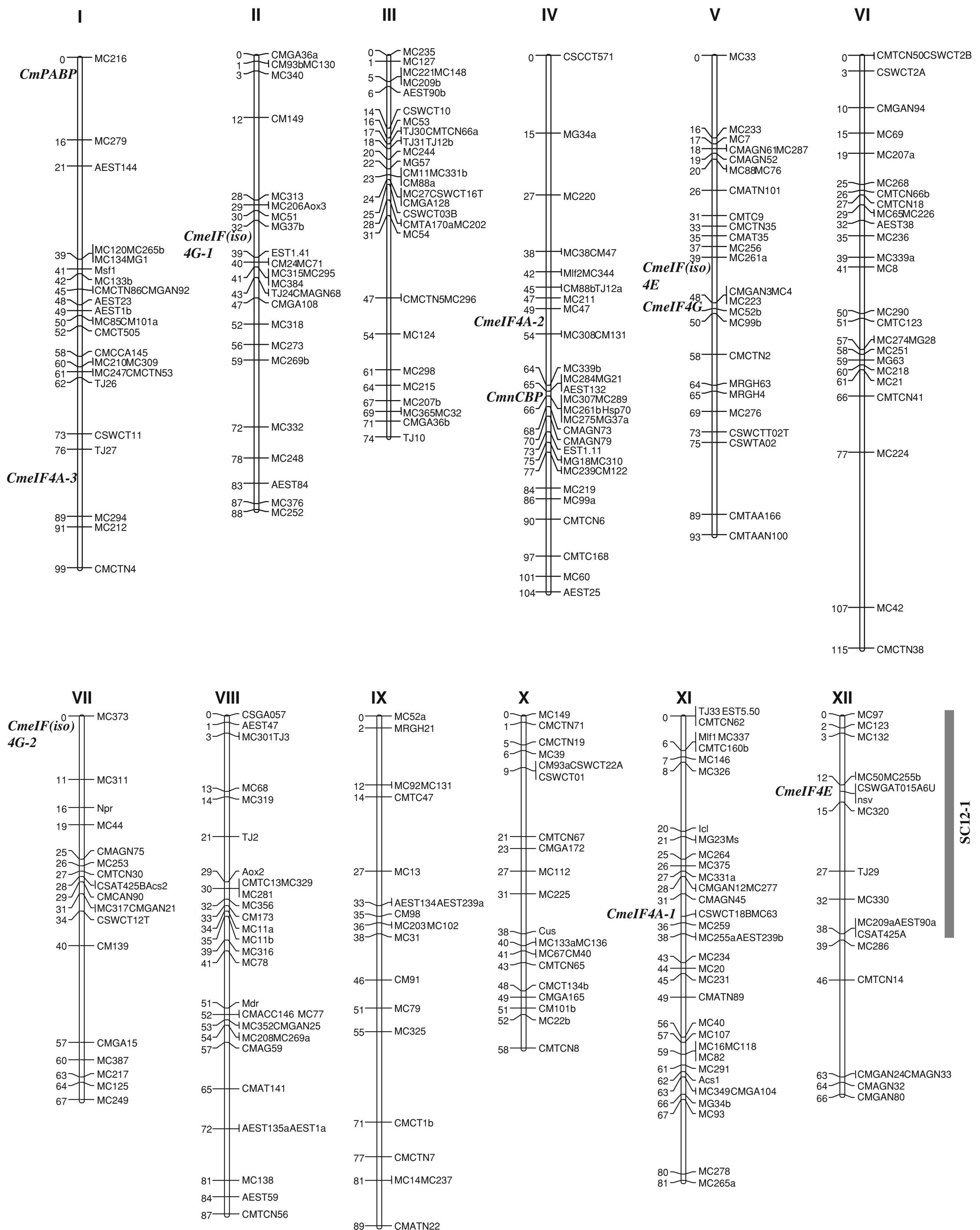

Fig. 3 Candidate genes mapping in the genetic map of *Cucumis melo*. Linkage groups are named according to Perin et al. (2002). The introgression of SC in NIL SC12-1 is indicated as a *grey bar*

