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Incidence and genetic diversity of cucurbit viruses in the Spanish Mediterranean area

María López-Martín[1] | Alicia Sifres[1] | Maria Luisa Gómez-Guillamón[2] | Belén Picó[1] | Ana Pérez-de-Castro[1]

[1]COMAV, Instituto de Conservación y Mejora de la Agrodiversidad Valenciana, Universitat Politècnica de València, València, Spain

[2]Instituto de Hortofruticultura Subtropical y Mediterránea La Mayora, CSIC-UMA, Málaga, Spain

Correspondence
Ana Pérez-de-Castro, COMAV, Instituto de Conservación y Mejora de la Agrodiversidad Valenciana, Universitat Politècnica de València, Cno. de Vera, s/n, 46022 València, Spain.
Email: anpede1@btc.upv.es

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Abstract

Viral infections on cucurbit fields can cause major economic losses. Monitoring of the main producing areas is essential to identify both prevalent and emerging viruses. For two consecutive years (2019–2020), the presence and molecular diversity of nine aphid- and whitefly-transmitted viruses in the main cucurbit-producing areas of the Spanish Mediterranean basin and other important regions were studied. In analyses of symptomatic plants, watermelon mosaic virus (WMV), cucurbit aphid-borne yellows virus (CABYV) and cucumber mosaic virus (CMV) were found to be prevalent in all the monitored areas, regardless of the crop and the farming conditions. Moroccan watermelon mosaic virus (MWMV) and tomato leaf curl New Delhi virus (ToLCNDV) were also found at lower rates, mainly in mixed infections with WMV. Phylogenetic analyses were conducted to determine the molecular variability of the different isolates. Whereas the sequences of CABYV, MWMV and ToLCNDV isolates all clustered within their corresponding Mediterranean clade, new viral variants of WMV and CMV were found. Seven new WMV profiles and a reassorting CMV isolate (IB-IB-IA) were observed. Moreover, the complete genome of the newly described WMV isolates was sequenced. Further studies should be done to determine if these new variants spread to new areas and if they can overcome the previously described resistances.

KEYWORDS
CMV, cucurbits, epidemiology, phylogenetics, recombination, WMV

1 | INTRODUCTION

The cultivation of cucurbits is of major economic importance in Spain, which is the principal exporter of these crops in Europe, with an annual production of more than 3.5 million tonnes. Melon (*Cucumis melo*), watermelon (*Citrullus lanatus*), zucchini (*Cucurbita pepo*) and pumpkin (*Cucurbita maxima* and *C. moschata*) are widely cultivated in the Spanish Mediterranean area. Unfortunately, the good climatic conditions that allow Spain to be considered the vegetable garden of Europe also favour the spread of different pests and diseases. The incidence of viral infections might threaten the growth of cucurbits in this region, affecting yield as well as the organoleptic quality of the collected fruits. To date, 59 different viruses have been described infecting curcurbits and at least 28 of them have been reported in the Mediterranean basin (Desbiez & Lecoq, 2012). The potyviruses watermelon mosaic virus (WMV) and zucchini yellow mosaic virus (ZYMV), the polerovirus cucurbit aphid-borne yellows virus (CABYV) and the cucumovirus

cucumber mosaic virus (CMV), which are aphid-transmitted, are the most important viruses in open fields, because they have been detected in more than half of the Mediterranean countries, causing major economic losses (Desbiez & Lecoq, 2012). Additionally, Moroccan watermelon mosaic virus (MWMV) was first described affecting zucchini crops in Europe more than 20 years ago, but it has not spread as much as other potyviruses (De Moya-Ruiz et al., 2021; Desbiez et al., 2020).

Some whitefly-transmitted viruses, such as the crinivirus beet pseudo-yellows virus (BPYV), have been present in the Mediterranean basin for many years, but the emergence of the crinivirus cucurbits yellow stunting disorder virus (CYSDV) in the late 1990s rapidly replaced BPYV in some areas. CYSDV causes major economic losses, and is considered one of the most damaging recent emerging viruses in cucurbits (Desbiez & Lecoq, 2012). In 2011 the crinivirus cucurbit chlorotic yellows virus (CCYV) was first reported in Europe, specifically in Greece, causing symptoms similar to those produced by CYSDV (Orfanidou et al., 2014). Even though CCYV has spread to other Mediterranean countries (Maachi et al., 2022), it has not yet been as problematic in open field conditions as it is in the United States, where both CCYV and CYSDV have been detected in a high percentage of samples tested on both the East and West Coasts (Kavalappara et al., 2021; Mondal et al., 2023). Another whitefly-transmitted virus that causes severe economic losses in greenhouse and open-field cucurbit crops is the tomato leaf curl New Delhi virus (ToLCNDV), that was first reported in Europe in 2012 in Spain (Juárez et al., 2014) and is rapidly spreading. Contact- and seed-transmitted viruses from the genus *Tobamovirus* are also emerging worldwide. Among them, cucumber green mottle mosaic virus (CGMMV) is the most economically important and has achieved a global distribution. This virus has been present in southern Spain since the 1990s and lately different outbreaks have taken place in greenhouses located in that area (Crespo et al., 2017).

The already complex epidemiological situation can be complicated even more as a consequence of the appearance of new viral strains that may arise from recombination events, or introduced by international trade, because they might cause more severe symptoms and reduce the efficiency of genetic resistances (Desbiez et al., 2021). This is the case of WMV, in which the 'emergent' groups (EM) that were first reported in France in 2000 have completely displaced the 'classic' strains that used to be predominant in the Mediterranean region (Bertin et al., 2020; Desbiez et al., 2020). These 'emergent' isolates have been connected with more severe symptoms observed since 2000 in open-field conditions. Moreover, new genetic variants have been found within the WMV groups (Desbiez et al., 2020), and some of them seem to overcome the genetic resistances previously described (Desbiez et al., 2021). New recombinant strains of CABYV causing more severe yellowing symptoms have recently been detected (Rabadán et al., 2021), and they have had an important economic impact on the production of cucurbits. Also, introduction of new strains of CMV (Jacquemond, 2012), CGMMV (Crespo et al., 2017) and ZYMV (Desbiez & Lecoq, 2012) with Asian origin have been reported during recent decades.

Conducting virus incidence surveys to monitor introduction/spread of viruses in the main cucurbit-producing areas is essential to detect the main viral infections and to study the genetic variability of the viruses. This allows the mitigation of threats for agriculture, by designing the most effective methods to manage these viruses and their vectors.

Historically, CMV, WMV and CABYV have been the most prevalent viruses in Spain, affecting all the main cucurbit crops and producing areas (Juárez et al., 2013; Kassem et al., 2007). ZYMV has also been detected in previous studies, but at lower infection rates (Juárez et al., 2013; Kassem et al., 2007) whereas CYSDV caused important economic losses during the late 1990s but its incidence decreased in the early 2000s (Juárez et al., 2013). Recent surveys carried out on watermelon and pumpkin fields in 2018–2020 summer seasons showed that WMV and CABYV were still the most common viruses found in plants from both crops, with mixed infections being common (De Moya-Ruiz et al., 2021; Rabadán et al., 2021, 2023). MWMV, CMV and ZYMV were also found in these surveys but at much lower rates. Nevertheless, these surveys did not analyse the presence of whitefly-transmitted viruses such as CYSDV, ToLCNDV or CCYV, the latter detected for the first time in Spain in 2018 (Chynoweth et al., 2021) nor the seed- and contact-transmitted virus CGMMV.

The aim of this study was to increase the knowledge of the current status of viral infections in melon, watermelon, zucchini and pumpkin crops in Spain. The occurrence of nine different viruses in the main cucurbit-producing areas of the Spanish Mediterranean region was monitored. Additionally, the molecular diversity of these viruses in the different regions was further studied.

2 | MATERIALS AND METHODS

2.1 | Sample collection

Surveys in conventional farming fields were carried out during early and late July in 2019 and 2020, corresponding to the middle and end of the main growing season of cucurbit crops in eastern Spain. In 2020, surveys were also carried out in September, the harvesting time for squashes and pumpkins. In 2019, a total of 10 locations were monitored in six provinces (Valencia, Castellón, Málaga, Almería, Murcia and Ciudad Real) belonging to four regions (Comunidad Valenciana, Andalucía, Región de Murcia and Castilla la Mancha) and during 2020 samples were collected in five locations distributed in four provinces (Valencia, Málaga, Ciudad Real and Badajoz) of four Spanish regions (Comunidad Valenciana, Andalucía, Castilla la Mancha and Extremadura) (Table S1). During 2019 and 2020, a total of 170 and 132 apical leaf samples, respectively, with symptoms of virus infection, such as mosaic, yellowing or foliar deformation, were collected from melon, zucchini and pumpkin grown in open fields. Moreover, 323 apical leaves of symptomatic plants were also collected in an organic melon field (one plot) located in La Punta (province of Valencia in Comunidad Valenciana) in 2019, to explore whether there might be

different results depending on the farming system. Additionally, plots located in Museros and La Punta (province of Valencia in Comunidad Valenciana) were monitored on three and four different dates, respectively, during the summer season to evaluate the viral incidence changes over time.

