# Outbreak of cucumber mosaic virus subgroup IB in pepper from the Espelette area (Basque Country, southwestern France) and first report of five taxa as natural hosts of CMV

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**Outbreak of cucumber mosaic virus subgroup IB in pepper from the Espelette area (Basque Country, southwestern France) and first report of five taxa as natural hosts of CMV**

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Short title: Outbreak of CMV in the Espelette area

Keywords: emergence, cucumovirus, reservoir, viral plant disease, weed

## Abstract

To better understand the emergence of cucumber mosaic virus (CMV) in the protected designation of origin of Espelette pepper (southwestern France), more than 7,300 samples were collected in and around 36 pepper fields in 2021 and 2022, and diagnosed using ELISA, RT-PCR and partial Sanger sequencing of viral RNAs. This allowed the identification of five new host genera or species among the natural hosts of CMV: *Arum italicum*, *Cerastium glomeratum*, *Hyacinthoides* sp., *Lysimachia arvensis* and *Trifolium incarnatum*. A CMV variant belonging to subgroup IB and presenting a low molecular diversity was highly prevalent in the pepper crops (78% of the pepper samples) as well as in naturally growing plants (8% of the non-pepper samples) within the fields. CMV isolates from group II were detected in a single pepper plant as well as in *Hyacinthoides* sp. (3 samples), *Capsella bursa-pastoris* (2 samples) and *Stachys arvensis* (1 sample). To our knowledge, this is the second report of the occurrence of subgroup IB of CMV in France. Investigation of old pepper samples indicate that it was present at least since 2009.

## Main text

Cucumber mosaic virus (CMV, genus *Cucumovirus*, family *Bromoviridae*) is frequent in crops and has a host range of over 1,000 species (Hirsch and Moury 2021). On cultivated hosts such as pepper, CMV can trigger severe leaf symptoms like mosaic, vein-clearing, mottling, filiformism and necrosis. It also considerably reduces both the quantity and quality of fruits (Avilla et al. 1997). CMV has a tripartite positive-sense RNA genome, the three fragments being encapsidated separately in identical icosahedral particles. CMV isolates belong to two main molecular groups, namely groups I and II. Within group I, two subgroups have been described based on coat protein sequences, namely IA and IB. In complete genome sequences, the IA-IB distinction remains relevant for RNAs 2 and 3 but is less clear-cut for RNA1, where subgroup IB does not appear monophyletic (Hirsch and Moury 2021; Jacquemond 2012). Subgroup IA and group II are present worldwide, although group II is mostly restricted to cooler areas or temperate regions. Subgroup

IB is of Asian origin and several introductions to other parts of the world have taken place, such as California, Brazil, Africa, Australia (Hirsch and Moury 2021), and Italy (Gallitelli 2000). Reassortants between the different groups and subgroups have also been described (Ohshima et al. 2016). CMV is transmitted by more than 80 species of aphids in a non-persistent non-circulative manner, and, for some host species, by the seed of infected plants (Hirsch and Moury 2021; Pagán 2019).

In the past, CMV had little impact on pepper crops in the Espelette protected designation of origin (PDO) area. This crop represents more than 5 million plants each year in a 300 ha zone of the French Basque Country (southwestern France), and is famous for three types of products: whole fresh (non-transformed) Espelette peppers, fresh or dried cords of Espelette peppers (strings composed of 20 to 100 fruits), and powders of dried Espelette peppers. However, CMV epidemics have recently gained momentum and severity, inducing yield and quality losses. Despite the considerable damage caused by CMV, no effective control method has yet been developed.

