BIOLOGIA PLANTARUM 35(1): 131-135, 1993

BRIEF COMMUNICATION

PSTV infection in tobacco (*Nicotiana tabacum* L.) transformed with PSTV cDNA

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Abstract

Tobacco cv. White Burley was transformed with disarmed expression vector pCB1314 containing dimeric cDNA of potato spindle tuber viroid (PSTV, severe strain) in plus orientation regulated from the mannopine promoter. Amount of PSTV specific (-) and (+) sequences and PSTV circular forms was measured in transformed tobacco stock and compared with PSTV content in untransformed tomato and tobacco grafts. It follows from the results that lower rate of accumulation of PSTV in tobacco as compared with tomato is due to less intensive viroid transportation through the cytoplasm and/or cell to cell movement, whereas both, viroid replication and processing showed comparable characteristics in tomato and tobacco with respect to accumulation of minus and plus strands and circular forms in infected tissues. Despite of accumulation of viroid in comparable amount in both transformed tobacco and infected tomato, no expression of any morphological symptom of disease was observed in transgenic tobacco.

Although PSTV causes severe developmental distortions in some host species such as tomato and potato, it is considered to be unable to infect other members of the same family (Singh 1973). Tobacco (*Nicotiana tabacum* L.) is known to be host species for PSTV, but no obvious symptoms of disease were described for PSTV-infected tobacco. This could be due to either absence of interaction of PSTV with specific cell compartment(s) to promote pathogenesis and/or unsufficient accumulation of PSTV in tobacco cells due to some barrier(s) for viroid propagation in this host species.

Received 28 November 1991, *accepted* 15 January 1992.
Contribution presented to the 5th Czechoslovak Seminar "Plant Gene Engineering" organized by the Institute of Plant Molecular Biology in České Budějovice, 2 - 13 September 1991.

Such barrier(s) (including *e.g.* replication, processing, transportation) could be partly overcome by transformation of plants with infectious cDNA constructs (*e.g.* Gardner *et al.* 1986). It is also possible that some processes of viroid propagation in tobacco differ in details from those in tomato or potato. In the present work we attempted to compare some aspects of viroid infection in tobacco transformed with PSTV cDNAs with infections in untransformed tobacco and tomato.

We transformed tobacco cv. White Burley with vector pCB1413 (Matoušek *et al.* 1989) containing dimer of PSTV cDNA obtained by insertion of cDNA PSTV (severe) into unique BamHI site in front of weak mannopine (dual) promoter from T_R-DNA of octopine Ti plasmid. Neomycin phosphotransferase II gene expresses in the opposite direction. PSTV cDNA was those described originally by Van Wezenbeek (1982) except for composition of upper part of premelting loop 1. According to our sequencing data the sequence of the insert is fully consistent with the sequence described by Schnölzer *et al.* (1985) and corresponds to PSTV severe. All the experiments were performed under aseptic conditions. *In vitro* plants were supplied with light (16 h - 35 μmol m^{-2} s^{-1} PAR) and day/night temperature was 25/18 °C.

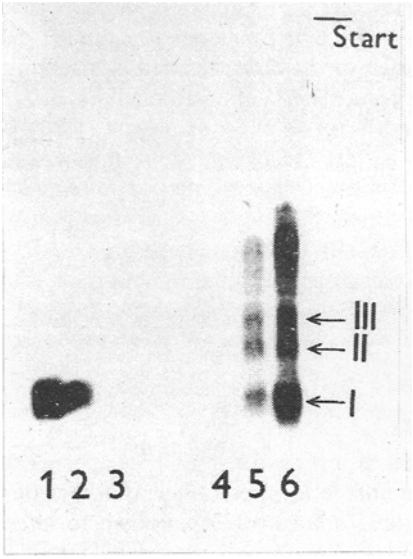

Fig. 1. Northern blot analysis of PSTV-specific sequences from tobacco. 20 μg total nucleic acids isolated from transformed tobacco (clone 107^+) were applied to lanes 1 and 6; the same amount of total nucleic acids from infected tobacco (clone 107^-) were applied to lanes 2 and 5. Lanes 3 and 4 represent nucleic acids (20 μg) from uninfected and untransformed control tobacco plants. Samples 1, 2 and 3 were analyzed for the presence of (+)PSTV sequences using ^{32}P-labelled (-)RNA (spec. activity 4.5×10^4 cps ml^{-1}, exposure 8 h). Samples 4, 5 and 6 were analyzed for (-)PSTV sequences using (+)RNA as a probe (spec. activity 4.5×10^4 cps ml^{-1}, exposure 48 h). Positions of trimeric (III), dimeric (II) and monomeric (I) transcripts are indicated by the arrows.

