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Molecular characterization of viroid associated with tapping panel dryness syndrome of *Hevea brasiliensis* from India

Ajay Kumar[1,2], D. M. Pandey[1], Thomson Abraham[3], Jacob Mathew[3], P. Jyothsna[2], Padma Ramachandran[2] and V. G. Malathi[2,*]

[1]Birla Institute of Technology, Deemed University, Mesra, Ranchi 835 215, India
[2]Advanced Centre for Plant Virology, Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi 110 012, India
[3]Rubber Research Institute of India, Kottayam 686 009, India

Tapping panel dryness (TPD) disease is the most economically important malady affecting the quality and quantity of yield of rubber latex. The etiological agent of the disease has not yet been characterized. In the present communication, we report the association of a viroid belonging to potato spindle tuber viroid group (PSTVd). The isolates cloned from TPD-affected rubber samples and tomato leaves infected by the extract from TPD-affected rubber showed more than 95% identity with PSTVd.

Keywords: Rubber latex, tapping panel dryness disease, tomato leaves, viroids.

VIROIDS are unique plant pathogens consisting of low molecular weight (LMW), non-encapsidated, autonomously replicating single-stranded RNA molecules (~246 to 425 nucleotides (nt)) without any functional open reading frames (ORFs) in their genome[1]. Ever since the first report of a viroid on potato[2], about 32 viroid species and more than 340 viroid variants have been recorded. In the 'Subviral RNA Database' (http://subviral.med.uottawa.ca/), more than 1700 viroid sequences are available. They are the etiologic agents of diverse diseases affecting food, industrial and ornamental herbaceous and lignaceous plants. Viroid-induced symptoms range from necrosis to less severe developmental disorders, including leaf chlorosis, necrosis, stunting, flowering alterations, and fruit and seed deformations. They are more prevalent in vegetatively propagated plants and in high-yielding clones and varieties of plants developed.

Potato spindle tuber viroid (PSTVd) belonging to the family Pospiviroidae, has a wide host range and has been reported from a wide variety of ornamental and horticultural crops; to name a few, capsicum[3], potato[4,5], gooseberry[6–8], *Brugmansia* spp[9], *Solanum jasminoides*[9], petunia[10], tomato[11], cestrum[7] and dahlia[12]. Avocado is by far the only tree species where PSTVd has been

*For correspondence. (e-mail: vgmalathi@rediffmail.com)

recorded[13]. Different variants of PSTVd range in size from 359 to 370 nt with a well-conserved central domain. In India, occurrence of potato spindle tuber disease in potato was reported in 1989 (ref. 14) and its viroid-like nature was confirmed later[15]. The viroid causes typical necrotic lesions on leaves, stunting of plants and spindle-shaped potato tubers. It is easily sap and graft transmitted to both potato and tomato.

Tapping panel dryness (TPD) syndrome of rubber tree (*Hevea brasiliensis*) is widespread in all rubber-growing areas and is a matter of serious concern to rubber industry. It was first observed in Malaysia, affecting mostly high-yielding clones of rubber[16]. The disease derives its name from the effect it causes on the rubber tree, namely reduction in latex yield leading to partial and eventually total dryness of the tapping panel. One of the causes that have impeded progress in the management of TPD is the fact that the syndrome becomes evident, about six to seven years after transplanting, only when the trees reach maturity for tapping. Many evidences had been put forward to show that the syndrome may be of abiotic nature, particularly due to physiological changes or nutritional deficiency[17,18]. However, involvement of different biotic agents with the syndrome has also been reported from time to time, viz. a virus[19], a rickkettsia-like organism[20], a phytoplasma[21] and a viroid[22,23].

Association of a viroid RNA with the TPD syndrome of rubber was first reported by Ramachandran *et al.*[22] on the basis return polyacrylamide gel electrophoresis (R-PAGE), and RT-PCR using primers specific to PSTVd. But its sequences and relationship with other viroids were not reported. Using R-PAGE[24], LMW RNA has been detected in leaf, bark and root of TPD-affected trees of different rubber clones grown in different locations, in seedlings as well as in bud grafted plants[23]. The infectious nature of the isolated LMW RNA from TPD-affected rubber plants was shown on healthy tomato seedlings cv. Pusa Ruby by sap inoculation. The LMW RNA was reisolated from symptomatic tomato leaves which reinfected healthy tomato seedlings, thus proving the biotic etiology of TPD[23].