To characterise this outbreak and possible natural reservoirs of CMV, cultivated (pepper) and wild plants were sampled in and around 36 pepper plots in the PDO area. Leaves of 2684 pepper plants (*Capsicum annuum*) and 4653 other plants from 241 species, 164 genera and 58 families were collected in March (before pepper planting) and September (during the cropping season) over two consecutive years (2021-2022). Three environments were monitored: pepper plots (5365 samples), edges (1555 samples of wild plants naturally growing close to pepper plots), and the natural neighborhood up to 100 m away from the plot (417 samples). Most pepper plants (74% in 2021 and 49% in 2022) as well as a few wild plants (2.5% of total collected samples both years) showed mosaics and leaf deformations (**Fig. 1**), but the majority of wild plants were asymptomatic. All samples were tested by double-antibody sandwich enzyme-linked immunosorbent assays (DAS-ELISA), using antisera provided by J. C. Devergne (INRA, Antibes) and directed against the D isolate of CMV (Devergne and Cardin 1973). Although isolate D belongs to subgroup IA, these antisera are able to detect isolates from all groups of CMV. The DAS-ELISA revealed the presence of CMV in pepper (2088 positive/2684 tested; 77.8%) and in 37 other species (or genera when the species taxon could not be identified; 393 positive/4653 tested; 8.4%), mostly within the area of pepper plots (i.e. pepper plots and their edges) (detailed results are presented in **Supplementary Table S1**). This was confirmed for a subset of samples by Reverse-Transcription Polymerase Chain Reaction (RT-PCR, 368 tested samples), and partial Sanger (in 2021, 163 samples) or high-throughput (in 2022, 473 samples) genetic sequencing of viral RNAs 1, 2 and 3 (**Table 1**). Primers for viral fragment sequencing as well as the strategy and procedures for Illumina sequencing of amplified fragments were as described in (Desbiez et al. 2020). Briefly, the primers target a fragment of the replicase gene in RNA 1 (376 nt), of the RNA-dependent RNA polymerase gene in RNA 2 (446 nt) and of the coat protein gene and 3' non coding region in RNA 3 (436 or 451 nt depending on the CMV group) (**Supplementary Fig. S1**). These primers allow amplification of isolates belonging to all groups of CMV (Desbiez et al. 2020). After extraction of total RNAs from frozen samples with TRI-reagent (Molecular Research Center Inc., Cincinnati, OH) according to the manufacturer's instructions, the targeted regions were amplified via a two-step protocol of RT-PCR. Most of the 37 species or genera infected were already known as natural hosts of CMV, except five: *Lysimachia arvensis* (8 positive/21 tested), *Hyacinthoides* sp. (*H. hispanica* and/or *H. non-scripta* and/or their hybrid *H. x massartiana*; 3/11), *Arum* spp. (*A. italicum* and/or *A. maculatum* and/or their hybrid; 8/273), *Cerastium glomeratum* (2/43) and *Trifolium incarnatum* (12/123). Distinguishing the three species among the genus

*Arum* was often impossible (since the hybrid may present characteristics of its two parents), but three of the eight infected plants could formally be identified as *Arum italicum* (via expertise and a determination key: presence of white veins and absence of brown macula on the limb of well-developed leaves). Similarly, the three species among the genus *Hyacinthoides* that are present in the Basque Country can only be distinguished based on morphological differences on flower parts, but these were not available at the time of sampling.

These findings extend the natural host range of CMV, noting that *Arum italicum*, *Cerastium glomeratum* and *Trifolium incarnatum* were already known to be experimental hosts (Douine et al. 1979; Edwardson and Christie 1997; Palukaitis and García-Arenal 2019). Viral isolates from pepper and the five new host species or genera (at least one isolate per species) were mechanically inoculated to *Nicotiana benthamiana* and *N. tabacum* cv. Xanthi at the 2-4 leaf stage. The plants expressed mosaic symptoms (without noticeable difference between isolates) three weeks after inoculation under greenhouse conditions and CMV infection was confirmed by DAS-ELISA.

