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REVIEW ARTICLE

The discovery and eradication of potato spindle tuber viroid in Canada

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Abstract In 1960s, potato spindle tuber was thought to be a viral disease. In 1971, the agent of the disease was characterised as a low-molecular weight infectious ribonucleic acid (RNA), which was named as 'viroid', specifically *Potato spindle tuber viroid* (PSTVd)). Since then, more than 30 plant diseases in horticultural and ornamental plants have been shown to be caused by different viroids globally. Viroids are single-stranded RNA, covalently closed circular molecule, without any protein coat. They are the smallest known plant pathogen containing RNA genome ranging from 246 nucleotides (*Coconut cadang–cadang viroid*) to 399 nucleotides (*Chrysanthemum chlorotic mottle viroid*). Some viroids are located in the plant cell nucleus (pospiviroids) and others in the chloroplast (avsunviroids). With the recognition of pathogenic nature of viroid, specific detection methodologies were developed, which enabled detection of PSTVd in seed-potato tubers prior to their planting in the field, and thus PSTVd was prevented from spreading the disease. As a result, PSTVd was eradicated from Canada in late 1980s. Viroids similar to PSTVd (Pospiviroid) have been discovered and they are detected in symptomless ornamental plants. Although, PSTVd has been eradicated from Canada, there is a strong possibility of viroid introduction from other plants besides potato and tomato and causing PSTVd like diseases.

Keywords Potato Spindle tuber viroid · Discovery of viroid · Eradication of PSTVd

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Introduction

Viroids disguised as "viruses" have probably existed as long as the plant viruses, considering that they do not differ significantly from viruses in their pathological effects on plants or in their modes of natural transmission [77]. Relative to virus, viroid is a newly described plant pathogen. Discovery of a novel class of pathogen is quite rare. Economic devastation through crop failures or significant losses in quality of agricultural products for human consumption takes place prior to attracting the attention of researchers such as plant pathologists. The discovery of viroids was no exception in that their presence remained undetected until they had plenty of impact on agricultural and fruit crops. Approximately a century ago (1917), Werner in Nebraska [91] was working on a degeneration disease of potato (probably potato spindle tuber). However, the first report of the disease and the use of name "spindle tuber" are credited to Martin [34]. Unaware of the naming of the disease by Martin, Schultz and Folsom [53] demonstrated the transmissibility of what they termed "spindling tuber" disease (Fig. 1). This disease was studied in New Brunswick, Canada, as early as 1925 by MacLeod [33]. Potatoes in the USA and Canada have suffered the outbreaks of potato spindle tuber virus (PSTV) with infection as high as 25–50 %. Low yields and long-pointed tubers with an excessive number of deep eyes were characteristic symptoms of the novel disease as described from the several areas of USA and Canada. In Canada potatoes exhibiting characteristic symptoms were observed from 1918 to 1921 mainly in the province of New Brunswick and in subsequent years in other provinces of Canada. Besides North America, in the 1960s and 1970s over half of the plants in some stands were infected in certain states of the Ukraine [27] and in China [42]. However, it was the

Fig. 1 Potato plant with PSTVd infection. Upright growth and upward pointed leaves are the identifying symptoms in potato plants. The tubers are spindly with deep cracks and unfit for human consumption

high rejection rate of the potato-seed fields due to PSTV in the mid-1950s in Canada [32] which led to the renewed interest in the study of PSTV in Canadian and US laboratories.

On a personal note, my journey into the world of PSTV began in 1962 as I was accepted into PhD program at North Dakota State University, Fargo, USA and was assigned the PSTV as my research subject. By mid 1960s, two government laboratories with long-time virus-researchers [13, 59] and four universities with PhD students [1, 28, 68, 92], were investigating the potato spindle tuber disease in the northeast USA and Canada. In 1964 and 1966, investigators from Nebraska and North Dakota in USA claimed that rod-shaped [1] or spherical-particles [68] were associated with the spindle tuber disease. Although, both claims later turned out to be wrong, these failures compelled us to try other approaches. Finally, the PSTV was identified as a unique phytopathogen named as *Potato spindle tuber viroid* (PSTVd). In this review, the discovery of PSTVd and its eradication in Canada are summerasied.

