**SHORT COMMUNICATION**

# IDENTIFICATION AND CHARACTERIZATION OF *POTATO SPINDLE TUBER VIROID* INFECTING TOMATO IN ITALY

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**SUMMARY**

*Potato spindle tuber viroid* (PSTVd), a quarantine plant pathogen in Europe, was detected in tomato plants in northern Italy. Infected plants, showing shortened internodes, deformed and necrotic leaves, and abnormal fruit maturation, were growing close to symptomless *Solanum jasminoides* plants also infected by PSTVd. Molecular characterization of field isolates, biological assays and sequence analyses support the likelihood of PSTVd transmission to tomato plants from the neighbouring infected *S. jasminoides* plants. These data provide the first evidence of the risk of PSTVd spread from symptomless ornamental *Solanaceae* to susceptible horticultural crops when prophylactic measures are not enforced, suggesting that occasional PSTVd outbreaks in tomato crops reported from several European countries in the last two decades could have a similar origin. This report appears also to be the first of PSTVd infections of tomato in Italy.

*Key words*: tomato, viroids, *Solanum jasminoides,* detection.

*Potato spindle tuber viroid* (PSTVd) is the agent of a disease that may entail relevant economic losses to potato crops (Diener, 2003). Natural infection of tomato plants has also been observed in a few occasions (Puchta *et al*., 1990; Elliot *et al.*, 2001; EPPO, 2003a, 2003b, 2004; Verhoeven *et al.*, 2004), on plants showing stunting and curled, chlorotic and/or necrotic leaves. The economic impact of PSTVd to tomato crops has been quite limited so far, mainly because of the prophylactic measures adopted to avoid its introduction and spread in tomato-growing areas. Since PSTVd is absent in most European countries, it is included in the EPPO list of quarantine pests.

From the end of 1980s, several viroids, including PSTVd, were identified in tomato crops in the Netherlands and other countries, but the origin of these infections was not conclusively identified (Verhoeven *et al*., 2004). These findings stimulated an extensive survey on ornamental plants in the Netherlands that allowed the identification of *Brugmansia suaveolens* and *Solanum jasminoides* as non-symptomatic natural hosts of PSTVd, and *Cestrum* spp. and *Verbena* spp. as non-symptomatic natural hosts of *Tomato apical stunt viroid* and *Citrus exocortis viroid*, respectively (Verhoeven *et al.,* 2008). These studies showed that several damaging viroids can be introduced into new areas by symptomlessly infected ornamental species and raised again the still unanswered question as to whether the occasional PSTVd outbreaks in tomatoes is related with its presence in ornamental species (de Hoop *et al.*, 2008). Moreover, recent reports of symptomless ornamental *Solanaceae* infected by PSTVd in several European countries, including, Germany (EPPO, 2006a), Italy (Di Serio, 2007), Slovenia (IPPC, 2007), Czech Republic (EPPO, 2008a) and Austria (EPPO, 2008b), and again the Netherlands (EPPO, 2006b), revealed that the presence of PSTVd in Europe is wider than thought, determining a general alert on the epidemiological risk for horticultural crops. These considerations are of particular relevance considering that PSTVd is transmissible by seeds and pollen (Fernow *et al.*, 1970; Singh, 1970; Singh *et al.*, 1992), as well as by contact, mainly through contaminated tools. A possible insect-mediated transmission route due to heterologous encapsidation of PSTVd in particles of *Potato leafroll virus* has been also reported (Querci *et al.*, 1997).

We now report the incidental finding of tomato plants infected by PSTVd in Italy and provide biological and molecular evidence that this viroid was transmitted from infected *S. jasminoides* cultivated in their close proximity. These findings constitute the first well-supported evidence that non-symptomatic ornamental *Solanaceae* can be the source of PSTVd outbreaks in horticultural crops in Europe.

In September 2008, during a survey for the assessment of the sanitary status of ornamental *Solanaceae* plants in Liguria (Northern Italy), severely stunted tomato plants of undetermined cultivar showing curled, chlorotic and necrotic leaves, and discoloured fruits,

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were observed in a small family garden close to a field where *S. jasminoides* plants were grown. Only three of the *ca.* 60 tomato plants had the described symptoms that appeared in a late developmental stage. All plants had been bought in a local market as seedlings prior to transplanting in the garden.

Tissue printing hybridization assays with a PSTVd specific DIG-labelled riboprobe (Di Serio, 2007), revealed that the three symptomatic tomato and ten *S. jasminoides* plants growing nearby were infected by PSTVd, whereas symptomless tomato plants tested negative (data not shown). These data were confirmed analyzing the same plants by RT-PCR as described (Di Serio, 2007) using primers PSTVd-32 (5'-AAACCCTGTTTCGGCGGGAATTAC-3') and PSTVd-33 (5'-TCACCCTTCCTTTCTTCGGGTGTC-3'), complementary and identical to positions 179-156 and 180-203 of the PSTVd reference variant (accession number: NC_002030), respectively. Because the two primers are adjacent, a cDNA corresponding to the PSTVd full length genome is amplified (Di Serio, 2007).

