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RESEARCH PAPER

WMV resistance in melon induced by substitutions in VPS4

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A single substitution in *Vacuolar protein sorting 4* is responsible for resistance to *Watermelon mosaic virus* in melon

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Highlight

Virus infections are responsible for substantial yield losses. This study identified a virus resistance gene in melon and highlights a new family of *Potyvirus* resistance genes in plants.

Abstract

In plants, introgression of genetic resistance is a proven strategy for developing new resistant lines. While host proteins involved in genome replication and cell to cell movement are widely studied, other cell mechanisms responsible for virus infection remain underinvestigated. Endosomal sorting complexes required for transport (ESCRT) play a key role in membrane trafficking in plants and are involved in the replication of several plant RNA viruses. In this work, we describe the role of the ESCRT protein CmVPS4 as a new

susceptibility factor to the *Potyvirus Watermelon mosaic virus* (WMV) in melon. Using a worldwide collection of melons, we identified three different alleles carrying non-synonymous substitutions in *CmVps4*. Two of these alleles were shown to be associated with WMV resistance. Using a complementation approach, we demonstrated that resistance is due to a single non-synonymous substitution in the allele $CmVps4^{P30R}$. This work opens up new avenues of research on a new family of host factors required for virus infection and new targets for resistance.

Keywords: *Cucumis melo*, ESCRT, plant, *Potyvirus*, resistance, VPS4, WMV.

Introduction

Plant viruses are considered to be responsible for half of the emerging infectious plant diseases worldwide (Anderson *et al.*, 2004). These pathogens can severely affect the yield and quality of crops, and cause substantial economic losses (McKirdy *et al.*, 2002; Gray *et al.*, 2010). Most of the plant viruses are insect transmitted (Whitfield *et al.*, 2015). One of the strategies for controlling viruses while limiting the use of insecticides is the use of genetically resistant crops (Gómez *et al.*, 2009; Kaur and Garg, 2014). In plant–virus pathosystems, two major groups of resistance are usually considered. Plant viruses are obligate parasites and require various host-encoded proteins (host factors) to complete the steps of their life cycle (Heinlein, 2015). Therefore, the absence of appropriate host factors or inhibition of the interactions between viral proteins and corresponding host factors confers recessively inherited resistance (Truniger and Aranda, 2009). The second major type of resistance is dominantly inherited. In that case, the interaction between a viral avirulence factor and a host-encoded resistance protein (R) triggers downstream defense responses, resulting in induction of plant immunity (Truniger and Aranda, 2009). It has been suggested that genetic resistance pyramiding and mosaic deployment strategies are more sustainable than using a single resistance (Mundt, 2014; Djidjou-Demasse *et al.*, 2017). However, the very limited number of

resistances available against certain viruses limits the development of these strategies. Thus, focusing research on new resistance genes is an essential tool to control plant diseases. Recessively inherited resistances account for almost half of the 200 virus resistance genes known so far in plants (Kang *et al.*, 2005; Garcia-Ruiz, 2019; Mäkinen, 2020). Up to now, the vast majority of the recessive resistance genes identified in plants encode eukaryotic translation initiation factors (eIFs) (Nieto *et al.*, 2006; Piron *et al.*, 2010; Sanfaçon, 2015; Bastet *et al.*, 2017; Shopan *et al.*, 2017). Some other plant cell mechanisms have been involved in recessive resistance to plant viruses, mainly in the model plant *Arabidopsis thaliana*, such as mutations in *cum1-1* reducing cell to cell movement of *Cucumber mosaic virus* (CMV) or the ethylene-inducible transcription factor RAV2 which is required for suppression of RNA silencing by the *Potyvirus* HC-Pro and *Carmovirus* p38 viral proteins (Yoshii *et al.*, 1998; Endres *et al.*, 2010; Ouibrahim *et al.*, 2014; Wu *et al.*, 2020), but novel mechanisms remain to be discovered. In order to extend our understanding of plant–pathogen interactions and to identify new targets for the development of resistant cultivars, the identification of host factors different from those of the eIF protein family, involved in other stages of the viral cycles, is a timely strategy (Nicaise, 2014; Hashimoto *et al.*, 2016).

In melon (*Cucumis melo* L., family *Cucurbitaceae*), genes conferring recessive resistance to *Melon necrotic spot virus* (MNSV; genus *Carmovirus*) and CMV (genus *Cucumovirus*) have been isolated. They correspond to modifications in the translation initiation factor gene *eIF4e* and the vesicle transport protein SNARE encoded by *Vps41*, respectively (Nieto *et al.*, 2006; Giner *et al.*, 2017; Pascual *et al.*, 2019). A dominant NBS-LRR-coding gene that controls resistance to *Papaya ringspot virus* (PRSV; genus *Potyvirus*) has been isolated (Brotman *et al.*, 2013). Complete or partial (quantitative) resistance to several other viruses, as well as their inheritance, have been described in melon (Dogimont, 2011). Nevertheless, the genes responsible for these resistances are not known.

Amano *et al.* (2013) identified in cucumber (*Cucumis sativus*), a species closely related to melon, a genetic region responsible for *Zucchini yellow mosaic virus* (ZYMV; genus *Potyvirus*) resistance. Using a map-based approach, they identified six candidate genes. The gene *CsVPS4* was identified as the most likely candidate due to two non-synonymous substitutions at positions T86C and G99A corresponding to the F29S and M33I amino acid changes, respectively, distinguishing resistant and susceptible genotypes, while the other genes did not show any substitution or expression modification (Amano *et al.*, 2013). Very recently, Soler-Garzón *et al.* (2021) identified two homologs of *VPS4* as the most likely candidate genes for resistance to potyviruses in common bean. Interestingly, a non-synonymous substitution leading to the M33R amino acid change was a candidate for the resistance allele *bc-4.*

The VPS4 protein belongs to the endosomal sorting complex required for transport (ESCRT) endomembrane trafficking mechanism, which has been mainly described in mammals and is involved in the formation of vesicles in multivesicular bodies (MVBs), cell division, and HIV replication (Perez and Nolan, 2001; Davies *et al.*, 2009; Hwang and Robinson, 2009; Schmidt and Teis, 2012). In plants, VPS4 and ESCRT are mainly known for the formation of MVBs (Haas *et al.*, 2007), an essential step in protein recycling and autophagy. Thus, loss of ESCRT function through *VPS4* or other plant genes involved in biogenesis of MVBs leads to lethality (C. Gao *et al.*, 2015; Lefebvre *et al.*, 2017). Some interactions between ESCRT and RNA plant viruses have been recently reported (Barajas *et al.*, 2009; Diaz *et al.*, 2015). VPS4 contains three domains, namely the microtubule interacting and transport (MIT) domain, the AAA+ ATPase domain, and the C-terminal domain (Scott *et al.*, 2005a; Haas *et al.*, 2007). The MIT domain is characterized by three antiparallel α-helices (Takasu *et al.*, 2005).

In the present study, we demonstrate the function of VPS4 protein in *Potyvirus* resistance in melon. Using a collection representative of the natural diversity in melon, we identified non-synonymous variants of *CmVps4* in the MIT domain and assessed their susceptibility to the potyviruses ZYMV and *Watermelon mosaic virus* (WMV). Finally, we used WMV as a vector to restore the viral susceptibility in a resistant accession, demonstrating the involvement of *CmVPS4* in WMV susceptibility in melon.

Materials and methods

Plant material and culture conditions

Natural melon diversity used in this study was provided by the Biological Resources Center for Vegetables (CRB-Leg, INRAE) in Avignon, France. A total of 652 accessions of melon from different geographic origins, representative of the genetic diversity of the species, were used for PCR and high-throughput sequencing. Seedlings used for inoculations and plants used for multiplication were grown in an insect-proof greenhouse. Plants inoculated with a viral strain were placed into growth chambers over a day/night period of 14 h/10 h at 22 °C/16 °C. Germination of each accession occurred under the same temperature conditions as in the growth chambers except for HSD 2441, HSD 2445, Humaid 93-4, and Humaid 93-10, which were incubated at 30 °C half submerged with water for 4–7 d. Plants inoculated with genetically modified viruses were placed in a biosecurity level C3 growth chamber over a day/night period of 14 h/10 h at 22 °C/16 °C.

CmVps4 gene extraction, sequencing, and data analysis

CmVps4 from melons Charentais Monoecious (CM) and TGR-1551 were amplified by reverse transcription–PCR (RT–PCR). First leaf samples were frozen in liquid nitrogen. Total RNA was extracted with Tri-reagent (Molecular Research Center, Cincinnati, OH, USA) following the manufacturer's recommendations. Reverse transcription was performed with SuperScript III Reverse Transcriptase and poly(dT) primer. *CmVps4* was amplified using

Herculase II Fusion DNA polymerase (Agilent) using forward and reverse primers 5′-ATGTATAGCAATTTCAAGGAGCAAGC-3′ and 5′-TCAACCTTCCTCCCCAAACTC-3′, respectively.

The *CmVps4* gene from the melon collection was sequenced using a next-generation sequencing (NGS) strategy. Samples from the first leaf were frozen in liquid nitrogen. Each sample was ground before adding 140 µl of extraction buffer EBp (200 mM Tris–HCl pH 7.5, 250 mM NaCl, 25 mM EDTA, and 0.5% SDS) and placed at 50 °C for 10 min. After centrifugation at 14 000 *g* for 5 min, the supernatant was retrieved. DNA was precipitated using 120 µl of cold isopropanol. The DNA was pelleted by centrifugation and washed twice with 400 µl of 75% ethanol before resuspension in 100 µl of H_2O miliQ.

CmVps4 amplification from the melon collection was performed using five amplicons with primer pairs overlapping each exon. Each forward primer had the tag sequence 5′-TTCCCTACACGACGCTCTTCCGATCT-3′ at its 5′ extremity, whereas reverse primers had the tag 5′-AGTTCAGACGTGTGCTCTTCCGATCTG-3′ (Supplementary Table S1). Illumina NGS MiSeq sequencing allowed sequencing of each amplicon in a single run (Metzker, 2010). Sequenced DNA data were collected in one compiled file. Data decompilation was performed using internal software. The assembly was carried out by aligning the reads. In addition to the 652 accessions sequenced here, data from the re-sequencing of 1175 accessions of melons from Zhao *et al.* (2019) were aligned with the DHL92 melon reference genome.

Viral material and mechanical inoculation

The virus strains used in this work are WMV-Fr, WMV-FMF00-LL1, and ZYMV-R5A (Luis Arteaga and Quiot, 1976; Baker *et al.*, 1992; Granier *et al.*, 1993; Desbiez and Lecoq, 2004; Desbiez *et al.*, 2012a). They were propagated using zucchini squash cv. Diamant (Seminis) plants. For mechanical inoculations of plants, infected leaves were ground in a mortar with 4

ml of inoculation solution (EBv) [75 mM Na_2HPO_4 and 0.2% diethyldithiocarbamic acid (DIECA)] per 1 g of fresh weight, 0.5 g of activated carbon, and 0.4 g of carborundum. The extracts were gently rubbed onto the cotyledons of young melons at the spreading cotyledon stage. The plantlets were rinsed with tap water and incubated in growth chambers.