Isolate ES223617 from pepper was sent to DSMZ (Braunschweig, Germany) for high-throughput sequencing with Illumina NextSeq 2000, 2x150 paired-end reads. The full-length genome was assembled (28,675,068 trimmed reads; 16,941,696 viral reads; average coverage 1966 reads/base), yielding 3 fragments of 3357 nt, 3046 nt and 2212 nt corresponding to CMV RNAs 1 to 3, respectively (GenBank accession numbers OR355463, OR355464, OR355465). No other virus was detected in the sample. Isolate ES223617 was closely related (97.5-99% nucleotide identity, depending on the segment) to pepper isolate R1 from Rwanda (accessions MG470798-MG470800) and isolates WF-Ch (PP083007-PP083009) and CH99 (MW926531-MW926533) from China, all belonging to subgroup IB (**Fig. 2**). Isolate ES223617 clustered unambiguously with subgroup IB, whatever the RNA segment (**Fig. 2**).

Partial sequences were obtained for 252 isolates from *C. annuum* and 384 isolates from other species collected in 2021 and 2022 (**Table 1**). Even though Sanger sequences of RNA3 were often of low quality (**Table 1**), most isolates were identical or closely related to isolate ES223617 (1 to 3 differences in the sequenced fragments of RNAs 1 and 2, up to 4 differences in the more variable RNA 3). In contrast, the three isolates from *Hyacinthoides* sp. (including isolate ES210111, GenBank accessions OR490418, OR490419 and OR490420 for RNAs 1-3), two isolates from *Capsella bursa-pastoris*, a single one from *Stachys arvensis* and a single one from pepper had only 73 % nucleotide identity with ES223617 in the sequenced regions but shared 99.1 to 99.4% identity between themselves and with isolate R from France (accessions HE793685, HE793686, Y18138) belonging to group II of CMV (Jacquemond 2012). There was no evidence for the presence of subgroup IA isolates, nor of IA-IB or I-II reassortants in the Espelette samples. In order to confirm the taxonomic status of isolate ES210111, it was sent to DSMZ for Illumina 2x150 paired-end sequencing. The full-length genome was assembled (51,723,560 trimmed reads, 722,154 reads matching with CMV), yielding three RNAs of 3391 nt, 3039 nt and 2209 nt corresponding to RNAs 1 to 3, respectively (Genbank accessions PP731930-PP721932). These three RNAs present 98.8%, 99.1% and 97.5% identity, respectively, with isolate R. Therefore, the full-genome sequence data confirm unambiguously that isolate ES210111 from *Hyacinthoides* sp. belongs to CMV group II (**Fig. 2**). No satellite RNA was detected in the Illumina sequences of ES223617 (subgroup IB) and ES210111 (group II).

To our knowledge, this is the first description of *Arum italicum*, *Cerastium glomeratum*, *Hyacinthoides sp.*, *Lysimachia arvensis* and *Trifolium incarnatum* as natural hosts of CMV. Since no seed transmission of CMV was detected in the local pepper cultivar (Hirsch et al. 2023), further analyses will investigate the epidemiological role of these new hosts. This is also the second report of the occurrence of CMV subgroup IB in France and the first one in the Southwest. Indeed, the large majority of CMV isolates characterized so far in France, as well as in many European countries, belong to subgroup IA (Desbiez et al. 2020; Hirsch and Moury 2021; Jacquemond 2012; López-Martín et al. 2024; Sacristán et al. 2004), whereas group II is occasionally observed in crops and weeds and does not cause severe epidemics (Desbiez et al. 2020). Subgroup IB isolates were observed on tomato and pepper in one farm in Southeastern France in 2017, and a IA-IA-IB reassortant was detected in 2017 in tomato in another field about 20 km away (Desbiez et al. 2020), but these different IB sequences are genetically distinct from the Espelette variant (**Fig. 3**), indicating a lack of direct spread between the different locations in France where CMV IB was described. In order to get insights into the early stages of CMV emergence in the PDO area of Espelette pepper, we collected old commercial products (29 powders and 1 cord produced by 30 different growers from 2009 to 2022) and evaluated the presence and phylogenetic group of CMV by RT-PCR and partial Sanger sequencing (exactly as described before for leaf samples). CMV was detected in all collected samples, indicating that the virus has been present at least since 2009. The sequences were of low quality but the isolate from 2009 (1 isolate) as well as all isolates from 2016 to 2022 belonged to CMV subgroup IB (0 or 1 nucleotide differences in a 248-nt fragment of RNA 1 with isolate ES223617), whereas those from years 2011, 2013 and 2014 belonged to CMV group II (2 to 4 differences with isolate ES210111 in a 248-nt fragment of RNA 1) (**Supplementary Table S2**).