2.2 | Virus detection

Reverse transcription-PCRs were carried out to detect all the studied RNA viruses (WMV, MWMV, CMV, CYSDV, ZYMV, CGMMV, CCYV and CABYV). Total RNA was extracted from young leaves using Extrazol EM30 (BLIRT S.A.) following the manufacturer's instructions. RNA concentration was measured using spectrophotometry in a NanoDrop ND-1000 spectrophotometer and 1 μg of RNA was reverse-transcribed with the RevertAid RT First Strand cDNA Synthesis Kit (ThermoFisher Scientific) using random primers and following the manufacturer's recommendations. The obtained cDNA was directly used for the PCR amplification of the coat protein (CP) gene regions of WMV, MWMV, CMV, CYSDV, ZYMV, CGMMV, CCYV and CABYV (Table S2). To make the results comparable with previous studies carried out in France, the primers described by Desbiez et al. (2020) to sequence different regions of WMV, CMV and CABYV genomes were also used (Table S2) for both PCR amplification and sequencing (see section below). All the PCRs were carried out with the DreamTaq Green PCR Master Mix (ThermoFisher Scientific) following the same cycling conditions: 94°C for 5 min; 32 cycles of 94°C 30 s, 55°C 30 s, 72°C 45 s; and 72°C 5 min. We used 1 μL of template, 1× DreamTaq Green PCR Master Mix, 0.1 μM of each primer, 0.14 mM of dNTPs and 0.01 U/μL of Taq DNA polymerase in a final volume of 30 μL. Two independent replicates were performed for each cDNA sample to reduce the risk of false negatives. In order to detect ToLCNDV, the only DNA virus screened during the surveys, a tissue printing assay was performed as described by Sáez et al. (2021). Total DNA was also extracted from samples from conventional farming fields where ToLCNDV had been detected in the tissue-printing analysis and from some random symptomatic samples from the organic farming field in which ToLCNDV had also been detected. Total DNA extraction was carried out using the CTAB method with minor modifications (Sáez et al., 2021). DNA concentration was measured using spectrophotometry in a NanoDrop ND-1000 spectrophotometer and the DNA was diluted to 50 ng/μL. PCR amplification of the DNA-A coat protein gene of ToLCNDV was done with the same primers used to synthesize the corresponding probe (Table S2) and following the conditions previously indicated.

2.3 | Sequencing of viruses in cucurbits

For all the viruses found to be present in the surveys, one positive sample of every crop in each location and plot at different dates was chosen, and the corresponding PCR product was purified using the EXTRACTME DNA CLEAN-UP KIT (BLIRT S.A.) and paired-end sequenced by the Sanger method (Secuenciación de ADN y análisis de la expression génica, Instituto de Biología Molecular y Celular de Plantas [IBMCP], Valencia, Spain) (Tables S2, S3, S4, S5, S6 and S7). Moreover, in the case of WMV, one isolate for each of the different detected profiles (see phylogenetic analysis) was chosen to be fully sequenced. The full-length WMV genome was paired-end sequenced by the Sanger method using a set of 19 internal primers (Tables S4 and S8) that produced overlapping sequences of 300–900 bp. The PCRs were conducted as previously described. The quality of the obtained fragment sequences was manually checked using Chromas (Technelysium DNA Sequencing Software) and the consensus sequence of the complete genome of the WMV isolates was obtained using CAP3 sequence assembly program (https://doua.prabi.fr/software/cap3).

2.4 | Sequence analysis

The obtained sequences of the different viruses were aligned using MUSCLE, included in MEGA 11 (Tamura et al., 2021). Worldwide reference sequences of the same viruses were retrieved from GenBank (www.ncbi.nlm.nih.gov/genbank/) and included in the alignments and phylogenetic analysis. MODELTEST, included in MEGA 11, was used to calculate the best model of multiple substitutions. Maximum-likelihood trees were constructed with MEGA 11, using the previously selected substitution model and 500 bootstrap replicates. Clusters were defined based on the structure of the constructed trees. 'Profiles' were defined as the combination of groups for the different genetic fragments, as described by Desbiez et al. (2020).

The detection of potential recombination sites within the complete WMV sequences was performed with RDP4 (Martin et al., 2015). The program was used with the default settings and a Bonferroni corrected p value ≤0.01. Different analytical methods were used: RDP, GENECONV, Chimaera, MaxChi, BOOTSCAN, 3Seq and SISCAN. Only those recombination events detected by four or more methods were considered as significant. For WMV isolates, pairwise identities of nucleotide and translated amino acid sequences were determined using MEGA 11 (Tamura et al., 2021). Furthermore, DNAsp 6 (Rozas et al., 2017) was used to calculate the dN/dS ratios (ω), which compares the synonymous substitution rates (dS)—assumed to be neutral—with the nonsynonymous substitution rates (dN), which are exposed to selection as they change the amino acid composition of a protein.

3 | RESULTS

3.1 | Virus occurrence in Comunidad Valenciana

Among the 121 symptomatic samples collected under conventional farming conditions in Comunidad Valenciana during the summer season of 2019, 76 of them (62.8%) were infected by, at least, one of the viruses identified in this area (WMV, MWMV, CMV, CABYV)

TABLE 1 Proportion of samples infected by the studied viruses in 2019 and 2020 in all the monitored areas under conventional farming conditions.

Year	Region	Host	Infection rate		WMV	CMV	CABYV	ToLCNDV	MWMV
			No. positive/ no. samples	%					
2019	C. Valenciana	Melon	9/18	50.0	7	2	2	0	1
		Watermelon	26/42	61.9	19	14	8	0	0
		Squash	37/54	68.5	34	8	11	0	2
		Zucchini	4/7	57.1	0	2	3	0	0
		Total	76/121	62.8	60	26	24	0	3
	Andalucía	Melon	17/19	89.5	12	13	13	1	0
		Watermelon	2/4	50.0	2	0	2	0	0
		Squash	1/1	100	1	0	1	0	0
		Zucchini	2/2	100	2	2	2	0	0
		Total	22/26	84.6	17	15	18	1	0
	Murcia	Melon	11/12	91.7	3	2	8	1	0
		Watermelon	2/8	25.0	1	1	0	0	0
		Total	13/20	65.0	4	3	8	1	0
	Castilla la Mancha	Melon	3/3	100	3	2	3	2	0
		Total	3/3	100	3	2	3	2	0
2020	C. Valenciana	Melon	41/42	99.2	41	28	29	0	0
		Watermelon	12/14	85.7	10	9	6	0	0
		Squash	62/65	95.4	52	47	42	0	1
		Total	115/121	95.0	103	84	77	0	1
	Andalucía	Melon	5/6	83.3	5	2	2	0	0
		Zucchini	2/2	100	2	1	0	0	0
		Total	7/8	97.9	7	3	2	0	0
	Extremadura	Melon	2/2	100	2	0	1	0	0
		Watermelon	1/1	100	0	0	1	0	0
		Total	3/3	100	2	0	2	0	0
	Castilla la Mancha	Melon	3/3	100	0	3	3	0	0
		Total	3/3	100	0	3	3	0	0

Abbreviations: CMV, cucumber mosaic virus, CABYV, cucurbit aphid-borne yellows virus, MWMV, Moroccan watermelon mosaic virus; ToLCNDV, tomato leaf curl New Delhi virus; WMV, watermelon mosaic virus.

(Table 1; Figures 1a and S1a). The tested viruses were most common in symptomatic squash plants compared to symptomatic plants of other crops with 68.5% of infected plants, followed by watermelon (61.9%) and zucchini (57.1%), whereas only 50.0% of the melon samples were diagnosed as infected (Table 1; Figure 1a). In 2020, 115 of the 121 tested samples (95.0%) were infected (Table 1; Figures 1b and S1b). The percentage of infected melon and squash samples was similar (99.2% and 95.4%, respectively), while it was lower among watermelons (85.7%; Table 1).

