New Disease Reports

Melon yellow spot virus identified in China for the first time

Q.S. Gu [1]*, H.J. Wu [1], H.Y. Chen [2], X.J. Zhang [3], M.Z. Wu [3], D.M. Wang [3], B. Peng [1], X.Y. Kong [4] and T.J. Liu [4]

[1] *Henan Key Laboratory of Fruit and Cucurbit Biology, Zhengzhou Fruit Research Institute, Chinese Academy of Agricultural Sciences, Zhengzhou 450009, Henan Province, P.R. China*; [2] *Xiamen Entry-Exit Inspection and Quarantine Bureau, Xiamen 361026, Fujian Province, China*; [3] *Hami-melon Research Center, Xinjiang Academy of Agricultural Sciences, Urumqi 830000, Xinjiang Autonomous Region, China*; [4] *Sanya Science and Technology Academy for Crop Winter Multiplication, Sanya 572000, China*

*E-mail: guqsh@126.com

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During the spring of 2009, a number of outbreaks of a new disease were observed on melon (*Cucumis melo*) grown in plastic houses in Sanya City, Hainan Province, China. Symptoms included numerous yellow, necrotic leaf spots (Fig. 1) and subsequent leaf yellowing (Fig. 2). By mid-April disease incidence in different houses ranged from 30% to 100% of plants. Suspecting a tospovirus infection, DAS-ELISA was performed using specific antisera for *Tomato spotted wilt virus* (TSWV), *Groundnut ringspot virus* (GRSV), *Impatiens necrotic spot virus* (INSV) and *Watermelon silver mottle virus* (WSMoV) following the manufacturer's instructions (Agdia, USA). No positive reaction was observed for any of these viruses. Given the ELISA results and the symptoms observed, infection by *Melon yellow spot virus* (MYSV), a further serologically distinct tospovirus was suspected, and additional testing was carried out using RT-PCR.

Based on a previously published sequence (GenBank Accession No. AB038343.1), a primer set MYSV-CP-5' (TTA AAC TTC AAT GGA CTT AGA TC) and MYSV-CP-3' (AAT TCA ACA TCA GCA AGT CAA) was designed to amplify a 885 bp region of the MYSV coat protein gene. Total RNA from fresh leaves with symptoms was extracted using Trizol (TAKARA, Dalian, China). For cDNA synthesis, 2 µg of total RNA with 10 µM of MYSV-CP-3' primer was heated at 70°C for 5 min and immediately placed on ice. The reaction was completed by adding 2 µl 5x reaction buffer, 2 µl dNTPs (2.5 mM each), 0.3 µl RNase Inhibitor (40 U/µl), 0.3 µl M-MLV and double distilled water to a total volume of 10 µl and incubating at 42°C for one hour. The PCR reaction was performed as follows: 2 µl 10x PCR buffer, 2 µl forward and reverse primer (10 µM), 0.2 µl Taq DNA polymerase (5 U/µl), 2 µl cDNA synthesis product, 1.8 µl dNTPs (2.5 mM each) and double distilled water to 20 µl. Thermal cycling profile included one cycle of 94°C for 3 min; 35 cycles of 94°C for 30 s, 60°C for 30 s, 72°C for 40 s; and a final step at 72°C for 8 min. PCR products of the expected size were amplified from total RNA extracted from leaves with symptoms but none were amplified from extracts taken from healthy control material. Sequence analysis of the amplified DNA fragment revealed that this virus is an isolate of MYSV, denoted as MYSV-SY.

To further characterise the new isolate, the complete nucleotide sequence of MYSV-SY S RNA was obtained. It contains 3257 nts (GQ397254) and has two open reading fames (ORF), in an ambisense coding strategy, separated by an A-U-rich IGR of 872 nts. The 5' and 3' untranslated regions of the S RNA (68 nts and 67 nts, respectively) can form a panhandle structure. Phylogenetic analysis with other completely sequenced S RNAs from MYSV isolates showed that MYSV-SY was most closely related to the isolate from *Physalis* (3257 nts; AF067151), with 97.0% nt identity in the genomic RNA sequence, and a distant relationship to isolates Tospo-melo (95.3%; AB038343) and TW (94.9%; FJ386391). The ORF (nt 3190-2351) on the viral complementary strand encoded N protein of 279 aa with a molecular mass of 31.0 kDa. The N protein shared 97.5-99.6% identities with those of other MYSV isolates. The other ORF (nt 69-1478) on the viral strand encoded NSs protein of 469 aa with a predicted molecular mass of 53.1 kDa. The NSs protein shared relatively higher identities (95.9-98.1%) with those of other isolates. The comparison results of N and NSs proteins with the corresponding sequences of other isolates revealed the closest relationship between MYSV-SY and the *Physalis* isolate, in accordance with that of genomic RNA sequence. MYSV has been previously reported in Japan (Kato *et al*., 2000), Thailand (Bhunchote *et al*., 2005) and Taiwan (Chen *et al*., 2008). To the best of our knowledge, this is the first report of MYSV in China. In addition to the samples taken from Hainan Province, further MYSV-positives have been confirmed in distinct areas in Guangxi province.

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Figure 1

Figure 2

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