Viroid discovery

After completing my studies at North Dakota State University in early 1966, I joined the Canada Department of Agriculture as a National Research Council, post-doctorate fellow, to continue work on PSTV with Dr. R.H. Bagnall. By 1967–1968, it became clear to us, that the agent causing potato spindle tuber disease was not a conventional "virus" but a unencapsidated free ribonucleic acid (RNA) [13, 59]. Continued investigations in the two laboratories, one in USA [11] and the other in Canada [62], using the density-gradient centrifugation and the mobility in polyacrylamide gel electrophoresis showed that the infectious entity of PSTV was an extremely low-molecular weight RNA, compared to the conventional viral RNA [11, 62]. For the infectious RNA, names like 'viroid' [11] or "low-molecular weight RNA" [62] or "metavirus" [25] were used. After a few discussions at the Potato Association of America meetings, the "viroid" name was accepted.

Viroid discovery controversy

Viroid, being one of the newest discovered plant pathogen, was extensively reviewed in the early 1970–1980 s. For example, within the first 12 years of discovery there were 48 review articles dealing with viroid [58]. About 80 % of these articles were contributed by two groups, namely; T.O. Diener and associates (60 %) (USA) and H. L. Sänger and associates (20 %) (Germany). As high as 10 reviews were published in the years 1979 and 1983. However, most of the reviews by Diener have made an issue of the viroid-discovery aspect. Although the viroid or low-molecular weight nature of PSTVd was demonstrated by Diener [11], and Singh and Clark [62] in 1971, and has been referred in some books and review articles dealing with viroids and additionally pointed out to Diener the "real" circumstances of discovery in exchanges of *Letter to the Editor to Plant Disease* [56, 57], the treatment of our work has been either omitted or distorted by Diener. For example, in his review article 30 years later [12], he made misleading statements and quoted non-existent '*Singh personal communication*' as the evidence. Finally, he has eliminated the reference of our 1971 paper [62] altogether from the Table 1 in the same 2001 review article by his 'arbitrary' decision. Therefore, the circumstances dealing with the viroid discovery have been elaborated below.

Our first paper reporting the isolation of RNA from PSTVd infected-plants was accepted for publication in *Phytopathology* in 1967 [59]. It was in reviewer's hand before the publication of Diener and Raymer [13] paper. The reviewer of our paper, in this case happen to "see" a galley-proof of Diener and Raymer's [13] paper. To our amazement, this reviewer recommended to the editor of the Phytopathology that "…… Singh and Bagnall, (1967) manuscript [59] is acceptable for publication (29 November 1967) but

Table 1 Families, Genera and Species of viroids

Families	Genera	Species
Avsunviroidae	Avsunviroid	Avocado sunblotch viroid
	Elaviroid	Eggplant latent viroid
	Pelamoviroid	Chrysanthemum chlorotic mottle viroid Peach latent mosaic viroid
Pospiviroidae	Apscaviroid	Apple dimple fruit viroid
		Applefruit crinkle viroid
		Apple scar skin viroid
		Australian grapevine viroid
		Citrus bent leaf viroid
		Citrus viroid III
		Citrus viroid V
		Citrus viroid original source
		Grapevine yellow speckle viroid 1
		Grapevine yellow speckle viroid 2
		Grapevine yellow speckle viroid 3
		Pear blister canker viroid
		Persimmon viroid
	Cocadviroid	Coconut cadang-cadang viroid
		Coconut tinangaja viroid
		Citrus bark cracking viroid
		Hop latent viroid
	Coleviroid	Coleus blumei viroid I
		Coleus blumei viroid II
		Coleus blumei viroid III
		Coleus blumei viroid IV
		Coleus blumei viroid V
		Coleus blumei viroid VI
	Hostuviroid	Hop stunt viroid
	Pospiviroid	Chrysanthemum stunt viroid
		Citrus exocortis viroid
		Columnea latent viroid
		Irsine viroid 1
		Mexican Papita viroid
		Pepper chat fruit viriod
		Potato spindle tuber viroid
		Tomato apical stunt viroid
		Tomato planta macho viroid
		Tomato chlorotic dwarf viroid

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"I" would strongly urge that the authors withhold submitting until they had an opportunity to evaluate more recent results….. the results which I have mentioned". This "unusual recommendation" by a reviewer of *Phytopathology* clearly shows that our work [59] was done independently and at the same time as *Diener and Raymer's* [13].