traits under polygenic control (Zamir 2001). Our results show that in fact, the major QTL present in LGXII explained the whole resistance to strains P9 and P104.82, but did not confer any resistance to strains TL and M6. Therefore, the resistance present in this linkage group, which could be mapped in a 2.2 cM interval flanked by two SSR markers, is controlled by one unique locus, instead of being polygenic. Likewise, for strains TL and M6, there should be one or more loci responsible for the resistance, defined in other genomic regions which should be further studied. Therefore, our results suggest that we can re-define the resistance to CMV as oligogenic for the whole species, but monogenic—governed by independent loci—for each set of strains, being at least one of these loci—the one present in LGXII—independent of the other(s). This new finding would be important for breeding programmes, since historically, breeding resistances to CMV in melon have been very difficult due to its oligogenic nature. Being able to dissect it into independent monogenic characters would allow breeders to piramide these loci to produce cultivars resistant to different CMV strains. Monogenic resistances controlled by several independent loci have already been described in *C. melo* against the foliar pathogen *Didymella bryoniae*, the agent of gummy stem blight (Frantz and Jahn 2004), where resistance to this pathogen is controlled by four dominant and one recessive-independent genes. Similarly, in pea (*Pisum sativum* L.), independent recessive resistances against different pathotypes of PsbMV and other potyviruses have been mapped in linkage groups II and VI (reviewed in Provvidenti and Hampton 1992) and in *Brassica napa*, two genes control broad-spectrum resistance to TuMV (Rusholme et al. 2007).

Recessive resistance genes against viruses described to date encode for eukaryotic translation initiation factors. Although only four of them [eIF4E, eIF4G, eIF(iso)4E and eIF(iso)4G] have been reported to date as resistance genes in plants, we can not discard this role for other initiation factors. For example, eIF4A is a helicase that participates in unfolding the 5′UTR of capped or uncapped RNAs. In yeast, a DED1 helicase, like eIF4A, regulates translation of Brome mosaic virus RNA2 (Noueiry et al. 2000), thus in plants eIF4A could be involved in virus resistance. According to this idea, we have mapped in the genetic melon map several translation initiation factors whose sequences are in the melon EST database (MELOGEN) in order to identify those mapping in the area of the resistance to CMV present in the NIL SC12-1. Out of ten candidate genes, only eIF4E was found to reside in LGXII. However, according to our results, eIF4E maps in LGXII in a region different from the locus *cmv1*, which is defined by markers CMN61_44 and CMN21_55. Nevertheless, we cannot discard the possibility that the resistance described here might be encoded by another translation initiation factor gene whose sequence is not present in our EST collection. In *Arabidopsis* there are three eIF4E genes, one eIF(iso)4E, one eIF4G, two eIF(iso)4G, three additional eIF4G-like genes, nine putative PABPs, two eIF4A genes and four eIF4A-like sequences (TAIR, http://www.arabidopsis.org/browse/genefamily/eIF.jsp), suggesting that additional work is needed because we may be underestimating the number of translation initiation factors present in the melon genome and more will have to be mapped and analysed as candidates for *cmv1*. Alternatively, other type of plant host factors required for CMV life cycle steps other than translation of the viral genome might be involved, such as factors for short or long-distance movement or suppression of gene silencing (Voinnet 2001). For example, a phloem protein in cucumber has been reported to be involved in long-distance movement of CMV (Requena et al. 2006).

Future work will focus in fine mapping the locus *cmv1*. Synteny has already been described between several regions of the melon genome and *Arabidopsis thaliana*, *Medicago truncatula* and *Populus trichocarpa* (van Leeuwen et al. 2003; Deleu et al. 2007), leading to the cloning of the *nsv* gene (Nieto et al. 2006). It would be interesting to look for a possible synteny between the region surrounding the *cmv1* locus and model species. Additionally, the identification of the other genes for resistance to other CMV strains, present in SC, will require a screening of the whole NILs collection in order to more precisely dissect the nature of the interactions between each set of strains and their determinants of resistance.

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Note added in proofs During the publishing procedure a paper concerning the mapping of markers ECM67 and ECM105 has been published. The reference is Fernández-Silva, I Moreno E, Eduardo I, Arús P, Alvarez JM, Monforte AJ. (2008) On the Genetic Control of Heterosis for Fruit Shape in Melon (*Cucumis Melo* L.). J. of Heredity DOI 10.1007/s00122-008-0883-3.

References

Albar L, Bangratz-Reyser M, Hebrard E, Ndjiondjop MN, Jones M, Ghesquiere A (2006) Mutations in the eIF(iso)4G translation initiation factor confer high resistance of rice to Rice yellow mottle virus. Plant J 47:417–426

Caranta C, Pflieger S, Lefebvre V, Daubeze AM, Thabuis A, Palloix A (2002) QTLs involved in the restriction of *Cucumber mosaic*

virus (CMV) long-distance movement in pepper. Theor Appl Genet 104:586–591

Deleu W, Gonzalez V, Monfort A, Bendahmane A, Puigdomenech P, Arus P, Garcia-Mas J (2007) Structure of two melon regions reveals high microsynteny with sequenced plant species. Mol Genet Genomics 278:611–622