WMV was the most prevalent virus, followed by CMV and CABYV. Only three and one samples were diagnosed as infected by MWMV in 2019 and 2020, respectively, while ZYMV, CGMMV, ToLCNDV, CYSDV and CCYV were not detected in the monitored areas (Table 1; Figure 1). Mixed infections were common, representing 24.8% and 79.3% of the total samples in 2019 and 2020, respectively. Mixed infections of WMV and CABYV or WMV and CMV were frequently observed (Figure 1). Triple infection by WMV, CABYV and CMV represented 4.1% of cases during 2019, while in 2020 it increased to 79.3%. Only one squash sample was diagnosed with the four detected viruses (WMV, CABYV, CMV and MWMV; Figure 1). When infection over time was studied at Museros (Province of Valencia in Comunidad Valenciana), it was noticed that single infections were more common during mid-July and the proportion of mixed infections increased over time (Figure S2). WMV was the most prevalent virus in all the studied crops in early and mid-July, maintaining its relative importance over time, while CABYV, CMV and MWMV mostly appeared in mixed infections with WMV in late July (Figure S2).

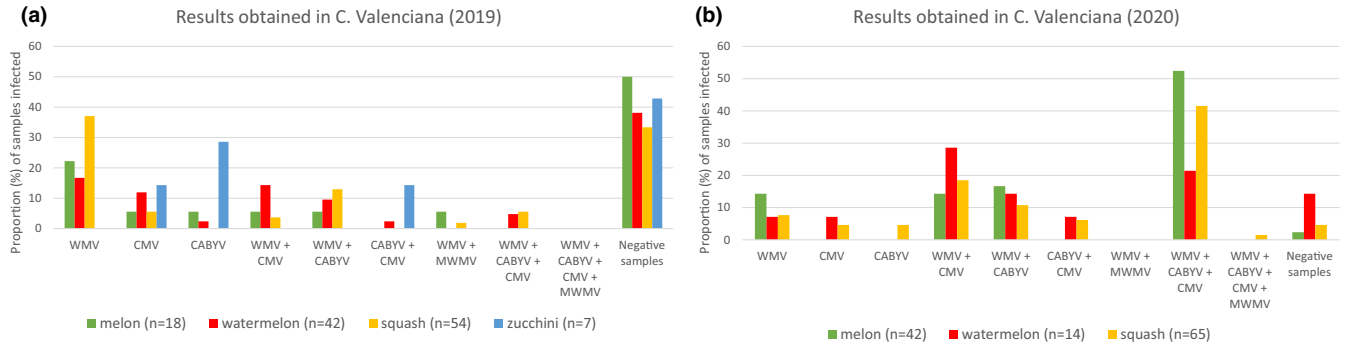


FIGURE 1 Detailed results of viral infections detected in Comunidad Valenciana during 2019 (a) and 2020 (b), indicating the proportion of single and mixed infections observed per crop. The number of samples tested is indicated for each crop. CMV, cucumber mosaic virus; CABYV, cucurbit aphid-borne yellows virus; MWMV, Moroccan watermelon mosaic virus; WMV, watermelon mosaic virus.

TABLE 2 Proportion of positive samples for each of the viruses detected in the organic melon farming field at different dates.

Date	Infection rate		WMV	CABYV	ToLCNDV	CMV	MWMV
	No. positive/no. samples	%					
June	104/105	99.0	104/105	15/105	11/105	12/105	5/105
Early july	94/94	100	94/94	40/94	47/94	4/94	0/94
Mid july	16/16	100	16/16	12/16	12/16	0/16	0/16
Early august	104/108	96.3	99/108	69/108	44/108	27/108	0/108
Total	318/323	98.5	313/323	136/323	114/323	44/323	5/323

Abbreviations: CABYV, cucurbit aphid-borne yellows virus; CMV, cucumber mosaic virus; MWMV, Moroccan watermelon mosaic virus; ToLCNDV, tomato leaf curl New Delhi virus; WMV, watermelon mosaic virus.

3.2 | Virus occurrence in other areas

In order to determine the genetic variability of the detected viruses in different geographical regions, surveys were also carried out in other major producing areas. Hence, during 2019–2020, 26 and 3 symptomatic cucurbit samples were collected from open fields in Murcia, Andalucía and Castilla la Mancha, respectively. In these areas CABYV was the most prevalent virus, followed by WMV and CMV. No samples were infected by MWMV, ZYMV, CGMMV, CYSDV or CCYV, while one melon sample in Andalucía, one in Murcia and two melons from Castilla la Mancha were diagnosed as infected by ToLCNDV (Table 1). Mixed infections were also common in these areas. During 2020, eight, three and three symptomatic samples were collected from Andalucía, Extremadura and Castilla la Mancha, respectively (Table 1). Once again, WMV, CMV and CABYV were the prevalent viruses, although CMV was not detected in Extremadura nor WMV in Castilla la Mancha, probably due to the low number of collected samples.

3.3 | Virus occurrence under organic farming conditions

A melon field located in La Punta (Province of Valencia in Comunidad Valenciana) and cultivated under organic farming conditions was monitored on four different dates during the 2019 summer season. Whiteflies and aphids were frequently observed in the surveys. While acknowledging that our study was conducted solely in a single organic field, it serves as an initial exploration to assess the incidence of different viruses in organic fields compared to conventional ones. In the studied field, 98.5% of the collected symptomatic samples were diagnosed as infected by, at least, one of the tested viruses. WMV was the most prevalent virus (96.6% of the samples were infected by WMV), followed by CABYV, ToLCNDV and CMV. Only five samples were infected by MWMV, while CYSDV, CCYV, CGMMV and ZYMV were not detected in the survey (Table 2; Figure 2).

In late June, most samples were infected only by WMV (67.6%), but at later dates the proportion of mixed infections increased. In early July, infections only by WMV were the most common (27.7%), followed by mixed infections by WMV and ToLCNDV (26.6%), WMV and CABYV (21.3%) or triple infection caused by WMV, CABYV and ToLCNDV (20.2%). In mid-July, the proportion of samples infected only by WMV decreased to 18.75% while the rate of mixed infections caused by WMV and ToLCNDV or WMV, ToLCNDV and CABYV both increased to 37.5%. In early August, a lower rate of plants infected only by WMV was found (11.1%), while mixed infections by WMV and CABYV, and WMV, CABYV and ToLCNDV (31.5 and 16.7%, respectively) remained predominant. At this date, CMV gained importance, as 25% of the samples were infected with this virus, appearing especially in mixed infections. Surprisingly, it was observed that the

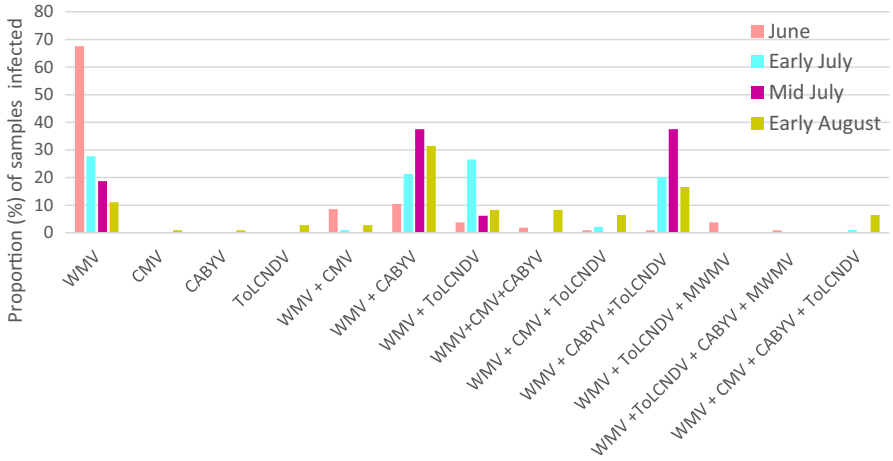


FIGURE 2 Temporal evolution of single and mixed infections during the summer season of 2019 in an organic melon field located in Comunidad Valenciana. CMV, cucumber mosaic virus; CABYV, cucurbit aphid-borne yellows virus; ToLCNDV, tomato leaf curl New Delhi virus; MWMV, Moroccan watermelon mosaic virus; WMV, watermelon mosaic virus.