These findings on the detection of new CMV host species and the characterisation of emerging CMV isolates belonging to subgroup IB are essential to better understand and control the current outbreak which threatens this renowned pepper crop. Further research focusing on the epidemiological role of these new hosts as well as the role of aphids in the spread of CMV subgroup IB will be crucial for devising effective control strategies. Additionally, our findings alert us to potential risks, should CMV subgroup IB spread beyond the Espelette area.

## Acknowledgments

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## Literature Cited:

Avilla, C., Collar, J. L., Duque, M., and Fereres, A. 1997. Yield of bell pepper (Capsicum annuum) inoculated with CMV and/or PVY at different time intervals / Ertrag von Paprika (Capsicum annuum) nach Inokulation mit CMV und/oder PVY in verschiedenen Zeitintervallen. J. Plant Dis. Prot. 104:1-8.

Desbiez, C., Wipf-Scheibel, C., Millot, P., Berthier, K., Girardot, G., Gognalons, P., Hirsch, J., Moury, B., Nozeran, K., Piry, S., Schoeny, A., and Verdin, E. 2020. Distribution and evolution of the major

viruses infecting cucurbitaceous and solanaceous crops in the French Mediterranean area. Virus Res. 286:198042.

Devergne, J.-C., and Cardin, L. 1973. Contribution to the study of cucumber mosaic virus (CMV). IV. A tentative classification of several

isolates on the basis of their antigenic structure. Ann. Phytopathol. 5:409-430.

Douine, L., Quiot, J. B., Marchoux, G., and Archange, P. 1979. Recensement des espèces végétales sensibles au virus de la mosaïque du concombre (CMV). Etude bibliographique. Ann. Phytopathol. 11:439-475.

Edwardson, J. R., and Christie, R. G. 1997. Cucumoviruses. Pages 129-203, in: Viruses Infecting Peppers and Other Solanaceous Crops. Gainesville, University of Florida, USA.

Gallitelli, D. 2000. The ecology of *Cucumber mosaic virus* and sustainable agriculture. Virus Res. 71:9-21.

Hirsch, J., and Moury, B. 2021. Cucumber mosaic virus (*Bromoviridae*). Pages 371-382, in: Encyclopedia of Virology (Fourth Edition) D. H. Bamford, and M. Zuckerman, eds. Academic Press, Oxford.

Hirsch, J., Szadkowski, M., Wipf-Scheibel, C., Lepage, E., Dumeaux, P., Nguyen, E., Verdin, E., Moury, B., and Rimbaud, L. 2023. Absence of seed-mediated transmission of cucumber mosaic virus in Espelette pepper crops despite widespread and recurrent epidemics. Viruses 15:2159.

Jacquemond, M. 2012. Chapter 13 - Cucumber Mosaic Virus. Pages 439-504, in: Advances in Virus Research G. Loebenstein, and H. Lecoq, eds. Academic Press.

López-Martín, M., Sifres, A., Gómez-Guillamón, M. L., Picó, B., and Pérez-de-Castro, A. 2024. Incidence and genetic diversity of cucurbit viruses in the Spanish Mediterranean area. Plant Pathol. 73:431-443.

Ohshima, K., Matsumoto, K., Yasaka, R., Nishiyama, M., Soejima, K., Korkmaz, S., Ho, S. Y. W., Gibbs, A. J., and Takeshita, M. 2016. Temporal analysis of reassortment and molecular evolution of *Cucumber mosaic virus*: Extra clues from its segmented genome. Virology 487:188-197.

Pagán, I. 2019. Movement between plants: Vertical transmission. Pages 185-198, in: Cucumber Mosaic Virus P. Palukaitis, and F. García-Arenal, eds. The American Phytopathology Society.