The present study reports cloning, sequencing and identification of the LMW RNA associated with TPD-affected rubber trees as well as tomato inoculated with LMW RNA from rubber that showed symptoms of epinasty and curling. A diagnostic probe for quick detection of TPD has also been developed and validated with field samples of rubber.

Leaf and bark samples of all the clones of rubber used in the present study were collected from rubber trees growing in the Rubber Research Institute of India (RRII) farms at Kottayam, Kerala, India. The samples were immediately frozen and the nucleic acid was extracted from 2–5 g of tissue using the protocol described by Kumar *et al.*[23]. The total nucleic acid (TNA) precipitated by this method yields unsheared LMW RNA, which is further purified using RNeasy plant minikit (Qiagen, USA) following the manufacturers' protocol. The purified RNA thus obtained was used to inoculate healthy tomato plants. Nucleic acid extraction was also performed with leaves of healthy asymptomatic plants of rubber clone RRII 105.

The nucleic acid prepared from TPD-affected rubber plants was inoculated on tomato. The LMV RNA was extracted 21 days post-inoculation from healthy uninoculated and inoculated symptomatic tomato plants, as described earlier[23].

A total of 27 samples from TPD-affected rubber plants were analysed along with eight samples from asymptomatic rubber tree and two samples of tomato plants, inoculated with TNA from healthy and TPD-affected rubber trees, and with uninoculated tomato plants serving as control.

The total RNA thus isolated from different samples served as a template for cDNA synthesis using Qiagen omniscript RT kit (Qiagen, USA). The first strand cDNA was synthesized in a total reaction volume of 25 µl (10 µl of total RNA, 2 µl dNTP mix (40 mM), 4 µl 5 × first strand cDNA buffer, 1 µl of DEPC-treated sterile distilled water, 1 µl RNase inhibitor (30 U µl), 200 pmol/µl of each reverse primers, and 1 µl of M-MLV (200 U µl) reverse transcriptase) and the reaction mix was incubated at 42°C for 60 min and then at 72°C for 5 min (for enzyme inactivation). The cDNA was amplified by PCR (50 µl reaction volume containing 5 µl of cDNA, 5 µl (10×) PCR reaction buffer, 4 µl (2.5 mM) dNTP mix, 4 µl $MgCl_2$ (25 mM), 200 pmol/µl each of forward and reverse primers, 1 µl (2.5 units) of *Taq* DNA polymerase and 29 µl RNase free water) following the phenol : isoamyl alcohol extraction protocol[25]. Two different sets of primers specific to potato spindle tuber viroid were designed from the consensus sequences in the central conserved region and used. They are: set I Abt C-F, CAACTGAAGCTCCCGAGAACCGCT (nt position 273–296) Abt C-R, TTTCCACCGGGTAGTAGCCGAAGCGAC (nt position 272–246); set II PSTVF CCGGTGGAAACAACTGAAGCTCCCGAGAAC (nt position 263–292) PSTVR GTAGTAGCCGAAGCGACAGCGCAAAGGGGG (nt position 262–233). The PCR amplicons were expected to be of 350–370 bp length. PCR products were purified from the gel using a Gel Extraction Kit (Qiagen, USA). The double-stranded cDNA was cloned in pGEM-T Easy vector (Promega). The *Escherichia coli* strain DH5α cells were transformed with the recombinant plasmids and selected clones were sequenced at the facility at Delhi University, South Campus, New Delhi, India. Sequence data were edited and aligned according to the convention followed in viroid genome editing.

The viroid sequences obtained in the present study are available in NCBI GenBank database under accession nos HM107843 to HM107848. To understand the relationships

with other viroids, the sequences were compared with other viroid sequences obtained from NCBI database in multiple sequences alignments done using the CLUSTAL X program[26]. A phylogenetic tree was constructed using MEGA 4.0 bootstrapping from 1000 replicates. Multiple alignment of nucleotide sequences of the viroids in the present study was done with PSTVd sequence available in database in Bioedit sequence alignment editor version 5.09, to know the sequence identity between them. For nucleic acid spot hybridization (NASH) tests, plant extract (PE) and nucleic acid extract (NAE) were prepared individually and dotted onto nylon membrane. Hybridization was carried out using α^{32}P-labelled cDNA probe as described previously[27]. The RNA extract from TPD-infected rubber samples (2, 4/4, 19, 44, 50) was subjected to real-time PCR analysis. The RNA extracted from healthy rubber samples (24H) served as negative control, while plasmid DNA of the viriod clone (PSTVd-RubberKER1) served as positive control. The qRT-PCR reaction mixture (20 µl) consisted of 2× SYBR Green Master Mix (Roche Diagnostics GmbH, Penzberg, Germany). 0.2 µM primers and 500 ng of the template. The PCR conditions were kept as 95°C for 10 min, followed by 40 cycles of 10 sec at 95°C, 30 sec at 58°C and 30 sec at 72°C for amplification. The mixture was then subjected to qRT-PCR using LightCyclerR 480 Real-Time PCR System (Roche Diagnostic GmbH, Germany) coupled with the DNA-binding flouresecent dye SYBR Green I. All amplified products were analysed by recording fluorescence. The threshold cycle (C_t) at which significant increase in fluorescence occurs was calculated using software version LCS480 1.5.0.39 provided with the LightCycler R 480. Melting curve analyses were performed to verify the specific product formation.

