New Disease Reports

First report of *Chickpea chlorotic dwarf virus* in watermelon (*Citrullus lanatus*) in Morocco

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Watermelon (*Citrullus lanatus*) is one of the most important cucurbit crops in the world, with a total production of c. 118 million tons (FAO, 2017). It is a popular fruit in many Mediterranean countries and is widely cultivated. In Morocco an area of 16,680 ha is grown annually with a production of 619,322 tonnes in 2017.

During 2015 watermelon plants with fruits showing cracking, yellowing and brown areas in the flesh were observed in some areas in Morocco. During 2017, similar symptoms were again observed in the regions of Marrakech, Tifelt and Zagoura. The symptoms observed (Fig. 1) were similar to those caused by *Chickpea chlorotic dwarf virus* (CpCDV, genus *Mastrevirus*, family *Geminiviridae*) which was recently reported affecting watermelon in Tunisia inducing "hard fruit syndrome" (Zaagueri *et al*., 2017a).

Seventeen samples of watermelon leaves from symptomatic plants were collected (six from Marrakech, four from Tifelt and seven from Zagoura) and were subjected to total nucleic acid extraction by the TLES buffer-based method (50 mM Tris-HCl, pH 9, 150 mM LiCl, 5 mM EDTA, and 5% SDS), according to Noris *et al.* (1996). CpCDV infection was detected by dot-blot hybridization, using a digoxigenin-labeled probe targeting the coat protein gene (Zaagueri *et al*., 2017b) and was confirmed by PCR using a novel primer pair CpCDV-SEQ2 (5'-CGACACATAAGGTTCAGGTTG-3') and CpCDV-Tu-1145-R (5'-AGGCAACCCTTGGGAGTCA-3'), amplifying a 544 bp fragment in the Rep gene. Four samples out of fifteen collected in 2017 (samples 11, 12, 14 and 15) and one sample out of two collected in 2015 (Sample 'R') were positive for CpCDV by PCR (Fig. 2, upper panel). All five infected samples were collected in the Zagoura area, south-eastern Morocco. Infection in three of five of the samples was confirmed by dot-blotting (Fig. 2, lower panel). To further confirm CpCDV detection, three positive samples collected in 2017 were amplified by PCR using the CP-targeting primer pair CpCDV-CP-F/R (Zaagueri *et al*., 2017b) and the 501 bp products were sequenced on both strands. The three sequences (GenBank Accession Nos. MH500777 - MH500779) were almost identical except for four mismatches. BLAST analysis identified a CpCDV isolate from squash in Egypt (KF692356) as the best match in the NCBI database.

These results constitute the first record of CpCDV infecting watermelon in Morocco and indicate that the virus has been present in the country since at least 2015. In the Mediterranean region, CpCDV has only been previously reported in Egypt (Fahmy *et al*., 2015) and Tunisia (Zaagueri *et al*., 2017b), and this report indicates that the virus may be more widely distributed than indicated by published records.

Figure 1

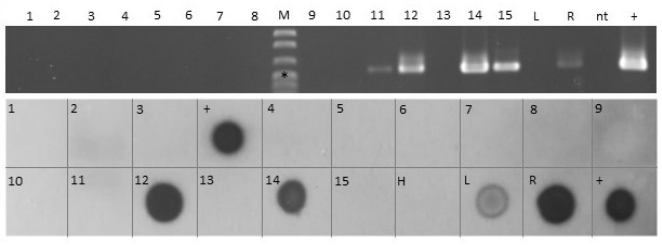

Figure 2

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