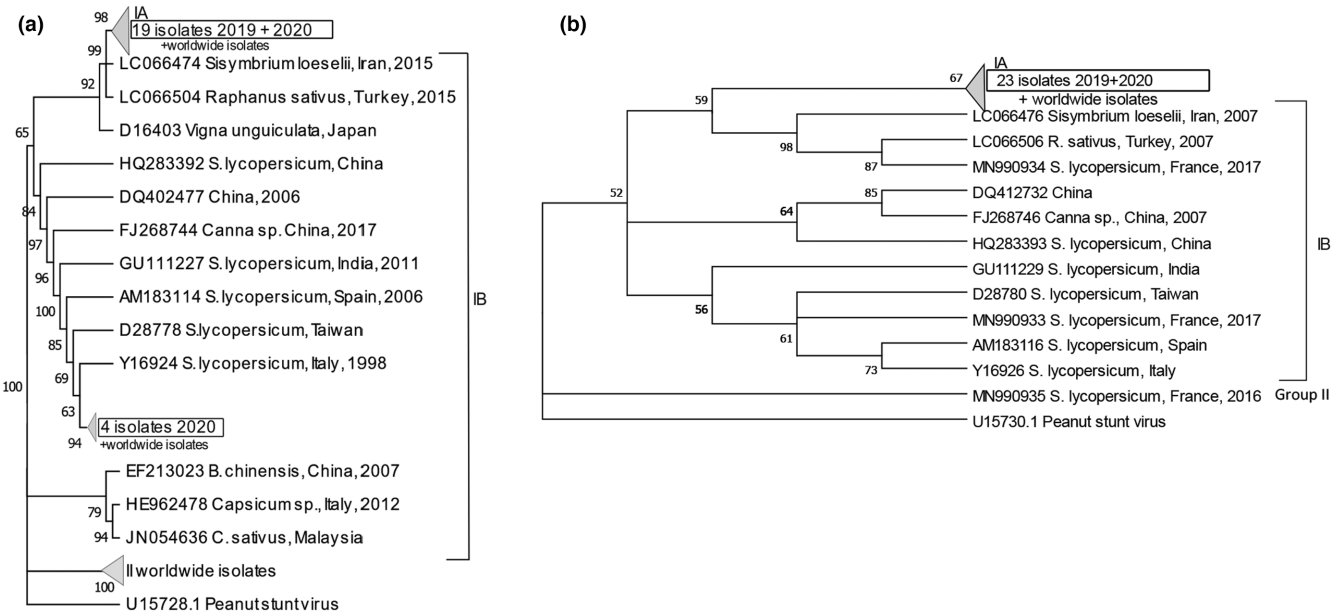


FIGURE 3 Maximum-likelihood trees obtained for cucumber mosaic virus (CMV) RNA1 (a) and RNA3 (b) partial sequences. Bootstrap values ($n = 500$ bootstrap replicates) above 50% are indicated for each node. For RNA2 the same result was obtained as for RNA1. Sequences corresponding to isolates from the survey are boxed. Peanut stunt virus was used as an outgroup. The isolates sequenced in this work included within each cluster are reported in Table S3. The worldwide isolates used to construct the tree are reported in Table S9.

percentage of plants infected by WMV, CMV, CABYV and ToLCNDV was maintained or even increased throughout the study period. In contrast, the presence of MWMV was only detected in the month of June. Further sampling in organic fields is warranted to facilitate a more robust comparison with conventional fields.

3.4 | Molecular diversity of viruses in crops

3.4.1 | CMV

To study the molecular diversity of CMV, 376, 446 and 434 or 451 bp long partial sequences of the three RNAs that comprise the genome of this virus were obtained from 23 samples that represented all the crops and locations monitored (Table S3). Phylogenetic analyses were performed including isolates retrieved from GenBank belonging to the three main CMV groups that have been described: IA, IB and II. None of the PCR products for viral isolates was classified within Group II and 19 of the sequenced CMV isolates belonged to Group IA (Figure 3; Table S3). Moreover, four samples (one watermelon and three melons) collected in Museros (Valencia, Comunidad Valenciana) and Alhaurín el Grande (Málaga, Andalucía) in 2020 were infected with a reassorting isolate. For RNA1 and RNA2, these isolates clustered within Group IB, whereas for RNA3, they grouped within Group IA (Figure 3a,b; Table S3).

3.4.2 | WMV

The molecular diversity of WMV was analysed by sequencing the P3-CI (452 bp) and NIb-CP (450 or 456 bp) coding regions of 21 and

17 isolates in 2019 and 2020, respectively (Table S4). Phylogenetic analyses were conducted including sequences retrieved from GenBank of the 'classic' (CL) and 'emerging' (EM 1–4 and Profiles 5–11) isolates of the WMV groups that had previously been established (Desbiez et al., 2020). This study revealed a high molecular diversity among the sequenced isolates, as they were classified within nine different profiles (combinations of P3-CI and NIb-CP groups based on the phylogenetic tree structure; Figure 4; Table S4). None of the partially amplified genomes were catalogued within the 'classic' group and only two isolates collected in Murcia and one isolate collected in the province of Badajoz (Extremadura) belonged to one of the 11 profiles previously described by Desbiez et al. (2020). The WMV PCR products amplified from samples collected in Murcia were clearly classified within the EM4 group and the isolate from Extremadura belonged to Profile 10 (Table S4). Thereby, seven new profiles (A–G) were established for the first time.

When the geographical origin of the new profiles was studied, a certain degree of co-localization of the isolates clustered within the same profile was observed (Figure S3; Table S4). Profiles A, C and D were only detected in Comunidad Valenciana: all the viral isolates sequenced from samples collected in Museros in 2019 grouped within Profile D, while those collected in the same place but in 2020 were distributed between Profiles A and D; WMV isolates obtained from samples collected in Benicarló (Castellón, Comunidad Valenciana) in 2019 were classified as Profile A, while Profile C was only detected within the samples collected in Gandía (Valencia, Comunidad Valenciana) in 2019. In the south, all the isolates sequenced from the province of Málaga (Andalucía), except one, were classified as Profile E, and the remaining isolate, collected in 2020, was classified as Profile G. Finally, only one isolate collected in Castilla la Mancha in 2019 was classified as Profile B.

As apparent discrepancies between P3-CI and NIb-CP clustering suggested recombination events, the complete WMV genome sequence of one isolate of each profile was obtained to study the possible recombination sites, as well as the nucleotide and amino acid identity between isolates (Table S4). Isolates EM190179, EM190155, EM190221, EM190185, EM190182 and EM190275 were further identified as putative recombinants (Table S10). Two breakpoints within the CI coding region (nucleotide positions 4281–5637) were identified for isolates EM190221 and EM190182, suggesting isolate EM190275 as the major parent and isolate FBR04-37 (sequence EU660586) as the minor parent. On the other hand, two breakpoints were detected between HC-Pro and P3 genes (nucleotide positions 1831–3775) in isolates EM190185 and EM190275, suggesting isolate FBR04-37 as the major parent and isolate CHI87-620 (sequence EU660580) as the minor parent. Two additional breakpoints were detected in isolate EM190179 within the P1-HC-Pro region (nucleotide positions 1137–2027), suggesting isolate FBR04-37 as major