In this study, a total of 27 samples of rubber trees were analysed, most of which showed symptoms of panel dryness as they were in the different stages of tapping. Among the 27 samples analysed, LMW RNA were detected in 21 samples by R-PAGE, reconfirming the application of electrophoresis under denaturing condition to facilitate efficient detection. The extracted LMW RNA was used as template for cDNA synthesis and PCR amplicon of ~400 bp was observed in 15 out of 22 TPD-affected samples tested with both the sets of primers.

No such amplicon was observed with nucleic acid extract from healthy asymptomatic rubber seedlings (Figure 1). The details of PCR results are provided in Table 1, from which it is clear that PCR amplicons were observed in 56% of symptomatic plants analysed, irrespective of the stage of the tapping.

Inoculation of LMW RNA extract onto tomato seedling led to symptom expression in tomato seedlings about 21 days post-inoculation, as reported earlier[23]. The typical symptoms were epinasty, mild drooping of top leaves, and overall chlorotic and stunted appearance of plants. The nucleic acid extract from such symptomatic tomato leaves gave rise to ~400 bp amplicon with both sets of primers. No such amplicons were obtained with nucleic acid extract from uninoculated healthy tomato seedlings.

The PCR products from eight samples (chosen randomly marked * in Table 1) were cloned in pGEM-T easy vector. In all the clones 359–400 bp fragments were released when restricted with *Eco*RI, indicating the insertion of anticipated viroid-like fragment. The insert in

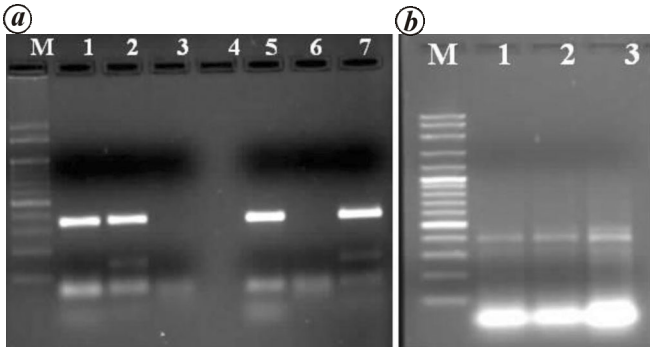

Figure 1. Agarose gel electrophoresis of RT-PCR amplicons obtained with RNA extract from (***a***) samples tomato and (***b***) rubber. Lane M, Molecular weight marker (1 M); lanes 1–7, RNA extract from tomato plants inoculated with extract from the following TPD-affected rubber samples: lane 1, 2, TPD; lane 2, 4/4TPD; lane 3, 95H; lane 4, no sample; lane 5, 2TPD; lane 6, 95H; lane 7, 4/4TPD. ***b***, Lane M, 100 bp marker; lanes 1–3, amplicon from TPD-affected rubber samples; lane 1, 6/15 and lane 2, 6/15 and lane 3, 95.

Table 1. Detection of LMW RNA viroid in TPD-affected rubber plants by R-PAGE and PCR

Rubber sample ID		R-PAGE	PCR
*4/4 TPD	B	+ ve	
	L	+ ve	+ ve
24 H	L	– ve	– ve
	B	– ve	
62 TPD	L	+ ve	+ ve (400 bp)
	B	+ ve	
120 TPD	L	+ ve	+ ve (400 bp)
	B	+ ve	
*6/15 TPD	L	+ ve	+ ve (400 bp)
	B	+ ve	+ ve (400 bp)
*2 TPD	L	+ ve	+ ve (400 bp)
	B	+ ve	+ ve (400 bp)
11 H	L	+ ve	+ ve (400 bp)
	B	+ ve	
36 H	L	– ve	– ve
	B	– ve	
116 TPD	L	+ ve	+ ve (350 bp)
	B	+ ve	
*95 TPD	L	+ ve	+ ve (400 bp)
98 H	L	– ve	–ve
103 H	L	– ve	
*50	L	+ ve	+ ve (400 bp)
*44	L	+ ve	+ ve (400 bp)
*19	L	+ ve	+ ve (400 bp)
09	L	+ ve	+ ve (400 bp)
15	L	+ ve	+ ve (400 bp)
*19	L	+ ve	+ ve (400 bp)

*Samples which were cloned. L, Leaf samples; B, bark samples.