Diaz JA, Mallor C, Soria C, Camero R, Garzo E, Fereres A, Alvarez JM, Gómez-Guillamón ML, Luis-Arteaga M, Moriones E (2003) Potential sources of resistance for melon to nonpersistently aphid-borne viruses. Plant Dis 87:960–964

Dogimont C, Leconte L, Perin C, Thabuis A, Lecoq H, Pitrat M (2000) Identification of QTLs contributing to resistance to different strains of *Cucumber Mosaic cucumovirus* in melon. Acta Hortic 510:391–398

Doyle JJ, Doyle JL (1990) Isolation of plant DNA from fresh tissue. Focus 12:13–15

Eduardo I, Arus P, Monforte AJ (2005) Development of a genomic library of near isogenic lines (NILs) in melon (*Cucumis melo* L.) from the exotic accession PI161375. Theor Appl Genet 112:139–148

Frantz JD, Jahn MM (2004) Five independent loci each control monogenic resistance to gummy stem blight in melon (*Cucumis melo* L.). Theor Appl Genet 108:1033–1038

Fraser R (1990) The genetics of resistance to plant viruses. Annu Rev Phytopathol 28:179–200

Fukino N, Sakata Y, Kunihisa M, Matsumoto S (2007) Characterisation of novel simple sequence repeat (SSR) markers for melon (*Cucumis melo* L.) and their use for genotype identification. J Hortic Sci Biotechnol 82:330–334

Gao Z, Johansen E, Eyers S, Thomas CL, Noel Ellis TH, Maule AJ (2004) The potyvirus recessive resistance gene, sbm1, identifies a novel role for translation initiation factor eIF4E in cell-to-cell trafficking. Plant J 40:376–385

Garcia-Mas J, Oliver M, Gómez-Paniagua H, de Vicente MC (2000) Comparing AFLP, RAPD and RFLP markers for measuring genetic diversity in melon. Theor Appl Genet 101:860–864

Gonzalez-Ibeas D, Blanca J, Roig C, González-To M, Picó B, Truniger V, Gómez P, Deleu W, Caño-Delgado A, Arús P, Nuez F, Garcia-Mas J, Puigdomènech P, Aranda MA (2007) MELOGEN: an EST database for melon functional genomics. BMC Genomics 8:306

Gonzalo MJ, Oliver M, Garcia-Mas J, Monfort A, Dolcet-Sanjuan R, Katzir N, Arus P, Monforte AJ (2005) Simple-sequence repeat markers used in merging linkage maps of melon (*Cucumis melo* L.). Theor Appl Genet 110:802–811

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser 41:95–98

Howad W, Yamamoto T, Dirlewanger E, Testolin R, Cosson P, Cipriani G, Monforte AJ, Georgi L, Abbott AG, Arus P (2005) Mapping with a few plants: using selective mapping for microsatellite saturation of the Prunus reference map. Genetics 171:1305–1309

Karchi Z, Cohen S, Govers A (1975) Inheritance of resistance to *Cucumber mosaic virus* in melons. Phytopathology 65:479–481

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg L (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181

Lellis AD, Kasschau KD, Whitham SA, Carrington JC (2002) Loss-of-susceptibility mutants of *Arabidopsis thaliana* reveal an essential role for eIF(iso)4E during potyvirus infection. Curr Biol 12:1046–1051

Leroux JP, Quiot JB, Lecoq H, Pitrat M (1979) Mise en évidence et répartition dans le Sud-Est de la France d'un pathotype particulier du Virus de la Mosaïque du Cocombre. Ann Phytopathol 11:431–438

Maule AJ, Caranta C, Boulton MI (2007) Sources of natural resistance to plant viruses: status and prospects. Mol Plant Pathol 8:223–233

Morales M, Roig E, Monforte AJ, Arus P, Garcia-Mas J (2004) Single-nucleotide polymorphisms detected in expressed sequence tags of melon (*Cucumis melo* L.). Genome 47:352–360

Morales M, Orjeda G, Nieto C, van Leeuwen H, Monfort A, Charpentier M, Caboche M, Arus P, Puigdomenech P, Aranda MA, Dogimont C, Bendahmane A, Garcia-Mas J (2005) A physical map covering the nsv locus that confers resistance to Melon necrotic spot virus in melon (*Cucumis melo* L.). Theor Appl Genet 111:914–922

Moreno E, Obando JM, Dos-Santos N, Fernández-Trujillo JP, Monforte AJ, Garcia-Mas J (2007) Candidate genes and QTLs for fruit ripening and softening in melon. Theor Appl Genet 116:589–602

Nieto C, Morales M, Orjeda G, Clepet C, Monfort A, Sturbois B, Puigdomenech P, Pitrat M, Caboche M, Dogimont C, Garcia-Mas J, Aranda MA, Bendahmane A (2006) An eIF4E allele confers resistance to an uncapped and non-polyadenylated RNA virus in melon. Plant J 48:452–462