Palukaitis, P., and García-Arenal, F. 2019. Host list of cucumber mosaic virus. Pages 287-367, in: Cucumber Mosaic Virus. The American Phytopathology Society.

Sacristán, S., Fraile, A., and García-Arenal, F. 2004. Population dynamics of *Cucumber mosaic virus* in melon Crops and in weeds in central Spain. Phytopathology 94:992-998.

Tepfer, M., Girardot, G., Fénéant, L., Ben Tamarzizt, H., Verdin, E., Moury, B., and Jacquemond, M. 2016. A genetically novel, narrow-host-range isolate of cucumber mosaic virus (CMV) from rosemary. Arch. Virol. 161:2013-7.

## Conflict of interest

All authors have approved the final version of this manuscript and declare they have no conflict of interest.

## Table

**Table 1**. Summary of genetic analyses of CMV isolates found in Espelette.

| Year of sample | Sequencing method | Number of samples (host species) | Number of obtained sequences | | |
|---|---|---|---|---|---|
| | | | **$RNA_1$** | **$RNA_2$** | **$RNA_3$** |
| 2021 | Partial (Sanger)[a] | 83 (pepper) | 77 | 79 | 20 |
| | | 80 (weeds[c]) | 75 | 64 | 63 |
| | Complete (DSMZ) | 1 (*Hyacinthoides* sp.) | 1 | 1 | 1 |
| 2022 | Partial (High Throughput Sequencing)[b] | 169 (pepper) | 178 | 245 | 161 |
| | | 304 (weeds[d]) | 364 | 347 | 326 |
| | Complete (DSMZ) | 1 (pepper) | 1 | 1 | 1 |

[a] all samples but 3 yielded a sequence for at least one RNA.

[b] note that high-throughput sequencing yields more than one sequence per sample and RNA.

[c] including 1 *Arum italicum*, 1 *Cerastium glomeratum*, 1 *Hyacinthoides* sp., 2 *Lysimachia arvensis*.

[d] including 1 *Arum italicum*, 2 *Hyacinthoides* sp., 6 *Lysimachia arvensis*, 13 *Trifolium incarnatum*.

## Figure captions

**Figure 1**. Mosaics and leaf deformations observed in pepper (A, *Capsicum annuum*), Cerastium (B, *Cerastium glomeratum*), bluebell (C, *Hyacinthoides* sp.), arum (D, *Arum italicum*) and clover (E & F, *Trifolium spp.*).

**Figure 2**. Maximum-likelihood tree of complete sequences of CMV RNA 1 (A), 2 (B) and 3 (C) representative of worldwide genetic diversity (Jacquemond 2012; Tepfer et al. 2016), with a GTR + G + I substitution model. Bootstrap values above 70% (n=500 bootstraps) are indicated for each node. The scale bar represents a genetic distance of 0.1. Red arrows indicate isolates from Espelette, belonging to subgroup IB and group II. Names of CMV isolates are preceded by accession numbers.

**Figure 3**. Maximum-likelihood tree of partial sequences of CMV RNA 1 (A), 2 (B) and 3 (C) representative of worldwide genetic diversity (Jacquemond 2012; Tepfer et al. 2016), with a GTR + G + I substitution model. Bootstrap values above 70% (n=500 bootstraps) are indicated for each node. The scale bar represents a genetic distance of 0.1. Red arrows indicate isolates from Espelette, belonging to subgroup IB and group II; blue stars indicate isolates found in Southeastern France during surveys performed in 2016-2017 (Desbiez et al. 2020). Names of CMV isolates are preceded by accession numbers.


Figure 1. Mosaics and leaf deformations observed in pepper (A, Capsicum annuum), Cerastium (B, Cerastium glomeratum), bluebell (C, Hyacinthoides sp.), arum (D, Arum italicum) and clover (E & F, Trifolium spp.).