Again in 1971, our continuing research demonstrating that the PSTVd was a low- molecular weight infectious RNA [62], was presented at a meeting of the *American Phytopathological Society* on August 17, 1971, in Philadelphia, USA. The abstract of this paper could have been available to the society members earlier than the 17th of August. The fact that Diener was aware of our work in advance of its publication was shown in US press release (source, Washington UPI) and published in Toronto Telegram (Canada), and St. Luis Post-Dispatch (USA), dated August 14, 1971. The release stated: "Agriculture officials (USDA) hurried the announcement of Diener's discovery [11] yesterday because a similar announcement is expected from a Canadian scientist next week. Officials said Dr. R. P. Singh of the Canadian Department of Agriculture was planning to deliver a paper on the subject at a meeting in Philadelphia." In light of the above statement, it is unfortunate that later reviews by Diener put a different slant on the discovery of viroid.

Viroid, a novel phytopathogen

At present more than 30 plant diseases [16–18] have been recognized as being caused by viroids in agricultural, horticultural and ornamental plants throughout the world (Table 1).

Viroids are molecularly a novel class of plant pathogen compared to viruses, where they were previously grouped. Viroids are considered as the most efficient and sophisticated 'molecular plant parasites' apparently shedding the need to synthesize structural or functional proteins by manipulating host enzymes for their replication and are capable of destroying trees, including coconut palms [44]. Viroids are highly contagious, particularly members of the genus *Pospiviroid* [77]. Some viroids have a wide host-range and are transmitted readily by mechanical means, including foliage contact, handling during cultivation, and contamination of cutting knives or other tools, e.g., 80 % of potato plants were infected by PSTVd when infected leaves were rubbed in the field against healthy plant leaves [40]. All viroids are transmissible by grafting, while a number of reports are for transmission through seeds and pollen of the infected plants [3, 55, 65, 71, 72, 73, 87]. There are a few reports of transmission of pospiviroid by insects [10, 21]. Despite the free RNA nature of viroids, PSTVd can survive in freeze-dried tomato leaves at room temperature for several years [66] or in true potato seeds for over 20 years [72]. Most cultivated plants have no natural resistance to viroid diseases and transgenic plant approaches have had limited success [8].

Although viroids multiply faster at high temperatures and under high light-intensities [8, 48], however, repeated exposures to freezing temperature has also been found to eliminate PSTVd from potato tubers [61] and Tomato chlorotic dwarf viroid (TCDVd), which is closely related to

PSTVd, can flourish in symptomless plants of *Vinca minor* at subzero temperatures (−12 °C) [65].

Besides PSTVd, the low-molecular weight nature of citrus exocortis viroid (CEVd) was reported in 1972 in Germany [47]. Within 5 years of viroid-discovery in North America, the German researchers elucidated the complete nucleotide sequence of PSTVd and titled their paper as "Viroids are single stranded covalently closed circular RNA molecules existing as highly base-paired rod-like structures" [49]. By the year 1982 the German team had described other structural properties of viroids [22–24, 26, 45]. They showed that the viroids consist of a single unit of single-stranded, covalently closed, circular RNA that ranges in size from 246 to 399 nucleotides, depending on the viroid species. The nucleotide chain length of various strains of a viroid could vary 2–5 nucleotides in length, for example in PSTVd [26], CEVd [86] and hop stunt (HSVd) [50].

The German team also provided the details of viroid structure and function. They demonstrated that due to the extensive regions of intra-molecular complementarity a viroid molecule under native conditions is double-stranded and rod-like, but when denatured, it becomes a circular, single-stranded molecule. The circles have been shown to elongate and form a defective double-helix, in which double helical segments are separated by short unpaired stretches. In conditions where free energy is low, viroids form a rod-like native conformation as a secondary structure, in which loops and bulges of unpaired nucleotides separate double-stranded regions. The secondary structure is assumed to be the key for the biological activity by being functional as such or by providing binding signals to specific host factors.

