Potato Spindle Tuber Viroid Does Not Complement Tobacco Mosaic Virus Temperature-sensitive Transport Function

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SUMMARY

In mixed infections, the potato spindle tuber viroid (PSTV) does not complement the transport function of the tobacco mosaic virus (TMV) Lsl mutant, which has a temperature-sensitive transport function. This appears to be due to the fundamentally different mechanisms of virus and viroid transport. However, PSTV significantly enhances the accumulation of temperature-resistant TMV in mixed infections at a temperature non-permissive for Lsl.

When a plant is inoculated with a virus, the latter replicates in the primarily infected cells and then moves into neighbouring healthy ones. The process of systemic spread of viral infection in a plant is controlled by a special transport function (TF) encoded in the virus genome (for review, see Atabekov & Dorokhov, 1984). Tobacco mosaic virus (TMV) mutants Ni2519 (Zimmern & Hunter, 1983) and Lsl (Ohno et al., 1983), temperature-sensitive (ts) in systemic spread (transport) through infected plants, possess mutations in the gene coding for the 30K protein that is responsible for the TF (Deom et al., 1987; Meshi et al., 1987). Viroids do not code for transport proteins yet are also capable of systemic spread (Palukaitis, 1987), but it is evident that movement of viruses and viroids through the plant occurs in fundamentally different ways.

The virus-encoded TF can be regarded as a factor controlling the resistance of a plant to a virus (Taliansky et al., 1982c). In many virus–host combinations a virus is restricted to replication only in primarily infected cells or in isolated protoplasts because TF is somehow blocked (e.g. the transport protein is not functional in the given type of plant or it becomes non-functional at a non-permissive temperature). This type of resistance can be overcome by TF complementation under conditions of mixed infection when transport of a virus that is deficient in TF is provided by a helper virus. It is of note that complementation can occur not only between related viruses but also between unrelated ones (Taliansky et al., 1982b, c; Carr & Kim, 1983; Atabekov et al., 1984; Barker, 1987; Malyshenko et al., 1987, 1988). Although the molecular mechanisms of viral TF are still obscure, it can be suggested that the virus-specific transport protein plays a significant role in this process.

In this connection the possibility of complementation in TF between viruses and viroids was of interest. The possibility could not be ruled out that the viroid might promote the movement of the virus, causing some general physiological effects in the plant cells. However in this work we show that the systemic spread of TMV ts transport mutant Lsl cannot be complemented by the potato spindle tuber viroid (PSTV).

Tomato plants (cv. Rutgers) were first infected with PSTV (isolate from Armenia); a total preparation of low M~ RNA (1 mg/ml) from PSTV-infected Scopolia plants was used for inoculations (Morris & Wright, 1975). Three to 4 weeks after the inoculation of tomato plants with PSTV, the uninoculated leaves showing symptoms of PSTV disease from systemic infection were superinoculated with the TMV ts mutant Lsl (10 µg/ml) (the second inoculation)
Table 1. Accumulation of the TMV ts mutant Lsl in tomato plants preinoculated with PSTV

<table>
<thead>
<tr>
<th>Inoculum</th>
<th>1st inoculation</th>
<th>2nd inoculation*</th>
<th>Temperature (°C)</th>
<th>Amount of TMV (ng/g of leaf tissue)$</th>
<th>Infectivity (results of TST)‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>I§ II</td>
<td>I II III</td>
</tr>
<tr>
<td>Lsl</td>
<td>33</td>
<td>24</td>
<td>&lt;10</td>
<td>1000</td>
<td>77 (s) 123 (s) 78 (s)</td>
</tr>
<tr>
<td>PSTV Lsl</td>
<td>33</td>
<td>24</td>
<td>1000</td>
<td>490</td>
<td>2 (s) + 123 (l) 8(s) + 142 (l) 7(s) + 108 (l)</td>
</tr>
</tbody>
</table>

* Concentration of Lsl inoculum was 10 μg/ml.
† Detected by ELISA.
‡ The virus progeny was analysed under conditions of TST (24 °C → 33 °C → 24 °C) (see text); (s) denotes small lesions, (l) large lesions. The average numbers of local lesions per half-leaf of N. tabacum are shown.
§ Roman numerals indicate individual experiments.

and incubated at 24 °C or 33 °C. Lsl was supplied by Dr N. Oshima and purified as described earlier (Atabekov et al., 1970). Some plants were infected with Lsl in the absence of PSTV and were used as a control. Four days later the leaves inoculated with Lsl were harvested and treated with anti-TMV serum to remove any surface virus particles. After 15 min antiserum was removed by washing the leaves with water. The leaves were then homogenized in 0.01 M-phosphate buffer pH 7.2. The virus content in the extracts was determined by the double antibody sandwich method of ELISA using serial dilutions of purified TMV as concentration standards (Malyshenko et al., 1985). TMV infectivity was determined as the average number of local lesions per half-leaf of Nicotiana tabacum cv. Samsun NN. Temperature sensitivity of the virus in TF was determined by temperature shift treatment (TST) (Jockusch, 1968). The inoculated leaves of N. tabacum were kept at 24 °C for 2 days to allow the growth of primary local lesions and then transferred to 33 °C for 1 day. The formation of lesions was blocked at 33 °C and the virus spread systemically only if it was not ts in transport. During the subsequent transfer to 24 °C, the infected parts of the leaf became necrotic. As a result the TMV mutant ts in transport induces small lesions whereas temperature-resistant (tr) TMV produces large lesions with a characteristic halo of necrotic tissue.