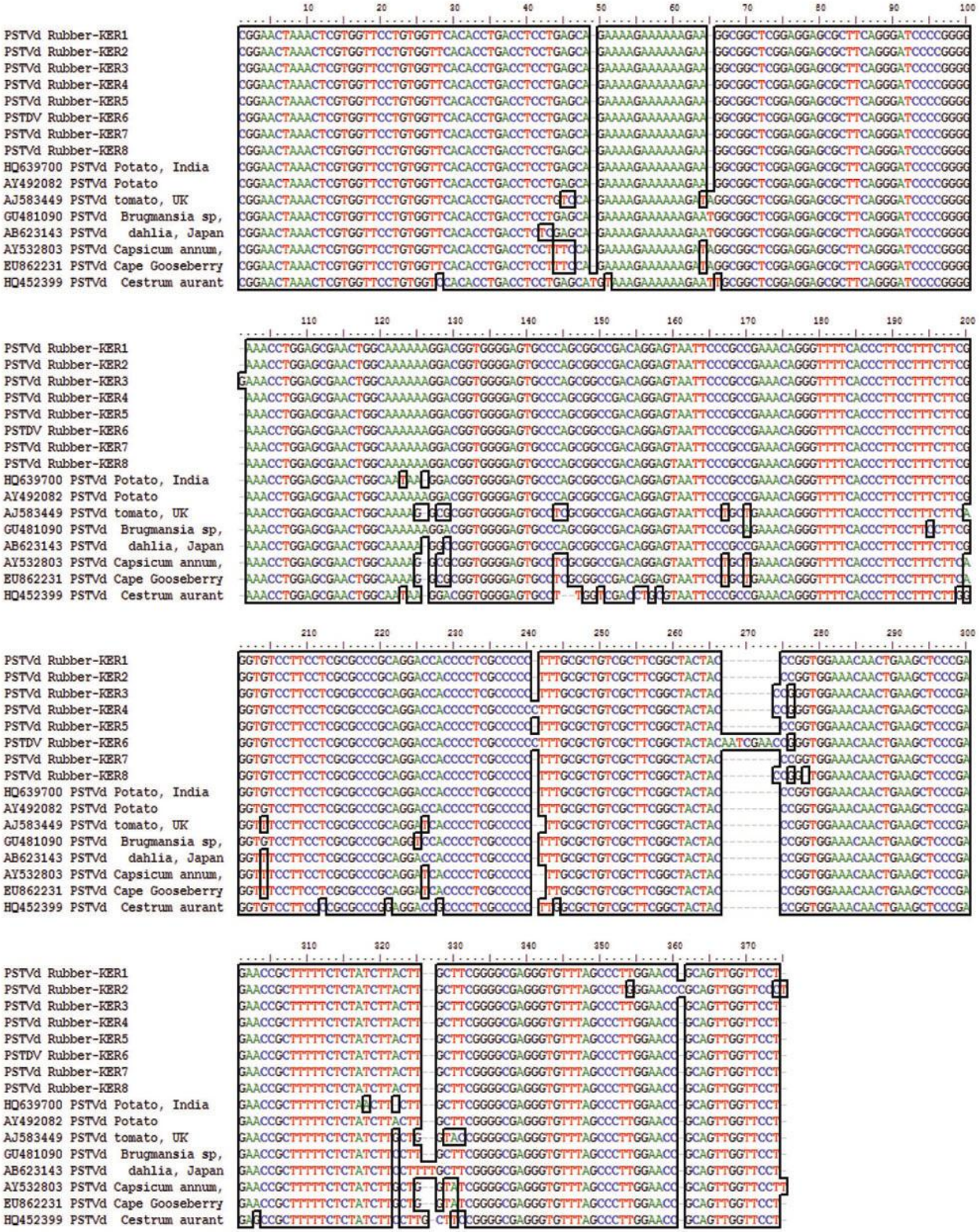

Figure 2. Multiple nucleotide sequence alignment of PSTVd isolates. PSTVd-Rubber-KER-1-8 isolates compared with those of PSTVd variants from other host species and countries.