For detection of PSTV we utilized either RNA-DNA hybridization (Palukaitis *et al.* 1985) using ^{32}P labelled BamH1 insert of PSTV cDNA, or RNA-RNA hybridization (Mühlbach *et al.* 1983). For detection of plus and minus strands standard capillary blots from 1.5 % agarose onto *Biodyne A* nylon membrane were performed and ^{32}P labelled T7 RNA transcripts of EcoRI linearized plasmids pRH703 and pRH704 (Hecker 1989) were used, respectively. All autoradiograms were scanned using an *ULTROSCAN* (*LKB*) and quantified using the *Gelscan 2D-spots* computer program version 2.1 (*LKB*).

As expected, all kanamycin resistant tobacco regenerants were simultanously viroid-infected. In F_2 generation we found segregation closest to ratio 3:1 suggesting monogene inheritance of the PSTV cDNA. Some of F_2 plants which were originaly PSTV negative became PSTV infected after longer time of cultivation probably due to secondary infection through seed tissue. These plants showed low level of PSTV as usually expected for tobacco inoculated with PSTV by conventional way (using carborundum). We selected PSTV segregants containing high and low level of PSTV for further experiments and designated them as 107^+ and 107^- (Fig. 1), respectively. Clone 107^+ was used for grafting experiments (Table 1).

Table 1. Amount of PSTV in grafted transgenic tobacco.

Tissue analyzed		Amount of PSTV specific sequences relative tomato graft* [%] hybridization for strands (+)	(-)	ratio (+)/(-)	ratio circular/linear PSTV**
Graft	untransformed tomato (stems)	100.0	100.0	16.9	1.4
Stock	transformed tobacco (stems)	91.5	206.1	7.9	4.0
Stock	(roots)	143.3	277.8	8.0	5.9

* estimated by the method of RNA-RNA hydridization

**after electrophoresis of PSTV under denaturating conditions (Schumacher *et al.* 1986) and electroblott onto Biodyne nylon A membrane $PSTV_C$ $PSTV_L$ forms were detected by RNA-DNA hybridization

In these experiments we grafted stock 107^+ with tomato cv. Rutgers graft. After one month nucleic acids were extracted from chimeric plants and analyzed for presence of PSTV(+) or (-) strands using Northern blot-analysis, and for presence of circular or linear forms using gel electrophoresis under denaturating conditions (Schumacher *et al.* 1986). We found in these experiments that level of PSTV, which was achieved in transgenic tobacco clone 107^+ is comparable with the level in tomato graft, more (up to 143 %) of viroids was usually detected in the roots than in the leaves of transgenic tobacco. Despite of the fact that high amount of PSTV can be accumulated in transformed tobacco clone 107^+, no morphological symptoms of the disease were observed on plants of this clone. That means that absence of symptoms in infected tobacco is rather due to absence of specific target to promote pathogenesis than due to low level of PSTV accumulation in infected tobacco. It

should be noted that viroid inoculum prepared from clone 107$^+$ caused strong symptoms on tomato. No difference between viroid sequence isolated from transformed tobacco or infected tomato was observed in our experiments using electrophoresis of RNA heteroduplexes under non-denaturated conditions (Zimmat *et al.* 1990), suggesting that the viroid sequence did not change in these hosts. High level of PSTV (-) strands in transgenic tobacco and of circular forms, which even exceeded the level in tomato (Table 1) suggests that processes of PSTV replication and processing are not blocked in tobacco. Ratios between plus and minus strands (+/-) and $PSTV_C/PSTV_L$ forms were very similar for clones 107$^+$ and 107$^-$ suggesting no significant difference between transformed and infected tobacco in this respect. The ratio +/- was 7.4 and 7.8 for clones 107$^+$ and 107$^-$, respectively. $PSTV_C/PSTV_L$ ratio was 4.32 and 3.47 for 107$^+$ and 107$^-$, respectively. It seems, therefore, that there is no significant difference in viroid replication and processing in transformed and secondary infected tobacco. In order to compare the rate of viroid transportation in tomato and tobacco, we used regenerants of the transformed tobacco as a stock and untransformed tobacco and tomato as grafts (Table 2).

Table 2. Accumulation of PSTV in grafted tobacco.

Tissue analyzed		Level of PSTV relative to transformed tobacco [%]* time after grafting [d]	
		40	90
Stock	transformed tobacco (stems)	100	100
Graft	untransformed tomato (stems)	225	n.d.
Graft	untranfromed tobacco (stems)	5	46

*amount of PSTV was assayed according to RNA-DNA hydridization
n.d. - non determined

Analysis of PSTV in the grafts after different postgrafting period suggests that PSTV is much quickly translocated in tomato than in tobacco graft. It can be supposed therefore, that under our experimental conditions transport and translocation were less intensive in tobacco than in tomato.

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