Symptom ratings and GFP visualization

To observe the course of infection in the newly formed tissues, virus inoculation was performed at the spreading cotyledon stage. Two rating scales were used for WMV and ZYMV following the appearance of the strongest symptoms in a susceptible accession. Both scales are based on leaf tissue development following infection: (0) absence of symptoms, (1) chlorotic spots on the leaf, (2) mosaic symptoms on the leaves and first blisters, and (3) deformation, reduced size, and a large number of blisters. The average score of the three youngest leaves was used to determine the score of each plant. A mean score of ≤1 was considered resistant. A mean score >1 was considered susceptible. Green fluorescent protein (GFP; see below) was visualized on leaves and cotyledons at 6 d after infection, using a 488 nm laser and a 510/10 nm filter in a dark room. Each experiment was carried out twice independently with at least eight plants per accession. Significant differences from control were estimated by a Kruskal–Wallis statistical test and indicated by asterisks for $P<0.05$ (*).

ELISAs

For each sample, 0.5 g of the youngest leaves was taken and crushed using a roller press. A 4 ml aliquot of solution EBv was added to the ground material. The ELISA was carried out with the 'ELISA Reagent set for *Potyvirus* group (Poty)' kit (Agdia®) following the manufacturer's protocol. Each experiment was carried out twice independently with a least eight plants per accession. Significant differences from control were estimated by a Kruskal–Wallis statistical test and indicated by asterisks for $P<0.05$ (*).

Construction of WMV vector and of WMV-*CmVps4* clones

The enhanced GFP (eGFP)-coding sequence was introduced in the pWMV-LL1A clone using homologous recombination in the yeast *Saccharomyces cerevisiae*. First, the pWMV-LL1A clone was digested with restriction enzymes *Nru*I (unique site at position 416 in WMV-LL1A) and *Avr*II (unique site at position 1476), and the linearized vector without the 417–1476 fragment was purified from an agarose gel. The eGFP- (Siemering *et al.*, 1996) coding region was amplified by PCR with the Pfu DNA polymerase (Promega, France) using primers P1-GFP-5 and GFP-HC-3. To re-insert the deleted *Nru*I–*Avr*II fragment with the GFP fragment, an overlapping region in WMV-LL1A was amplified by PCR with primers W-1-Hind-5 and W-P1-GFP-3, complementary to P1-GFP-5. The P1 and GFP fragments purified from the agarose gel as well as the digested pWMV-LL1A were transformed into competent yeast YHP501 cells, extracted as described (Desbiez *et al.*, 2012), and used for transformation of *Escherichia. coli* DH5α. The presence of the GFP in the resulting clones was confirmed by PCR with primers P1-GFP-5 and GFP-HC-3 and sequencing of the GFP-coding region, as well as digestion with *Nco*I and *Pvu*II.

To build a vector allowing the expression of different proteins, a unique *Not*I site was added in the WMV-FMF00-LL1A clone. Two fragments were amplified with Pfu DNA polymerase, using pWMV- LL1A-GFP as a template, with primer pairs (i) W-1-Hind-5 and W-P1HC-RV and (ii) W-P1HC-FW+W-1700-3. The two fragments purified from the agarose gel and pWMV-LL1A digested with *Nru*I and *Avr*II were transformed into competent yeast YHP501 as above. Yeast DNA extraction, *E. coli* transformation, and bacterial plasmid DNA extraction were as above. The presence of the *Not*I site in the constructs was checked by partial sequencing (Siemering *et al.*, 1996).

The $CmVps4^{Wt}$ and $CmVps4^{P30R}$ genes were inserted in the viral vector as follows: the WMV-LL1A-Not clone was linearized using the unique *Not*I restriction enzyme. The restriction enzyme and undigested plasmids were eliminated using gel migration and the gel

extract kit from QIAGEN®. The cDNAs of the alleles *CmVps4*^{Wt} and *CmVps4*^{P30R} were amplified from the accessions CM and TGR-1551, respectively, with primers containing 30 nt extensions corresponding to the extremities of the WMV-LL1A-Not linearized plasmid. Linear plasmids and the purified PCR fragments *CmVps4*^{Wt} or *CmVps4*^{P30R} were transformed in the diploid *S. cerevisiae* yeast strain YPH501. Yeast DNA was extracted as described and used for transformation of thermocompetent bacteria *E. coli* Stellar™ HST08 (Clontech®) according to the manufacturer's recommendations. Liquid culture of the bacteria grown at 37 °C in LB media for 24 h was used for DNA extraction with the Qiaprep® Extraction Kit (Qiagen™). Storage of bacteria was carried out by adding 50% glycerol at a 1:1 volume of liquid culture (medium:glycerol) before freezing at –80 °C. The insertion of *CmVps4* in the constructs was confirmed by PCR amplification using primers 5′-GGAGATATTCAACACTATTCCC-3′/5′-GTGTCCGGACTGCAGGGACAC-3′ and partial Sanger sequencing.

Inoculation of the WMV-LL1A, WMV-LL1A-GFP, WMV-LL1A-*CmVps4*^{P30R}, and WMV-LL1A-Cm*Vps4*^{Wt} cDNAs was performed by biolistic bombardment on zucchini squash as previously described (Ueki *et al.*, 2013). In a second step, melon plants were infected by mechanical inoculation using infected tissues of zucchini.

Bio-informatics tools

The amino acid sequence of Gy14 *C. sativus* CsVPS4 was obtained from CuGenDB (http://cucurbitgenomics.org/). The amino acid sequence of A192-18 *C. sativus* CsVPS4 was obtained from Amano *et al.* (2013) (GenBank: AB819729.1). The amino acid sequences of *CmVPS4* from the melon accessions CM, TGR-1551, and Humaid 93-4 come from this study. Multiple alignments were performed using Clustal Omega (https://www.ebi.ac.uk/Tools/msa/clustalo/). The amino acid sequence of DHL92 *C. melo* CmVPS4 was taken from CuGenDB. Modeling of the MIT domain of CmVPS4 was realized

using SWISS-MODEL and the picture was extracted from Pdb-Viewer (Guex *et al.*, 2009; Bertoni *et al.*, 2017; Bienert *et al.*, 2017; Waterhouse *et al.*, 2018; Studer *et al.*, 2020).

Results

VPS4 is highly conserved across cucumber and melon

To identify homologous sequence of the cucumber *CsVps4* gene in melon, we aligned the Csa6G152960 gene sequence encoding the CsVPS4 protein from the fully sequenced cucumber genome Gy14 with the DHL92 whole melon genome (http://cucurbitgenomics.org/) (Huang *et al.*, 2009; Garcia-Mas *et al.*, 2012). We identified the nucleic acid sequence of MELO3C021413, sharing 98.1% identity with Csa6G152960. The predicted encoded protein from MELO3C021413 shares 99.1% identity with CsVPS4. The gene MELO3C021413 will be named herein as *CmVps4*. *CmVps4* mapped to chromosome 11 of melon, in a syntenic region to chromosome 6 of cucumber Gy14. The *CmVps4* gene is 5597 bp long and comprises seven introns and eight exons (Fig. 1A). The predicted protein sequence comprises 433 amino acids, and protein structure prediction shows the presence of the three expected VPS4 domains: MIT, AAA+ ATPase, and C-terminal domains.

Sequencing of the MIT domain of *CmVPS4* in a large collection of melon reveals non-synonymous diversity

In order to identify variations in *CmVPS4*, we sequenced the coding sequence for the MIT and AAA+ ATPase domains of *CmVps4* in 652 accessions from all parts of the world representative of cultivated and wild melon diversity. The *CmVps4* allele carried by the CM breeding line (INRAE) was used as a reference and named $CmVps4^{Wt}$. Sequence alignments revealed that most accessions (639/652) carry the $CmVps4^{Wt}$ allele. In the remaining accessions (13/652), we identified three different alleles carrying the non-synonymous substitutions C88T, C89G, or A119G, which induce the P30S, P30R, or K40R amino acid

changes, respectively, on the MIT domain (Fig. 1B, C). Alleles carrying P30S, P30R, or K40R, namely *CmVps4*^{P30S}, *CmVps4*^{P30R}, and *CmVps4*^{K40R}, were observed in two, four, and seven accessions, respectively.

The allele *CmVps4*^{P30S} is carried by the accession PI 161375, originating from Korea. The same allele is carried by the completely sequenced DHL92 accession, which derives from PI 161375 (Garcia-Mas *et al.*, 2012). The allele *CmVps4*^{P30R} was found in the four accessions TGR-1551, IVT 2365, PI 482398, and PI 482399, all originating from Africa. The accessions TGR-1551, PI 482398, and PI 482399 were collected in Zimbabwe, while the precise origin of IVT 2365 is uncertain. The allele *CmVps4*^{K40R} was found in the accessions Humaid 93-4, Humaid 93-10, HSD 2441, HSD 2445, HSD 2446, HSD 2447, and HSD 2458, all originating from Sudan. Besides these data, we analyzed the resequencing data of 1175 melon accessions from Zhao *et al.* (2019). We identified the allele carried by CM (*CmVps4*^{Wt}) in 1125 accessions and the *CmVps4*^{P30S} allele carried by PI 161375 and DHL92 in 34 additional accessions. In 16 accessions, the sequencing data did not cover the *CmVps4* gene. No new non-synonymous substitutions could be detected among these 1125 melon accessions.

Using structure prediction of the entire VPS4 protein or the MIT domain only, the amino acid changes produced by the three alleles, *CmVps4*^{P30S}, *CmVps4*^{P30R}, and *CmVps4*^{K40R}, were located on the second helix of the MIT domain (Fig. 1C), as well as those previously identified in cucumber *CsVps4*$^{F29S/M33I}$.

Amino acid changes in the three alleles were not predicted to alter the structure of the MIT domain or the entire protein, compared with *CmVps4*^{Wt}. However, modeling references are based on human VPS4, whose MIT domain only shares 26.3%/56.3% identity/similarity with that of melon (human HsVPS4A/CmVPS4^{Wt}) (Scott *et al.*, 2005b; Takasu *et al.*, 2005).

Proline is considered a highly structuring amino acid (MacArthur and Thornton, 1991). Its replacement by any other amino acid could result in local structural changes in

CmVSP4^{P30S} and CmVPS4^{P30R}. Moreover, the replacement of a proline by an arginine in CmVPS4^{P30R} induces a strong steric obstruction and charge modification, in contrast to the replacement of a proline by a serine in CmVPS4^{P30S} (Kobayashi *et al.*, 1990; Ogura *et al.*, 2004). The replacement of a lysine by an arginine in CmVPS4^{K40R} is considered as a conservative modification, despite the hydrophobic properties of lysine and the hydrophilic properties of arginine.

Accessions carrying the *CmVps4^{P30R}* and *CmVps4^{K40R}* alleles are resistant to WMV

The melon accession TGR-1551, which carries the *CmVps4^{P30R}* allele, has been previously reported to be resistant to several pests and diseases, including WMV (López-Sesé and Gómez-Guillamón, 2000; Yuste-Lisbona *et al.*, 2010; Peng and Walker, 2020). WMV resistance in TGR-1551 has been shown to be mainly controlled by a recessive locus mapped to chromosome 11. The genetic region involved in WMV resistance comprises >100 candidate genes so far (Palomares-Rius *et al.*, 2011; Pérez-de-Castro *et al.*, 2019). We located *CmVps4* in this interval.

