Impeded transmission of defective isolates of *Tomato spotted wilt virus* by *Frankliniella occidentalis*

Tatsuya Nagata[1], Alice K. Inoue-Nagata[2], Marcel Prins[3], Rob Goldbach[3] and Dick Peters[3]
[1]Biotecnologia Genômica, Universidade Católica de Brasília, Campo II, 916 Norte, 70790-160, Brasilia, DF, Brazil. [2]Departamento de Biologia Molecular, EMBRAPA/Hortaliças, C.P. 218, 70359-970, Brasília, [3]Department of Virology, Wageningen University & Research, Binnenhaven 11, 6709 PD Wageningen, The Netherlands.
E-mail: tatsuya@pos.ucb.br

Tomato spotted wilt virus (TSWV), the type species of the genus *Tospovirus* in the *Bunyaviridae*, is transmitted by several thrips species. *Frankliniella occidentalis* is considered to be the most efficient vector. Replication of TSWV in thrips has been demonstrated by several immunocytochemical studies (Wijkamp *et al.,* 1993; Ullman *et al.,* 1993). The ability to acquire virus decreases with the development of the larvae, and is completely lost when the thrips become adult (van de Wetering *et al.,* 1996; Nagata *et al.*, 1999).

Two distinct types of TSWV mutants, generated during multiple mechanical passages in plants, have been described (Resende *et al.,* 1991). One type contains a defective RNA segment that is generated by a deletion of a sequence in the polymerase-encoding L RNA segment (Resende *et al.,* 1991; Inoue-Nagata *et al.,* 1997 and 1998). Most of these defective RNAs cause symptom attenuation in plants. They represent true defective interfering (DI) RNAs as they interfere with the synthesis of L RNA. However, some defective RNAs exist that hardly interfere with the synthesis of this RNA segment (Inoue-Nagata *et al.,* 1997). The location of the deleted region in the L RNA varies, but always occurs internally, preserving both termini of the molecule (Resende *et al.,* 1992; Inoue-Nagata *et al.,* 1998). The second type of mutant is characterised by the lack of the viral envelope that contains the viral encoded glycoproteins (Ie, 1982; Resende *et al.,* 1991).

Acquisition of both types of TSWV mutant, infection of thrips organs by these mutants after acquisition, and their transmission have been studied. The defective RNA isolates, Pe-1 and 16-2, (Inoue-Nagata *et al.*, 1997, 1998) derived from the BR-01 isolate, and an envelope deficient (env$^-$) isolate derived from the TSWV NL-04 isolate (Resende *et al.*, 1991), were selected for this study. The ability of these mutants to infect thrips larvae after acquisition was analysed using a whole mount immunofluorescent staining technique (WMIS, Nagata *et al.*, 1999) and inoculation of thrips primary cell cultures (Nagata *et al.*, 1997).

The Pe-1 contained a truncated L RNA segment, which barely interfered with symptom expression and replication of the wild type (wt) L RNA segment. This isolate was transmitted with an efficiency of 51%, a value comparable to that found for wt TSWV (54%). The isolate 16-2 containing a genuine defective interfering (DI) L RNA, as concluded from its ability to suppress wt L RNA synthesis and attenuation of symptom expression, was not transmitted at all.

The midguts of all larvae that ingested Pe-1 became infected, whereas limited midgut infections were found in 24 % of the larvae that ingested 16-2. This difference in infection could be explained by the presence of the low number of infectious units in the inoculum, as demonstrated by the number of local lesions on inoculated leaves and verified by Northern blot analysis.

The env$^-$ isolate failed to infect the midgut after ingestion, and could not be transmitted by either second instar larvae nor adults. This result, and the observation that this TSWV isolate can also not infect primary cell cultures (Nagata *et al.*, 1997), demonstrates that the determinants required for binding and subsequent infection of the midgut epithelium cells or thrips cells by the virus are located on the envelope. The current study shows that thrips completely fail to transmit the DI isolate 16-2 despite limited initial replication in the midgut epithelium. On the other hand, the isolate Pe-1 accumulated at a somewhat slower rate but was transmitted at a similar rate as the wt virus. These observations suggest that a dose-dependent process regulates virus accumulation in the midgut.

The results of this study suggest that thrips can play an important role in the elimination from natural virus reservoirs of TSWV mutants, since these will be eliminated or suppressed during the replication of the vital wild type isolates.

References

Ie TS. 1982. A sap-transmissible, defective form of tomato spotted wilt virus in plant cells. *J. General Virology* **59,** 387-391.

Inoue-Nagata AK, Kormelink R, Nagata T, Kitajima EW, Goldbach R and Peters D. 1997. Effects of temperature and host on the generation of tomato spotted wilt virus defective interfering RNAs. *Phytopathology* **87,** 1168-1173.

Inoue-Nagata AK, Kormelink R, Sgro J-Y, Nagata T, Kitajima EW, Goldbach R and Peters D. 1998. Molecular characterization of tomato spotted wilt virus defective interfering RNAs and detection of truncated L proteins. *Virology* **248**, 342-356.

Nagata T, Storms MMH, Goldbach R and Peters D. 1997. Multiplication of tomato spotted wilt virus in primary cell cultures derived from two thrips species. *Virus Research* **49,** 59-66.

Nagata T, Inoue-Nagata AK, Smid H, Goldbach R and Peters D. 1999. Tissue tropism related to vector competence of *Frankliniella occidentalis* for tomato spotted wilt virus. *Journal of General Virology* **80**, 507-515.

Resende R de O, De Haan P, De Ávila AC, Kitajima EW, Kormelink R, Goldbach R and Peters D. 1991. Generation of envelop and defective interfering RNA mutants of tomato spotted wilt virus by mechanical passage. *Journal of General Virology* **72,** 2375-2383.

Resende R de O, De Haan P, Van de Vossen E, De Ávila AC, Goldbach R and Peters D. 1992. Defective interfering RNA segments of tomato spotted wilt virus retain both virus genome termini and have extensive internal deletions. *Journal of General Virology* **73,** 2509-2516.

Ullman DE, German TL, Sherwood JL, Westcot DM and Cantone FA. 1993. Tospovirus replication in insect vector cells: Immunocytochemical evidence that the nonstructural protein encoded by the S RNA of tomato spotted wilt tospovirus is present in thrips vector cells. *Phytopathology* **83**, 456-463.

Van de Wetering F, Goldbach R and Peters D. 1996. Tomato spotted wilt tospovirus ingestion by first instar larvae of *Frankliniella occidentalis* is a prerequisite for transmission. *Phytopathology* **86,** 900-905.

Wijkamp I, Van Lent J, Kormelink R, Goldbach R and Peters D. 1993. Multiplication of tomato spotted wilt virus in its vector, *Frankliniella occidentalis*. *Journal of General Virology* **74**, 341-349.