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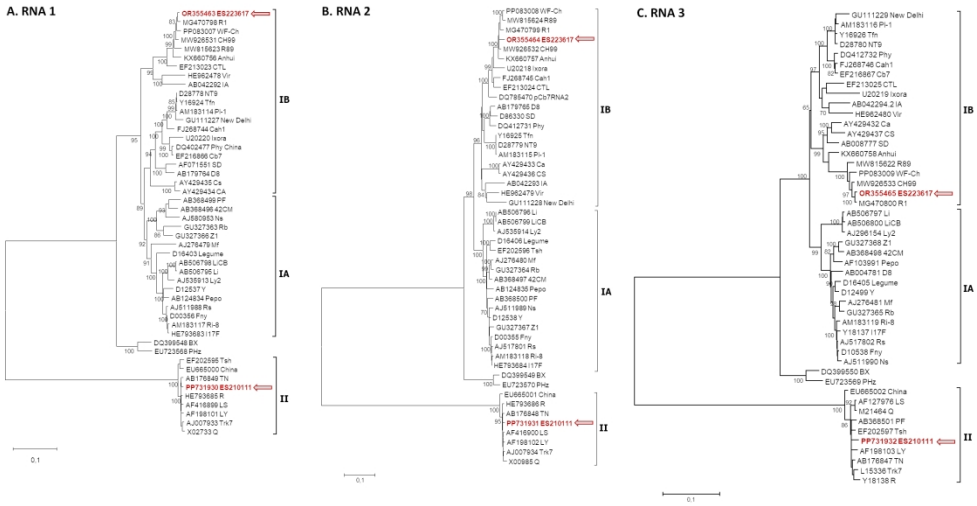


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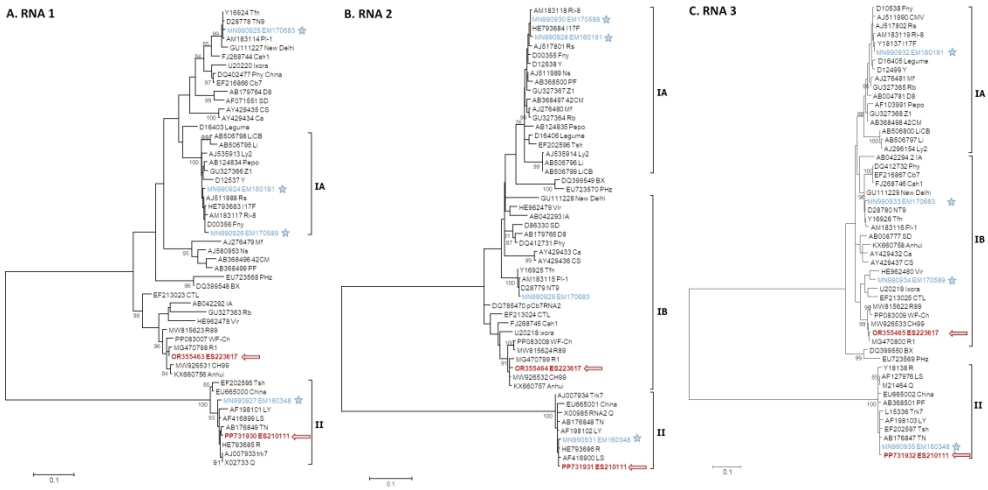
235x119mm (300 x 300 DPI)

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238x117mm (300 x 300 DPI)

# Supplementary material

**Outbreak of cucumber mosaic virus subgroup IB in pepper from the Espelette area (Basque Country, southwestern France) and first report of five taxa as natural hosts of CMV**