Noueiry AO, Chen J, Ahlquist P (2000) A mutant allele of essential, general translation initiation factor DED1 selectively inhibits translation of a viral mRNA. Proc Natl Acad Sci USA 97:12985–12990

Palukaitis P, Roossinck MJ, Dietzgen RG, Francki RIB (1992) *Cucumber mosaic virus*. Adv Virus Res 41:281–348

Perin C, Hagen S, De Conto V, Katzir N, Danin-Poleg Y, Portnoy V, Baudracco-Arnas S, Chadoeuf J, Dogimont C, Pitrat M (2002) A reference map of *Cucumis melo* based on two recombinant inbred line populations. Theor Appl Genet 104:1017–1034

Provvidenti R, Hampton RO (1992) Sources of resistance to viruses in the Potyviridae. Arch Virol Suppl 5:189–211

Requena A, Simon-Buela L, Salcedo G, Garcia-Arenal F (2006) Potential involvement of a cucumber homolog of phloem protein 1 in the long-distance movement of *Cucumber mosaic virus* particles. Mol Plant Microbe Interact 19:734–746

Risser G, Pitrat M, Rode JC (1977) Etude de la résistance du melon (*Cucumis melo* L.) au virus de la mosaïque du concombre. Ann Amél Plant 27:509–522

Robaglia C, Caranta C (2006) Translation initiation factors: a weak link in plant RNA virus infection. Trends Plant Sci 11:40–45

Rozen S, Skaletsky H (2000) Primer3 on the WWW for general users and for biologist programmers. Methods Mol Biol 132:365–386

Ruffel S, Gallois JL, Moury B, Robaglia C, Palloix A, Caranta C (2006) Simultaneous mutations in translation initiation factors eIF4E and eIF(iso)4E are required to prevent pepper veinal mottle virus infection of pepper. J Gen Virol 87:2089–2098

Rusholme RL, Higgins EE, Walsh JA, Lydiate DJ (2007) Genetic control of broad-spectrum resistance to turnip mosaic virus in Brassica rapa (Chinese cabbage). J Gen Virol 88:3177–3186

Seo YS, Rojas MR, Lee JY, Lee SW, Jeon JS, Ronald P, Lucas WJ, Gilbertson RL (2006) A viral resistance gene from common bean functions across plant families and is up-regulated in a non-virus-specific manner. Proc Natl Acad Sci USA 103:11856–11861

Stamova BS, Chatelat RT (2000) Inheritance and genetic mapping of *Cucumber mosaic virus* resistance introgressed from *Lycopersicon chilense* into tomato. Theor Appl Genet 1001:527–537

Stein N, Perovic D, Kumlehn J, Pellio B, Stracke S, Streng S, Ordon F, Graner A (2005) The eukaryotic translation initiation factor 4E confers multiallelic recessive Bymovirus resistance in *Hordeum vulgare* (L.). Plant J 42:912–922

Takahashi H, Suzuki M, Natsuaki K, Shigyo T, Hino K, Teraoka T, Hosokawa D, Ehara Y (2001) Mapping the virus and host genes involved in the resistance response in *Cucumber mosaic virus*-infected *Arabidopsis thaliana*. Plant Cell Physiol 42:340–347

Valkonen JP, Watanabe KN (1999) Autonomous cell death, temperature sensitivity and the genetic control associated with resistance to *Cucumber mosaic virus* (CMV) in diploid potatoes (*Solanum* spp). Theor Appl Genet 99:996–1005

van Leeuwen H, Monfort A, Zhang HB, Puigdomenech P (2003) Identification and characterisation of a melon genomic region containing a resistance gene cluster from a constructed BAC library. Microcolinearity between *Cucumis melo* and *Arabidopsis thaliana*. Plant Mol Biol 51:703–718

Voinnet O (2001) RNA silencing as a plant immune system against viruses. Trends Genet 17:449–459

Yoshii M, Yoshioka N, Ishikawa M, Naito S (1998) Isolation of an *Arabidopsis thaliana* mutant in which the multiplication of both *Cucumber mosaic virus* and *Turnip Crinkle virus* is affected. J Virol 72:8731–8737

Yoshii M, Nishikiori M, Tomita K, Yoshioka N, Kozuka R, Naito S, Ishikawa M (2004) The *Arabidopsis cucumovirus* multiplication 1 and 2 loci encode translation initiation factors 4E and 4G. J Virol 78:6102–6111

Zamir D (2001) Improving plant breeding with exotic genetic libraries. Nat Rev Genet 2:983–989