To test the impact of *CmVps4* alleles on WMV susceptibility, we inoculated accessions carrying the *CmVps4^{P30S}*, *CmVps4^{P30R}*, and *CmVps4^{K40R}* alleles with the WMV-Fr isolate (Fig. 2). Twenty days after inoculation, the susceptible control CM showed severe mosaic symptoms. PI 414723 was used as the resistant control with a full recovery of phenotype 10 d after infection (Gilbert *et al.*, 1994). This resistance is independent of *Vps4* as PI 414723 carries the *Vps4^{Wt}* allele. In comparison with CM, accession TGR-1551 showed a significant reduction of symptoms as previously reported (Díaz-Pendón *et al.*, 2005) (Fig. 2A, B). The phenotype is characterized by an almost complete absence of symptoms, except for rare chlorotic spots, which indicate that the virus is present in the plant. The other identified accessions carrying the same allele, IVT 2365, PI 482399, and PI 482398, also showed the

same phenotype with strongly reduced symptom intensity compared with the CM control and had rare chlorotic spots, like those observed in TGR-1551 (Fig. 2B). Small variations in notations can occur due to differences in plant phenotype and physiology. Thus, some chlorotic spots and yellowing can be mistaken for virus symptoms, as can be seen in the resistant control. This explains the small difference observed between TGR-1551/IVT 2365 and PI 482398/PI 482399. The PI 161375 accession, which carries the *CmVps4*^{P30S} allele, was also inoculated with WMV-Fr. It exhibited clear symptoms which distinguished it from TGR-1551 resistance.

To evaluate the replication level of the virus in each plant, we measured the capsid accumulation in the youngest leaf. The viral load in the different accessions was assessed 20 d after inoculation and compared with samples from the susceptible control CM (Fig. 2C). A significant decrease in virus accumulation was observed in the four accessions carrying the *CmVps4*^{P30R} allele. These results showed that all the accessions carrying the *CmVps4*^{P30R} allele are resistant to WMV. The substitution identified in the *CmVps4* gene, which is present in the genetic region involved in WMV resistance in TGR-1551, is thus a major candidate to control resistance to WMV.

Accessions carrying the *CmVps4*^{K40R} allele have a slow germination rate and need specific temperature conditions. As PI 414723 did not germinate in these conditions, we had to switch our resistant control to Pastis1, an INRAE breeding line resistant to WMV, which carries the *Vps4*^{Wt} allele. Twenty days after inoculation with WMV-Fr, the accessions HSD 2441, HSD 2445, Humaid 93-4, and Humaid 93-10, carrying the *CmVps4*^{K40R} allele, showed significantly lower symptoms (Fig. 2D) and a lower viral load compared with CM. They also showed a lower viral load (Fig. 2E) compared with CM. The other three accessions HSD 2446, HSD 2447, and HSD 2458 that carry the *CmVps4*^{K40R} allele could not be tested due to a

growth defect under growth chamber conditions. These results show a complete association between the resistance to WMV and the presence of *CmVps4*^{P30R} or *CmVps4*^{K40R} alleles.

Accessions carrying the *CmVps4*^{P30R} and *CmVps4*^{K40R} alleles are not resistant to ZYMV

To test the impact of the *CmVps4* alleles on ZYMV replication in melon, we inoculated accessions carrying the *CmVps4*^{P30S}, *CmVps4*^{P30R}, and *CmVps4*^{K40R} alleles with the R5A isolate of ZYMV and compared them with the susceptible control CM (Fig. 3). We used the PI 414723 accession as a ZYMV-resistant control. As noted previously, PI 414723 carries the same *CmVps4* allele as CM (*CmVps4*^{Wt}). Resistance to ZYMV is conferred by a dominant gene (*Zym*), not linked to *CmVps4* (Pitrat and Lecoq, 1984; Adler-Berke *et al.*, 2021). Following ZYMV inoculation, accessions carrying the *CmVps4*^{P30R} allele showed symptoms similar to those of the susceptible CM control (Fig. 3A). TGR-1551, PI 482398, and PI 482399 showed severe typical mosaic symptoms 14 d after inoculation. The accession IVT 2365 showed symptoms of necrosis and wilting, which may be consistent with a hypersensitive immune response leading to the death of infected tissues (Camagna and Takemoto, 2018). Similarly, PI 161375, carrying the *CmVps4*^{P30S} allele, and the accessions HSD 2441, HSD 2445, and Humaid 93-10, carrying the *CmVps4*^{K40R} allele, showed no reduction in symptom intensity after ZYMV inoculation compared with CM (Fig. 3B). Only the accession Humaid 93-4, which also carries the *CmVps4*^{K40R} allele, showed a complete absence of symptoms. Therefore, the observed ZYMV resistance in only one of the four tested accessions cannot be associated with the *CmVps4*^{K40R} allele. In contrast to the *CsVps4*$^{F29S/M33I}$ resistance allele in cucumber, the *CmVps4*^{P30S}, *CmVps4*^{P30R}, and *CmVps4*^{K40R} alleles did not confer resistance to ZYMV in melon under the conditions used and with the isolate tested.

The *CmVps4*^{P30R} allele in the TGR-1551 accession is responsible for WMV resistance

Next, to determine the impact of the P30R amino acid change of CmVPS4 on the resistance to WMV, we proceeded with a functional complementation to restore the WMV susceptibility in TGR-1551. For this approach, we used the WMV-LL1A cloned strain adapted for genetic transformation (Desbiez *et al.*, 2012). As this WMV strain is a different isolate (WMV-LL1A) from that used to phenotype melon accessions in previous experiments (WMV-Fr), we checked that inoculation of CM and TGR-1551 with WMV-LL1A induces a viral accumulation similar to that observed with WMV-Fr (Fig. 4A). As expected, CM showed a high viral load and TGR-1551 showed a greatly reduced viral load compared with CM, after inoculation with WMV-LL1A.

To control the infectivity of the modified virus and the expression of the transgene, we used a recombinant WMV strain expressing the GFP (WMV-LL1A-GFP) to infect CM and TGR-1551 plants by mechanical inoculation. We compared the number of GFP dots on infected cotyledons between both accessions. Six days after inoculation, we observed GFP dots on cotyledons of CM and extensive GFP on leaves (Fig. 4B). The dots correspond to virus-infected cells expressing GFP. In TGR-1551, we also observed GFP dots on cotyledons but no GFP in leaves after 6 d (Fig. 4C). Comparison of the number of GFP dots showed that TGR-1551 had a significantly reduced number of dots compared with CM (Fig. 4D). Visualization of GFP in plant leaves attested to the expression of the transgene and virus infectivity. These data also show that resistance to WMV in TGR-1551 is expressed at an early stage of infection and reduces the number of infection sites after mechanical inoculation.

Finally, for functional complementation, we modified the genome of the viral strain WMV-LL1A with *CmVps4*^{Wt} or *CmVps4*^{P30R} from the CM and TGR-1551 accessions to obtain the constructs named WMV-LL1A-*CmVps4*^{Wt} and WMV-LL1A-*CmVps4*^{P30R},

respectively. This strategy allows transient expression of VPS4^{Wt} or VPS4^{P30R} in a coordinated way with WMV infection. Inoculation of the susceptible cultivar CM with both constructs, WMV-LL1A-*CmVps4^{Wt}* and WMV-LL1A-*CmVps4^{P30R}*, led to viral accumulation in the leaves 20 d after inoculation (Fig. 5A). This result shows that both modified virus strains coding for *CmVPS4^{Wt}* or *CmVPS4^{P30R}* were able to replicate and infect melon. We did not observe a significant difference in the infectivity of the two strains in CM.

Based on these results, it was possible to assess whether the WMV construct carrying the *CmVps4^{Wt}* allele was able to restore virus susceptibility in the resistant TGR-1551 accession but not in that carrying the *CmVps4^{P30R}* allele. Symptoms and virus accumulation in TGR-1551 were assessed after inoculation with the WMV-LL1A-*CmVPS4^{Wt}* and WMV-LL1A-*CmVps4^{P30R}* constructs. Plants of accession TGR-1551 showed mosaic symptoms and a high viral load 20 d after inoculation with WMV-LL1A-*CmVps4^{Wt}*, while plants inoculated with WMV-LL1A-*CmVps4^{P30R}* were almost asymptomatic and showed low virus accumulation (Fig. 5B–D). These results clearly show that the expression of CmVPS4^{Wt} in TGR-1551 is sufficient to restore WMV susceptibility, whereas the expression of the CmVPS4^{P30R} protein carrying the single amino acid substitution P30R does not allow efficient virus replication. Thus, the *CmVps4^{P30R}* allele is responsible for WMV resistance in TGR-1551.

Discussion

Plants viruses have small genomes and hijack the plant cellular machinery to complete their infection cycle. These cellular mechanisms and underlying genes are all potential targets for recessive resistance by loss of susceptibility. Further characterization of the natural variability is greatly needed to identify new host factors conferring recessive resistance. In this study, we identified CmVPS4 as responsible for recessive resistance to WMV in melon. This gene encodes a protein involved in vesicle formation at the MVB compartment. We validated

CmVps4^{P30R} as a resistance allele both by phenotyping all accessions carrying this allele and by expressing the susceptibility allele in the resistant accession TGR-1551. For that, we used an infectious clone of WMV as a vector (Fig. 5). Our results demonstrate a major role for VPS4 in WMV infection and complement our understanding of the diversity of functions of natural resistance genes.

In plant cells, and more broadly in eukaryotic cells, the VPS4 protein has been involved in the formation of internal vesicles resulting in the formation of MVBs through the ESCRT pathway (Piper and Katzmann, 2007; Azmi *et al.*, 2008). The ESCRT complex is a multiprotein complex composed of five subunits. The first proteins of this complex are recruited to endosomes by lipidic and ubiquitin signals (Kutateladze *et al.*, 1999; Hofmann and Falquet, 2001; Prag *et al.*, 2007). Then, each protein is recruited one by one, inducing membrane deformation (Henne *et al.*, 2011; Schmidt and Teis, 2012). VPS4 is the final component of the ESCRT complex and is essential for the release of vesicles into the endosome (Scott *et al*., 2005a, b; Davies *et al.*, 2008). In recent years, the major role of ESCRT proteins in viral infection has been highlighted in plants (Agaoua *et al.*, 2021). The replication of most single-stranded positive RNA [ssRNA(+)] viruses has been associated with structural changes in intracellular membranes. The viruses form vesicles and quasi-organelles, made up of viral replication complexes (VRCs), which combine all the elements necessary for the replication of viral RNA and the translation of viral proteins (Jiang and Laliberte, 2016; Lõhmus *et al.*, 2016; Nagy *et al.*, 2016). VRCs are confined in defined structures, increasing the efficiency of replication and preventing the activation of cellular defense mechanisms such as recognition of dsRNA during replication (Verchot, 2011; Kovalev *et al.*, 2017). Membranes are an integral part of the formation of VRCs. Their origins and final destinations are different depending on the virus. Studies seem to show that most cell compartments can be a target for the formation of VRCs but that each virus uses a defined

host compartment (Hwang *et al.*, 2008; Laliberté and Sanfaçon, 2010; Cao *et al.*, 2015; Jin *et al.*, 2018). The essential character of the anchoring of ssRNA(+) VRCs in the membrane has been demonstrated repeatedly in yeast by knockout approaches of the mechanisms of endomembrane trafficking (Panavas *et al.*, 2005; Rapaport, 2005; Nagy *et al.*, 2014; Diaz *et al.*, 2015; Cross *et al.*, 2016). In plants, transient inhibition of these mechanisms has shown a similar reduction in the efficiency of viral replication (Barajas *et al.*, 2009; Richardson *et al.*, 2014).

