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Key words: *Cucumis melo*, begomovirus, MeCMV, ToLCNDV, genetic control, inheritance

Abstract

Thirty-one melon accessions were screened for resistance to the begomoviruses *Melon chlorotic mosaic virus* (MeCMV) and *Tomato leaf curl New Delhi virus* (ToLCNDV). Five accessions presented nearly complete resistance to both viruses. Accession IC-274014, showing the highest level of resistance to both viruses, was crossed with the susceptible cultivar Védrantais. The F_1, F_2, F_3/F_4 and both back-cross progenies were mechanically inoculated with MeCMV. Plants without symptoms nor virus detection by ELISA and/or PCR were considered as resistant. The segregations were compatible with two recessive and one

dominant independent genes simultaneously required for resistance. Inheritance of resistance to ToLCNDV in the F_2 was best explained by one recessive gene and two independent dominant genes simultaneously required. Some F_3 and F_4 families selected for resistance to MeCMV were also resistant to ToLCNDV, suggesting that common or tightly linked genes were involved in resistance to both viruses. We propose the names *begomovirus resistance-1* and *Begomovirus resistance-2* for these genes (symbols *bgm-1* and *Bgm-2*). Resistance to MeCMV in IC-274014 was controlled by *bgm-1*, *Bgm-2* and the recessive gene *melon chlorotic mosaic virus resistance* (*mecmv*); resistance to ToLCNDV was controlled by *bgm-1*, *Bgm-2* and the dominant gene *Tomato leaf curl New Delhi virus resistance* (*Tolcndv*).

Introduction

Melon (*Cucumis melo* L.) is one of the major crop species in the family *Cucurbitaceae.* Global production of melon is circa 30,000,000 tons per year (www.fao.org/faostat). Cucurbit diseases caused by begomoviruses (genus *Begomovirus*, family *Geminiviridae*) are a new threat to cucurbit production worldwide. At least six and eight begomovirus species from the New World (NW) and Old World (OW), respectively, have been described infecting cucurbits in the past 30 years (Brown *et al.* 2015). Some of them have a narrow geographic distribution despite a high local prevalence and agronomic impact, like the NW begomovirus *Melon chlorotic mosaic virus* (MeCMV) observed only in Venezuela so far (Romay et al. 2010). Others have a larger geographic range: the NW begomovirus *Squash leaf curl virus* (SLCV) and the OW begomovirus *Watermelon chlorotic stunt virus* (WmCSV) were introduced into the Eastern Mediterranean Basin in the early 2000s (Lapidot et al. 2014).The OW *Tomato leaf curl New Delhi virus* (ToLCNDV), first described in India, was more recently

reported in Europe and in the north of Africa (Fortes et al. 2016) where it has rapidly become a major agronomic problem (Moriones et al. 2017). Control of begomoviruses relies on the limitation of their vector *Bemisia tabaci* –mostly by intensive insecticide treatments-, and on genetic resistance when available. Considering the frequent emergence of highly damaging begomoviruses, breeding for resistance is a key factor for a durable control, particularly if broad-spectrum resistances effective against several viruses at once can be found.

In melon, resistances to different viruses and pathogens are often found in the same genotype even when the resistance factors are distinct (Lecoq et al. 1998, Table 1). Resistances to WmCSV (Yousif et al. 2007), cucurbit leaf crumple virus (CuLCrV) (Hagen et al. 2008b; McCreight et al. 2008), and more recently ToLCNDV (Lopez et al. 2015), have been described. However there is no evidence for general resistance factors against several of these viruses

In this work, we looked for resistance against the genetically distant MeCMV (NW) and ToLCNDV (OW) and checked whether common genetic factors could target these two viruses. In a first step we screened 31 melon accessions, previously described as resistant to at least one virus or representative of the phenotypic melon diversity. We also tested the inheritance of resistance to MeCMV in the most resistant accession IC-274014 and its relationship with resistance to ToLCNDV, in order to find efficient and generic resistance factors that could be used in breeding programs.

Material and methods

Virus strains and infectious clones

MeCMV was collected from watermelon (*Citrullus lanatus* (Thumb.) Matsum. and Nakai) in Zulia, Venezuela. A multimeric infectious clone of this virus was obtained in a previous work (Romay et al. 2015). A ToLCNDV (ES13-35) isolate was obtained from a leaf sample of zucchini squash (*Cucurbita pepo* L) from Murcia, Spain. Total DNA was extracted from the infected plant as described by Gilbertson et al. 1991, and ToLCNDV detection was confirmed by sequencing of a PCR fragment amplified using universal primers for begomoviruses (Wyatt and Brown, 1996). The full-length genome sequence of this isolate was obtained for further constructions of a multimeric infectious clone.

The complete genome of ES13-35 isolate was amplified by rolling circle amplification (RCA) according to the manufacturer's recommendations (Romay et al. 2015). The DNA-A fragment generated by RCA was digested with *XbaI* and *XhoI* (~1 kb) and inserted into pBluescript II SK (+) (Stratagene). Then, a complete DNA-A was linearized with *BamH*I and inserted into the plasmid containing the *Xba*I-*Xho*I fragment. To generate a multimeric clone of DNA-B, a 1.6kb fragment was obtained by digestion of RCA product with *Pst*I and insertion into pBlueScript. Then, a complete DNA-B fragment after *Mlu*I digestion of the RCA product was ligated to the previously clone containing the 1.6kb fragment and linearized with *Mlu*I. For cloning of both DNA components, *Escherichia coli* strain DH5 alpha was used as host of plasmid constructs.

The infectivity of the clones was confirmed by bombardment of melon seedlings according to Romay et al. 2015.

Viral inoculation assays

Melon seedlings of the susceptible cultivar Védrantais, inoculated with infectious clones of ToLCNDV and MeCMV, were used as virus sources for mechanical inoculations. One g of

young infected leaf tissue was ground in 5 ml of an ice-cold solution of 0.03M Na_2HPO_4 + 0.2% DIECA (diethyldithiocarbamate). Activated charcoal and carborundum were added to the sap extract (Romay et al. 2015). Two successive inoculations were performed for each melon seedling: a first one on the cotyledons at 6 days post sowing, followed by a second inoculation onto the first true leaf six days later, as described by Romay et al. 2015. Symptoms were checked visually at weekly intervals. At 30 days post-inoculation (dpi), young leaves were collected from all plants for accessions that did not show any symptoms, and one plant per accession in the accessions that displayed symptoms, for serological or molecular virus detection. All inoculated plants were kept in biosafety level 3 (S3) greenhouse.