The rod-like conformation has been shown to contain five structural domains based on nucleotide sequence analysis [30]. These domains are referred to as central (CD), pathogenic (PD), variable (VD), terminal left (TLD) and terminal right (TRD). Initially, the domains were presumed to have individual functional roles. However, later it was shown [80] that pathogenicity was not only controlled by PD but also by TLD, VD and TRD. In our studies, a seven-nucleotide substitution in the TRD has been correlated with severe stunting symptoms of potato and tomato plants (Fig. 2) infected with a strain of TCDVd [80]. In addition, it has been shown that even substitutions of single nucleotide in the lower CD could have a dramatic impact on pathogenicity [88]. Besides the rod-like structure, members of the family *Pospiviroidae* can form metastable secondary structures, e.g., hairpins, tetra-loops and stems. Many members of *Pospiviroidae* have been shown to contain an internal loop in the CCR, which appears similar to the loop E of the eukaryotic 5S rRNA [2, 6]. The E-loop is involved in the synthesis and transport of 5S rRNA. A similar role has been assumed for the E-loop-like structure of the *Pospiviroidae* [2].

Fig. 2 Tomato chlorotic dwarf viroid in a greenhouse tomato production system (From Singh et al. 1999)

An orderly grouping of different viroids in genera and families, based on their genome structure and replication features has been established [9]. Viroid species have been divided into *Pospiviroidae* and *Avsunviroidae* families. The viroids, which possess specific sequences within their molecular structure, known as central conserved region (CCR) and are located in the plant cell nucleus, are in the *Pospiviroidae* and those viroids which possess a hammerhead ribozyme structure and are located in the plant chloroplast are in the family *Avsunviroidae*. The PSTVd has been recognized as a distinct species of viroid under the genus *Pospoviroid*, class *Pospiviroinae* and family *Pospiviroidae*.

Both groups of viroids have different replication modes. In *Pospiviroidae* the replication takes place in the nucleus. The replication pathway is an asymmetrical cycle, and the enzyme involved is DNA dependent RNA polymerase II and no self-cleavage is involved. The steps involved are as follows: The (+) circular RNA is transcribed into an oligomeric linear (-) strand RNA. The (-) strands then serve as intermediate for the synthesis of oligomeric (+) strand RNAs, which are cleaved into unit length monomers and then ligated into circles, the active viroid molecule [5]. Members of the family *Avsunviroidae* have a quasi-rod like or branched-secondary structure. They replicate in chloroplast by a symmetric rolling circle mechanism using chloroplastic encoded RNA polymerase and are self-cleaved via hammerhead structures [9]. Beside this simple outline, different enzymes are involved in transcription, cleavage and ligation and have not been confirmed.

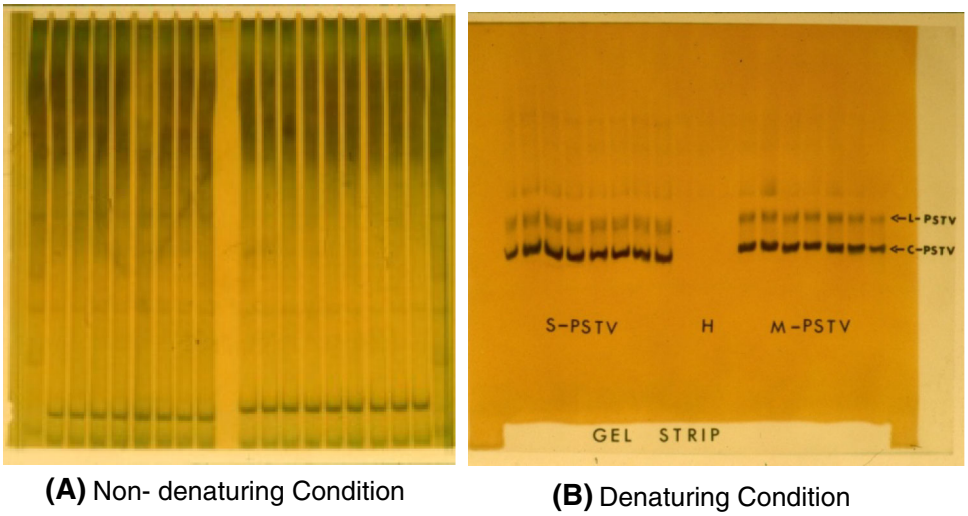

Fig. 3 Return-Polyacrylamide Gel Electrophoresis. First electrophoresis run is in non-denaturing gel (**a**). Lower part of gel-strip is cut out and placed in the bottom of the gel (**b**). Polarity is reversed and electrophoresis is run as before but at higher temperature