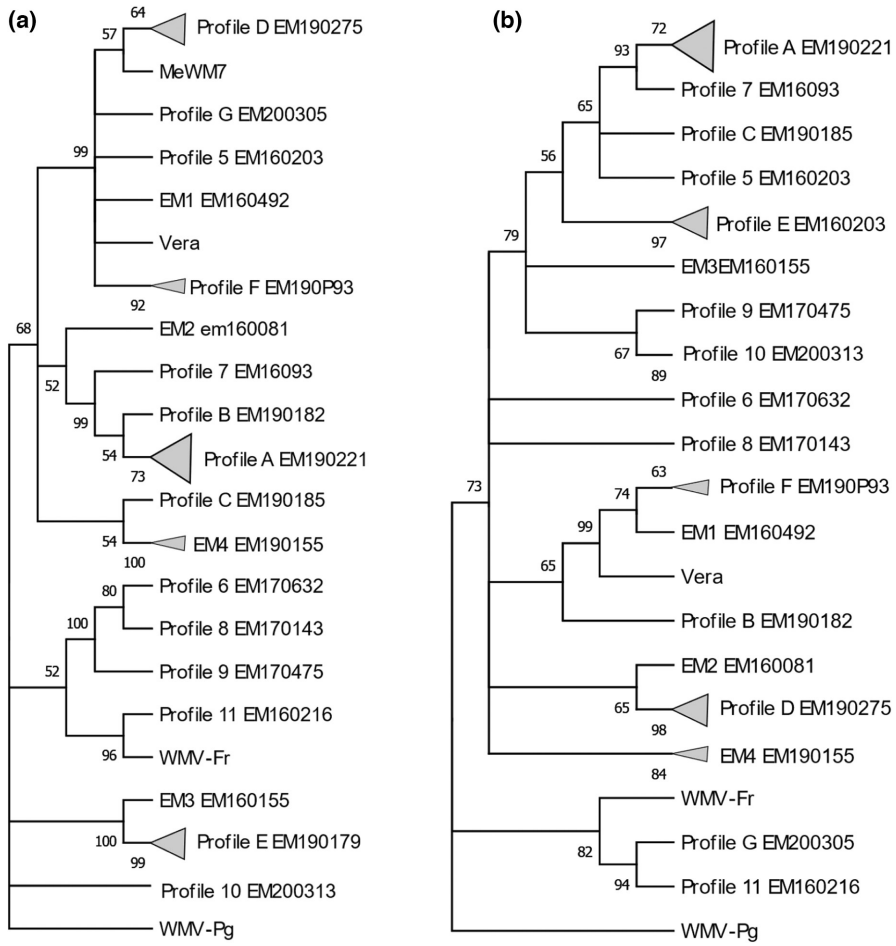
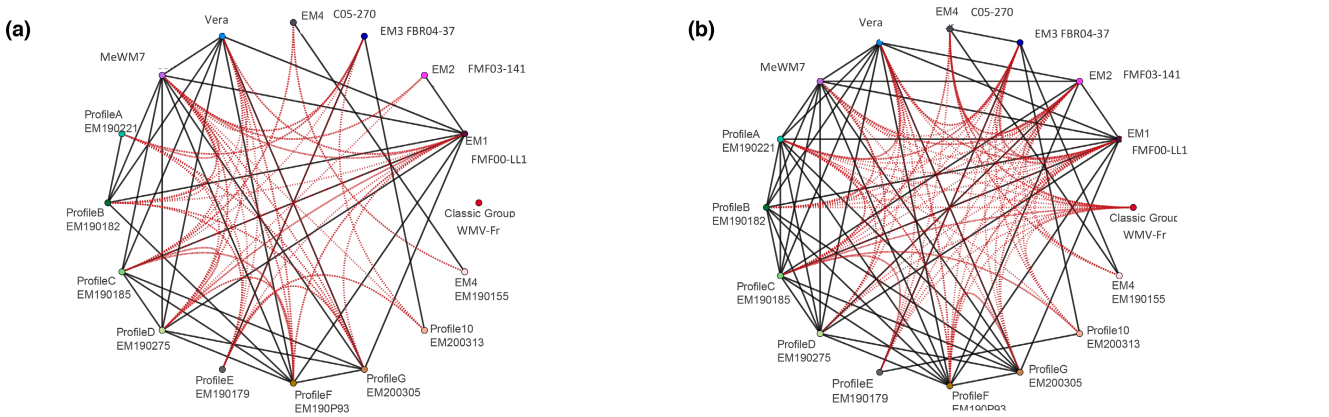


FIGURE 4 Maximum-likelihood trees obtained for partial nucleotide sequences of the watermelon mosaic virus (WMV) P3-CI (a) and NIb-CP (b) gene regions. Bootstrap values ($n = 500$ bootstrap replicates) above 50% are indicated for each node. The highly divergent sequence of isolate WMV-Pg was used as an outgroup. Profile names (A–G) and the name of the reference isolate for each profile, as defined in Table S4, are indicated for each branch tip. The isolates included within each profile are also indicated in Table S4.

parent and isolate EM190182 as the minor parent. The two last breakpoints were detected in isolate EM190155, within the HC-Pro coding region (nucleotide positions 1950–2344), suggesting isolate FBR04-37 as the major parent and isolate FMF03-141 (sequence EU660583) as the minor parent. The 5′ end of the HC-Pro coding region and the 3′ end of the CI coding region had been described as recombination hotspots in previous studies (Desbiez et al., 2020). Pairwise comparisons for the complete genome sequence of the analysed profiles were carried out (Figure 5a,b and S4). Sequences from isolates FMF00-LL1 (Group EM1), FMF03-141 (Group EM2), FBR04-37 (Group EM3), C05-270 (Group EM4) and WMV-Fr ('classic' group) (sequences EU660581, EU660583, EU660586, EU660585 and AY437609, respectively) and from the WMV Spanish isolates most recently described, Vera and MeWM7 (sequences MH469650 and MW147356, respectively), were also included in the analysis. The nucleotide sequence identity ranged from 89.6% to 99.5%, whereas the amino acidic sequence identity was between 94.1% and 99.7% (Figure S4), which is consistent with the observed low dN/dS ratios (ω) (Figure 5c). The highest identity, at both nucleotide and amino acid levels, was between some of the isolates infecting samples collected in Comunidad Valenciana (Profiles C, D and F) and Andalucía (Profile G), and these sequences were also highly similar to Vera and MeWM7 isolates, and to the isolate FMF00-LL1, which belongs to the EM1 group (Figures 5a,b and S4). Isolates of Profile B, which were found infecting samples collected in Castilla la Mancha, also showed similar identity ratios. Considering isolates of Profile A, which were detected in plants collected in both Benicarló and Museros, a lower identity level was observed with the previously named isolates (Profiles C, D, F, G and B) (Figures 5a,b and S4). On the other hand, the genome sequence of Profile E, collected in Málaga, and Profile 10, collected in Badajoz, showed a high similarity between them and with EM3 sequences (Figure 5a,b). Isolates of EM4 and Profile 10 were the most divergent at both nucleotide and amino acid levels, showing, on average, higher nonsynonymous substitutions rates (Figures 5c and S4). However, it should be considered that these analyses have been conducted using only one isolate per group or profile. Hence, slight differences could be obtained when analysing a greater variability within each cluster. In any case, these results constitute an initial approximation to better understand the variability between profiles.

The position within the sequences in which the amino acid changes were taking place was further analysed. It was observed that most of the amino acid sequence differences detected between the sequenced isolates were located within the P1, HC-Pro and P3 gene regions and a lower number of differences were also detected at the CI and CP gene regions (Figure S5). This accumulation of differences within the P1 sequences had already been observed when the complete sequence of the Spanish isolates MeWM7 and Vera



(c)

Location			C. Valenciana	Castilla la Mancha	C. Valenciana	C. Valenciana	Andalucía	C. Valenciana	Andalucía	Extremadura	Región de Murcia	C. Valenciana (2016)	Región de Murcia (2019)	France (2000)	France (2003)	France (2004)	France (2005)
	Profile	Isolate	Profile A EM190221	Profile B EM190182	Profile C EM190185	Profile D EM190275	Profile E EM190179	Profile F EM190P93	Profile G EM200305	Profile 10 EM200313	EM4 EM190155	Vera	MeWM7	EM1 FMF00-LL1	FMF03-141	FBR04-37	C05-270
C. Valenciana	Profile A	EM190221															
Castilla la Mancha	Profile B	EM190182	0.06														
C. Valenciana	Profile C	EM190185	0.04	0.04													
C. Valenciana	Profile D	EM190275	0.05	0.04	0.03												
Andalucía	Profile E	EM190179	0.06	0.05	0.05	0.05											
C. Valenciana	Profile F	EM190P93	0.04	0.04	0.02	0.04	0.05										
Andalucía	Profile G	EM200305	0.04	0.04	0.03	0.05	0.05	0.04									
Extremadura	Profile 10	EM200313	0.05	0.05	0.05	0.05	0.05	0.05	0.05								
Región de Murcia	EM4	EM190155	0.05	0.05	0.06	0.06	0.04	0.06	0.06	0.04							
C. Valenciana (2016)		Vera	0.04	0.04	0.02	0.04	0.05	0.04	0.04	0.05	0,057						
Región de Murcia (2019)		MeWM7	0.05	0.04	0.03	0.1	0.05	0.04	0.05	0.05	0.06	0.04					
France (2000)	EM1	FMF00-LL1	0.05	0.04	0.03	0.05	0.05	0,070	0.04	0.05	0.06	0.09	0.05				
France (2003)	EM2	FMF03-141	0.05	0.05	0.05	0.06	0.05	0.05	0.05	0.05	0.05	0.06	0.06	0.06			
France (2004)	EM3	FBR04-37	0.05	0.05	0.04	0.05	0.05	0.05	0.04	0.05	0.04	0.04	0.05	0.05	0.04		
France (2005)	EM4	C05-270	0.05	0.05	0.06	0.06	0.04	0.06	0.06	0.04	0.06	0.06	0.06	0.06	0.06	0.03	
France	Classic Group	WMV-Fr	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.04	0.05