Virus detection (ELISA, PCR)

Begomovirus detection was performed by PCR as described in (Romay et al. 2015), with primers 64ACR5 and 64debAC3 (Romay et al. 2014) for MeCMV and primers TLCNDV-CP-5' (5'-ATGKYGAAGCGACCAGCAGA-3') and TLCNDV-CP-3' (5'-CCGAATCATARAARTAGATCCG-3') for ToLCNDV.

For the studies on F_2 and backcrosses (BC) with MeCMV, all plants were tested in DAS-ELISA with an antiserum against SLCV (Cohen et al. 1983; courtesy of J.E. Duffus) that presents a strong cross-reactivity with MeCMV.

For the studies on F_3 and F_4 progenies, asymptomatic plants were tested by DAS-ELISA with a ToLCNDV antiserum (Agdia EMEA, Grigny, France) also presenting a strong cross-reactivity with MeCMV (data not shown).

Plant material

Screening for resistance

Thirty one melon accessions were selected from a germplasm collection at Institut National de la Recherche Agronomique (INRA, Avignon, France) for the resistance screening experiments. Most of the selected accessions had been previously reported to be resistant or tolerant to at least one plant pathogen; others were representative of the phenotypic melon diversity (Pitrat, 2016a,b, Table 1). Ten plants of each melon accession were used for each virus in two independent tests.

Inheritance studies

The melon accession IC-274014 belongs to the group momordica (Pitrat, 2016b) and was supplied by N.P.S. Dhillon (Dhillon et al. 2007). It was crossed with the cultivar Védrantais belonging to the group cantalupensis sub-group charentais (obt. Vilmorin). The F_1 hybrid was selfed to obtain the F_2 progeny and backcrossed to IC-274014 or Védrantais to obtain respectively the BC_R and BC_S progenies. The parents, F_1, F_2 and both BC were mechanically inoculated with MeCMV in two independent tests. A third test was conducted for MeCMV with only the susceptible parent Védrantais and the F_2 progeny. Ten resistant F_2 plants (no symptom, no virus detected by ELISA and PCR after inoculation with MeCMV) were selfed to produce ten F_3 progenies. As only few seeds with low germination ability have been obtained in the F_3 progeny, two to four plants, without selection for virus resistance or susceptibility, from each F_3 progeny were selfed to obtain F_4 families.

The F_1 and F_2 progenies (IC-274014 × Védrantais) were mechanically inoculated with ToLCNDV. Besides, plantlets of the F_3 progenies deriving from the F_2 selected for resistance to MeCMV, and of the corresponding F_4, were mechanically inoculated with ToLCNDV to determine if the same genes were involved in resistance to both viruses.

144

145 Correlation with resistance to other cucurbit-infecting viruses

146 *Resistance of the F_4 (IC-274014 × Védrantais) progenies to zucchini yellow mosaic virus*
147 *(ZYMV)*

148 Ten plantlets of Védrantais, IC 274014 and of the F_4 progenies at the cotyledonary stage
149 were mechanically inoculated with strain E15 of ZYMV (Lecoq and Pitrat, 1984). Symptoms
150 were recorded weekly and an ELISA test was performed 4 weeks after inoculation on the
151 asymptomatic plants.

152

153 *Behaviour of melon accession and of 6 of the F_4 (IC-274014 × Védrantais) progenies towards*
154 *cucurbit aphid-borne yellows virus (CABYV)*

155 A field assay was performed to test for the resistance to CABYV. An experimental plot was
156 planted in July 2018 with 20 plants of Védrantais and 10 plants each of 11 accessions (HSD
157 2445-005, IC-274014, PI 164323, PI 164723, PI 179901, PI 234607, PI 236355, PI 414723, PI
158 482420, WM7 and WM9), the F_1 hybrid (IC-274014 × Védrantais) and 6 of the F_4 (IC-
159 274014 × Védrantais) progenies. The plantlets were planted in 4 rows of 50 plants each,
160 with 1.5 m between ranks and 0.8 m between plants in a rank. Two blocks of 5 plants each
161 were planted for each accession including the resistant control PI 414723, and 4 blocks for
162 the susceptible control Védrantais. Two months after planting, an ELISA test was performed
163 with antisera against CABYV, ZYMV, watermelon mosaic virus (WMV) and cucumber mosaic
164 virus (CMV).

165

166

167 **Results**

168 Infectivity of MeCMV and ToLCNDV clones

169 The full-length genome of the ToLCNDV isolate ES13-35 (GenBank accession numbers
170 MK279352 and MK279353 for DNA-A and DNA-B, respectively) showed at least 99%
171 nucleotide identity with isolates previously reported in southern Spain (Juarez et al. 2014;
172 Moriones et al. 2017; Ruiz et al. 2015). After biolistic inoculation of seedlings of the
173 susceptible cultivar Védrantais with multimeric clones of ToLCNDV and MeCMV, viral
174 symptoms could be observed between the first and second week post-inoculation: severe
175 mosaics, leaf crumpling and stunting (more severe for MeCMV than for ToLCNDV) with
176 occasional enations on the lower leaf surface for MeCMV only. The presence of each virus
177 was confirmed by PCR at 30 dpi. Mechanical inoculation of both begomoviruses from these
178 sources yielded 100% infection in Védrantais in all experiments.
179
180 Resistance to MeCMV and ToLCNDV in melon accessions
181 Among the 31 melon lines tested, 17 were susceptible to both MeCMV and ToLCNDV
182 (Table 1). In the susceptible accessions as well as in Védrantais, symptoms induced by
183 MeCMV were usually more severe than those of ToLCNDV with severe stunting, leaf
184 crispation and occasionally leaf enations (data not shown).
185 Among the 14 remaining accessions, heterogeneous response
186 (susceptibility/intermediate resistance/resistance) or intermediate resistance was observed
187 in 8 melon lines for MeCMV and in 7 melon lines for ToLCNDV. The symptoms that were
188 predominantly observed in intermediate resistant melon were systemic chlorotic spots.
189 Interestingly, several accessions exhibited a “recovery” phenotype, with more symptoms in
190 the first leaves at 7 dpi than in young leaves at 30 dpi. This was the case for the melon lines
191 PI 164723, PI 414723 and 90625 for ToLCNDV, AM 87, PI 414723 and PI 179901 when

