New Disease Reports

First report of *Tomato brown rugose fruit virus* infecting tomato in Germany

W. Menzel [1]*, D. Knierim [1], S. Winter [1], J. Hamacher [2] and M. Heupel [3]

[1] *Leibniz Institute DSMZ - German Collection of Microorganisms and Cell Cultures, Plant Virus Department, Inhoffenstraße 7 B, 38124 Braunschweig, Germany*; [2] *University of Bonn, Department of Phytomedicine, Institute of Crop Science and Resource Conservation, Nußallee 9, 53115 Bonn, Germany*; [3] *Landwirtschaftskammer Nordrhein-Westfalen, Pflanzenschutzdienst, Gartenstrasse 11, 50765 Köln-Auweiler, Germany*

*E-mail: wulf.menzel@dsmz.de

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In July 2018, unusual fruit and leaf symptoms were observed in numerous greenhouses cultivating tomatoes commercially in the Lower Rhine region of North-Rhine Westphalia, Germany. Foliar symptoms included chlorosis, mosaic with dark green bulges and narrowing (Fig.1). Fruit symptoms consisted of yellow spots, often concentrated around the calyx (Fig. 2) with occasional rugose symptoms rendering the fruits non-marketable (Fig. 3). In total, more than 25 ha of greenhouse tomatoes were affected.

Initial investigation by electron microscopy showed the presence of filamentous virus-like particles, later identified as the Chile 2 strain of *Pepino mosic virus* (PepMV) and, in addition, particles with a tobamovirus-like structure. PepMV Chile 2 preparations are commonly used as inoculum for cross-protection against severe strains of PepMV, whereas infections with tobamoviruses are particularly rare since commercially grown tomato varieties harbour effective resistance genes (e.g. *Tm-2*^{2}). RT-PCR using a primer pair (514 Tobamo-s1: 5'-GGGAATCAGTTTCAAACRCA-3'; 515 Tobamo-as1: 5'-GGGGGGATTCGAACCYCT-3') designed for universal detection of tobamoviruses (unpublished) resulted in amplicons of the expected size, c. 560 bp, for all symptomatic samples. Blastn analysis of the sequenced PCR products revealed 100% nucleotide identity (GenBank Accession Nos. KX619418 and KT383474) to *Tomato brown rugose fruit virus* (ToBRFV), a tobamovirus that was recently found in Jordan (Salem *et al.*, 2016) and Israel (Luria *et al.*, 2017). The resistance-breaking property of ToBRFV has already been observed by Luria *et al.* (2017) and was cited as the reason for the rapid spread of the virus in Israel, indicating the importance for commercial tomato cultivation worldwide. Total RNA preparations of two samples from Germany (P12-3G, P12-3H) were subjected to Illumina miSeq sequencing, revealing besides PepMV (MK133092 - P12-3G; MK133094 - P12-3H), the genome sequences of ToBRFV isolates (MK133093 - P12-3G; MK133095 - P12-3H) showing 99.6% nucleotide sequence identity to each other. The sequence identities to the isolates from Jordan and Israel were in the same range (99.7-99.8%) which points to a common origin.

ToBRFV was isolated from mixed infection with PepMV by passage on *Chenopodium murale*. The tomato plants subsequently inoculated with *C. murale* plant sap showed the same leaf symptoms as those originally observed (Fig. 4). The ToBRFV isolate P12-3G was deposited as PV-1236 in the DSMZ Plant Virus Collection. This is the first report of ToBRFV in Europe and outside countries of the Near East.

References

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Figure 1

Figure 2

Figure 3

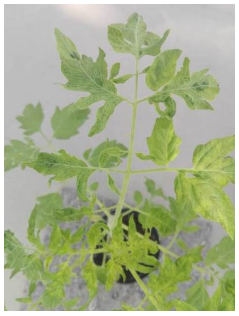

Figure 4

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