Plant ESCRT proteins have been shown to directly interact with viral proteins of viruses belonging to the families *Tombusviridae* and *Bromoviridae* (Barajas *et al.*, 2009; Richardson *et al.*, 2014; Diaz *et al.*, 2015; Nagy and Feng, 2021). The VPS4 protein of *A. thaliana* interacts directly with the p33 protein of *Tomato bushy stunt virus* (TBSV; genus *Tombusvirus*) (Barajas *et al.*, 2014). According to our data, a single amino acid change (P30R) in the CmVPS4 protein is responsible for recessive WMV resistance. This suggests that CmVPS4 protein modification could prevent an interaction with a so far undetermined WMV protein. In potyviruses, VRC formation and membrane deformation have been observed associated with endoplasmic reticulum and then moving to chloroplasts (Grangeon *et al.*, 2012). VRCs have also been observed in MVBs moving to apoplasta (Movahed *et al.*, 2019). Therefore, in a resistant plant, this putative interaction would not happen, reducing the capability of the virus to induce vesicle formation. However, VPS4 is the final component of the vesicle formation mediated by ESCRT proteins and is responsible for the release of vesicles (Haas *et al.*, 2007). This indicates that VPS4 alone is not sufficient to induce vesicle formation. Thus, other ESCRT proteins could be recruited by WMV to initiate ESCRT vesicles, as observed for *Brome mosaic virus* (BMV; genus *Bromovirus*), which recruits early and late components of the ESCRT (Diaz *et al.*, 2015). Another possibility is that VPS4 may

be used for the regulation of vesicle formation in order to keep open pores to the cytosol, as observed for TBSV (McCartney *et al.*, 2005).

In this work, we showed a reduced number of GFP dots in the TGR-1551 resistant accession after inoculation with a GFP-WMV strain (Fig. 4. This suggests that the number of infection sites is reduced and that resistance affects the ability of the virus to replicate in the host but does not completely suppress it. We can relate this result to those of Gonzalez-Ibeas *et al.* (2012), who showed that the expression of defense response genes is activated in TGR-1551 after WMV inoculation. Taken together, these results suggest a scenario in which VRC formation and sheltering of WMV in TGR-1551 would not be fully successful, allowing the cell to detect the virus and activate its defenses. Finally, although our results reveal a strong impact of the P30R substitution encoded by the allele Cm*Vps4*^{P30R} on susceptibility to WMV in melon, we cannot exclude a role for other genes, as proposed by Pérez-de-Castro *et al.* (2019).

From two melon collections gathering >1500 accessions from natural and cultivated diversity, we identified three alleles presenting an amino acid change in VPS4 (Fig. 1). Two of them (*CmVps4*^{P30R} and *CmVps4*^{K40R}) showed a strong but not fully resistant phenotype to WMV. The last allele (*CmVps4*^{P30S}) did not show any altered phenotype (Fig. 2). The scarcity of the accessions carrying each of these alleles could indicate a recent and independent evolution of the allelic forms. WMV-resistant accessions carrying the *CmVps4*^{P30R} allele were collected in Zimbabwe, where WMV has been reported. In contrast, the accessions carrying the *CmVps4*^{K40R} allele, which are also resistant to WMV, originate from Sudan, where WMV has not been identified so far (Desbiez and Lecoq, 1997; Kheyr-Pour *et al.*, 2000; Mohammed *et al.*, 2012). This suggests that the *CmVps4*^{K40R} non-synonymous substitution could be responsible for resistance to other viruses exerting selection pressure in East Africa and induce a serendipitous resistance to WMV. All accessions carrying the *CmVps4*^{P30S} allele

originate from Asia, where WMV strains are found (Q. Gao *et al.*, 2015). Since this substitution does not induce WMV resistance, following the same scenario, it could be involved in resistance to another disease.

Our results reveal that none of the three *CmVps4* alleles induced resistance to ZYMV, while their non-synonymous substitutions are located in close proximity to the two non-synonymous substitutions present in the $CsVps4^{F29S/M33I}$ candidate allele for resistance to ZYMV in cucumber (Fig. 3). Using the same approach as we did in this work, the use of a ZYMV vector to restore susceptibility in resistant cucumber could determine the involvement of $CsVps4^{F29S/M33I}$ in ZYMV resistance. Variations within a single host protein, independently responsible for resistance to distinct potyviruses, have been already observed. Associated with each other, such substitutions confer resistance to several potyviruses in pea (Bastet *et al.*, 2019). Thus, we can hypothesize that mimicking the $CsVps4^{F29S/M33I}$ allele could induce ZYMV resistance in melon. Moreover, accumulation of $CsVps4^{F29S/M33I}$ and $CmVps4^{P30R}$ substitutions in a single allele could induce resistance to both viruses in melon and cucumber.

All our results point to a novel mechanism of natural recessive resistance. The candidate gene approach that we have adopted here can also be used for all the susceptibility genes identified, using translational biology or protein interaction approaches. This could already be the case for other ESCRT proteins, such as SNF7 or VPS23, which have been shown to interact directly with a BMV and TBSV protein, respectively (Diaz *et al.*, 2015; Kovalev *et al.*, 2016). Furthermore, the identification of the causative substitutions in *CmVps4* enables directed gene editing by CRISPR/Cas9 to generate melon plants resistant to multiple potyviruses and this possibility could also be explored in other crop species.

Further efforts are needed to determine the molecular mechanism of resistance conferred by the *CmVps4* gene and its relationship to WMV proteins. The identification and cloning of *CmVps4* open up the possibility of addressing new questions related to the precise

mechanism of resistance, such as how and where the interaction between CmVPS4 and viral proteins occurs and how the P30R amino acid change is responsible for the induction of defense gene expression. Finally, it remains to be seen whether this new knowledge of resistance can be transferred to other crop species, such as cucumber and pea, and towards a larger spectrum of viruses (Amano *et al.*, 2013; Soler-Garzón *et al*., 2021).

Supplementary data

The following supplementary data are available at *JXB* online.

Table S1. List of primers used in this study.

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Authors contributions

AA, CDE, AB, FM and CDO: conceptualization;

AA: investigation, formal analysis, visualization and writing-original draft;

CDE, AB, FM and CD: methodology and resources;

AA, CDE, AB, FM and CD: writing-review and editing;

FM and CDO: project administration, supervision, funding acquisition.

Conflict of interest

The authors declare no conflict of interest.

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Data availability

The data supporting the findings of this study are available from the corresponding author, Catherine Dogimont, upon request.

References

Adler-Berke N, Goldenberg Y, Brotman Y, *et al.* 2021. The melon *Zym* locus conferring resistance to ZYMV: high resolution mapping and candidate gene identification. Agronomy **11**, 2427.

Agaoua A, **Bendahmane A**, **Dogimont C**, **Moquet F**. 2021. Membrane trafficking proteins: a new target to identify resistance to viruses in plants. Plants **10**, 2139.

Amano M, **Mochizuki A**, **Kawagoe Y**, **Iwahori K**, **Niwa K**, **Svoboda J**, **Maeda T**, **Imura Y**. 2013. High-resolution mapping of *zym*, a recessive gene for *Zucchini yellow mosaic virus* resistance in cucumber. Theoretical and Applied Genetics **126**, 2983–2993.

Anderson PK, **Cunningham AA**, **Patel NG**, **Morales FJ**, **Epstein PR**, **Daszak P**. 2004. Emerging infectious diseases of plants: pathogen pollution, climate change and agrotechnology drivers. Trends in Ecology and Evolution **19**, 535–544.

Azmi IF, **Davies BA**, **Xiao J**, **Babst M**, **Xu Z**, **Katzmann DJ**. 2008. ESCRT-III family members stimulate Vps4 ATPase activity directly or via Vta1. Developmental Cell **14**, 50–61.

Baker CA, **Hiebert E**, **Marlow GC**, **Wisler GC**. 1992. Comparative sequence analysis of the Reunion isolate of *Zucchini yellow mosaic virus*. Phytopathology **82**, 1176.

Barajas D, **de Castro Martin IF**, **Pogany J**, **Risco C**, **Nagy PD**. 2014. Noncanonical role for the host Vps4 AAA+ ATPase ESCRT protein in the formation of *Tomato bushy stunt virus* replicase. PLoS Pathogens **10**, 24–32.

Barajas D, **Jiang Y**, **Nagy PD**. 2009. A unique role for the host ESCRT proteins in replication of *Tomato bushy stunt virus*. PLoS Pathogens **5**, e1000705.

Bastet A, **Robaglia C**, **Gallois JL**. 2017. eIF4E resistance: natural variation should guide

gene editing. Trends in Plant Science **22**, 411–419.

Bastet A, **Zafirov D**, **Giovinazzo N**, **Guyon-Debast A**, **Nogué F**, **Robaglia C**, **Gallois JL**. 2019. Mimicking natural polymorphism in eIF4E by CRISPR-Cas9 base editing is associated with resistance to potyviruses. Plant Biotechnology Journal **17**, 1736–1750.

Bertoni M, **Kiefer F**, **Biasini M**, **Bordoli L**, **Schwede T**. 2017. Modeling protein quaternary structure of homo- and hetero- oligomers beyond binary interactions by homology. Scientific Reports **7**, 10480.

Bienert S, Waterhouse A, Beer TAP De, Tauriello G, Studer G, Bordoli L, Schwede T. 2017. The SWISS-MODEL Repository—new features and functionality. Nucleic Acids Research **45**, 313–319.

Brotman Y, **Normantovich M**, **Goldenberg Z**, ***et al***. 2013. Dual resistance of melon to *Fusarium oxysporum* races 0 and 2 and to *Papaya ring-spot virus* is controlled by a pair of head-to-head-oriented NB-LRR genes of unusual architecture. Molecular Plant **6**, 235–238.

Camagna M, Takemoto D. 2018. Hypersensitive response in plants. eLS, 1–7.

Cao X, **Jin X**, **Zhang X**, **Li Y**, **Wang C**, **Wang X**, **Hong J**, **Wang X**, **Li D**, **Zhang Y**. 2015. Morphogenesis of endoplasmic reticulum membrane-invaginated vesicles during *Beet black scorch virus* infection: role of auxiliary replication protein and new implications of three-dimensional architecture. Journal of Virology **89**, 6184–6195.