192 inoculated with MeCMV. PCR analyses confirmed the presence of the viruses in all
193 symptomatic plants –including the "recovered" ones that showed almost no symptoms at
194 their apex, but also in a few asymptomatic ones (data not shown).
195 Accessions IC-274014, WM 7, WM 9, PI 124112 and PI 282448 did not show any
196 symptoms after inoculation by ToLCNDV or MeCMV. PCR results indicated the occasional
197 presence of viruses in asymptomatic plants (data not shown). Accessions AM 87 and PI
198 179901 presented a complete resistance to ToLCNDV but a heterogeneous behaviour
199 towards MeCMV, with some plants displaying yellow spots or mosaics (Table 1).
200
201 Determinism of IC-274014 resistance to MeCMV
202 Védrantais exhibited severe mosaic symptoms, stunting and enations when inoculated
203 with MeCMV and the virus was clearly detected by ELISA or PCR. IC-274014 exhibited no
204 symptom and the virus was not detected. The F_1 (IC-274014 × Védrantais) exhibited
205 systemic chlorotic spots and the virus was detected in apical leaves (Figure 1), indicating a
206 recessive genetic control for virus infection. As expected for a recessive genetic control, the
207 BC_S was fully susceptible (Table 2). All the BC_S plants exhibited a range of symptoms: severe
208 mosaic and stunting, mild mosaic or mottle or systemic chlorotic spots and the virus was
209 detected by ELISA in all the plants. In the BC_R progeny, some plants had no symptoms but
210 the majority exhibited symptoms varying from systemic chlorotic spots to mottle; the virus
211 was not detected by ELISA and/or PCR in 29/95 plants (Table 2). This observed segregation
212 fitted a 3 susceptible *:* 1 resistant ratio expected for two independent recessive genes, both
213 required to confer resistance.
214 In the F_2 progeny, all types of symptoms were observed (mosaic, mottle, a majority with
215 systemic chlorotic spots) and some plants had no visible symptoms. The virus was detected

by ELISA and/or PCR in all the plants with symptoms and in a few plants without clear symptoms. The observed segregation (409 susceptible *vs.* 20 resistant plants) was compatible with a 15 susceptible (positive in ELISA or PCR) *vs.*1 resistant (negative in ELISA or PCR) ratio (χ^2 = 1.846; df = 1; Prob = 17%) corresponding to a genetic control by two independent recessive genes required simultaneously for resistance.

After selfing F_2 resistant plants, two F_3 families (1A6 and 1A8) appeared resistant to MeCMV (Table 3). The 8 other F_3 families obtained by selfing of resistant F_2 plants were segregating, with some plants showing symptoms of mottling or systemic chlorotic spots and virus systemic spread as detected by ELISA. If only two recessive genes were required and sufficient to confer resistance to MeCMV, all the F_3 and F_4 plants issued from resistant F_2 plants should be resistant. It is the case only for 1A8 and the associated F_4 progenies. The F_3 1A6 appeared fully resistant but only six plants were tested and the corresponding F_4 were either segregating (1A6A and 1A6B) or susceptible (1A6C). The F_3 1A6 can therefore be considered as also segregating. The high frequency of segregating F_3 could be explained by the need of a third dominant gene. This hypothesis of a third dominant gene does not change the expected segregation in the F_1, BC_S and BC_R progenies. Indeed, the F_1 and BC_S are all susceptible because the recessive resistance alleles are not present in a homozygous state whatever the state of the dominant allele. For the BC_R, the dominant resistance allele is present in all plants either in a homozygous or a heterozygous state, so the resistance or susceptibility depends only on the state of the two recessive alleles. Under the hypothesis of two recessive and one dominant genes simultaneously required for resistance, the expected segregation in the F_2 would be 61 susceptible *vs.* 3 resistant. The observed segregation (409 susceptible *vs.* 20 resistant plants) fits this hypothesis (Table 2).

In the case of two recessive and one dominant genes simultaneously required for resistance, the F_2 resistant plants must be homozygous for the two recessive genes but can be homozygous or heterozygous for the third dominant gene. If the 3 genes are independent, 1/3 of the F_2 resistant plants should be homozygous for the dominant gene producing homogeneous resistant F_3 progenies and 2/3 of the F_2 should be heterozygous, producing segregating F_3 progenies. The observed segregation (1 homozygous F_3 progeny *vs.* 9 segregating ones) fits this hypothesis: $\chi^2 = 2.450$ (df = 1; Prob = 12%) even if the power of the statistical analysis is low due to the small number of F_3 families available. Excluding the resistant 1A8 family, the observed segregation for the number of susceptible or resistant plants (43 susceptible *vs* 93 resistant, Table 3) among the 9 segregating F_3 families fits the hypothesis of 1 susceptible *vs* 3 resistant corresponding to the expected segregation for a single dominant gene ($\chi^2 = 3.177$; df = 1; Prob = 7%).

Similarly, in the F_4 generation obtained by selfing F_3 plants without selection for resistance or susceptibility, it was expected a 3 susceptible *vs.* 5 resistant ratio in the number of F_4 plants. As the number of plants in the F_4 families varies from 20 (corresponding to the F_3 1A4 or 1A11) to 80 (corresponding to the F_3 1A5), it had to be adjusted to the same number of F_4 plants for each F_3 progeny in order to test the 3:5 segregation (Supplementary Table S1). The observed segregation fits this hypothesis ($\chi^2 = 0.085$, df = 1; Prob = 77.1%).

In summary, the segregations observed for resistance to MeCMV in all the progenies (F_1, BC, F_2, F_3 and F_4) fitted the hypothesis of two recessive and one dominant independent genes which can be provisionally symbolized *a*, *b* and *C*.

