New Disease Reports

First report of *Pepino mosaic virus* in tomato in Morocco

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Pepino mosaic virus (PepMV) is a member of the *Potexvirus* genus (Martelli *et al*., 2007) that infects a relatively broad host range of plants in the Solanaceae, particularly tomato (*Solanum lycopersicum*) and causes significant losses. The virus was first reported in Europe in 2000 (van der Vlugt *et al*., 2000). Subsequently, it has been identified in Canada and the United States (French *et al*., 2001) and has become widespread on greenhouse tomatoes in many countries. In Morocco the occurrence of PepMV is unrecorded as no surveys on its incidence have been performed until now. Notwithstanding this there are several interception reports of PepMV infection in exported Moroccan tomato fruit suggesting the presence of the virus in the production area (European and Mediterranean Plant Protection Organization, 2014).

Early in 2009, possible disease symptoms were observed on a number of tomato plants growing in greenhouses in the Souss region of Morocco. Plants exhibited a chlorotic mosaic on leaves, necrotic stems and typical fruit marbling (Fig. 1). Based on these observations viral infection was suspected. Seven tomato samples were tested for the presence of PepMV using a DAS-ELISA kit (Agdia, USA). The presence of the symptoms correlated with positive ELISA results. In 2014 five tomato fruits grown near the towns of El Jadida and Skhirat were sampled from markets in Rabat, three of which displayed symptoms typical of PepMV infection and two had non-typical symptoms. Extracts from all five tomatoes tested positive for PepMV infection following DAS-ELISA using monoclonal antibodies raised against a PepMV isolate (Souiri *et al*., 2013).

The presence of PepMV in samples from both surveys was confirmed by RT-PCR amplification. Total RNA was isolated from all diseased fruit and one healthy tomato using TRIzol® (Invitrogen, USA) according to the manufacturer's instructions and subjected to RT-PCR using a PepMV-specific primer pair Pep3 (5′-ATGAGGTTGTCTGGTGAA-3′) and Pep4 (5′-AATTCCGTGCACAACTAT-3′) targeting a conserved part of the RNA-dependent RNA polymerase gene (Aguilar *et al*., 2002). The cycling regime included reverse transcription for 30 min at 50°C, denaturation for 2 min at 94°C, followed by 40 cycles of denaturation at 94°C for 15 s, annealing at 45°C for 30 s and an extension cycle at 60°C for 45 s with a final extension cycle of 7 min at 68°C. PCR amplicons were fractionated by electrophoresis on 1.5% agarose gels and viewed with a UV transilluminator. Amplicons of the anticipated size (624 bp) were produced from all 12 diseased samples, confirming PepMV infection, whilst no amplicons were produced from virus-free controls. RT-PCR products were sequenced in both directions and the sequences were deposited in GenBank under Accession Nos. KP761701 - KP761712. BLAST analysis of the sequences confirmed the presence of PepMV in the diseased samples which was 99% identical to other tomato PepMV isolates (e.g. EF599605 and JX866666).

To our knowledge this is the first report of PepMV incidence in Moroccan tomatoes. Since PepMV has become an endemic disease in many countries and is possibly seed transmitted, efforts should focus on phytosanitary control for both import and export of both tomato fruit and seeds.

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Figure 1

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