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First Report of Tomato Brown Rugose Fruit Virus Naturally Infecting *Chrysanthemum Indicum* in Sicily, Italy

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Tomato brown rugose fruit virus (ToBRFV, *Tobamovirus fructirugosum*), a member of the genus *Tobamovirus* (family *Virgaviridae*) was first detected in April 2015 in greenhouse-grown tomato plants in Jordan (Salem et al. 2016). In Italy, it was reported in October 2018 in tomato production areas of the Ragusa province (Sicily) (Panno et al. 2019). Since its introduction, ToBRFV has rapidly spread throughout the island, causing significant concern for tomato cultivation, due to its high transmissibility through mechanical contact, contaminated seeds, pollination by bumblebees (Levitzky et al. 2019), human-mediated activities, and the tomato leafminer *Tuta absoluta* (Caruso et al. 2024).

In June 2025, chrysanthemum plants (*Chrysanthemum indicum*, family Asteraceae) cultivated in two greenhouses in Ragusa province exhibited typical viral symptoms, that is leaf deformation and interveinal discolouration (Figure 1). Twenty diseased and ten asymptomatic plants were collected, and total RNA was extracted using a NucleoSpin RNA Plant kit (Macherey-Nagel, Germany). Based on the symptoms observed and the incidence of ToBRFV in this area, samples were tested for ToBRFV using RT-qPCR (ISHI-Veg protocol; European and Mediterranean Plant Protection Organization 2021). All 20 diseased samples tested positive for ToBRFV, whereas the 10 asymptomatic samples were negative. To confirm these findings, a conventional RT-PCR (Alkowni et al. 2019) was performed. Two of the PCR products obtained were purified using ExoSAP-IT (Thermo Fisher Scientific, USA) and Sanger sequenced in both directions by Macrogen Europe (The Netherlands). The resulting sequences were deposited in GenBank as isolates ToBRFV_M3-RdRp and ToBRFV_M4-RdRp (GenBank Acc. Nos. PX314972 and PX361033). BLASTn analysis showed a 99.89% nucleotide identity between the two isolates and 98.66% identity with the Sicilian ToBRFV isolate ToB-SIC01/19 (MN167466).

To assess transmission of ToBRFV to this new host, mechanical inoculation assays were performed. Ten tomato and ten chrysanthemum plants were mechanically inoculated on the surface of leaves which had been sprinkled with 320 mesh carborundum, using infected chrysanthemum leaves homogenised in phosphate buffer pH 7; two uninoculated plants of each species were used as negative controls. At 30 days post-inoculation, the leaves of inoculated tomato plants showed typical ToBRFV symptoms, and the leaves of chrysanthemum had interveinal discolouration and a light mosaic. Newly developed leaves were collected and tested by RT-PCR, as previously described. All inoculated plants tested positive for ToBRFV, confirming successful transmission, while control plants tested negative.

Chrysanthemum is widely cultivated as an ornamental plant in Italy and across Europe, and is generally propagated through cuttings. To the best of our knowledge, this is the first report of ToBRFV naturally infecting chrysanthemum. Ongoing surveillance is required to determine the potential impact of ToBRFV on chrysanthemum cultivation.

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FIGURE 1 | Interveinal yellowing and mosaic in young chrysanthemum leaves.

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