Resistance to ToLCNDV

After mechanical inoculation with ToLCNDV, Védrantais exhibited severe mosaic symptoms and the virus was detected in ELISA, while IC-274014 exhibited no symptom and the virus was not detected in ELISA. As for MeCMV, the F_1 was considered as susceptible since the virus was detected in all inoculated plants, indicating a recessive genetic control but the symptoms were only mild chlorotic spots.

In the F_2 progeny, 25 plants among 200 inoculated plants exhibited no symptom and were negative in ELISA, what did not correspond to a mono-or digenic inheritance (Supplementary Table S2). However this observed segregation fitted a 55 susceptible *vs.* 9 resistant expected segregation corresponding to one recessive and two dominant genes simultaneously required for resistance ($\chi^2 = 0.404$; df = 1; Prob = 52.5%). These genes can be provisionally symbolized *x*, *Y* and *Z*.

Relationship between resistance to MeCMV and ToLCNDV

The F_3 and F_4 progenies derived from F_2 plants resistant to MeCMV were inoculated with ToLCNDV (Table 3). Among 157 F_3 and 250 F_4 plants, 95 and 70 respectively were resistant to ToLCNDV. The F_3 1A8 and the two corresponding F_4 1A8A and 1A8B which were homogeneously resistant to MeCMV were also homogeneously resistant to ToLCNDV. This suggested a linkage or common genes for resistance to both viruses. A first hypothesis was a linkage between one of the genes for MeCMV resistance and one of the genes for ToLCNDV resistance with four subcases: (i) *a* (or *b*) linked with *x*, (ii) *a* (or *b*) linked with *Y* (or *Z*), (iii) *C* linked with *x*, and (iv) *C* linked with *Y*(or *Z*). A second hypothesis was two linkages with five subcases: (i) *a* linked with *x* and *b* linked with *Y* (or *Z*), (ii) *a* linked with *Y* and *b* linked with *Z*, (iii) *a* linked with *Y* (or *Z*) and *C* linked with *x*, (iv) *a* linked with *Y* and *C* linked with *Z*, and (v) *a* linked with *x* and *C* linked with *Y* (or *Z*). The expected frequencies of resistant and

susceptible plants to ToLCNDV were calculated under the conditions that *a* and *b* were homozygous and that *C* was heterozygous *CC*$^{+}$ or homozygous *CC* for respectively 2/3 or 1/3 of the F_3 progenies. Among these nine possibilities, two fit the observed segregation in the F_3 progenies: linkages between *a* and *x* and between *b* and *Y* (Prob χ^2 = 54.7%) and linkages between *a* and *x* and between *C* and *Y* (Prob χ^2 = 20.5%) (Supplementary table S3). For the F_4 progenies, the probabilities of the χ^2 were respectively 2.9 10^{-7} and 4.3% % with in both cases an excess of observed susceptible plants (Supplementary Table S3). The best hypothesis is thus: *a* linked with *x*, and *C* linked with *Y,* whereas gene *b* involved in MeCMV resistance and gene *Z* involved in ToLCNDV resistance are independent. F_3 plants homozygous resistant to both viruses such as the F_3 1A8 could be expected with a frequency of 1/12 (1/3 for *C*-*Y* and 1/4 for *Z*, *a*-*x* and *b* being homozygous for resistance to MeCMV) which corresponds to the observed frequency of one homozygous resistant F_3 out of 10. The expected segregation for the number of susceptible : segregating : resistant F_4 families towards ToLCNDV was 23 : 16 : 9. The observed values (respectively 8:13:2 in Table 3) fit this hypothesis (χ^2 = 5.778; df = 2; Prob = 5.6%).

Correlation with resistance to other viruses

IC-274014 was found resistant to ZYMV after mechanical inoculation (Supplementary Table S4). Among the 23 F_4 (IC-274014 × Védrantais) progenies, 14 were symptomless, 5 displayed more or less severe symptoms of mosaics, yellowing and stunting, and 4 had an heterogeneous behaviour (no symptoms, chlorotic spots or mosaics). Among the 14 symptomless F_4 progenies, 5 were positive in ELISA for at least one plant, being thus considered as heterogeneous (F_4 progenies 1A1A, 1A3B, 1A5D and 1A7B) or susceptible (F_4 progeny 1A1B) (Supplementary Table S4 and data not shown).

In conditions of natural infection, IC-274014 was resistant to CABYV whereas Védrantais, the F_1 (IC-274014 × Védrantais) and the six tested F_4 progenies were susceptible to the virus based on ELISA (Table 4). Among the tested accessions, HSD 2445-005, PI 414723 and WM9 were resistant to CABYV, whereas PI 164323, PI 164723, PI 179901, PI 234607, PI 236355, PI 482420, Védrantais and WM7 were susceptible based on ELISA (Table 4). PI 414723 and Védrantais were confirmed as resistant and susceptible respectively. All accessions were susceptible to CMV and WMV, whereas ZYMV was not present in the experimental plot (data not shown)

Discussion and Conclusions

Among the 31 melon accessions tested against MeCMV and ToLCNDV, most had similar behaviours towards both viruses. MeCMV and ToLCNDV are quite distant molecularly (less than 60% and 50% identity in DNA-A and DNA-B, respectively), and belong to different clades (NW vs. OW). However, MeCMV and ToLCNDV share a high infectivity when mechanically inoculated, a rare trait among begomoviruses even though it has been described in a few ones particularly in the NW clade (Morales and Niessen, 1988; Wege and Pohl, 2007). This suggests that, like a few other begomoviruses, they are not exclusively phloem-limited, at least at some stages of their host development (Sudarshana et al. 1998). A common resistance mechanism may thus target the same key parameter in the cycle of these two viruses. Both viruses were also found associated with atypical alphasatellites in natural conditions (Anwar, 2017; Romay et al. 2010). ToLCNDV has also been found associated with a betasatellite (Anwar, 2017), contrary to MeCMV. The two viruses differ strikingly by their host range. MeCMV appears restricted to a few cucurbit hosts (Romay et al. 2015), whereas ToLCNDV has a broad host range including more than 40 species from a

range of families including *Cucurbitaceae*, *Solanaceae*, *Malvaceae, Amaranthaceae*, *Euphorbiaceae* and *Fabaceae* (Zaidi et al. 2017). In several accessions, intermediate resistance mechanisms appeared related to a "recovery" phenotype, as observed after melon or watermelon infection with CuLCrV (Hagen et al. 2008a). This suggests that the resistance could be related in these cases to RNA silencing (Ghoshal and Sanfaçon, 2015; Hagen et al. 2008a) although the actual mechanism remains unknown.