FIGURE 5 Pairwise identities found for the different watermelon mosaic virus (WMV) profiles sequenced. Reference isolates were also included in the analysis. (a) Proportion (%) of identity between nucleotide sequences: black lines indicate a global identity >95% and red lines show a local 100% identity between regions >500 bp. (b) Proportion (%) of identity between amino acid sequences: black lines indicate a global identity >97% and red lines show a local 100% identity between local sequence regions >500 amino acids. (c) Pairwise comparisons of the dN/dS ratio (nonsynonymous substitution rates:synonymous substitution rates). The origin of the reference isolates is indicated, as well as the profile they are clustered in. EM 1–4: 'emerging' groups 1–4.

were studied (De Moya-Ruiz et al., 2021). The dN/dS ratio was then studied separately for each gene of the WMV genome. No signs of positive selection were observed in any of the genes. However, the ratios obtained for the P1 gene were the highest (ranging from 0.11 to 0.60). When the amino acid sequences were compared, it was observed that most of the differences not previously described in the newly sequenced Spanish isolates (Vera and MeWM7) or in 'classic' isolates (WMV-Fr) were already present in the sequences of the 'emerging' isolates (groups EM1–EM4) previously detected in France (FMF00-LL1, FBR04-37, C05-270 and FMF03-141). However, several amino acid sequence differences with respect to the Spanish, the 'classic' and 'emerging' isolates were detected for the first time (Table 3). Furthermore, based on the BLOSUM 62 matrix scores, a significant number of these recently identified amino acid modifications were categorized as non-conservative. The BLOSUM matrix scores quantify the likelihood (log-odds) of one amino acid being replaced by another within a collection of protein sequence alignments. Negative BLOSUM scores correspond to amino acid pairs with a low likelihood of substitution, while a positive score denotes commonly observed amino acid pairs.

3.4.3 | CABYV

Partial sequences of CABYV CP (423 bp) and RdRp (419 bp) genes were obtained for 17 samples in 2019 and 10 samples in 2020 (Table S5). The variability for the sequenced region of the RdRp gene (maximum sequence divergence 9.3%) was much higher than for the sequenced region of the CP gene (up to 4.0% divergence). Compared to the worldwide diversity, the phylogenetic analysis showed that all the partially sequenced isolates belonged to the 'European-African' clade and they were highly similar to isolates previously described in Spain (Rabadán et al., 2021) and France (Desbiez et al., 2020). Four molecular clusters were defined for the sequenced region of the RdRp gene and two clusters for the sequenced region of the CP gene, but with a low bootstrap support (data not shown). The same problem had been observed in previous works (Desbiez et al., 2020).

3.4.4 | Other viruses

The other two viruses observed in the survey, MWMV and ToLCNDV, showed a low prevalence and partial CP gene sequences (638 and 505 bp, respectively) were obtained for four and five isolates, respectively (Tables S6 and S7). Regarding the four MWMV isolates, the identity between the sequences was approximately 99% and they shared >98.0% of identity with isolates previously detected in Spain (ZuM10 and ESP; sequences MW161172 and EF579944, respectively). Consequently, they were clustered within the 'Mediterranean' clade in the phylogenetic tree (Figure S6a). Regarding the ToLCNDV isolates, all of them clustered within the ToLCNDV-ES clade (Figure S6b). The isolates that belong to the ToLCNDV-ES strain have been present in the Mediterranean area since it was detected in Spain in 2012 (Juárez et al., 2014). The sequence identity between the isolates was >99.5% and it was also >99.5% when comparing the studied sequences with the most recently described Spanish isolates ES-Alm-Mel-16, ES-Alm-Zuc-16 and ES-Alm-Cuc-16 (sequences LC596381, LC596382 and LC596380, respectively).

4 | DISCUSSION

In order to characterize the principal viruses affecting cucurbit crops in Spain, extensive surveys in south-eastern regions and other important areas for cultivation of cucurbits were conducted in 2019 and 2020. The data presented here shows that aphid-transmitted viruses were more established than those transmitted by whiteflies and that the newly discovered viruses had not yet spread. WMV and CABYV were the most prevalent viruses in all the crops and monitored areas regardless of the farming system. Both viruses were found in single and mixed infections, with WMV appearing in single infections earlier in the growing season. These results are consistent with those observed in melon and squash fields in the same regions in 2003–2006 (Juárez et al., 2013; Kassem et al., 2007) and also in melon, watermelon and squash fields monitored in other areas in 2018–2020 (De Moya-Ruiz et al., 2021; Maachi et al., 2022; Rabadán et al., 2021). This same trend was also observed in other Mediterranean countries (Desbiez et al., 2020). Nevertheless, several differences concerning the other studied viruses were observed. It was remarkable that while CMV was present only at a low incidence or not even detected in other surveys (De Moya-Ruiz et al., 2021), it was the third most important virus in our surveys, affecting 26.0% and 66.6% of the tested symptomatic plants grown in conventional farming fields in 2019 and 2020, respectively. Regarding the studied organic farming field, 13.6% of the symptomatic plants were infected with CMV in 2019. This situation contrasted with the results obtained in relation to ZYMV and MWMV, the other two aphid-transmitted viruses studied. On the one hand, ZYMV was not detected in this work, which is consistent with the low infection rates observed in previous studies (De Moya-Ruiz et al., 2021). On the other hand, MWMV was detected in a low number of samples in both organic and conventional farming fields. This virus was detected in Spain for the first time in 2012, but it has not become established until now. This trend had also been observed in other areas of Spanish and different Mediterranean countries (De Moya-Ruiz et al., 2021; Desbiez et al., 2020). It seems that mixed infections might be the key to explain the different infection rates observed. It has been proven that the combination of a potyvirus and a non-related virus has a synergistic effect on the unrelated virus, as it can take advantage of the RNA silencing suppression function of HC-Pro of the potyvirus (Valli et al., 2018). In mixed infections with CMV--WMV and CMV--ZYMV, the viral load of the cucumovirus and its long-distance movement in the plant were shown to increase (Mochizuki et al., 2016). In contrast, the combination

TABLE 3 New amino acid sequence differences within each protein from the complete genomes of the different watermelon mosaic virus (WMV) profiles sequenced.

Protein	Profile A[a] (EM190221)	Profile B (EM190182)	Profile C (EM190185)
P1	V113I[b], H131Q*, E135K, K261L***, S278F***, K298R, D307N, T331M**, K386R, S410A, E437K	N274S, T275A, T279I**, F285L, S292L***, I438V	
HC-Pro	Q226K	T77I**	
P3	K217R, N293M***	I81V, A207V, N293M***	V149L, K281R, P332S**
6K1	L19M	L19M	
CI	N484S, T557I**	N484G, T557I**	D225E, G249_del[c]***, R250_del[c]***, P486S**
6K2			
NIa-VPg			
NIa-Pro			
NIb	M400I	V31I, M400T**	
CP		Q92P**, K113R, V114T*	

[a] Amino acid sequence differences represented are between each of the profiles sequenced in this work and reference isolates from the 'classic' (sequence AY437609) and 'emerging' groups (EM1–4; sequences EU660581, EU660583, EU660586, EU660585), as well as in other Spanish isolates previously sequenced (sequences MH469650 and MW147356). Only changes different from those previously described in the reference isolates are presented.

[b] The sequence difference is expressed including the most frequent amino acid previously described for each of the positions; that is, in the change V113I for PI, the most frequent amino acid in the reference isolates used in the comparison was V, and I was first reported in the isolates sequenced.

[c] Deletion of one or several amino acids.

* Amino acid changes punctuated as 0 as a function of the BLOSUM 62 matrix. ** Amino acid changes punctuated as −1 as a function of the BLOSUM 62 matrix. *** Amino acid changes punctuated as −2 as a function of the BLOSUM 62 matrix.

of two potyviruses seems to be neutral or beneficial for one virus with an antagonistic effect on the other virus. In mixed infections between ZYMV and WMV, the latter virus accumulated to significantly lower levels (Salvaudon et al., 2013). However, WMV could benefit from the increased vector traffic for its own transmission (Salvaudon et al., 2013). This differed from mixed infections with WMV and MWMV in zucchini, where the titre of WMV appeared to be steady while MWMV was antagonized (De Moya-Ruiz et al., 2021). Moreover, MWMV is principally transmitted by *Myzus persicae* and to a lesser extent by *Aphis gossypii*, which is more likely to be found in cucurbits fields. These data, in addition to the fact that MWMV is not able to overwinter in weeds (Desbiez et al., 2020), would explain the low incidence of this potyvirus.