Cross LL, **Ebeed HT**, **Baker A**. 2016. Peroxisome biogenesis, protein targeting mechanisms and PEX gene functions in plants. Biochimica et Biophysica Acta **1863**, 850–862.

Davies BA, Babst M, Katzmann DJ. 2011. Regulation of Vps4 during MVB sorting and cytokinesis. Traffic **12**, 1298-1305.

Davies BA, **Lee JRE**, **Oestreich AJ**, **Katzmann DJ**. 2009. Membrane protein targeting to the MVB/Lysosome. Chemical Reviews **109**, 1575–1586.

Desbiez C, **Chandeysson C**, **Lecoq H**, **Moury B**. 2012. A simple, rapid and efficient way to obtain infectious clones of potyviruses. Journal of Virological Methods **183**, 94–97.

Desbiez C, **Lecoq H**. 1997. Zucchini yellow mosaic virus. Plant Pathology **46**, 809–829.

Desbiez C, **Lecoq H**. 2004. The nucleotide sequence of *Watermelon mosaic virus* (WMV, *Potyvirus*) reveals interspecific recombination between two related potyviruses in the 5′ part of the genome. Archives of Virology **149**, 1619–1632.

Díaz-Pendón JA, Fernández-Muñoz R, Gómez-Guillamón ML, Moriones E. 2005. Inheritance of resistance to *Watermelon mosaic virus* in *Cucumis melo* that impairs virus accumulation, symptom expression, and aphid transmission. Phytopathology **95**, 840–846.

Diaz A, **Zhang J**, **Ollwerther A**, **Wang X**, **Ahlquist P**. 2015. Host ESCRT proteins are required for bromovirus RNA replication compartment assembly and function. PLoS Pathogens **11**, e1004742.

Djidjou-Demasse R, **Moury B**, **Fabre F**. 2017. Mosaics often outperform pyramids: insights from a model comparing strategies for the deployment of plant resistance genes against viruses in agricultural landscapes. New Phytologist **216**, 239–253.

Dogimont C. 2011. 2011 Gene list for melon. Cucurbit Genetics Cooperative Report **33–34**, 104–133.

Endres MW, Gregory BD, Gao Z, Foreman AW, Ge X, Pruss GJ, Ecker JR, Bowman LH, Vance V. 2010. Two plant viral suppressors of silencing require the ethylene-inducible host transcription factor RAV2 to block RNA silencing. PLoS Pathogenesis **6**, e1000729.

Gao C, Zhuang X, Cui Y, Fu X, He Y, Zhao Q, Zeng Y, Shen J, Luo M. 2015. Dual roles of an Arabidopsis ESCRT component FREE1 in regulating vacuolar protein transport and autophagic degradation. Proceedings of the National Academy of Sciences, USA

112, 1886–1991.

Gao Q, **Ren HL**, **Xiao WY**, **Zhang Y**, **Zhou B**, **Xu DL**. 2015. First report of *Watermelon mosaic virus* causing a mosaic disease on *Cucumis metuliferus* in China. Plant Disease **105**, 2025.

Garcia-Mas J, **Benjak A**, **Sanseverino W**, ***et al***. 2012. The genome of melon (*Cucumis melo* L.). Proceedings of the National Academy of Sciences, USA **109**, 11872–11877.

Garcia-Ruiz H. 2019. Host factors against plant viruses. Molecular Plant Pathology **20**, 1588–1601.

Gilbert RZ, Kyle MM, Munger HM, Gray SM. 1994. Inheritance of resistance to *Watermelon mosaic virus* in *Cucumis*. Hortscience **29**, 107–110.

Giner A, Pascual L, Bourgeois M, Gyetvai G, Rios P, Picó B, Troadec C, Bendahmane A, Garcia-Mas J, Martín-Hernández AM. 2017. A mutation in the melon Vacuolar Protein Sorting 41 prevents systemic infection of *Cucumber mosaic virus*. Scientific Reports **7**, 10471.

Gómez P, Rodríguez-Hernández AM, **Moury B**, **Aranda M**. 2009. Genetic resistance for the sustainable control of plant virus diseases: breeding, mechanisms and durability. European Journal of Plant Pathology **125**, 1–22.

Gonzalez-Ibeas D, Canizares J, Aranda MA. 2012. Microarray analysis shows that recessive resistance to *Watermelon mosaic virus* in melon is associated with the induction of defense response genes. Molecular Plant-Microbe Interactions **25**, 107–118.

Grangeon R, **Agbeci M**, **Chen J**, **Grondin G**, **Zheng H**, **Laliberte J-F**. 2012. Impact on the endoplasmic reticulum and Golgi apparatus of *Turnip mosaic virus infection*. Journal of Virology **86**, 9255–9265.

Granier F, **Durand-Tardif M**, **Casse-Delbart F**, **Lecoq H**, **Robaglia C**. 1993. Mutations in

Zucchini yellow mosaic virus helper component protein associated with loss of aphid transmissibility. Journal of General Virology **74**, 2737–2742.

Gray S, **De Boer S**, **Lorenzen J**, **Karasev A**, **Whitworth J**, **Nolte P**, **Singh R**, **Boucher A**, **Xu H**. 2010. Initial reports of necrotic strains of PVY in the United States and Canada. Plant Disease **94**, 1384–1397.

Guex N, **Peitsch MC**, **Schwede T**. 2009. Automated comparative protein structure modeling with SWISS-MODEL and Swiss-PdbViewer: a historical perspective. Electrophoresis **30**, 162–173.

Haas TJ, Sliwinski MK, Martinez DE, Preuss M, Ebine K, Ueda T, Nielsen E, Odorizzi G, Otegui MS. 2007. The Arabidopsis AAA ATPase SKD1 is involved in multivesicular endosome function and interacts with its positive regulator LYST-INTERACTING PROTEIN5. The Plant Cell **19**, 1295–1312.

Hashimoto M, Neriya Y, Yamaji Y, Namba S. 2016. Recessive resistance to plant viruses: potential resistance genes beyond translation initiation factors. Frontiers in Microbiology **7**, 1695.

Heinlein M. 2015. Plant virus replication and movement. Virology **479–480**, 657–671.

Henne WM, **Buchkovich NJ**, **Emr SD**. 2011. The ESCRT pathway. Developmental Cell **21**, 77–91.

Hofmann K, **Falquet L**. 2001. A ubiquitin-interacting motif conserved in components of the proteasomal and lysosomal protein degradation systems. Trends in Biochemical Sciences **26**, 347–350.

Huang S, **Li R**, **Zhang Z**, ***et al***. 2009. The genome of the cucumber, *Cucumis sativus* L. Nature Genetics **41**, 1275–1281.

Hwang I, **Robinson DG**. 2009. Transport vesicle formation in plant cells. Current Opinion in Plant Biology **12**, 660–669.

Hwang YT, **McCartney AW**, **Gidda SK**, **Mullen RT**. 2008. Localization of the *Carnation Italian ringspot virus* replication protein p36 to the mitochondrial outer membrane is mediated by an internal targeting signal and the TOM complex. BMC Cell Biology **9**, 1–26.

Jiang J, Laliberté J-F. 2016. Membrane association for plant virus replication and movement. In: Wang A, Zhou X, eds. Current research topics in plant virology. Cham: Springer, 67–85.

Jin X, **Jiang Z**, **Zhang K**, ***et al***. 2018. Three-dimensional analysis of chloroplast structures associated with virus infection. Plant Physiology **176**, 282–294.

Kang BC, **Yeam I**, **Jahn MM**. 2005. Genetics of plant virus resistance. Annual Review of Phytopathology **43**, 581–621.

Kaur H, Garg H. 2014. Pesticides: environmental impacts and management strategies. In: Larramendy ML, Soloneski A, eds. Pesticides—toxic aspects. InTech Open.

Kheyr-Pour A, **Bananej K**, **Dafalla GA**, **Caciagli P**, **Noris E**, **Ahoonmanesh A**, **Lecoq H**, **Gronenborn B**. 2000. *Watermelon chlorotic stunt virus* from the Sudan and Iran: sequence comparisons and identification of a whitefly-transmission determinant. Phytopathology **90**, 629–635.

Kobayashi Y, **Fukumaki Y**, **Yubisui T**, **Inoue J**, **Sakaki Y**. 1990. Serine–proline replacement at residue 127 of NADH-Cytochrome b5 reductase causes hereditary methemoglobinemia, generalized type. Blood **75**, 1408–1413.

Kovalev N, de Castro Martín IF, Pogany J, Barajas D, Pathak K, Risco C, Nagy PD. 2016. The role of viral RNA and co-opted cellular ESCRT-I and ESCRT-III factors in formation of tombusvirus spherules harboring the tombusvirus replicase. Journal of Virology **90**, 3611–3626.

Kovalev N, **Inaba JI**, **Li Z**, **Nagy PD**. 2017. The role of co-opted ESCRT proteins and lipid

factors in protection of tombusviral double-stranded RNA replication intermediate against reconstituted RNAi in yeast. PLoS Pathogens **13**, e1006520.

Kutateladze TG, **Ogburn KD**, **Watson WT**, **De Beer T**, **Emr SD**, **Burd CG**, **Overduin M**. 1999. Phosphatidylinositol 3-phosphate recognition by the FYVE domain. Molecular Cell **3**, 805–811.

Laliberté J-F, Sanfaçon H. 2010. Cellular remodeling during plant virus infection. Annual Review of Phytopathology **48**, 69–91.

Lefebvre C, **Legouis R**, **Culetto E**. 2017. ESCRT and autophagies: endosomal functions and beyond. Seminars in Cell & Developmental Biology **74**, 21–28.

Lõhmus A, Varjosalo M, Mäkinen K. 2016. Protein composition of 6K2-induced membrane structures formed during *Potato virus A* infection. Molecular Plant Pathology **17**, 943–958.

López-Sesé AI, Gómez-Guillamón ML. 2000. Resistance to *Cucurbit yellowing stunting disorder virus* (CYSDV) in *Cucumis melo* L. HortScience **35**, 110–113.

Luis Arteaga MP, Quiot JB Leroux JP. 1976. Mise en évidence d'une souche de *Watermelon mosaic virus* (WMV2) dans le Sud-Est de la France. Annales de Phytopathologie **8**, 347–353.

MacArthur MW, **Thornton JM**. 1991. Influence of proline residues on protein conformation. Journal of Molecular Biology **218**, 397–412.

Mäkinen K. 2020. Plant susceptibility genes as a source for potyvirus resistance. Annals of Applied Biology **176**, 122–129.

McCartney AW, **Greenwood JS**, **Fabian MR**, **White KA**, **Mullen RT**. 2005. Localization of the *Tomato bushy stunt virus* replication protein p33 reveals a peroxisome-to-endoplasmic reticulum sorting pathway. The Plant Cell **17**, 3513–3531.

McKirdy SJ, **Jones RAC**, **Nutter FW**. 2002. Quantification of yield losses caused by *Barley*

yellow dwarf virus in wheat and oats. Plant Disease **86**, 769–773.

Metzker ML. 2010. Sequencing technologies—the next generation. Nature Reviews Genetics **11**, 31–46.