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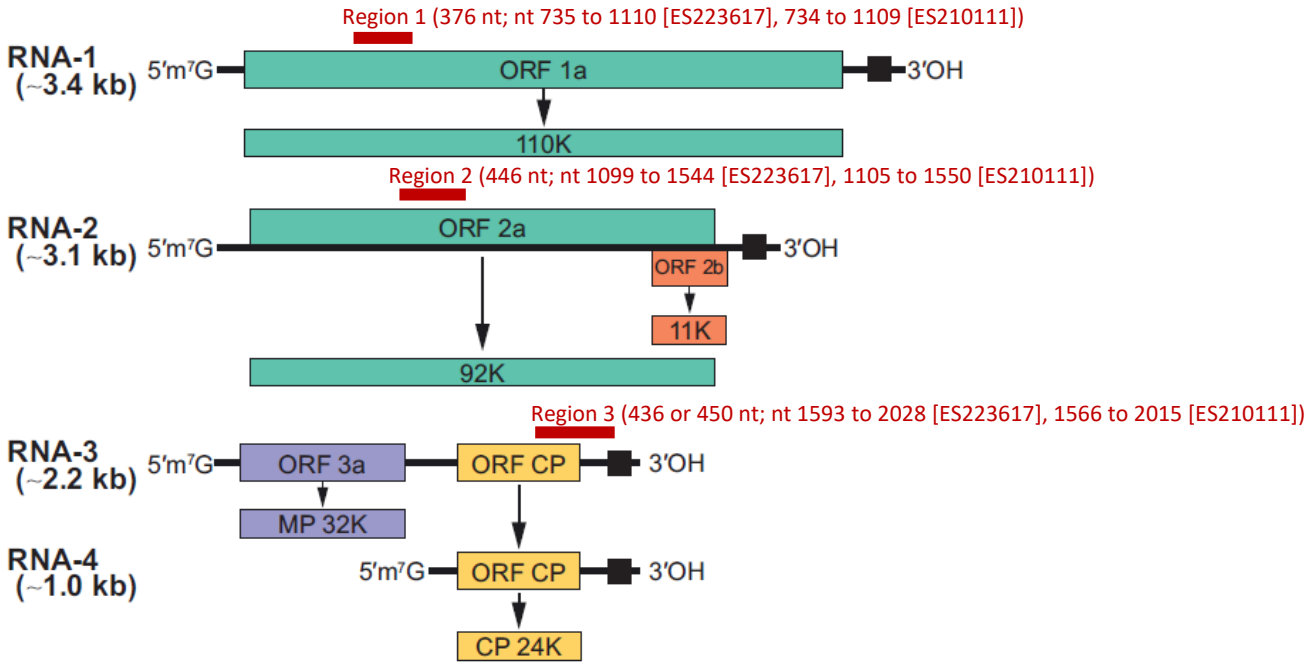


**Supplementary Figure S1**. Position of the 3 sequenced regions (red bars) in each RNA of the tripartite CMV genome. The representation of the Bromoviridae genome has been adapted from (ICTV 2011). Primer sequences are:

| Segment | Primer name | Primer sequence | Size of amplified region (nt) |
|---|---|---|---|
| RNA-1 | 1CMV703F | CGAYGGYGCKATGATGTTTGAC | 376 |
| RNA-1 | 1CMV1076R | AGAGGGGAACCARATRCAATG | |
| RNA-2 | 2CMV1063F | ACCGGGAGYGGTCACMAGAG | 446 |
| RNA-2 | 2CMV1510R | TCYCGAAGGCATCTCTGGAA | |
| RNA-3 | 3CMV1468F | CCTTTGCCGAAATTYGATTC | 436 or 450 |
| RNA-3 | 3CMV1889R | TGGAYGGACAACCCGTTC | |

**Supplementary Table S1**. Detailed numbers of plants tested with the different diagnosis techniques and found positive for CMV. All collected samples were tested by ELISA, whereas only a selected subset was tested by RT-PCR and genetic sequencing.