selected clones from eight samples was sequenced. The cloned fragment from eight samples (Table 2) was approximately 359–361 nt in length. Each sequence was individually analysed in BLAST to identify the sequence with which maximum identity is exhibited. All the clones showed more than 95% sequence identity with different PSTVd isolates. Maximum identity was observed with PSTVd isolates from USA, M16826 and AY937179

infecting potato. On the basis of nearly 100% identity with PSTVd isolates M16826 and AY937179, the viroid sequences characterized from rubber samples are considered as variants of PSTVd and the name proposed is PSTVd-Rubber-KER-1–8, to denote its origin from the rubber tree followed by clone id KER-1-8 indicating the geographic origin from Kerala.

The multiple alignment of nucleotide sequences of rubber isolates with PSTVd from other plant species is given in Figure 2. The viroid sequence from rubber varied in length from 359 to 361 nt. The viroid sequences exhibited 99–100% identity between themselves. The identity seen between rubber isolates and other PSTVd isolates was also high (Table 3). It was 100% with PSTVd M16826 and AY937179 from potato originating from USA, the lowest being 91.9% recorded with PSTVd isolate from *Solanum muricatum*, Slovenia and 93.6% from *Capsicum annuum*, New Zealand.

The region which is varying characteristically in rubber isolates compared to other PSTVd isolates is a segment from nt 323 to 329; there is deletion of nucleotides in all the rubber isolates at 328, 327 and 325 and substitution of 'A' instead of 'C' at position 323. There is an insertion of 'G' at position 61 in PSTVd-Rubber-KER8 similar to isolates from dahlia, tomato and capsicum. Whether variation in this region (which falls in the pathogenicity domain) will contribute to any specific pathogenicity criteria needs to be studied. Among the rubber isolates, the clone ID KER-6 showed insertion of seven nucleotides; 267 to 274 nt. One or two substitutions are also seen in PSTVd rubber isolates, perhaps contributing to some variability.

In a phylogenetic tree constructed on the basis of alignment of full-length sequence, the rubber isolates PSTVd-Rubber-KER-3, PSTVd-Rubber-KER-4, PSTVd-Rubber-KER-7 and PSTVd-Rubber-KER-6 clustered together as one group. These isolates along with PSTVd-Rubber-KER-1, KER-2, KER-5 formed a part of the major cluster comprising PSTVd potato isolates from Poland and Iran. However, PSTVd-KER-8 isolate occupied a branch separate from other rubber isolates (Figure 3).

Table 2. Details of PSTVd-rubber viroid characterized in the present study

Rubber sample ID	Clone ID	Length of genome (bp)	GenBank accession no.
19 Abt	PSTVd-Rubber KER1	359	HM107843
44 Abt	PSTVd-Rubber KER2	361	HM107844
95 PSTV	PSTVd-Rubber KER3	361	HM107845
6/15 PSTV	PSTVd-Rubber KER4	361	HM107846
2 PSTV	PSTVd-Rubber KER5	361	HM107847
4/4 PSTV	PSTVd-Rubber KER6	359	HM107848
50 Abt	PSTVd-Rubber KER7	359	
6/15 Abt	PSTVd-Rubber KER8	359	

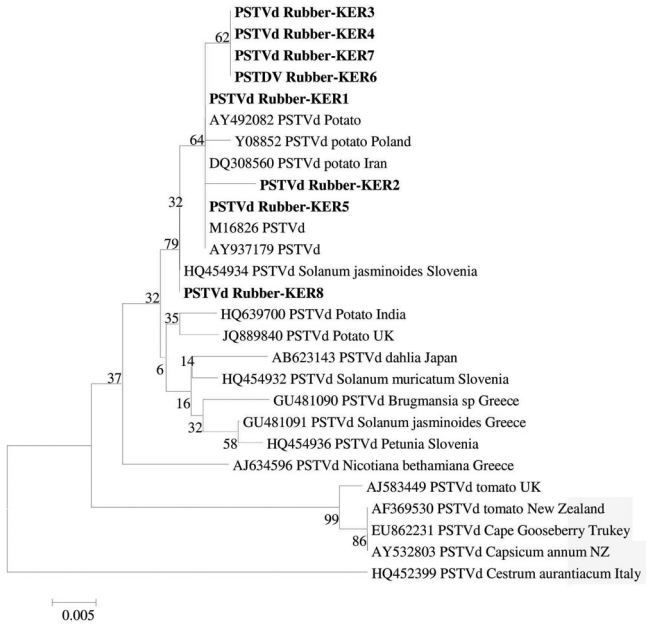

Figure 3. Phylogenetic relationship between viroid populations. Dendrogram constructed using the neighbour joining method with 1000 replications of bootstrap based on alignment of full-length nucleotide sequences. Alignments were produced with Clustal W MEGA 5. Numerals at the nodes refer to the number of times the branching was supported. The sequences of PSTVd isolates included in the analysis are given with their respective accession numbers.

