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New Disease Reports

First report of hard watermelon syndrome in Tunisia associated with *Tomato yellow leaf curl virus* infection

M. Mnari-Hattab*, S. Zammouri and M.R. Hajlaoui

Laboratoire de Biotechnologie Appliquée à l'Agriculture, INRA Tunisie, Université de Carthage, 2049 Ariana, Tunisie

*E-mail: hattab.monia@iresa.agrinet.tn

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In Tunisia, watermelon (*Citrullus lanatus*) is one of the most common and popular summer fruit crops, and is widely cultivated in the central and southern parts of the country. Since 1994, abnormal symptoms have been observed on fruits by the end of the cropping season grown in the regions of Kairouan, Sidi Bouzid and Gafsa. In some fields, watermelons harden precociously and, when ripe, present a white heart. Moreover, young leaves were mildly curled and stunted (Fig. 1). These symptoms were distributed randomly in the fields and were initially misdiagnosed and attributed to physiological and nutritional disorders.

Since the first reports of this syndrome in 1994, leaf and fruit samples with characteristic symptoms had been tested for the occurrence of any of the viruses known to affect cucurbits in Tunisia, but none showed any positive results (Mnari-Hattab *et al*., 2009). The frequency and distribution of the 'hard watermelon syndrome' suggested that virus infection might be responsible, particularly as *Tomato yellow leaf curl virus* (TYLCV), *Tomato yellow leaf curl Sardinia virus* (TYLCSV) and/or parental recombinant genus *Begomovirus* (family *Geminiviridae*) were widespread on tomato and on silverleaf nightshade (*Solanum elaeagnifoli*) growing in the same area (Mnari-Hattab *et al*., 2014; Zammouri & Mnari-Hattab, 2014).

In 2014, a total of 22 samples, eight samples of preserved vegetable material (i.e. leaves, peduncle and fruit bark) and 14 samples freshly collected from plants exhibiting the characteristic syndrome were tested for begomovirus infection. Genomic DNA was purified, and PCR amplification was performed using degenerate oligonucleotide primers based on the sequence in the *Begomovirus* coat protein genes (Wyatt & Brown, 1996; Mnari-Hattab *et al*., 2014). Amplicons *circa* 560 bp in size were obtained from all the tested symptom-bearing samples (plants with hardened fruit) but no amplicons were generated from symptomless plant samples. Three of the amplicons were cloned and sequenced, and the nucleotide sequences were deposited in GenBank (Accession Nos. KM042091 - KM042093). BLAST searches of the GenBank database revealed that the sequences from watermelon, which were 97.2 to 98.7% identical to one another other, shared 96% homology with a Turkish TYLCV isolate (Accession No. AJ867487). Similar TYLCV amplicons generated from tomato and silverleaf nightshade plants collected from open field tomato crops in Kairouan had similar sequences (results not shown). Further investigations using multiplex PCR amplification (Davino *et al*., 2008) revealed that all 22 samples referred to above were infected with TYLCV, and that two of the samples were doubly infected with TYLCV and TYLCSV following generation of respectively 570 bp and 800 bp amplicons for the two viruses. To the best of our knowledge, this is the first report that associates the watermelon hardness syndrome with *Tomato yellow leaf curl virus* infection. Based on our findings, further targeted surveys must therefore be undertaken to advance our knowledge on tomato yellow leaf curl disease epidemics on cucurbit crops in Tunisia.

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Figure 1

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