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DISEASE NOTES

Occurrence of the T36 Genotype of *Citrus tristeza virus* in Citrus Orchards in Sicily, Italy

G. Scuderi and **M. Russo**, Parco Scientifico e Tecnologico della Sicilia, and Agrobiotech Soc. Coop., 95121 Catania, Italy; **S. Davino**, Università degli Studi di Palermo, Dipartimento di Scienze Agrarie e Forestali (SAF), 90128 Palermo, Italy; **R. Ferraro** and **A. Catara**, Parco Scientifico e Tecnologico della Sicilia, 95121 Catania, Italy; and **G. Licciardello**, Parco Scientifico e Tecnologico della Sicilia, and Agrobiotech Soc. Coop., 95121 Catania, Italy.

Recent phylogenetic studies have shown that different lineages representing mild and virulent *Citrus tristeza virus* (CTV) isolates have been present in Italy for a long time (Davino et al. 2013). The genetic structure of CTV population, based on p23 and p27 genes sequences, highlighted two main clusters referred to as T30 and VT-like (Licciardello et al. 2012), as confirmed by the full genomes sequences of two representative isolates SG29 (KC748392) and Bau282 (KC748391) (Licciardello et al. 2015). On the basis of risk analyses, regulatory measures have been outlined, including the recommendation to replace sour orange (*C. aurantium*) with tolerant rootstocks and to monitor the spread and the presence (if any) of different isolates not controlled by the change of rootstock. In 2014, during a large survey covering more than 25,000 ha in eastern Sicily, a subset of 249 alemow (*C. macrophylla*) seedlings, which are the preferred host of many citrus aphid species, was tested for the presence of CTV by DAS-ELISA (Agdia, Elkhart, IN). A subset of 57 alemow seedlings infected by CTV, randomly selected, was transferred in a safe climate controlled greenhouse and grown to observe symptoms. Tissues from these alemow source plants were then used to bark-inoculate sour orange seedlings. Within 6 months, all alemow seedlings showed vein clearing and different degrees of stem pitting, whereas 36 sour orange seedlings were symptomless and 21 displayed a shortening of internodes and seedling yellows leaf discoloration typical of seedlings yellows phenotype (SY), associated with poor root systems. Indeed, they were similar to the phenotype observed in control sour orange seedlings inoculated with SG29, whereas seedlings inoculated with Bau282 were asymptomatic. Further characterizations by capillary electrophoresis SSCP (CE-SSCP) (Licciardello et al. 2012) and real time RT-PCR (Yokomi et al. 2010) were performed analyzing nucleic acids directly eluted from ELISA plates. CE-SSCP profile analysis on a variable region targeting ORFs 1b and 2, revealed that, among the SY isolates, Mac25 and

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Mac85 showed a different pattern from any others observed in previous surveys in Sicily. Moreover, real-time PCR revealed that both isolates reacted positively with CPi-T36 and T36-like probes specifically designed to discriminate T36 genotype. The Mac25 full genome sequence (KR263170) obtained by deep sequencing of small RNAs revealed a 99% homology with all isolates belonging to T36 CTV cluster. To our knowledge, this is the first report of a T36 CTV infection in Italy and the second from the Mediterranean area (after Qaha isolate in Egypt). Suspicious T36 genotype components, based on partial sequences of the CP and RdRp genes, have been also reported in Turkey (Çevik et al. 2013). Since the infection was spread by the prevalent local aphids population (*Aphis gossypii*), this report reinforces the risk of introduction to Italy of the brown citrus aphid (*Toxoptera citricidus*), already described in Spain and Portugal. A systemic surveillance is underway in citrus orchards close to the site of infected samples.

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Section: *Choose*

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