CGMMV was not detected in the monitored fields. The absence of this virus could be explained by two factors: its narrow host range is limited to cucurbits, and the fact that it is not transmitted by vectors, such as aphids or whiteflies. CGMMV is a seed-, soil- and mechanical-transmitted virus, which explains its higher incidence in crops grown under greenhouse conditions, where pruning and other cultural practices facilitate its propagation.

Regarding whitefly-transmitted viruses, their situation in cucurbit fields had not recently been studied in south-eastern Spain. The crinivirus CYSDV, which was prevalent in 2003–2004 in southern Spain (Kassem et al., 2007), was no longer detected. This contrasts with recent results obtained in Greece and Cyprus, where high infection rates were observed in both open field and greenhouses (Orfanidou et al., 2019). The emerging virus CCYV, detected in three independent cucumber greenhouses in southern Spain for the first time in 2018 and in one melon field in Murcia in 2020 (Chynoweth et al., 2021; Maachi et al., 2022), was also not detected in our surveys. Finally, regarding the geminivirus ToLCNDV, which has rapidly spread in different Spanish and Mediterranean regions since 2012 (Juárez et al., 2019; Maachi et al., 2022), a low incidence (2.4%) was detected in conventional farming fields, whereas its relative importance increased in a melon field grown under organic farming conditions (33.9%). As the studied organic field was in the same region as some of the monitored conventional fields, these significant differences might be due to the lack of efficient treatments against *B. tabaci* under organic farming conditions. A general low prevalence of whitefly-transmitted viruses has also been observed in conventional fields monitored in France (Desbiez et al., 2020). However, these results might be affected by the fact that no greenhouses were monitored during the surveys, as it is known that whitefly populations cause major damage under those conditions. Nevertheless, a high prevalence of ToLCNDV (79.7% of infected plants) has already been observed

Profile D (EM190275)	Profile E (EM190179)	Profile F (EM190P93)	Profile G (EM200305)	EM4 (EM190155)	Profile 10 (EM200313)
R286K, V349I, H364Y	V18A, P179S**, A153I, V154A, K157_del[c]***, S179N, I216V, N274D, A320V	A203V	K336R	H119Y	E93K, A153I**, V154A, S171F***, I216V, K241E, K261E, V302I, K361R, A387P**
T398I**					L76A**, R90Q, k100R
	F223I*	A230V	N133H		M36L, K217R, E317D, T338A
	I8M, S23N, R366K, L502_A517_del***			P361S**	I49V, I69T, G239S, K446N*, I471L
	K177R			Y119H	R90K
M400I		V94I	S145G*, K279R		R359K
G36D**, Q92P**, V114T*, M279K**	N30S	P28A**	G2K***, K3Q, D16E, G23D**, D26N, T33V*		

in melon fields located in Murcia, Castilla la Mancha and Alicante (Juárez et al., 2019), which might indicate that this virus could become established in Valencia and other important producing areas over the next few years.

Additionally, the molecular diversity of the detected viruses was analysed to study possible population changes. The phylogenetic analysis of CABYV, MWMV and ToLCNDV isolates showed that they clustered within their corresponding Mediterranean group, which is consistent with former studies (De Moya-Ruiz et al., 2021; Rabadán et al., 2021). On the other hand, WMV and CMV reflected a different picture, pointing out a dynamic status. Regarding the tripartite cucumovirus, CMV, sequence comparisons revealed that isolates of Group II were not present in the studied fields and that isolates of Group IA were predominant. This was not surprising, because isolates of Group II, which has a lower thermal optimum compared with Subgroup I, had only been found in Spain in pepper and tomato fields in northern areas (Fraile et al., 1997), whereas isolates of Group IA had previously been described as prevalent in Spanish fields (Fraile et al., 1997). Moreover, reassorting isolates IB-IB-IA were found infecting watermelon and melon in Comunidad Valenciana and Andalucía. Traditionally, cucurbit crops have always been infected by isolates classified within IA and II groups, or even with reassorting isolates (IA-II-II), whereas IB isolates, which are more prevalent in Asia (Jacquemond, 2012), were found infecting plants of other families. Nevertheless, in recent years different isolates whose CP genes were classified within Group IB (Ahsan et al., 2020; Xanthis et al., 2015), or even isolates with the three RNA segments that make up the virus genome clustered within that group (IB-IB-IB) (Nagendran et al., 2018), have been found infecting cucurbits in Bulgaria, Greece, Mexico, Pakistan and India. However, these kinds of isolates have not been detected infecting cucurbits in other surveys recently carried out in Europe (Desbiez et al., 2020; Valachas et al., 2021). The types of reassorting isolates (IB-IB-IA) described in this work had previously been detected infecting a zucchini plant in eastern Spain in 1995 (Bonnet et al., 2005). However, they were not detected again in subsequent studies. Other studies revealed that reassorting isolates were usually found in a low proportion and they did not become established in the population, due to selection against reassortment between IA and IB isolates (Fraile et al., 1997). Taking this into account, further surveys should be made to determine if isolates of the IB-IB-IA reassortment will be able to establish in this region, if it spreads to new areas and whether it is more or less virulent than previously detected isolates.

For WMV, none of the sequenced isolates clustered within the 'classic' group. The 'classic' isolates were already being displaced in Spain in 2005–2006 (Juárez et al., 2013) and they have now been

completely replaced by the EM groups in other Mediterranean countries (Bertin et al., 2020; Desbiez et al., 2020). The sequencing of both NIb-CP and P3-CI gene regions revealed a high molecular diversity among the WMV isolates, establishing seven new profiles. The sequencing of the complete genome for every detected profile showed that recombination was frequent among the isolates. Moreover, despite the fact that nonsynonymous mutations were not favoured by selection, which is consistent with previous studies (Rabadán et al., 2023), some new mutations were described for the first time in Spanish isolates, specially within the P1 protein coding sequence. This accumulation of changes within the P1 sequence had already been observed in the isolate MeWM7 when compared to isolate Vera (De Moya-Ruiz et al., 2021) and the highest dN/dS ratio was observed within this gene. P1, the first protein of the polyprotein, is the most divergent protein among potyviruses with regard to both length and amino acid sequence. Most of the observed amino acid changes between the sequenced isolates were detected in the N-terminal region of the P1 protein, whereas the C-terminal domain seems to be more conserved. Within this protein, the C-terminal sequence has a well-conserved serine protease domain and the N-terminal region is hypervariable, acting as a repressor of the protease activity. This seems to be an evolutionary step to keep viral amplification below levels detrimental to the host, to maintain a higher long-term replicative capacity (Pasin et al., 2014). Nevertheless, as it has been proposed that P1 protein could strengthen the ability of HC-Pro to suppress RNA silencing and enhance the pathogenicity of heterologous plant viruses (Valli et al., 2018), it will be necessary to further observe if the detected sequence differences could lead to an increase in symptom severity.

This work shows that, even though the new emerging viruses have not yet spread through the Spanish Mediterranean basin, virus populations can rapidly evolve as a result of mutations and recombination/reassortment events. These evolutionary mechanisms, in addition to long-distance introductions due to plant material exchanges and changing climate conditions, can lead to severe virus epidemics. In this sense, organic farming fields are especially vulnerable because there is a lack of effective treatments against viral vectors. Moreover, these new virus genotypes could overcome the previously described genetic resistances, as previously reported for different viruses (Desbiez et al., 2021; Giner et al., 2017). Integrated pest control, good agronomic practices, as well as the development of resistant cultivars will be necessary to improve the phytosanitary status of cucurbits fields, ensuring stable and high-quality production.

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CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article

ORCID

Ana Pérez-de-Castro https://orcid.org/0000-0002-4949-3323

REFERENCES

Ahsan, M., Ashfaq, M., Mukhtar, T. & Abbasi, N.A. (2020) Current status and genetic variability of cucumber mosaic cucumovirus (CMV) isolates infecting major cucurbits and solanaceous vegetables in Pothwar region of Pakistan. *Pakistan Journal of Agricultural Sciences*, 57, 1353–1361.

Bertin, S., Manglli, A., McLeish, M. & Tomassoli, L. (2020) Genetic variability of watermelon mosaic virus isolates infecting cucurbit crops in Italy. *Archives of Virology*, 165, 937–946.