Table 3. Nucleotide identity between PSTVd-Rubber-KER1 isolate and other PSTVd isolates

Viroid	PSTVd-Rubber-KER1 (%)
PSTVd-Rubber-KER1	100
PSTVd-Rubber-KER2	98.8
PSTVd-Rubber-KER3	99.1
PSTVd-Rubber-KER4	99.1
PSTVd-Rubber-KER5	100
PSTDV-Rubber-KER6	97.2
PSTVd-Rubber-KER7	100
PSTVd-Rubber-KER8	99.1
AY492082 PSTVd Potato, Poland	99.1
M16826 PSTVd	100
AY937179 PSTVd	100
HQ454934 PSTVd *Solanum jasminoides*, Slovenia	99.1
Y08852 PSTVd potato, Poland	99.4
DQ308560 PSTVd potato, Iran	99.4
GU481090 PSTVd Brugmansia sp., Greece	98
AB623143 PSTVd dahlia, Japan	95.3
AJ634596 PSTVd *Nicotiana bethamiana*, Germany	97.5
AJ583449 PSTVd tomato, UK	93.8
AF369530 PSTVd tomato, New Zealand	93.8
EU862231 PSTVd Cape Gooseberry, Trukey and Germany	93.8
AY532803 PSTVd *Capsicum annum*, New Zealand	93.6
GU481091 PSTVd *Solanum jasminoides*, Greece	95.5
HQ454936 PSTVd Petunia, Slovenia	95.2
HQ639700 PSTVd Potato, India	97.5
JQ889840 PSTVd Potato, UK	97.5
HQ454932 PSTVd *Solanum muricatum*, Slovenia	96.9
HQ452399 PSTVd *Cestrum aurantiacum*, Italy	91.9

Radiolabelled probe prepared from cloned DNA of TPD-affected rubber was used in nucleic acid spot hybridization test to detect the presence of viroid in field samples of rubber. The autoradiogram (Figure 4) showed the presence of viroid in TPD-affected samples in 1 : 1 and 1 : 10 dilution. Results showed that the radiolabelled probe could detect the presence of viroid in TPD-affected samples both in plant and nucleic acid extracts from rubber and TPD inoculated tomato. However, the intensity of the signal was low in some samples (Figure 4, lanes 5, 6, 8 and 9), which is attributed to the difference in the concentration of the viriods.

Melting profile analysis performed at the completion of amplification revealed T_m peaks of 88.29 ± 1°C for the plasmid DNA and for samples from TPD-affected rubber samples. The profile was similar in both confirming the presence of the same LMW RNA in all the samples. The healthy control did not show any increase in fluorescence, indicating the absence of non-specific amplification. The high C_t value varied from 27.79 to 29.30, which clearly indicated low concentration of viroid in the samples tested (Figure 5).

Reports on the biotic etiology of TPD of rubber are rather scanty. A recent report from our laboratory[23] has demonstrated the constant association of a LMW RNA with samples of TPD-affected rubber tissue (leaf, bark and root) and has further shown it to be infectious on inoculated young seedlings of tomato. The LMW RNA was reisolated from inoculated symptomatic tomato leaves, which further infected healthy tomato. However, its infectivity on rubber seedlings remains to be demonstrated.