Mohammed H, Manglli A, Zicca S, El Hussein A, Mohamed M, Tomassoli L. 2012. First report of *Papaya ringspot virus* in pumpkin in Sudan. New Disease Reports **26**, 26.

Movahed N, **Cabanillas DG**, **Wan J**, **Vali H**, **Laliberté JF**, **Zheng H**. 2019. *Turnip mosaic virus* components are released into the extracellular space by vesicles in infected leaves. Plant Physiology **180**, 1375–1388.

Mundt CC. 2014. Durable resistance: a key to sustainable management of pathogens and pests. Infection, Genetics and Evolution **27**, 446–455.

Nagy PD, **Feng Z**. 2021. Tombusviruses orchestrate the host endomembrane system to create elaborate membranous replication organelles. Current Opinion in Virology **48**, 30–41.

Nagy PD, **Pogany J**, **Lin JY**. 2014. How yeast can be used as a genetic platform to explore virus–host interactions: from 'omics' to functional studies. Trends in Microbiology **22**, 309–316.

Nagy PD, **Strating JRPM**, **van Kuppeveld FJM**. 2016. Building viral replication organelles: close encounters of the membrane types. PLoS Pathogens **12**, e1005912.

Nicaise V. 2014. Crop immunity against viruses: outcomes and future challenges. Frontiers in Plant Science **5**, 660.

Nieto C, **Morales M**, **Orjeda G**, ***et al***. 2006. An eIF4E allele confers resistance to an uncapped and non-polyadenylated RNA virus in melon. The Plant Journal **48**, 452–462.

Ogura T, **Whiteheart SW**, **Wilkinson AJ**. 2004. Conserved arginine residues implicated in ATP hydrolysis, nucleotide-sensing, and inter-subunit interactions in AAA and AAA+ ATPases. Journal of Structural Biology **146**, 106–112.

Ouibrahim L, **Mazier M**, **Estevan J**, **Pagny G**, **Decroocq V**, **Desbiez C**, **Moretti A**, **Gallois JL**, **Caranta C**. 2014. Cloning of the Arabidopsis *rwm1* gene for resistance to *Watermelon mosaic virus* points to a new function for natural virus resistance genes. The Plant Journal **79**, 705–716.

Palomares-Rius FJ, **Viruel MA**, **Yuste-Lisbona FJ**, **Lopez-Sese AI**, **Gomez-Guillamon ML**. 2011. Simple sequence repeat markers linked to QTL for resistance to *Watermelon mosaic virus* in melon. Theoretical and Applied Genetics **123**, 1207–1214.

Panavas T, **Serviene E**, **Brasher J**, **Nagy PD**. 2005. Yeast genome-wide screen reveals dissimilar sets of host genes affecting replication of RNA viruses. Proceedings of the National Academy of Sciences **102**, 7326–7331.

Pascual L, Yan J, Pujol M, Monforte AJ, Picó B. 2019. CmVPS41 is a general gatekeeper for resistance to cucumber mosaic virus phloem entry in melon. Frontiers in Plant Science **10**, 1219.

Peng HC, **Walker GP**. 2020. Sieve element occlusion provides resistance against *Aphis gossypii* in TGR-1551 melons. Insect Science **27**, 33–48.

Pérez-de-Castro A, Esteras C, Alfaro-Fernández A, Daròs JA, Monforte AJ, Picó B, Gómez-Guillamón ML. 2019. Fine mapping of *wmv*^{1551}, a resistance gene to *Watermelon mosaic virus* in melon. Molecular Breeding **39**, 93.

Perez OD, **Nolan GP**. 2001. Resistance is futile: assimilation of cellular machinery by HIV-1. Immunity **15**, 687–690.

Piper RC, **Katzmann DJ**. 2007. Biogenesis and function of multivesicular bodies. Annual Review of Cell and Developmental Biology **23**, 519–547.

Piron F, **Nicolaï M**, **Minoïa S**, **Piednoir E**, **Moretti A**, **Salgues A**, **Zamir D**, **Caranta C**, **Bendahmane A**. 2010. An induced mutation in tomato eiF4E leads to immunity to

two potyviruses. PLoS One **5**, e11313.

Pitrat M, **Lecoq H**. 1984. Inheritance of zucchini yellow mosaic virus resistance in *Cucumis melo* L. Euphytica **33**, 57–61.

Prag G, **Watson H**, **Kim YC**, **Beach BM**, **Ghirlando R**, **Hummer G**, **Bonifacino JSS**, **Hurley JH**. 2007. The Vps27/Hse1 complex is a GAT domain-based scaffold for ubiquitin-dependent sorting. Developmental Cell **12**, 973–986.

Rapaport D. 2005. How does the TOM complex mediate insertion of precursor proteins into the mitochondrial outer membrane? Journal of Cell Biology **171**, 419–423.

Richardson LGL, **Clendening EA**, **Sheen H**, **Gidda SK**, **White KA**, **Mullen RT**. 2014. A unique N-terminal sequence in the *Carnation Italian ringspot virus* p36 replicase-associated protein interacts with the host cell ESCRT-I component Vps23. Journal of Virology **88**, 6329–6344.

Sanfaçon H. 2015. Plant translation factors and virus resistance. Viruses **7**, 3392–3419.

Schmidt O, **Teis D**. 2012. The ESCRT machinery. Current Biology **22**, R116–R120.

Scott A, **Chung H-Y**, **Gonciarz-Swiatek M**, ***et al***. 2005a. Structural and mechanistic studies of VPS4 proteins. The EMBO Journal **24**, 3658–3669.

Scott A, **Gaspar J**, **Stuchell-Brereton MD**, **Alam SL**, **Skalicky JJ**, **Sundquist WI**. 2005b. Structure and ESCRT-III protein interactions of the MIT domain of human VPS4A. Proceedings of the National Academy of Sciences, USA **102**, 13813–13818.

Shopan J, **Mou H**, **Zhang L**, **Zhang C**, **Ma W**, **Walsh JA**, **Hu Z**, **Yang J**, **Zhang M**. 2017. Eukaryotic translation initiation factor 2B-beta (eIF2Bβ), a new class of plant virus resistance gene. The Plant Journal **90**, 929–940.

Siemering KR, **Golbik R**, **Sever R**, **Haseloff J**. 1996. Mutations that suppress the thermosensitivity of green fluorescent protein. Current Biology **6**, 1653–1663.

Soler-Garzón A, **McClean PE**, **Miklas PN**. **2021**. Coding mutations in vacuolar protein-

sorting 4 AAA + ATPase endosomal sorting complexes required for transport protein homologs underlie *bc-2* and new *bc-4* gene conferring resistance to *Bean common mosaic virus* in common bean. Frontiers in Plant Science **12**, 769247.

Studer G, Rempfer C, Waterhouse AM, Gumienny R, Haas J, Schwede T. 2020. Structural bioinformatics QMEANDisCo—distance constraints applied on model quality estimation. Bioinformatics **36**, 1765–1771.

Takasu H, Jee JG, Ohno A, Goda N, Fujiwara K, Tochio H, Shirakawa M, Hiroaki H. 2005. Structural characterization of the MIT domain from human Vps4b. Biochemical and Biophysical Research Communications **334**, 460–465.

Truniger V, **Aranda MA**. 2009. Recessive resistance to plant viruses. Advances in Virus Research **75**, 119–159.

Ueki S, Magori S, Lacroix B, Citovsky V. 2013. Transient gene expression in epidermal cells of plant leaves by biolistic DNA delivery. Methods in Molecular Biology **940**, 17–26.

Verchot J. 2011. Wrapping membranes around plant virus infection. Current Opinion in Virology **1**, 388–395.

Waterhouse A, Bertoni M, Bienert S, *et al.* 2018. SWISS-MODEL: homology modelling of protein structures and complexes. Nucleic Acids Research **46**, 296–303.

Whitfield AE, Falk BW, Rotenberg D. 2015. Insect vector-mediated transmission of plant viruses. Virology **479–480**, 278–289.

Wu G, **Cui X**, **Dai Z**, **He R**, **Li Y**, **Yu K**, **Bernards M**, **Chen X**, **Wang A**. 2020. A plant RNA virus hijacks endocytic proteins to establish its infection in plants. The Plant Journal **101**, 384–400.

Yoshii M, Yoshioka N, Ishikawa M, Naito S. 1998. Isolation of an *Arabidopsis thaliana* mutant in which accumulation of cucumber mosaic virus coat protein is delayed. The

Plant Journal **13**, 211–219.

Yuste-Lisbona FJ, López-Sesé AI, Gómez-Guillamón ML. 2010. Inheritance of resistance to races 1, 2 and 5 of powdery mildew in the melon TGR-1551. Plant Breeding **129**, 72–75.

Zhao G, **Lian Q**, **Zhang Z**, ***et al***. 2019. A comprehensive genome variation map of melon identifies multiple domestication events and loci influencing agronomic traits. Nature Genetics **51**, 1607–1615.

Fig. 1. Structure of the *CmVps4* gene and CmVPS4 protein in melon. (A) Intron–exon structure of the *CmVps4* gene and the predicted encoded protein. The yellow bars represent the 5′- and 3′-untranslated regions (UTRs) of the gene. The orange arrows represent the coding exons. The gray lines represent introns. (B) Protein alignment of the MIT domain of CsVPS4 and CmVPS4 protein variants. Gy14 and A192-18 accessions are from cucumber species (*Cucumis sativus*) and Charentais Monoecious (CM). TGR-1551 and Humaid 93-4 are from melon species (*C. melo*). Underlined amino acids represent variation from at least one protein sequence. Only one accession for each non-synonymous variant is represented. (C) Three helix structure of the CmVPS4 MIT domain (1–70) prediction. Positions of the CmVPS4 variants (P30S/P30R and K40R) are indicated in red.

Fig. 2. Symptoms and virus accumulation after WMV inoculation in melon. (A) Pictures of symptoms after WMV inoculation on leaves of Charentais Monoecious (CM) and TGR-1551. (B and D) Mean value of WMV symptoms on a 0–3 scale based on the viral severity of symptoms 20 d after WMV inoculation (DAI). (C and E) Average optical density relative to WMV accumulation in the youngest leaves at 20 DAI for each accession. Boxplots are designed from bottom to top with, respectively, the minimum value, Q1, median, Q3, and maximum value. Outliers are represented by filled diamonds. (B) Mean value of WMV symptoms on accessions carrying the $CmVps4^{P30R}$ and $CmVps4^{P30S}$ allele (plant numbers per

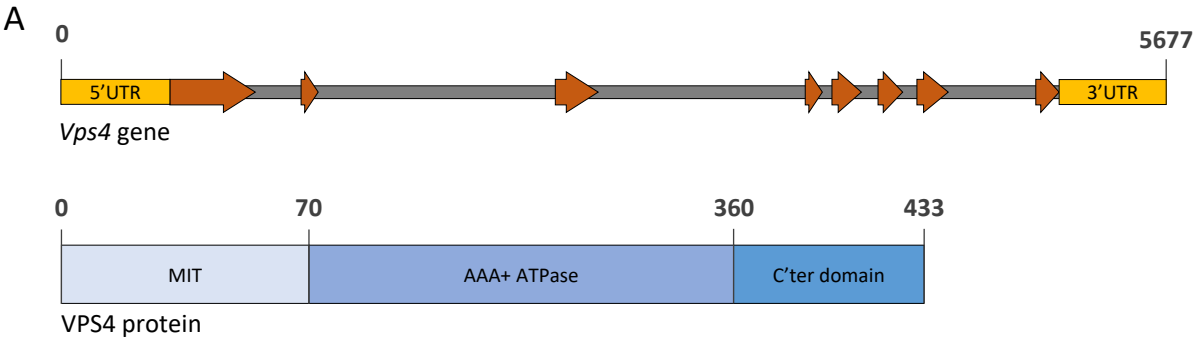
accession n≥8) compared with CM. (C) WMV accumulation in accessions carrying the CmVps4P30R allele (n≥8). (D) Mean value of WMV symptoms on accessions carrying the CmVps4K40R allele (n≥8) compared with CM. (E) WMV accumulation in accessions carrying the CmVps4K40R allele (n≥9). Kruskal–Wallis statistical analyses of these data are indicated by asterisks according to the significance of differences from the wild type at $P<0.05$ (*). (B and C) CM and PI 414723 were used as susceptible and resistant controls, respectively. Both carry the $CmVps4^{Wt}$ allele. (D and E) CM and Pastis1 were used as susceptible and resistant controls, respectively. Pastis1 also carries the $CmVps4^{Wt}$ allele.