Conclusive identification of the pathogen was obtained by cloning and sequencing the amplified products generated from one infected *S. jasminoides* and one symptomatic tomato plant. For cloning purposes, the Expand High Fidelity PCR System (Roche Diagnostics GmbH, Germany), composed of an enzyme mix containing *Taq* DNA polymerase and *Tgo* DNA polymerase with the 3'-5' proofreading activity, was used. Sequencing of four independent clones from each field isolate and multiple alignment of the resulting nucleotide sequences showed that the cloned inserts from both *S. jasminoides* and tomato were identical to each other and to the PSTVd sequence variant first isolated by Verhoeven *et al.* (2008) from *S. jasminoides* (accession No. EF192393) and later also found in other PSTVd isolates of ornamental *Solanaceae* (Verhoeven *et al.,* 2007; Di Serio, 2007). The sequence of the new tomato PSTVd variant was submitted to GenBank (accession No. FJ872823), and hereafter will be referred to as PSTVd-SjT.

The finding of identical PSTVd variants in both tomato and *S. jasminoides* plants grown in adjacent fields, the high incidence (100%) of PSTVd-infected *S. jasminoides* plants in the field, and the appearance of symptoms only in few and fully developed tomato plants, strongly support the notion that PSTVd had been transmitted to tomato from the neighbouring infected *S. jasminoides*. To our knowledge, this is the first report of PSTVd in tomato in Italy and the first evidence of natural transmission of PSTVd from ornamental *Solanaceae* to tomato.

To gain further insights into the biological and molecular properties of PSTVd-SjT, a plasmid containing the head-to-tail cDNA dimeric insert of this variant was generated by a PCR-based approach previoulsy described (Rodio *et al.,* 2006), and sequenced in both directions to confirm that no mutation had been introduced. *In vitro*-synthesized transcripts from this plasmid were mechanically inoculated to the cotyledons and the first true leaves of four tomato seedlings cv. Rutgers, which is the preferred indicator host for PSTVd (Raymer and O'Brien, 1962; Owens 2007). After one month in greenhouse at 30°C and 16 h photoperiod, indicator plants showed typical PSTVd symptoms and the presence of the viroid was confirmed by tissue printing hybridization and RT-PCR. Cloning and sequencing of progeny variants accumulating in two tomato plants showed a very low sequence variability with respect to the parental variant PSTVd-SjT: only two out of the eight sequenced clones showed one point mutation (a G to A substitution at position 18 and an A deletion at position 239 in the reference sequence). The sequences of the mutated variants from tomato cv Rutgers were submitted to GenBank (accession No. FJ872824 and FJ872825).

The data showing that the PSTVd-SjT, a variant identified as the master sequence in PSTVd populations infecting *S. jasminoides* and other ornamental *Solanaceae* species, was not substantially modified when experimentally transmitted to tomato, are in line with the sequence identity of variants cloned from *S. jasminoides* and tomato isolates reported herein, and further sustain the possibility that PSTVd infection of tomato originated from the adjacent infected *S. jasminoides* plants.

This conclusion is also supported by a multiple sequence alignment generated with CLUSTAL W (Thompson *et al.,* 1994) using the 144 PSTVd sequence variants deposited in databases. This analysis showed that the sequence variants from *S. jasminoides*, *S. rantonnetti* (syn. *Lycianthus rantonnetti*) and *Streptosolen jamesonii* differ from almost all the other PSTVd variants in at least three informative positions (Figure 1): an U insertion at position 64, and A and C deletions at positions 126 and 237, respectively. Besides the variants from the ornamental species reported above, only two additional variants (accession No. FJ872823 and AY372398) from two field tomato isolates, showed the same changes at the informative positions (Fig. 1). Interestingly, these isolates correspond to the one reported here and to an isolate identified in the Netherlands (Verhoeven *et al.*, 2004), where PSTVd on ornamental *Solanaceae* was first discovered, following surveys of these species stimulated by PSTVd outbreaks in tomato (de Hoope *et al.*, 2008).

Altogether, these data suggest that transmission from ornamental *Solanaceae* to horticultural crops could also be the origin of other outbreaks recently reported in Europe. They also highlight the need for surveys in the areas where ornamental and horticultural solanaceous crops are intensively grown, as well as for eradication efforts where PSTVd has been identified.