Methodologies for viroid detection

Unlike viruses, viroids are devoid of a protein-coat and they do not contain the machinery for protein synthesis. Therefore, the serological detection methods commonly used for viruses are not applicable to viroids. The polyacrylamide gel electrophoresis (PAGE), which initially enabled successful separation of viroids from other cellular constituents or from host RNAs [11, 62] was modified for large-scale diagnosis of PSTVd in various laboratories [19, 36, 37, 40, 41, 52, 60, 69, 73]. Extensive use of PAGE technique for viroid detection resulted in many modifications [52, 60, 69, 73]. The latter two modifications are known as "return" polyacrylamide gel electrophoresis (R-PAGE) [69, 73]. In R-PAGE the viroid molecules are first separated on 5 % non-denaturing gels in high salt buffer (Fig. 6). After the first electrophoresis, the buffer is exchanged for a heated (87–90 °C) low-salt buffer [69]. The polarity is reversed and a second electrophoresis is performed at 70–71 °C. The separation of viroid from other nucleic acids is achieved by the slower mobility of the denatured viroid molecules (Fig. 3). Extensive evaluation of R-PAGE using mild and severe PSTVd strains-infected potato leaves, tubers, pollen and true-potato seeds, and seeds from coleus infected with *coleus* viroids, showed that viroids can be reliably detected from individual seed despite the small seed size [19, 68, 73]. The R-PAGE detection technology was also adopted in Brazil [43], China [65] and India [39] to survey for the viroids from crop plants and ornamentals.

Although a sensitive technique for the detection of viroids was available [90] based on nucleic acid hybridization using radioactive-labelled probes, the use of radio-labelled probe is limited to centers with facilities which can handle storage and waste disposal of radioactive material. Therefore, highly sensitive digoxigenin-labelled DNA or RNA probes were developed [4, 75]. However, in addition to the R-PAGE and radiolabelled or digoxigenin probes, at present RT-PCR based methods for viroid detection are available [64, 78]. These RT-PCR methods can detect several members of a group. For this purpose RT-PCR based primer pairs are designed which can amplify more than one viroid [78] irrespective of the host species and viroid family (Fig. 4). The effectiveness of the primer pair for members of genus *Pospiviroid* has been demonstrated by the detection of PSTVd and TCDVd in potato; CSVd and IrVd in *Verbena* and *Vinca* species and CEVd from *Impatiens* [78]. Similar methods are also available in other countries [64]. To facilitate and simplify the viroid extraction from plant parts without organic solvents, NaOH-EDTA solution based protocol for viroid preparation from crop and ornamental plants has been developed. In this protocol plant tissues are homogenized in NaOH-EDTA (50 mM + 25 mM EDTA) with a tissue to solution

Pospiviroid	Forward	5' ATT	AAT	CCC	CGG	GGA	AAC	CTG	GAG 3'
PSTVd		---	---	---	---	---	---	---	---
TCDVd		---	---	---	---	---	---	---	---
TPMVd		---	---	---	---	---	---	---	---
MPVd		---	---	---	---	---	---	---	---
CSVd		---	---	---	---	---	---	---	---
CEVd		---	---	---	---	---	---	---	---
TASVd		---	---	---	---	---	---	---	---
IRVd		---	---	---	---	---	---	---	---
CLVd		--G	---	---	---	C--	---	TCA	--C