Accession IC-274014 was resistant (no symptoms and no virus detection by ELISA or PCR) to MeCMV. Different types of symptoms were observed in the F_1, F_2 and BC progenies between IC-274014 and the susceptible cultivar Védrantais: severe mosaic, mottle, systemic chlorotic spots. Inheritance of these different types of symptoms was difficult to analyse in terms of genetic control. The F_1 exhibited systemic chlorotic spots which were not observed in the parents. In the F_2 or BC progenies, "recovery" phenotypes were often observed, with much higher symptom severity in the old leaves than in young ones. In plants with the different types of symptoms, the virus was always detected by ELISA or PCR. When using this criterion (presence *vs.* absence of virus), the observed segregations in the analysed progenies fitted the hypothesis of one dominant and two recessive independent genes simultaneously required for resistance.

Resistance of IC-274014 to ToLCNDV could be controlled by one recessive and two dominant genes. As some progenies selected at the F_2 stage only for MeCMV resistance were also resistant to ToLCNDV, we concluded that common genes were involved in resistance to both viruses, even if the hypothesis of genes belonging to the same clusters cannot be completely ruled out, clusters of resistance genes being present in melon (Garcia-Mas et al. 2012; Gonzalez et al. 2014). We propose the names *begomovirus resistance-1*and *Begomovirus resistance-2* for these genes (symbols *bgm-1* and *Bgm-2*) corresponding

358 respectively to *a-x* and *C-Y* in the results section. For resistance to MeCMV, one more
359 recessive gene was required corresponding to the locus *b* in the results section; the name
360 *mecmv resistance* (symbol *mecmv*) is proposed. Full resistance to MeCMV was controlled by
361 *bgm-1*, *Bgm-2* and *mecmv*. Resistance to ToLCNDV was controlled by *bgm-1*, *Bgm-2* and an
362 additional dominant gene (proposed name *ToLCNDV resistance*, symbol *Tolcndv*)
363 corresponding to the gene *Z* in the results section.

364 The susceptible F_4 plants after inoculation with MeCMV or ToLCNDV exhibited mild
365 symptoms (mottling or systemic chlorotic lesions) and not the severe symptoms (mosaic and
366 stunting) observed in the susceptible control Védrantais. Consequently, *bgm-1* and/or *Bgm-2*
367 could be involved in the control of the severe symptoms.

368 Accession IC-274014 is also resistant to *Zucchini yellow mosaic virus* (ZYMV) and *Cucurbit*
369 *aphid-borne yellow virus* (CABYV), but no correlation was observed between resistance to
370 ZYMV or CABYV on the one hand, and to MeCMV or ToLCNDV on the other hand
371 (Supplementary Table S4).

372 Genes conferring resistance to several viruses from the same family, or even to
373 completely different pathogens, have already been described. This can be dominant genes
374 (e.g. the *L* gene conferring resistance to 7 tobamoviruses in pepper, see Moury and Verdin,
375 2012) or recessive ones. Recessive resistance is often related to the loss or modification of a
376 susceptibility factor required for the viral cycle, notably eukaryotic initiation factor 4E
377 (Kuwata, 2016; Robaglia and Caranta, 2006). A recessive resistance gene to the begomovirus
378 tomato yellow leaf curl virus, *ty-5*, corresponds to the tomato homolog of the messenger
379 RNA surveillance factor Pelota (Lapidot et al. 2015) and conferred resistance in field
380 conditions to at least five tomato-infecting begomovirus species (Al-Shihi et al. 2018). The
381 resistance abolished symptoms and reduced viral accumulation (Anbinder et al. 2009).

382 Besides *ty-5*, additional minor quantitative trait loci contributed to the resistance (Anbinder
383 et al. 2009). A similar situation may happen for MeCMV and ToLCNDV resistance in melon,
384 although the nature of *bgm-1* and the mechanisms of resistance remain unknown so far.
385
386 A pool of melon accessions with resistance to one or several begomoviruses is emerging
387 from the literature (Table 4). Most of them are from India and belong to the acidulus,
388 momordica or kachri groups (Pitrat, 2016b). The most recent publication on genetic control
389 was on the resistance to ToLCNDV of the accession WM 7 from India belonging to the kachri
390 group: a major dominant gene (on linkage group LG XI) and two minor quantitative trait loci
391 (QTLs) (on LG II and LG XII) (Saez et al. 2017). From our results, resistance in IC-274014 is also
392 controlled by three genes, one recessive (*bgm-1*) and two dominant ones (*Bgm-2* and
393 *Tolcndv*) suggesting that common genes could be involved in WM 7 and IC-274014. It would
394 be interesting to search for the candidate regions for the different resistance genes.
395 Several accessions belonging to different horticultural types (cantaloupes, honeydew,
396 cassaba...) were resistant to CuLCrV (Hagen et al. 2008b) after agroinoculation. Resistance in
397 the accession PI 313970 after inoculation by *B. tabaci* was controlled by one recessive gene,
398 *culcrv* (McCreight et al. 2008). Allelism tests indicate that the same gene was also probably
399 present in other resistant accessions such as MR-1, PI 124111, PI 124112, PI 179901, PI
400 234607, and PI 236355 (McCreight et al. 2008); PI 236355 which exhibited the best level of
401 resistance to CuLCrV was susceptible to MeCMV and ToLCNDV (Table 4). So the gene *culcrv*
402 is probably not involved in resistance to these two last viruses, and different from *bgm-1*or
403 *mecmv*.
404 Resistance to WmCSV after *Agrobacterium*-mediated inoculation was observed in several
405 accessions (Yousif et al. 2007). Accessions resistant to WmCSV were also partially or

completely resistant to MeCMV and ToLCNDV (Table 4). The inheritance of resistance to WmCSV has not been published. It would be interesting to test IC-274014 and the F_4 (IC-274014 × Védrantais) resistant or susceptible to MeCMV and ToLCNDV for their behaviour when inoculated with WmCSV.