The present report confirms that the LMW RNA isolated is a viroid, a distinct variant of the most widespread PSTVd. Although in our study we found tomato to be an experimental host of the rubber isolate, it is quite different from the naturally occurring PSTVd isolate infecting tomato (AJ 583449 from UK). The variations that have been observed in terms of addition and deletion of nucleotides are all either in the pathogenicity or the variable domains of the viroid, which can perhaps explain the altered pathogenicity of PSTVd to infect totally a new host causing the very characteristic symptoms on this host rubber. In recent years, PSTVd has been recorded from a wide variety of ornamental and horticultural plants[28]. Infection of tree species by PSTVd been recorded in avocado[29]. Incidentally, tomato and avocado have been reported as new emerging hosts of PSTVd in recent times[30]. Our results of multiple alignment of sequences of the clones of rubber with those of PSTVd on other hosts revealed only minor variations pertaining to one or more nucleotides. It is well known that a small variation in the case of viroid is sufficient to change the pathogenicity, virulence and host preference[31,32]. In the case of PSTVd, it was observed that even a single nucleotide substitution (C–U in position 259) changed the PSTVd from a non-infectious to an infectious RNA capable of infecting tobacco[33]. Similar variations have been reported for mild and severe variants of citrus exocortis viroid (CEVd)[34], and for CEVd variants infecting citrus and tomato in India[35]. In the present study, it was found that the rubber isolates show 97.5% identity with PSTVd

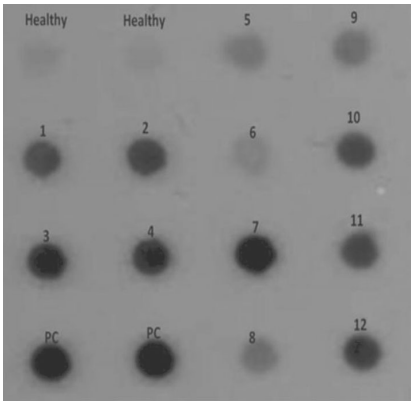

Figure 4. Results of nucleic acid spot hybridization test-^{32}P-labelled cDNA probe from rubber viroid clone was used to detect viroid in field samples of rubber L-R healthy (H) (24H), plasmid control positive control 4/4 TPD plasmid clone 1, 19 TPD; 2, 44 TPD; 3, 6/15 TPD (leaf); 4, 6/15 TPD (bark); 5, 95 TPD; 6, 9 TPD; 7, 2 TPD (leaf); 8, 50 TPD; 9, 4/4 TPD; 10, 2 TPD (bark); 11, 6/15 TPD (tomato inoculated) and 12, 2 TPD (tomato inoculated).

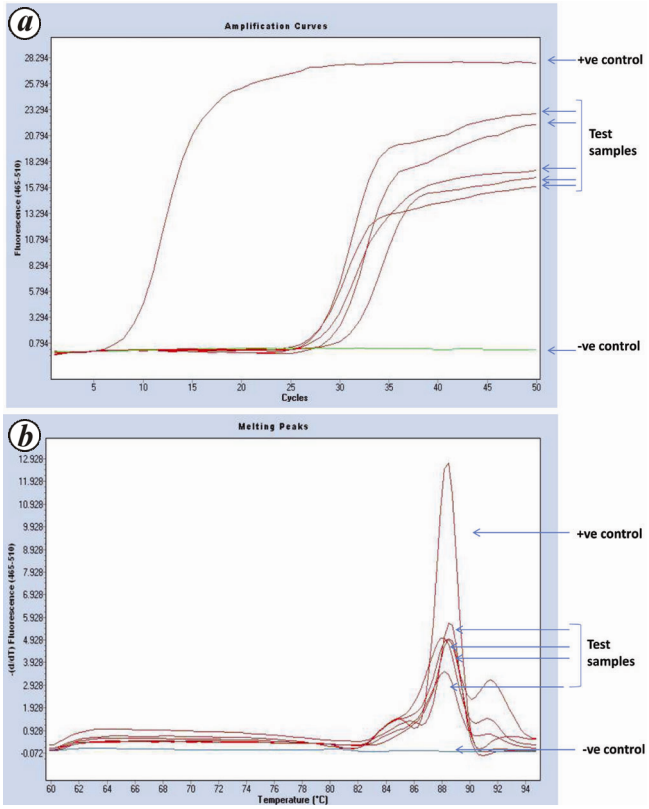

Figure 5. Amplification plot (***a***) and melting profile (***b***) generated by real-time PCR reactions performed with cDNA from TPD-affected test samples (2, 4/4, 19, 44, 50). RNA extract from healthy rubber samples served as negative control. The reaction was performed with 50 ng of plasmid DNA of the clone PSTVd-Rubber-KER1 as positive control.

reported on potato from India (seq. HQ639700). Apparently, the minor sequence variation coupled with host preference and other geographical conditions in which the two hosts are growing has possibly influenced pathogenicity of the viroid. At present, it is difficult to understand how PSTVd can be associated with a unique symptom that specifically impairs the function of laticiferous cells. However, symptoms of bark cracking, bulging and other abnormalities observed with TPD-affected plants are known to be associated with viroid infection in tree species[36]. Higher frequency of occurrence with high-yielding varieties/clones and vegetative propagation of the crop and seed transmission are some attributes of viroid diseases which rubber exhibits.