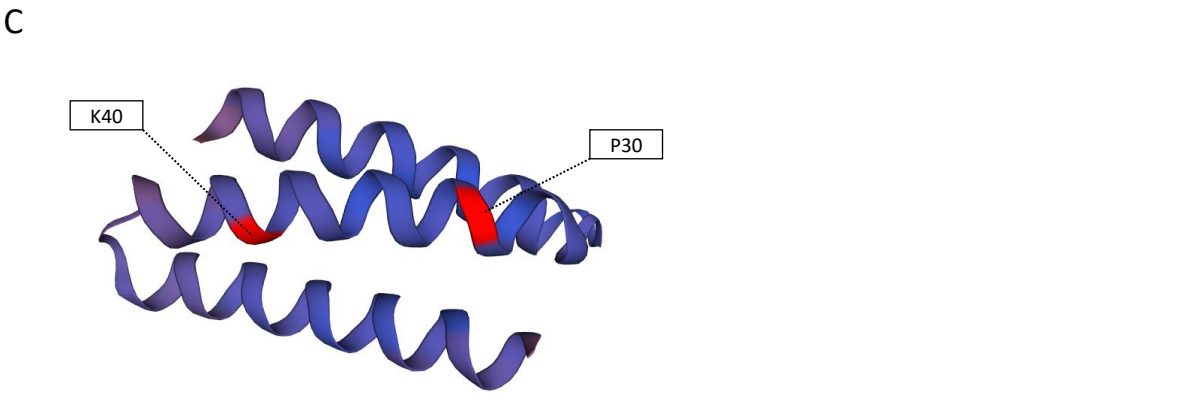
Fig. 3. Symptoms displayed after ZYMV inoculation in melon. ZYMV symptom scoring on a 0–3 scale based on the severity of viral symptoms 14 d after ZYMV inoculation (DAI) (0=absence of symptoms; 3=severe mosaic symptoms). Mean values are displayed. Charentais Monoecious (CM) and PI 414723 were used as susceptible and resistant controls, respectively; both carry the $CmVps4^{Wt}$ allele. (A) ZYMV symptom scoring on accessions carrying the Cm$Vps4^{P30R}$ and the Cm$Vps4^{P30S}$ alleles (plant numbers per accession n≥8) compared with those of CM. (B) ZYMV symptom scoring on accessions carrying the $CmVps4^{K40R}$ (K40R) allele (n≥9) compared with those of CM.

Fig. 4. Virus accumulation and symptoms displayed after WMV-LLA1 and WMV-LLA1-GFP inoculation in melon. (A) Average optical density relative to WMV-LLA1 strain accumulation in the youngest leaves of Charentais Monoecious (CM) and TGR-1551 at 20 d after inoculation (DAI) (plant numbers per accession n≥8). Kruskal–Wallis statistical analyses of these data are indicated by asterisks according to the significance of differences from the wild type of CM at $P<0.05$ (*). (B and C) Pictures of symptoms on cotyledons and first leaves of CM and TGR-1551 at 6 DAI. (D) Average number of GFP dots on mechanically inoculated cotyledons at 6 DAI. CM was used as the susceptible control. CM and TGR-1551 were inoculated with the modified strain WMV-LLA1-GFP (n=32). Boxplots are designed

from bottom to top with, respectively, minimum value, Q1, median, Q3, and maximum value. Outliers are represented by filled diamonds. Kruskal–Wallis statistical analyses of these data are indicated by asterisks according to the significance of differences from the wild type of CM at $P<0.05$ (*).

Fig. 5. Virus accumulation after WMV-LLA1-*CmVps4*^{Wt} and WMV-LLA1-*CmVps4*^{P30R} inoculation in melon TGR-1551. (A) Average optical density relative to accumulation of modified WMV-LLA1-*CmVps4*^{Wt} and WMV-LLA1-*CmVps4*^{P30R} strains in the youngest leaves of Charentais Monoecious (CM) at 20 DAI (plant number per accession $n\geq11$). (B and C) Illustrative pictures of symptoms on TGR-1551 after inoculation by WMV-LLA01-CmVps4P30R and WMV-LLA01-CmVps4Wt, respectively. (D) Virus accumulation in the TGR-1551 resistant accession after inoculation by the modified strains WMV-LLA1-CmVps4Wt and WMV-LLA01-CmVps4P30R (plant number inoculated per strain $n\geq11$). Boxplots are designed from bottom to top with, respectively, minimum value, Q1, median, Q3, and maximum value. Outliers are represented by filled diamonds. Kruskal–Wallis statistical analyses of these data are indicated by asterisks according to the significance of differences at $P<0.05$ (*). Each population has been compared two by two. Absence of an indication corresponds to no significative difference.





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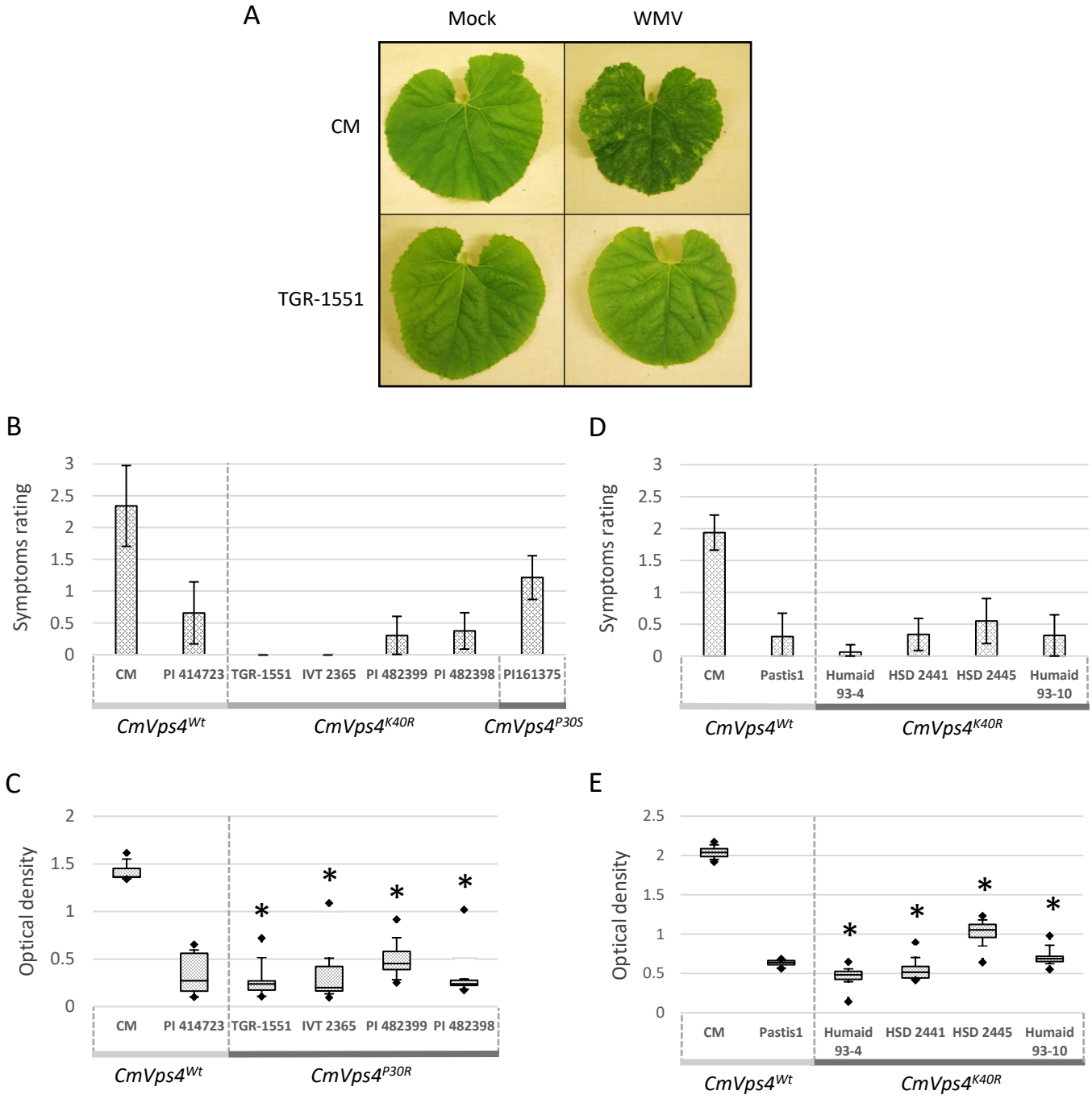
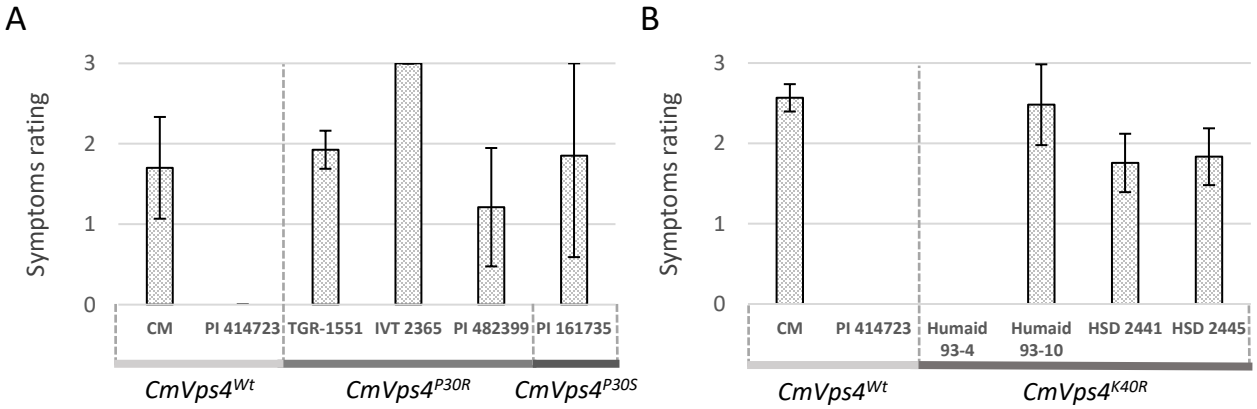


Figure 2: Symptoms and virus accumulation after WMV inoculation in melon.