Our results suggest that although some resistance factors to begomoviruses are specific, common broad-spectrum resistance genes may be present in melon germplasm and could constitute interesting tools for breeders.

Acknowledgements

We thank the Experimental Infrastructure team of INRA-Montfavet for their help in the greenhouse experiments. We thank D. Besombes for obtaining part of the melon accessions and crosses and Dr J.E. Duffus (USDA, Salinas) for providing the SLCV antiserum.

This work was funded in part through the EMERAMB project in the ARIMNet2 2015 Call. ARIMNet2 (2014-2017) is an ERA-NET coordinated by INRA (France). It has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement no. 618127.

The authors declare that they have no conflict of interest

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Melon accession	Known resistance against cucurbit viruses[b]	MeCMV[a]	ToLCNDV[a]
Védrantais		S	S
Ouzbèque 1		S	S
Ouzbèque 2		S	S
Isabelle		S	S
Anso 77		S	S
AR Hale's Best Jumbo		S	S
Canton		S	S
HSD 93-20-A	CVYV (systemic necrosis)[c]	S	S
PMR-5	MNSV[d]	S	S
WMR29	PRSV[e], MWMV[e], MRMV[e]	S	S
PI 161375	CMV[e], MNSV[e]	S	S
PI 164323	CVYV[c]	S	S
PI 179905	ZYMV[c]	S	S
PI 234607	CuLCrV[f]	S	S
PI 255478	CABYV[g]	S	S
PI 482420	CYSDV[e], WMV[c]	S	S
PI 236355	CuLCrV[f]	S	S
PI 164723	WmCSV[h]	S	IR
AM 87		H (S/IR/R)	**R**
HSD 2445-005	WmCSV[h]	H (S/R)	H (R/IR)
HSD 2458 B	CVYV[c]	H (S/IR/R)	H (IR/R)
Tibish Kordofan		H (S/R)	H (S/IR/R)
MR-1	CuLCrV[f], LIYV[e]	H (S/IR/R)	IR
PI 179901	CuLCrV[f]	H (S/IR/R)	**R**
PI 313970 (90625)	CABYV[g], CuLCrV[f], CYSDV[c], LIYV[c],WmCSV[h]	H (S/R)	H (S/IR/R)
PI 414723	CABYV[g],CuLCrV[f],PRSV[c] ,ToLCNDV[i],WmCSV[h],WMV[e],ZYMV[e]	H (S/IR/R)	H (IR/R)
IC-274014	CABYV[c], ZYMV[c]	**R**	**R**
PI 124112	CABYV[g], CuLCrV[f], PRSV[e], ToLCNDV[i], WmCSV[h]	**R**	**R**
PI 282448	CABYV[g],WmCSV[h]	**R**	**R**
WM7	ToLCNDV[i]	**R**	**R**
WM9	ToLCNDV[i]	**R**	**R**

Table 1. Behaviour of 31 melon accessions after artificial inoculation with two begomoviruses: melon chlorotic mottle virus and tomato leaf curl New Delhi virus.

[a] R: resistant; S: susceptible; IR: intermediate resistance; H: heterogeneous.

[b] CABYV: cucurbit aphid-borne yellow virus (polerovirus), CMV: cucumber mosaic virus (cucumovirus), CuLCrV: cucurbit leaf crumple virus (begomovirus), CYSDV: cucurbit yellow stunting disorder virus (crinivirus), CVYV: cucumber vein yellowing virus (ipomovirus), LIYV:

548 lettuce infectious yellows virus (crinivirus), MNSV: melon necrotic spot virus (carmovirus),
549 MRMV: melon rugose mosaic virus (tymovirus), MWMV: Moroccan watermelon mosaic virus
550 (potyvirus), PRSV: papaya ringspot virus (potyvirus), WMV: watermelon mosaic virus
551 (potyvirus), WmCSV: watermelon chlorotic stunt virus (begomovirus), ZYMV: zucchini yellow
552 mosaic virus (potyvirus). Begomoviruses (CuLCrV, MeCMV, ToLCNDV, WmCSV) are
553 underlined.
554 [c]Pitrat, 2016a
555 [d]Mallor et al. 2003
556 [e]Lecoq et al. 1998
557 [f]McCreight et al. 2008
558 [g]Dogimont et al. 1996
559 [h]Yousif et al. 2007
560 [i] Lopez et al. 2015
561

	Test	Total	Susc.[a]	Res.[a]	Segreg.[b]	Chi-square	
						Value	Probability
Védrantais	1	30	30	0			
	2	30	30	0			
	3	10	10	0			
	Total	70	70	0			
IC-274014	1	25	0	25			
	2	25	0	25			
	Total	50	0	50			
F_1 = IC-274014 × Védrantais	1	25	25	0	1:0		
	2	22	22	0	1:0		
	Total	47	47	0	1:0		
$F_2 = F_1 \times F_1$	1	99	97	2	61:3	1.577	21%
	2	80	78	2	61:3	0.857	35%
	3	250	234	16	61:3	1.641	20%
	Total	429	409	20	61:3	0.006	98%
$BC_S = F_1 \times$ Védrantais	1	50	50	0	1:0		
	2	47	47	0	1:0		
	Total	97	97	0	1:0		
$BC_R = F_1 \times$ IC-274014	1	50	40	10	3:1	0.667	41%
	2	45	26	19	3:1	7.119	0.8%
	Total	95	66	29	3:1	1.547	21%

Table 2. Number of plants observed after inoculation with MeCMV in the parents (Védrantais and IC-274014), F_1, F_2 and back-cross (BC) progenies

[a] Susceptible = with clear virus symptoms and/or positive detection by ELISA or PCR; Resistant = no symptom and no virus detection by ELISA or PCR.

[b] Expected segregation (Susceptible : Resistant) under the hypothesis of two recessive and one dominant genes.