Pospiviroid	Reverse	5' AGC	TTC	AGT	TGT	TTC	CAC	CGG	GT 3'
PSTVd		---	---	---	---	---	---	---	---
TCDVd		---	---	---	---	---	---	---	---
TPMVd		---	---	---	---	---	---	---	---
MPVd		---	---	---	---	---	---	---	---
CSVd		---	---	---	---	---	---	---	---
CEVd		---	---	---	---	---	---	---	---
TASVd		---	---	---	---	---	---	---	---
IRVd		---	---	---	---	---	---	---	---
CLVd		CCA	C--	GA-	G--	---	---	---	---

Fig. 4 A universal *Pospiviroid* primer pair, designed to amplify most viroids of the group (Bostan et al. [4])

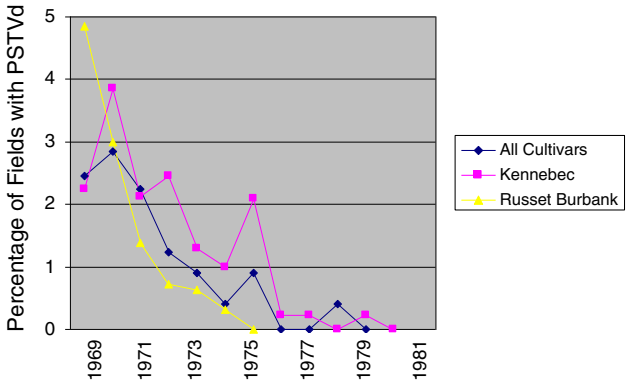

Fig. 5 Eradication of the PSTVd from Canada. Since visual observation of PSTVd is not reliable in all potato cultivars, three types of leaf samples were tested. All the samples were tested in the prescribed laboratories [64]

ratio of 1:4 (W/V) and incubated at room temperature for 15 min to settle the coarse plant material then the supernatant is used for RT-PCR [78].

Eradication of PSTVd from Canada

In Canada, a multifaceted eradication methodology was employed involving following steps: (1) Compulsory pre-planting testing of seed-potatoes for viroid to be used in commercial planting. (2) Establishment of specialized laboratories to carry out R-PAGE testing of seed potatoes throughout the country. (3) Requirement of viroid-testing of all parental material prior to their use in potato-breeding programs and (4) Viroid-freedom of the new cultivars before their release to potato seed growers. As a result PSTVd was eradicated from potato crops in Canada [64, 70] (Fig. 5). An analysis of the field inspection data over a period of 1969–1983 of seed potato crop in selected areas of Canada showed that the incidence of PSTVd by late 1980s had decreased to the point where it could not be detected by visual observation as well as by R-PAGE test. This eradication of PSTVd in the seed potato crop was attributed to the higher standards and stricter regulations in seed certification programs, use of viroid-free seed multiplied at Elite seed farms, enactment of provincial disease eradication acts and strict planting requirements by processing and seed growers in the region. With the eradication of PSTVd trade to customer countries resumed in the late 1980s.

Looming threats to crops from viroids in symptomless ornamental plants

Although PSTVd has been eradicated from Canada, there is a strong possibility of viroid introduction from other plants

Table 2 Pospiviroids and their economic and ornamental host plants

Viroids	Economic hosts	Ornamental hosts
Citrus exocortis viroid (CEVd)	Citrus	Glandularia pulchella
		Impatiens sp.
		Solanum jasminoides
		Verbena sp.
Potato spindle tuber viroid (PSTVd)	Potato	Brumansia × candida
	Tomato	Brugmansia × flava
		Brugmansia sanguinea
		Brugmansia suaveolens
		Calibrachoa sp
		Datura sp.
		Lycianthes rantonnetii
		Petunia sp.
		Solanum jasminoides
		Streptosolen jamesonii
Tomato apical stunt viroid (TASVd)	Tomato	Cestrum sp.
		Lycianthes rantonnetii
		Solanum jasminoides
		Solanum psecapsicumuedo
		Streptosolen jamesonii
Tomato chlorotic dwarf viroid (TCDVd)	Tomato	Brugmansia sanguinea
		Pittosporum tobira
		Verbena sp.
		Vinca minor