(A) Pictures of symptoms after WMV inoculation on leaves of Charentais Monoecious (CM) and TGR-1551. (B and D) Mean value of WMV symptoms on a 0 to 3 scale based of the viral severity of symptoms 14 day after WMV inoculation (DAI). (C and E) Average optical density relative to WMV accumulation in youngest leaves at 14 DAI for each accession. (B) Mean value of WMV symptoms on accessions carrying the *CmVps4^{P30R}* allele (plants number per accession n≥8) compared to CM. (C) WMV accumulation in accessions carrying the *CmVps4^{P30R}* allele (n≥8). (D) Mean value of WMV symptoms on accessions carrying the *CmVps4^{K40R}* allele (n≥8) compared to CM. (E) WMV accumulation in accessions carrying the *CmVps4^{K40R}* allele (n≥9). Kruskal–Wallis statistical analyses on these data are indicated by asterisks according to the significance of differences from the wild type at P <0.05 (*). (B and C) Charentais Monoecious (CM) and PI 414723 were used as susceptible and resistant controls. respectively; both carry the *CmVps4^{Wt}* allele (D and E) Charentais Monoecious (CM) and Pastis1 were used as susceptible and resistant controls. respectively. Pastis1 also carries the *CmVps4^{Wt}*.



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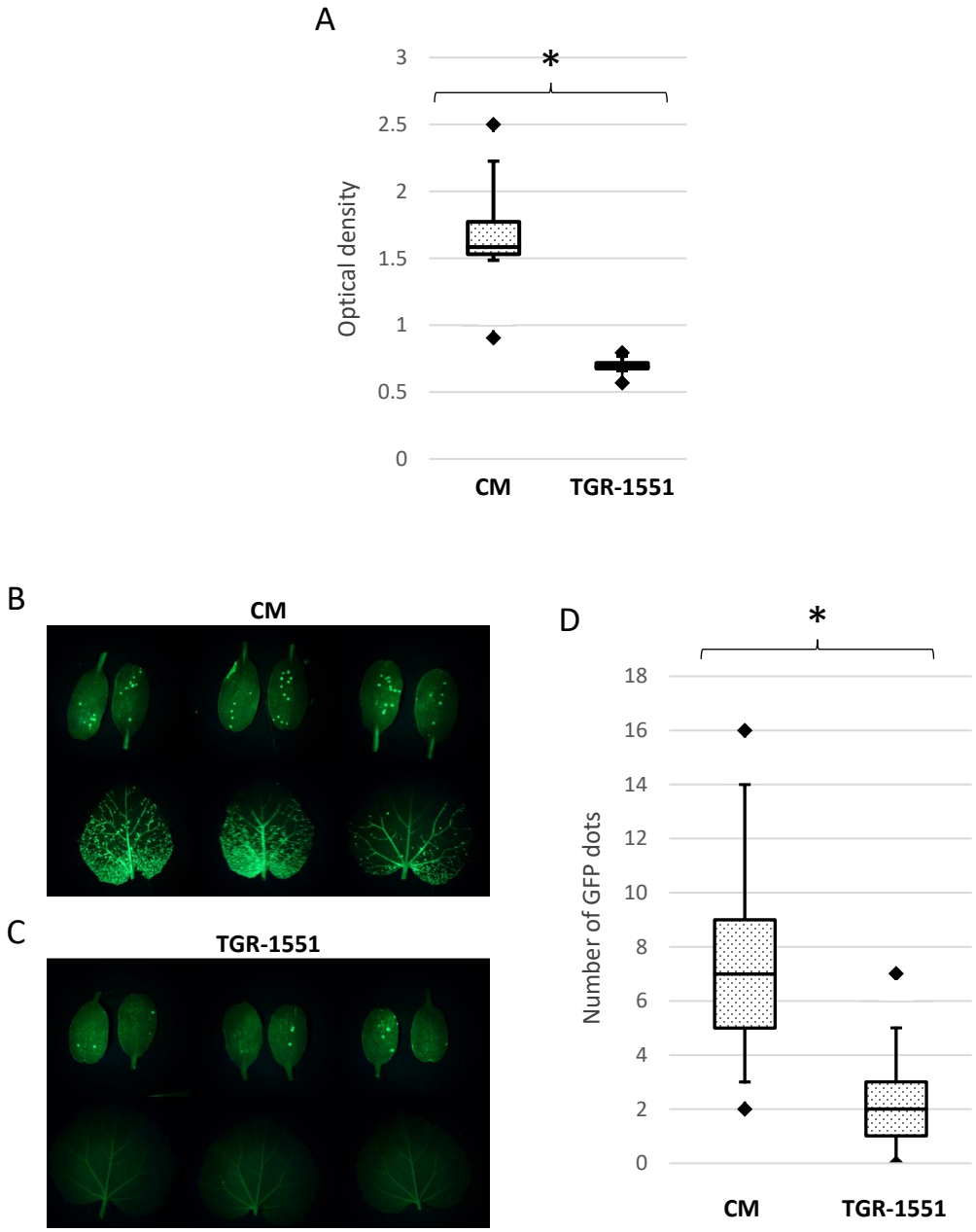

Figure 4: Virus accumulation and symptoms displayed after WMV-LLA1 and WMV-LLA1-GFP inoculation in melon.

(A) Average optical density relative to WMV-LLA1 strain accumulation in youngest leaves of Charentais Monoecious (CM) and TGR-1551 at 20 days after inoculation (DAI) (plant number per accession n≥8). Kruskal–Wallis statistical analyses on these data are indicated by asterisks according to the significance of differences from the wild type CM at P <0.05 (*). (B and C) Pictures of symptoms on cotyledons and first leaves of CM and TGR-1551 at 6 DAI. (D) Average number of GFP dots on mechanically inoculated cotyledons at 6 DAI. CM was used as susceptible control. CM and TGR-1551 were inoculated with the transformed strain WMV-LLA1-GFP (n=32). Kruskal–Wallis statistical analyses on these data are indicated by asterisks according to the significance of differences from the wild type CM at P <0.05 (*).

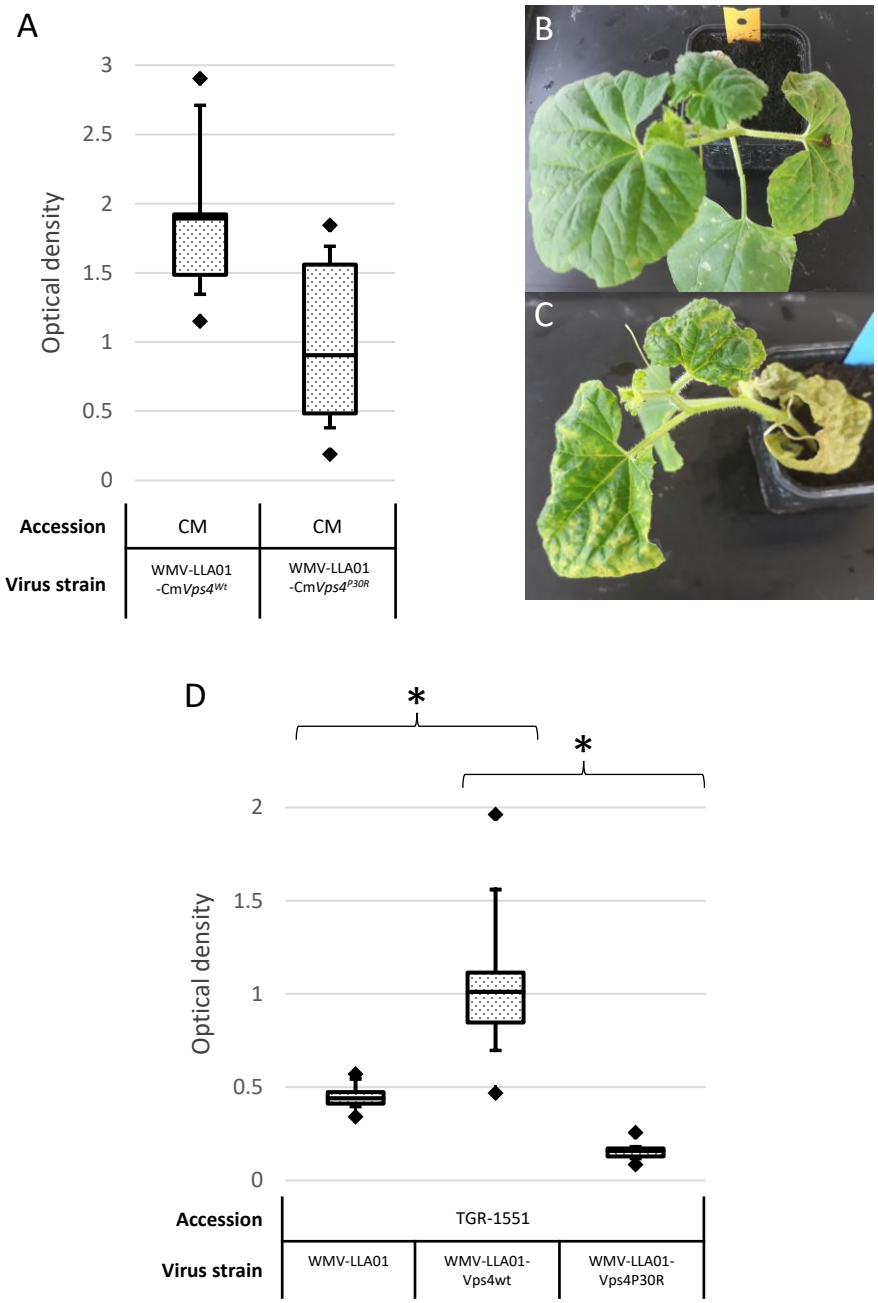

Figure 5: Virus accumulation after WMV-LLA1-*CmVps4^{Wt}* and WMV-LLA1-*CmVps4^{P30R}* inoculation in melon TGR-1551

(A) Average optical density relative to transformed *WMV-LLA1-CmVps4^{Wt} and WMV-LLA1-CmVps4^{P30R}* strains accumulation in youngest leaves of Charentais Monoecious (CM) at 20 DAI (plant number per accession n≥11). (B and C) Illustrative pictures of symptoms on TGR-1551 after inoculation by WMV-LLA01-*CmVps4^{P30R}* and WMV-LLA01-*CmVps4^{Wt}* respectively. (D) Virus accumulation in the TGR-1551 resistant accession after inoculation by the transformed strains WMV-LLA1-*CmVps4^{Wt}* and WMV-LLA01-*CmVps4^{P30R}* (plant number inoculated per strain (n≥11). Kruskal–Wallis statistical analyses on these data are indicated by asterisks according to the significance of differences at P <0.05 (*). Each population has been compared two at two. Absence of indication corresponding to no significative difference.