569

Melon genotypes	MeCMV		ToLCNDV		Melon genotypes	MeCMV		ToLCNDV	
	Susc.	Res.	Susc.	Res.		Susc.	Res.	Susc.	Res.
Védrantais	16	0	16	0	Védrantais	20	0	10	0
IC-274014	0	15	0	20	IC-274014	0	20	0	10
F_3 progenies					F_4 progenies				
1A1	3	7	4	6	1A1A	4	6	10	0
					1A1B	14	5	14	6
1A2	4	14	13	5	1A2A	17	3	20	0
					1A2B	1	9	9	1
1A3	5	15	7	13	1A3A	5	5	6	4
					1A3B	0	25	3	7
1A4	14	3	6	11	1A4A	3	7	10	0
					1A4B	7	3	8	2
1A5	5	4	5	4	1A5A	1	19	9	1
					1A5B	0	20	10	0
					1A5C	1	19	10	0
					1A5D	0	20	10	0
1A6	0	6	3	4	1A6A	1	7	10	0
					1A6B	1	4	9	1
					1A6C	16	4	10	0
1A7	6	14	6	14	1A7A	4	4	5	5
					1A7B	1	19	3	7
1A8	0	17	0	18	1A8A	0	20	0	10
					1A8B	0	20	0	10
1A10	2	17	5	15	1A10A	3	7	2	8
					1A10B	8	2	6	4
1A11	4	13	13	5	1A11A	1	19	7	3
					1A11B	3	13	9	1

570

571 Table 3: Number of plants susceptible or resistant to MeCMV and ToLCNDV in the parents
572 (Védrantais and IC-274014) and in the F_3 and F_4 progenies from F_2 plants resistant to
573 MeCMV.

574

Accession	MeCMV[a]	ToLCNDV[a]	CuLCrV[d]	WmCSV[e]	CABYV[a,b]
Védrantais	S	S	nt	S	S
HSD 2445-005	Segreg (R/S)	Segreg (R/S)	nt	R	R
IC-274014	R	R	nt	nt	R
Kharbuja	nt	IR[f]	nt	nt	nt
PI 124111 (MR-1)	IR	IR	R	nt	nt
PI 124112	R	IR[f]/R	IR	R	R[g]
PI 164323	S	S	nt	nt	S
PI 164723	S	IR	R/IR	R	S
PI 179901	IR	R	IR	nt	S
PI 234607	S	S	R	nt	S
PI 236355	S	S	R	nt	S
PI 282448	R	R	nt	R	R[g]
PI 313970 (90625)	R	IR	R	R	R[g]
PI 414723	IR	IR	IR	R	R
WM 7	R	R[f]/R	nt	nt	S[c]
WM 9	R	IR[f]/R	nt	nt	R

Table 4. Behaviour of melon accessions (S = susceptibility, R = resistance, IR = partial resistance, nt = not tested) towards the four Begomoviruses melon chlorotic mosaic virus (MeCMV), tomato leaf curl New Delhi virus (ToLCNDV), cucurbit leaf crumple virus (CuLCrV) and watermelon chlorotic spot virus (WmCSV) and the polerovirus cucurbit aphid-borne yellows virus (CABYV).

[a]This work, unless otherwise specified

[b] results from field trial based on ELISA test

[c] low ELISA values

[d]McCreight et al. 2008

[e] Yousif et al. 2007

[f]Lopez et al. 2015

[g] Dogimont et al. 1996

588 Figure 1: Symptoms of MeCMV: curling, yellowing and stunting in the susceptible cv.
589 Védrantais (a); no symptom and no virus multiplication in the resistant accession IC-274014
590 (b); systemic chlorotic spots in the F_1 (IC-274014 × Védrantais)
591
592

Supplementary Table S1: Observed number of plants susceptible and resistant to MeCMV in the F_4 (IC-274014 × Védrantais) progenies after selection of the F_2 for resistance to this virus, and adjusted number for the same size of F_4 plants for the different F_3 progenies.

Supplementary Table S2: Expected segregations for resistance to ToLCNDV in the F_2 (IC-274014 × Védrantais) under different hypotheses

Supplementary Table S3: Expected *vs*. observed frequency for susceptible *vs.* resistant plants to ToLCNDV in the F_3 and F_4 progenies derived from F_2 (IC-274014 × Védrantais) resistant to MeCMV, and probability of the chi-square under different hypotheses of linkage (or identity) of genes involved in resistance to MeCMV (*a*, *b* and *C*) and to ToLCNDV (*x*, *Y* and *Z*).

Supplementary table S4: behaviour of Védrantais, IC-274014, F_1 and F_4 (IC-274014 ×Védrantais) progenies towards CABYV and ZYMV, in relation to their susceptibility to the begomoviruses MeCMV and ToLCNDV. The F_4 progenies are from F_2 plants resistant to MeCMV.