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besides potato and tomato and causing PSTVd like diseases. For example, CEVd [76], and a viroid from *Nematanthus wettsteinii* plants, closely related to CLVd [74] have been shown to cause PSTVd like symptoms in potatoes. Recently, another pospiviroid, named Tomato chlorotic dwarf viroid (TCDVd), a viroid closely related to PSTVd was identified from the commercial greenhouse tomato Fig. 2 crops in Canada [76]. Finding of TCDVd in Canada was soon confirmed in other countries. For example TCDVd, infecting tomatoes in the Netherlands [84], Japan [35], USA [31] France [7] and Norway [20]. In addition to the infection of tomato plants, TCDVd has been isolated from symptomless ornamental plants from India [79], Netherlands [85], UK [29] and Japan [54] in the last few years. These rapid findings of TCDVd in tomato greenhouse crops and in open fields in ornamental plants

pose serious problems in keeping crops like potato and tomato free of viroids. Particularly the occurrences of TCDVd in tomato greenhouses could be due to the cultivation practices used at present in the greenhouses throughout the world. For example, continuous tomato production year round, tall plants, repeated pruning of new plant shoots during cultivation, high humidity and high temperature in the greenhouses provide ample chances of viroid contamination, survival and spread. However, the findings of TCDVd in symptomless ornamental plants [15, 29, 54, 65, 85] needs additional studies to determine the sources of viroids in the area. As shown (Table 2) at present the known pospiviroids have been detected from many more ornamental plants compared to the crop plants. It has also been shown that compared to PSTVd, the CLVD strains from ornamental plants can cause significant losses of potato tuber yields. Although most of the ornamental plants do not show symptoms when infected with viroids, they do cause severe symptoms, when transferred to potato and tomato plants and tubers (Fig. 6).

Fig. 6 Four symptomless Verbena selections, infected with chrysanthemum stunt, citrus exocortis or iresine viroids (*top panel*), when transferred to potato plants (only two potato plants are shown), caused severe stunting symptoms. Tubers were badly deformed, spindly and small (Nie et al. 2005)

Viroid as molecular tool

Attempts to isolate viroids from their host plant-tissues resulted in a new method of R-PAGE [52], which is increasingly used for viroid detection from plants [39, 43, 69, 73]. Similarly, another method of gel-electrophoresis, known as temperature-gradient gel electrophoresis (TGGE) [46] developed during the characterization of PSTVd, is used for determining the viroid conformal transactions, sequence variations and protein-nucleic acid interactions [46]. These by-products of viroid research have become the new tools for viroid characterization. Similarly, viroid replication strategies in their hosts have shown that both Pospiviroids and Avsunviroids, have to be transported into nucleus (Pospiviroids) and to the chloroplast (Avsunviroids) prior to their replication. In addition, the enzymes involved in viroid replication are host-coded. How the viroids manage these significantly important aspects of their movement and replication using enzymes from the host plants needs elucidation of the mechanisms. Understanding of this mode of pathogenicity would be a new tool for the plant molecular biology at large. Knowledge of how viroids accomplish these feats would go long way to show their real uniqueness as a plant pathogen. On these aspects, questions have been posed by viroid researchers dealing with the movement and replication [38]. What are the molecular signals from viroids, which make host enzymes to accept viroids as templates for the synthesis of complementary RNAs? [38]. It is worth noting, that the evidence of RNA-directed DNA methylation was discovered in tobacco plants, that contained multimeric genome-integrated copies of PSTVd cDNA [5], which is another tool to expand our understanding of viroid pathogenicity and may extend to other pathogens and to gene-silencing in host plants.

Concluding remarks

Transition from the dark phases of PSTV characterization in 1960s, to the viroid discovery in 1970s, created a competitive environment in viroid research. Just to illustrate how high was the "publication" pressure during that period, that some researchers were not thoroughly confirming their results before publication. For example, when one reads the variable molecular weight values of PSTVd from the same laboratory, one gets the idea of hurried publication path. For example, the molecular weight of PSTVd in 1971was 50,000 daltons [14], in 1973 it was 75,000–85,000 daltons [15], or 80–90,000 daltons [81] in North American publications. However, it was finally shown to be 127,000 daltons by European researchers in 1976 using novel technology [22]. The knowledge about the viroids has increased many-fold since then. However, more has to be done to learn how viroids manipulate the host plants to their advantage. Such knowledge could open up new avenues of disease management.

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