Our study thus confirms association of a PSTVd variant with the TPD syndrome of rubber, a century-old problem worldwide. It is proposed to designate the causal agent of TPD of rubber as PSTVd-Rubber-KER variant. That the cloned DNA could be used as a probe was also shown when PSTVd as a probe in dot-blot tests reacted specifically to nucleic acid extracts from TPD-affected rubber, but not from healthy trees. In the present study also, the cloned DNA was successfully used in spot hybridization for viroid detection from rubber samples. This will certainly prove to be a useful diagnostic tool for detection of the pathogen in planting material and help the orchardist in management of the problem. The study merits further investigations to prove the pathogenity of the isolated viroid on rubber in order to conclusively determine if the entire panel dryness symptom is caused by PSTVd alone, or there is involvement of other viroids/factors.

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Technology for rehabilitation of Yamuna ravines – cost-effective practices to conserve natural resources through bamboo plantation

A. K. Singh[1,*], S. Kala[1], S. K. Dubey[1], V. C. Pande[2], B. K. Rao[2], K. K. Sharma[1] and K. P. Mahapatra[3]

[1]Central Soil and Water Conservation Research and Training Institute, Research Centre, Chhalesar, Agra 282 006, India
[2]Central Soil and Water Conservation Research and Training Institute, Research Centre, Vasad 388 306, India
[3]ICAR Research complex for NEH Region, Shillong 793 001, India

The present study evaluated bamboo (*Dendrocalamus strictus*) based resource conservation in the Yamuna ravines at Central Soil and Water Conservation Research and Training Institute, Research Centre, Agra, Uttar Pradesh, India. Ravine lands are highly degraded dry lands and 3.97 m ha area is affected by ravines in India. One ravine micro watershed of 2.8 ha area was planted with two rows of bamboo in staggered manner as vegetative barrier for the analysis of hydrological and economic aspect of bamboo plantation. Hydrological results showed that runoff has been reduced from 9.6% to 1.8% and soil loss from 4.2 to 0.6 t/ha/year in the last 4 years. Based on bamboo growth performance, average value of culm height and culm collar diameter have been recorded as 3.80 m and 22.50 mm, the value of average crown size and number of culms per clump being 3.93 m and 18 numbers respectively. Further, the soils under bamboo plants improved in terms of decreased pH and enhanced soil organic carbon. The economic analysis suggested a cash outflow of Rs 48,000 ha^{-1} from 7th year onwards to the stakeholders in the region, in addition to the benefits accrued to society at large in terms of value of nutrient (Rs 2125–5555 ha^{-1}) saved through soil conservation. This study recommends bamboo plantation for productive and protective utilization of such degraded lands. It also suggests that the high cost of establishment for individual stakeholders can be met through subsidies and banks' financial inclusion programme in developing countries such as India. Further, public funding can also be routed through appropriate budgetary provisions in development plans of corporate entities involved in the rural development in the country.

Keywords: Bamboo plantation, degraded land, economic analysis, financial analysis, ravines.

RAVINE lands are highly degraded dry lands and 3.97 m ha area is affected by ravines in India. In Uttar Pradesh alone, 1.23 million ha (33.5%) of land is occupied by ravines, which are mainly found along the bank of river Yamuna and its tributaries. The severity of water erosion is found at the peak along the banks of Yamuna and Chambal rivers in the districts of Agra, Etawah, Kanpur, Fatehpur, etc. where terrain has completely deformed into ravines. Rao *et al.*[1] reported that stream bank erosion is a major cause of land degradation, leading to deteriorated drainage systems, which ultimately govern natural calamities in terms of floods, and non-point source pollution in ravine lands of India. Vegetation in these ravine regions suffers from a variety of unfavourable conditions such as nutrient deficiency, moisture stress and biotic interference. The inclement weather conditions coupled with very high summer temperature further aggravates the problem and makes farming uneconomical. In such situations, less water and nutrient demanding technologies hold a good promise to sustain the productivity and provide alternative source of income to the farmers. This vast tract of existing ravine lands poses potential threat to nearby productive lands because of overexploitation and poor management. Therefore, there is an urgent need to arrest degradation of these

*For correspondence. (e-mail: aksingh_1962@yahoo.co.in)