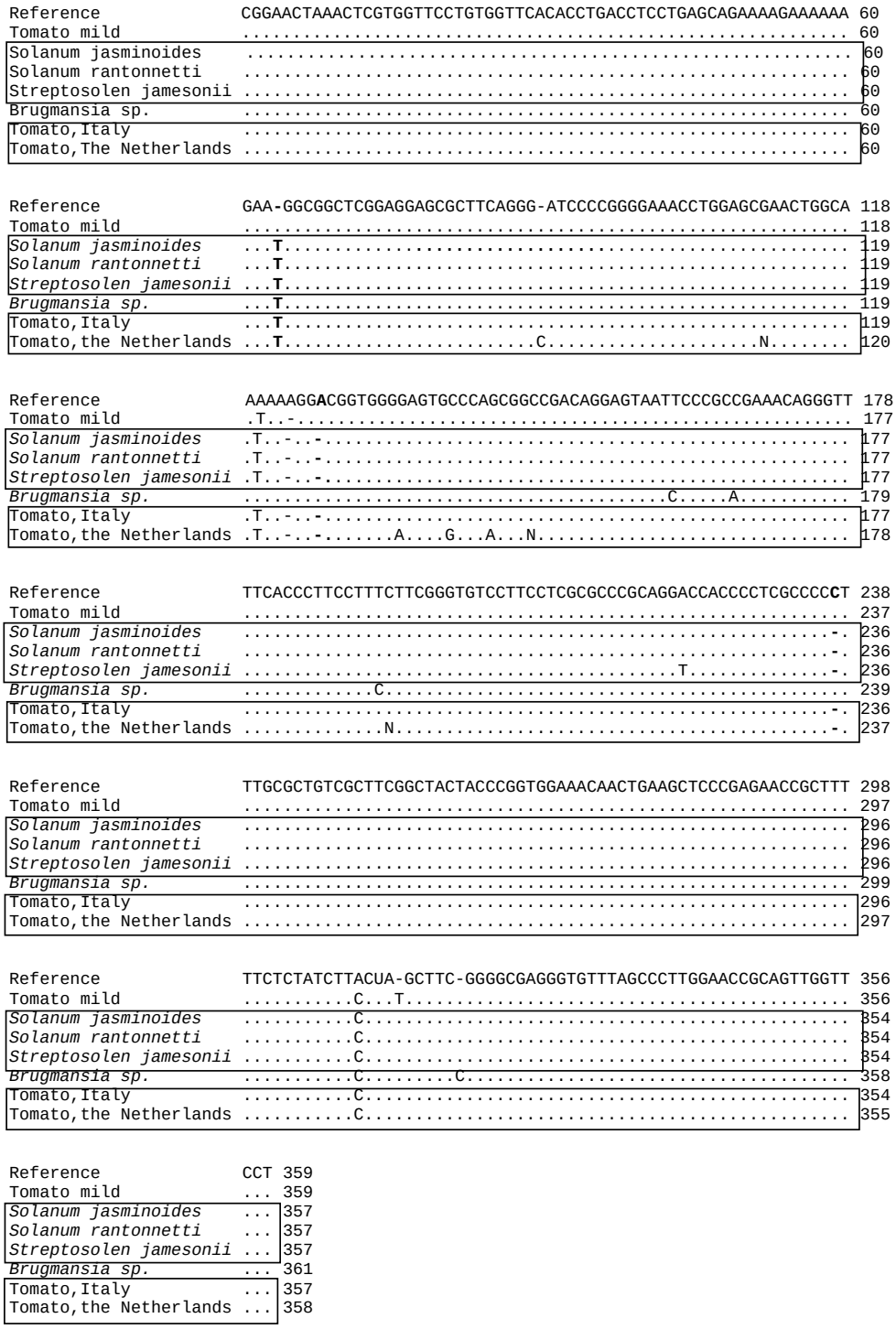


**Fig. 1.** Multiple sequence alignment of representative PSTVd-cDNA variants from the ornamental species *Solanum jasminoides, S. rantonnetti, Streptosolen jamesonii* and *Brugmansia suaveolens* (accession Nos EF192393, EF459700, FM998543 and FM998547, respectively) and from tomato, including a mild variant (accession No. X76844) and two additional variants (accession Nos FJ872823 and AY372398). The reference on the top correspond to the PSTVd reference variant with the accession No. NC_002030. Nucleotide identity with respect to the reference sequence is indicated by dots whereas gaps are denoted by slashes. Characters in bold mark the informative positions along the alignment in which specific modifications, characterized by an insertion (T64) and two deletions (A126 and C237), were contemporary identified exclusively in the variants from *S. jasminoides, S. rantonnetti, S. jamesonii* (also the variants not represented here) and in two variants from tomato isolates identified in Italy and the Netherlands (framed sequences). Possible relationships among these tomato isolates and ornamental *Solanaceae* infected by PSTVd are discussed in the text. Multiple sequence alignments with all PSTVd deposited in databanks confirmed that no other variants, beside those reported here, contemporary contain the informative changes. Moreover, all the PSTVd variants from ornamental *Solanaceae* have the same behaviour than their respective representative variants reported in the figure. Therefore, with respect to the informative positions discussed above, this figure can be considered as a synthetic view of multiple sequence alignments generated with all PSTVd deposited in databanks, which are difficult to show for space limitations.

Finally, although it is reasonable to assume that PSTVd could be accidentally transmitted to horticultural crops by contaminated tools previously used on infected ornamental *Solanaceae,* other transmission routes cannot be ruled out because data on natural spread of PSTVd among different host species and on the possible contribution of insect vectors are scanty (de Hoop *et al.*, 2008). Therefore, further investigations in this respect would strongly contribute to improve strategies for an efficient control of PSTVd epidemics in both ornamental and horticultural crops.

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