The TMV mutant Lsl (ts in TF) accumulated in very low amounts for 4 days in tomato plants at the non-permissive (33 °C) temperature in the absence of the viroid (Table 1). Unexpectedly, a significant amount of the virus progeny accumulated at the same temperature in Lsl-inoculated tomato plants preinfected with PSTV (Table 1). These results could have been interpreted as evidence for the ability of PSTV to complement the Lsl ts transport function. However the data from TST show that the virus progeny in these experiments differed from the initial TMV Lsl in that it was not ts but tr in TF (Table 1). We propose two suggestions to explain these unexpected results. Firstly, it is possible that the Lsl preparations contain a low level of contamination of tr TMV and tr revertants may be produced; these may have spread and accumulated selectively at the non-permissive high temperature. This suggestion is in accordance with the fact that upon prolonged incubation (8 to 10 days) in the control plants inoculated with TMV ts mutants the gradual accumulation of tr TMV occurs frequently, although it is not detectable in the usual 4 day experiments (Taliansky et al., 1982a, b). A similar effect has been detected by Bosch & Jockusch (1972) in work with Ni2519, another TMV mutant ts in TF. It is clear, however, that the proportion, if any, of the tr contaminant in the initial Lsl inoculum is extremely low according to two control tests the results of which are shown in Table 1 (Lsl only, at 33 °C and 24 °C). Another suggestion is that upon mixed infection, PSTV is capable of enhancing the reproduction of TMV particularly in the case of tr TMV accumulation at the temperature non-permissive for Lsl. The phenomenon of virus replication enhancement by a viroid has been demonstrated recently by Karosawa & Ehara (1988) in mixed infections of cucumber mosaic virus and the hop stunt viroid.
Table 2. Enhancement of reproduction of TMV tr strain vulgare by PSTV in mixedly infected tomato plants

<table>
<thead>
<tr>
<th>Inoculum</th>
<th>Amount of TMV (ng/g of leaf tissue)*</th>
<th>Infectivity (results of TST)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st inoculation</td>
<td>2nd inoculation</td>
<td>Temperature (°C)</td>
</tr>
<tr>
<td>10.0 24</td>
<td>1400</td>
<td>1100</td>
</tr>
<tr>
<td>10.0 24</td>
<td>1800</td>
<td>1300</td>
</tr>
<tr>
<td>10.0 33</td>
<td>1600</td>
<td>1200</td>
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<tr>
<td>10.0 33</td>
<td>1500</td>
<td>1300</td>
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<tr>
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<td>80</td>
<td>–</td>
</tr>
<tr>
<td>0.1 24</td>
<td>640</td>
<td>–</td>
</tr>
<tr>
<td>0.1 33</td>
<td>120</td>
<td>50</td>
</tr>
<tr>
<td>0.1 33</td>
<td>1400</td>
<td>800</td>
</tr>
</tbody>
</table>

* Detected by ELISA 4 days after inoculation with TMV.
† The virus progeny was analysed as described in the footnote of Table 1.
‡ Roman numerals indicate individual experiments.

A series of model experiments were performed to examine the second suggestion and to evaluate the enhancement of TMV accumulation in the presence of PSTV. The standard TMV tr strain vulgare (U1) was used in these experiments because the real nature and the origin of the accumulated tr TMV in plants mixedly infected with PSTV and Ls1 at 33 °C (Table 1) was obscure. Plants preinfected with PSTV were superinoculated with TMV vulgare at different concentrations and temperatures (Table 2). PSTV significantly enhanced the accumulation of tr TMV (at 24 °C as well as at 33 °C) when the concentration of TMV in the inoculum was quite low (0.1 µg/ml) (Table 2). When TMV concentration was increased (to 10 µg/ml) the enhancement was not detectable, which is probably due to the virus itself rapidly accumulating in this case.

It seems probable that at the temperature non-permissive for Ls1 transport, the conditions for selective spread of TMV tr contaminants (and/or revertants) are established and PSTV enhances their accumulation in mixedly infected tomato plants. It is important to note that systemic movement of Ls1 at 33 °C in the presence of viroid did not occur (Table 1), i.e. the TF of TMV was not complemented by PSTV. This finding may be due to differences in the mechanisms of TF between viruses and viroids in infected plants.

REFERENCES


Short communication


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