

**OP-07: Tomato torrado virus and Tomato marchitez virus, new plant picorna-like viruses infecting tomato**

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Tomato plants grown in the field in Murcia (Spain) in 2003 showed symptoms that could not be attributed to any known virus. The first systemic symptoms consisted of necrotic spots at the top of the plant, starting at the base of the leaflets. Necrotic spots expanded and were surrounded by a light-green or yellow area. Plants with severe pronounced necrotic spots on the leaves and obvious growth reduction were found. Locally the disease became known as 'torrado' (meaning roasted), which refers to the burn-like symptoms on the leaves. Electron microscopic investigations revealed the presence of spherical viral particles of approximately 28 nm in diameter in infected leaf tissue. A purification method was developed for the unknown virus and after density gradient centrifugation two virus bands were observed. PAGE showed that virus particles from both bands consisted of three capsid proteins of approximately 35, 26 and 23 kDa. The viral genome consists of two RNA molecules of approximately 8 (RNA1) and 5.5 kb (RNA2). Both RNA species were subjected to cDNA synthesis and cloning. Full nucleotide sequences were determined from cloned cDNA and PCR-generated fragments. Protein sequences of the three capsid proteins mapped them on RNA2. Phylogenetic analyses with the acquired sequence data clearly showed that this virus was not a member of any known plant virus genus. Mechanical inoculation of the isolated virus on tomato resulted in the typical 'torrado' symptoms. The name Tomato torrado virus (ToTV) was proposed for this new virus.

In 2005 it was found that tomato plants from the state of Sinaloa in Mexico, showed symptoms that were remarkably similar to those caused by ToTV; necrotic spots on the leaves, beginning at the base of the leaflets; and dark necrotic rings and patterns on fruit. In Mexico this new disease of tomatoes was commonly referred to as "marchitez", meaning withered or wilted, but an association with the Spanish disease caused by ToTV was never contemplated. Research conducted in the way as performed for ToTV described above showed that the material was infected with a very similar virus, resembling ToTV in both morphology (isometric particles of 28 nm in diameter), number and molecular sizes of coat proteins (35, 26 and 24 kDa) and the number of viral RNA's (two, with sizes of approximately 7.5 and 5.5 kb). Nucleotide sequencing of the full genome of this virus furthermore revealed a clear relationship with ToTV. Back inoculations of the isolated virus to tomato resulted in the typical symptoms as seen in the field. It could be concluded that the causal agent of marchitez disease was related to but distinct from ToTV and it was proposed to name it Tomato marchitez virus (ToMarV).

ToTV and ToMarV are now proposed as members of the new plant virus genus *Torradovirus*, of which ToTV is the type member.