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Additional Information

1 **First report of the US1 strain of *Pepino mosaic virus* in tomato in the Canary**
2 **Islands, Spain.**

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6
7 *Pepino mosaic virus* (PepMV), a member of the genus *Potexvirus*, was first described in
8 1974 on pepino (*Solanum muricatum* Ait.) in Peru. In 1999, PepMV was reported
9 affecting tomato (*Solanum lycopersicum* L.) (3), and currently, the virus is distributed
10 throughout many parts of the world causing economic losses in tomato crops. This virus
11 induces not only a high variability of symptoms on infected plants including distortion,
12 chlorosis, mosaic, blistering, and filiformity on leaves and marbling on fruits, but also
13 exhibits substantial genetic diversity. Five strains or genotypes of PepMV have been
14 described, including European tomato (EU), Peruvian (PE), Chilean 2 (CH2) and two
15 American strains, US1 (including CH1) and US2. No correlation has been found
16 between different genotypes and symptom expression of PepMV infection. Studies have
17 demonstrated that field populations of PepMV in Europe belong to EU and US2 or CH2
18 strains. Mixed infections between these strains and interstrain recombinant isolates are
19 also found (1,2). In Spain, PE strain was also described, but at lower relative frequency
20 than other strains (2). In February 2007, in the Canary Islands (Tenerife, Spain), a
21 PepMV isolate (PepMV-Can1) was collected showing the typical leaf symptoms of
22 blistering and mosaic. PepMV was first identified using double-antibody sandwich
23 enzyme-linked immunosorbent assay (DAS-ELISA) with specific antisera against
24 PepMV (DSMZ GMBH, Baunschweig, Germany) according to the manufacturer's
25 instructions. The serological identification was confirmed by reverse transcription-

1 polymerase chain reaction (RT-PCR) with two pairs of PepMV-specific primers
2 Pep3/Pep4 and CP-D/CP-R which amplify a fragment of the RNA dependent RNA
3 polymerase (RdRp) gene and the complete coat protein (CP) gene, respectively (2).
4 PCR products were purified and directly sequenced. The amplified RdRp fragment of
5 PepMV-Can1 (GenBank Accession No. EU791618) showed 82% nt identity with the
6 EU and PE strains (GenBank Accession Nos. AJ606360 and AM109896, respectively),
7 but more than 98% identity with the US2 and US1 strains (GenBank Accession Nos.
8 AY509927 and AY 509926, respectively). Sequence information obtained from the
9 amplified CP fragment (GenBank Accession No. EU797176) showed 99% nt identity
10 with US1 and less than 83% with EU, PE, CH2 (GenBank Accession No. DQ000985)
11 and US2. To confirm these results, specific primers for the triple gene block (TGB)
12 were designed using the sequence data from GenBank Accession Nos. AY509926,
13 AY509927, DQ000985, AJ606360 and AM109896. (PepTGB-D:5'
14 GATGAAGCTGAACAACATTC 3'and PepTGB-R: 5'
15 GGAGCTGTATTRGGATTTGA 3'). A 1437 bp fragment (GenBank Accession No.
16 EU797177) was obtained, sequenced and compared with the published sequences,
17 showing 98% nt identity with the US1 strain and less than 86% with the other strains of
18 PepMV. The highest sequence identity in all the studied regions of the PepMV-Can1
19 isolate was with the US1 strain of PepMV. To our knowledge, this is not only the first
20 report of an isolate of the US1 strain in the Canary Islands (Spain), but also the first
21 report of the presence of this genotype in a different location than its original report
22 (North America).

23 References: (1) I. Hanssen et al. Eur. J. Plant Pathol. 121 :131-146, 2008. (2) I. Pagán et
24 al. Phytopathology 96: 274-279, 2006. (3) R.A.R. Van der Vlugt et al. Plant Dis. 84 :
25 103, 2000.