| Species | FINAL DIAGNOSIS | | DAS-ELISA | | | RT-PCR | | SEQUENCING | |
|---|---|---|---|---|---|---|---|---|---|
| | Positive | Total sampled | Doubtful | Positive | Tested | Positive | Tested | Positive | Tested |
| **All** | **2481** | **7337** | **213** | **2404** | **7336** | **244** | **368** | **587** | **636** |
| ***Capsicum annuum*** | **2088** | **2684** | **107** | **1992** | **2684** | **140** | **153** | **249** | **252** |
| Pepper plot | 2088 | 2684 | 107 | 1992 | 2684 | 140 | 153 | 249 | 252 |
| **Weeds (all)** | **393** | **4653** | **106** | **412** | **4652** | **104** | **215** | **338** | **384** |
| Pepper plot | 329 | 2681 | 69 | 327 | 2681 | 88 | 158 | 280 | 302 |
| Edge | 54 | 1555 | 31 | 76[a] | 1554 | 9 | 46 | 53 | 77 |
| Neighborhood | 10 | 417 | 6 | 9 | 417 | 7 | 11 | 5 | 5 |
| ***Arum italicum*** | **3** | **3** | | **3** | **3** | **1** | **1** | **2** | **2** |
| Pepper plot | 1 | 1 | | 1 | 1 | | 0 | | 0 |
| Edge | 1 | 1 | | 1 | 1 | | 0 | 1 | 1 |
| Neighborhood | 1 | 1 | | 1 | 1 | 1 | 1 | 1 | 1 |
| ***Arum maculatum*** | **0** | **1** | | **0** | **1** | | **0** | | **0** |
| Pepper plot | 0 | 1 | | 0 | 1 | | 0 | | 0 |
| ***Arum spp. (not identified)*** | **5** | **269** | | **5** | **269** | **3** | **3** | **4** | **4** |
| Pepper plot | 3 | 21 | | 3 | 21 | 2 | 2 | 2 | 2 |
| Edge | 2 | 237 | | 2 | 237 | 1 | 1 | 2 | 2 |
| Neighborhood | 0 | 11 | | 0 | 11 | | 0 | | 0 |
| ***Cerastium glomeratum*** | **2** | **43** | | **2** | **43** | **1** | **1** | **1** | **1** |
| Pepper plot | 2 | 30 | | 2 | 30 | 1 | 1 | 1 | 1 |
| Edge | 0 | 11 | | 0 | 11 | | 0 | | 0 |
| Neighborhood | 0 | 2 | | 0 | 2 | | 0 | | 0 |
| ***Hyacinthoides sp.*** | **3** | **11** | | **3** | **11** | **3** | **3** | **3** | **3** |
| Edge | 2 | 8 | | 2 | 8 | 2 | 2 | 2 | 2 |
| Neighborhood | 1 | 3 | | 1 | 3 | 1 | 1 | 1 | 1 |
| ***Lysimachia arvensis*** | **8** | **21** | **2** | **8** | **21** | **2** | **3** | **5** | **8** |
| Pepper plot | 8 | 17 | 2 | 8 | 17 | 2 | 3 | 5 | 8 |
| Edge | 0 | 4 | | 0 | 4 | | 0 | | 0 |
| ***Trifolium incarnatum*** | **12** | **123** | **3** | **15[a]** | **123** | **0** | **3** | **12** | **13** |
| Pepper plot | 10 | 89 | | 12 | 89 | | 0 | 10 | 10 |
| Edge | 2 | 33 | 3 | 2 | 33 | 0 | 3 | 2 | 3 |
| Neighborhood | 0 | 1 | | 1 | 1 | | 0 | | 0 |

[a] The final number of positive samples may be lower than the number of positive ELISA because of cross-reactions of antibodies with the peanut stunt virus (PSV, *Cucumovirus*) infecting some *Trifolium* samples. PSV and CMV are very close and cannot be distinguished morphologically, chemically or serologically (Palukaitis & García-Arenal 2003), the final diagnostic of *Trifolium* samples was thus based on genetic sequencing.

**Supplementary Table S2**. Detection of CMV and phylogenetic group in samples from old commercial products: Espelette pepper powders and dried cord of fruits.

| Year of harvest | Type of sample | Number of samples (positive/tested) | Phylogenetic group |
|---|---|---|---|
| 2009 | Powder | 1/1 | IB |
| 2011 | Powder | 1/1 | II |
| 2013 | Powder | 1/1 | II |
| 2014 | Powder | 1/1 | II |
| 2016 | Powder | 21/21 | IB |
| 2017 | Cord | 1/1 | IB |
| 2018 | Powder | 2/2 | IB |
| 2021 | Powder | 1/1 | IB |
| 2022 | Powder | 1/1 | IB |