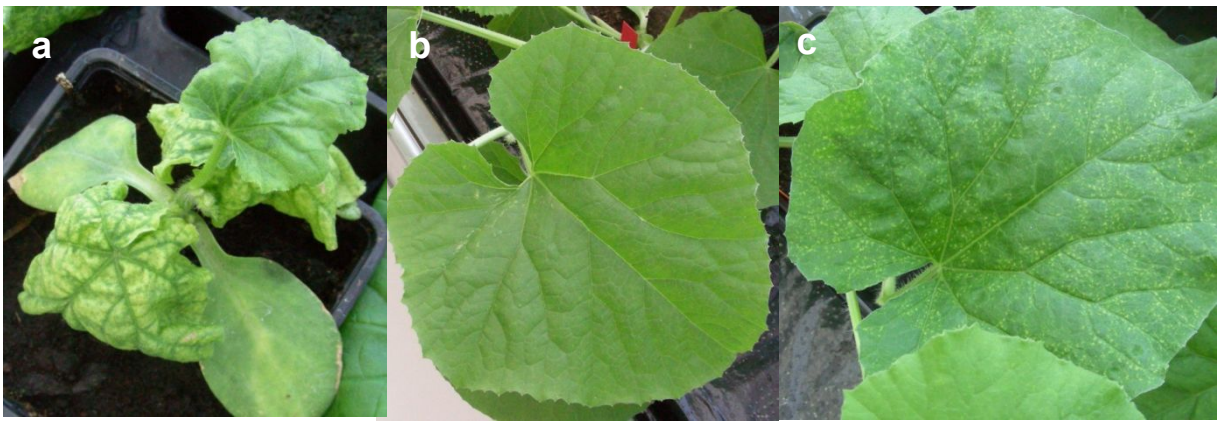

Figure 1

F_4 progeny	Observed			Adjusted		
	Susceptible	Resistant	Total	Susceptible	Resistant	Total
1A1A	4	6	10	21.8	13.3	35.1
1A1B	14	5	19			
1A2A	17	3	20	21.1	14.0	35.1
1A2B	1	9	10			
1A3A	5	5	10	5.0	30.1	35.1
1A3B	0	25	25			
1A4A	3	7	10	17.6	17.6	35.1
1A4B	7	3	10			
1A5A	1	19	20	0.9	34.2	35.1
1A5B	0	20	20			
1A5C	1	19	20			
1A5D	0	20	20			
1A6A	1	7	8	19.1	16.0	35.1
1A6B	1	4	5			
1A6C	16	4	20			
1A7A	4	4	8	6.3	28.8	35.1
1A7B	1	19	20			
1A10A	3	7	10	19.3	15.8	35.1
1A10B	8	2	10			
1A11A	1	19	20	3.9	31.2	35.1
1A11B	3	13	16			
Total	91	220	311	113.12	197.88	311

Supplementary Table S1: Observed number of plants susceptible and resistant to MeCMV in the F_4 (IC-274014 × Védrantais) progenies after selection of the F_2 for resistance to this virus, and adjusted number for the same size of F_4 plants for the different F_3 progenies.

	Number of plants		Segregation	Chi-square	
	Susceptible	Resistant		Value	Prob
Observed	175	25			
Hypothesis					
1 recessive	150	50	3 Sus:1 Res	16.667	< 0.0001
2 recessive	187.5	12.5	15 Sus:1 Res	13.333	0.0003
1 recessive+1 dominant	162.5	37.5	13 Sus:3 Res	5.128	0.0235
1 recessive + 2 dominant	171.875	28.125	55 Sus:9 Res	0.404	0.525

Supplementary Table S2: Expected segregations for resistance to ToLCNDV in the F_2 (IC-274014 × Védrantais) under different hypotheses

	F_3		F_4	
	Susceptible:Resistant	Proba χ^2	Susceptible:Resistant	Proba χ^2
Observed frequency				
-uncorrected	62:95		180:70	
-adjusted*	64.08:92.92		166.46:83.54	
One linkage				
C with *Y* (or *Z*)	103:25	$6.4\ 10^{-13}$	835:189	$1.36\ 10^{-4}$
a (or *b*) with *Y* (or *Z*)	49:15	$1.3\ 10^{-10}$	193:63	0.030
a (or *b*) with *x*	39:25	$3.6\ 10^{-4}$	175:81	0.67
C with *x*	103:25	$6.4\ 10^{-13}$	835:189	$1.36\ 10^{-4}$
Two linkages				
a with *x*	3:5	0.547	7:9	$2.9\ 10^{-7}$
and *b* with *Y* (or *Z*)				
a with *Y*	5:3	$1.2\ 10^{-4}$	9:7	0.018
and *b* with *Z*				
a (or *b*) with *Y* (or *Z*)	11:5	$6.6\ 10^{-7}$	43:21	0.886
and *C* with *x*				
a (or *b*) with *Y*	11:5	$6.6\ 10^{-7}$	43:21	0.886
and *C* with *Z*				
a (or *b*) with *x*	23:25	0.2054	37:27	0.0432
and *C* with *Y* (or *Z*)				

Supplementary Table S3: Expected *vs*. observed frequency for susceptible *vs.* resistant plants to ToLCNDV in the F_3 and F_4 progenies derived from F_2 (IC-274014 × Védrantais) resistant to MeCMV, and probability of the chi-square under different hypotheses of linkage (or identity) of genes involved in resistance to MeCMV (*a*, *b* and *C*) and to ToLCNDV (*x*, *Y* and *Z*).

*The observed frequencies were adjusted to take into account the difference in the number of plants tested for the different F_3 and F_4 progenies.

The F_2 plants used to obtain the F_3 and F_4 progenies were selected for MeCMV resistance, so they were homozygous for genes *a* and *b* (and for the linked or identical genes for ToLCNDV resistance) and homozygous *CC* (for 1/3 of the plants) or heterozygous *CC*$^+$ (for 2/3 of the plants) for the third dominant gene.

If there are some linkages between the genes involved in resistance to MeCMV and ToLCNDV, genes linked with *a* or *b* must be homozygous in the F_2 selected for resistance to MeCMV, and no more segregating in the F_3 and F_4 progenies. Genes linked with *C* are homozygous and heterozygous in 1/3 and 2/3 respectively of the MeCMV-resistant F_2. The other genes segregate independently, with frequencies of ¼ homozygous dominant, ½ heterozygous and ¼ homozygous recessive in the F_2. The expected frequencies in the F_3 and

F_4 progenies obtained without further selection for virus resistance were calculated based on these frequencies in the F_2 plants.

	MeCMV	ToLCNDV	ZYMV	CABYV
Védrantais	S[a]	S	S	S
IC-274014	R	R	R	R
F1 (IC× Ved)	IR	IR	R	S
1A1A	H	S	H	nt
1A1B	S	H	S	S
1A2A	H	S	S	nt
1A2B	H	H	S	nt
1A3A	H	H	R	nt
1A3B	R	H	H	S
1A4A	H	S	H	nt
1A4B	H	H	H	nt
1A5A	R/H	H	R	nt
1A5B	R	S	R	S
1A5C	R/H	S	R	nt
1A5D	R	S	H	S
1A6A	H	S	S	nt
1A6B	H	H	H	nt
1A6C	S/H	S	H	nt
1A7A	H	H	R	nt
1A7B	R/H	H	H	nt
1A8A	R	R	R	S
1A8B	R	R	R	S
1A10A	H	H	R	nt
1A10B	H	H	R	nt
1A11A	R/H	H	S	nt
1A11B	R/H	H	S	nt

Supplementary table S4: behaviour of Védrantais, IC-274014, F_1 and F_4 (IC-274014 ×Védrantais) progenies towards CABYV and ZYMV, in relation to their susceptibility to the begomoviruses MeCMV and ToLCNDV. The F_4 progenies are from F_2 plants resistant to MeCMV.

[a]S = with clear virus symptoms and/or positive detection by ELISA; R = no symptoms, no virus detection by ELISA; H= heterogeneous based on symptoms and/or ELISA.
nt = not tested