Bonnet, J., Fraile, A., Sacristán, S., Malpica, J.M. & García-Arenal, F. (2005) Role of recombination in the evolution of natural populations of cucumber mosaic virus, a tripartite RNA plant virus. *Virology*, 332, 359–368.

Chynoweth, R., Jimenez, D., Liberti, D., Bellon-Dona, D., Carralero, A., Crespo, A. et al. (2021) First report of cucurbit chlorotic yellows virus infecting cucumber plants in Spain. *Plant Disease*, 105, 2258.

Crespo, O., Janssen, D., García, C. & Ruiz, L. (2017) Biological and molecular diversity of cucumber green mottle mosaic virus in Spain. *Plant Disease*, 101, 977–984.

De Moya-Ruiz, C., Rabadán, P., Juárez, M. & Gómez, P. (2021) Assessment of the current status of potyviruses in watermelon and pumpkin crops in Spain: epidemiological impact of cultivated plants and mixed infections. *Plants*, 10, 138.

Desbiez, C., Wipf-Scheibel, C., Millot, P., Berthier, K., Girardot, G., Gognalons, P. et al. (2020) Distribution and evolution of the major viruses infecting cucurbitaceous and solanaceous crops in the French Mediterranean area. *Virus Research*, 286, 198042.

Desbiez, C., Wipf-Scheibel, C., Millot, P., Girardot, G. & Lecoq, H. (2021) Molecular epidemiology of cucurbit-infecting potyviruses: a rapid turnover of viral strains with a potential impact for resistance breeding. In: Gómez-Guillamón, M.L., Jamilena, M., Picó, M.B. & Pérez-de-Castro, A.M. (Eds.) *XIIth Eucarpia meeting on cucurbit genetics and breeding, 24–28 May 2021*. Spain: Almería, p. 69.

Fraile, A., Alonso-Prados, J.L., Aranda, M.A., Bernal, J.J., Malpica, J.M. & García-Arenal, F. (1997) Genetic exchange by recombination or reassortment is infrequent in natural populations of a tripartite RNA plant virus. *Journal of Virology*, 71, 934–940.

Giner, A., Pascual, L., Bourgeois, M., Gyetvai, G., Rios, P., Picó, B. et al. (2017) A mutation in the melon vacuolar protein sorting 41 prevents systemic infection of cucumber mosaic virus. *Scientific Reports*, 7, 10471.

Jacquemond, M. (2012) Cucumber mosaic virus. *Advances in Virus Research*, 84, 439–504.

Juárez, M., Legua, P., Mengual, C.M., Kassem, M.A., Sempere, R.N., Gómez, P. et al. (2013) Relative incidence, spatial distribution and genetic diversity of cucurbit viruses in eastern Spain. *Annals of Applied Biology*, 162, 362–370.

Juárez, M., Rabadán, M.P., Díaz-Martínez, L., Tayahi, M., Grande-Pérez, A. & Gómez, P. (2019) Natural hosts and genetic diversity of the emerging tomato leaf curl New Delhi virus in Spain. *Frontiers in Microbiology*, 10, 140.

Juárez, M., Tovar, R., Fiallo-Olivé, E., Aranda, M.A., Gosálvez, B., Castillo, P. et al. (2014) First detection of tomato leaf curl New Delhi virus infecting zucchini in Spain. *Plant Disease*, 98, 857.

Kassem, M.A., Sempere, R.N., Juárez, M., Aranda, M.A. & Truniger, V. (2007) Cucurbit aphid-borne yellows virus is prevalent in field-grown cucurbit crops of southeastern Spain. *Plant Disease*, 91, 232–238.

Kavalappara, S.R., Milner, H., Konakalla, N.C., Morgan, K., Sparks, A.N., McGregor, C. et al. (2021) High throughput sequencing-aided survey reveals widespread mixed infections of whitefly-transmitted viruses in cucurbits in Georgia, USA. *Viruses*, 13, 988.

Lecoq, H. & Desbiez, C. (2012) Viruses of cucurbit crops in the Mediterranean region. An ever-changing picture. *Advances in Virus Research*, 84, 67–126.

Maachi, A., Donaire, L., Hernando, Y. & Aranda, M.A. (2022) Genetic differentiation and migration fluxes of viruses from melon crops and crop edge weeds. *Journal of Virology*, 96, e00421–e00422.

Martin, D.P., Murrell, B., Golden, M., Khoosal, A. & Muhire, B. (2015) RDP4: detection and analysis of recombination patterns in virus genomes. *Virus Evolution*, 1, vev003.

Mochizuki, T., Nobuhara, S., Nishimura, M., Ryang, B.S., Naoe, M., Matsumoto, T. et al. (2016) The entry of cucumber mosaic virus into cucumber xylem is facilitated by co-infection with zucchini yellow mosaic virus. *Archives of Virology*, 161, 2683–2692.

Mondal, S., Hladky, L.J. & Wintermantel, W.M. (2023) Differential seasonal prevalence of yellowing viruses infecting melon crops in southern California and Arizona determined by multiplex RT-PCR and RT-qPCR. *Plant Disease*, 107, 2653–2664.

Nagendran, K., Priyanka, R., Aravintharaj, R., Balaji, C.G., Prashant, S., Basavaraj, B. et al. (2018) Characterization of cucumber mosaic virus infecting snake gourd and bottle gourd in India. *Physiological and Molecular Plant Pathology*, 103, 102–106.

Orfanidou, C., Maliogka, V.I. & Katis, N.I. (2014) First report of cucurbit chlorotic yellows virus in cucumber, melon, and watermelon in Greece. *Plant Disease*, 98, 1446.

Orfanidou, C.G., Papayiannis, L.C., Pappi, P.G., Katis, N.I. & Maliogka, V.I. (2019) Criniviruses associated with cucurbit yellows disease in Greece and Cyprus: an ever-changing scene. *Plant Pathology*, 68, 764–774.

Pasin, F., Simón-Mateo, C. & García, J.A. (2014) The hypervariable amino-terminus of P1 protease modulates potyviral replication and host defense responses. *PLoS Pathogens*, 10, e1003985.

Rabadán, M.P., Juárez, M., De Moya-Ruiz, C. & Gómez, P. (2021) Aphid-borne viruses infecting cultivated watermelon and squash in Spain: characterization of a variant of cucurbit aphid-borne yellows virus (CABYV). *Plant Pathology*, 70, 1476–1485.

Rabadán, M.P., Juarez, M. & Gomez, P. (2023) Long-term monitoring of aphid-transmitted viruses in melon and zucchini crops: genetic diversity and population structure of cucurbit aphid-borne yellows virus and watermelon mosaic virus. *Phytopathology*, 113, 1761–1772.

Rozas, J., Ferrer-Mata, A., Sanchez-DelBarrio, J.C., Guirao-Rico, S., Librado, P., Ramos-Onsins, S.E. et al. (2017) DnaSP 6: DNA sequence polymorphism analysis of large data sets. *Molecular Biology and Evolution*, 34, 3299–3302.

Sáez, C., Ambrosio, L.G.M., Miguel, S.M., Valcárcel, J.V., Díez, M.J., Picó, B. et al. (2021) Resistant sources and genetic control of resistance to ToLCNDV in cucumber. *Microorganisms*, 9, 913.

Salvaudon, L., de Moraes, C.M. & Mescher, M.C. (2013) Outcomes of co-infection by two potyviruses: implications for the evolution of manipulative strategies. *Proceedings of the Royal Society B: Biological Sciences*, 280, 2012295.

Tamura, K., Stecher, G. & Kumar, S. (2021) MEGA11: molecular evolutionary genetics analysis version 11. *Molecular Biology and Evolution*, 38, 3022–3027.

Valachas, C.A., Giantsis, I.A., Sareli, K., Winter, S., Zelezniakof, E., Pentheroudaki, Z. et al. (2021) Molecular analysis of Greek isolates of cucumber mosaic virus from vegetables shows a low prevalence of satellite RNAs and suggests the presence of host-associated virus strains. *Archives of Virology*, 166, 2199–2208.

Valli, A.A., Gallo, A., Rodamilans, B., López-Moya, J.J. & García, J.A. (2018) The HCPro from the *Potyviridae* family: an enviable multitasking helper component that every virus would like to have. *Molecular Plant Pathology*, 19, 744–763.

Xanthis, C., Maliogka, V.I., Lecoq, H., Desbiez, C., Tsvetkov, I. & Katis, N.I. (2015) First report of cucumber mosaic virus infecting watermelon in Greece and Bulgaria. *Journal of Plant Pathology*, 97, 399.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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