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Secondary structures of *Potato spindle tuber viroid* variants detected in Slovenia

Sekundarna struktura v Sloveniji najdenih genotipov viroida vretenatosti krompirjevih gomoljev

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Abstract: A survey of *Potato spindle tuber viroid* was initiated in Slovenia in 2006. Until the end of 2010, 100 samples of ornamental plants were found to be infected. Analysis of 96 complete master sequences of Slovene samples revealed new genetic variants. Our sequence variants showed considerable variation in their predicted secondary structure. The variability was observed in the pathogenic, central and variable domains. In several cases even single mutations were sufficient for the change in predicted secondary structure.

Keywords: *Potato spindle tuber viroid,* PSTVd, pospiviroid, predicted secondary structure, sequence variants

Izvleček: Leta 2006 smo v Sloveniji uvedli posebni nadzor viroida vretenatosti krompirjevih gomoljev (*Potato spindle tuber viroid*). Do konca leta 2010 smo okužbo potrdili v 100 vzorcih okrasnih rastlin. Analizirali smo 96 celotnih prevladujočih nukleotidnih zaporedij in predvideli njihove sekundarne strukture. Odkrili smo nove genotipe in znatno variabilnost sekundarnih struktur in sicer v patogeni, osrednji in variabilni domeni viroida. V določenih primerih je že mutacija enega samega nukleotida povzročila spremembo predvidene sekundarne strukture viroida.

Ključne besede: *Potato spindle tuber viroid,* PSTVd, pospiviroid, sekundarna struktura, različice nukleotidnega zaporedja

Introduction

Viroids are circular, covalently closed RNA molecules with a high degree of self-complementation resulting in compact folding. Approximately 30 viroids are known today, many of which cause serious losses of important crops. *Potato spindle tuber viroid* (PSTVd) is the type species of the genus *Pospiviroid* and can cause losses up to 64% in potato and up to 45% in tomato (Singh et al. 2003). The members of genus *Pospiviroid* show a rod-like secondary structure which is divided into 5 structural domains: terminal left, pathogenic, central, variable and terminal right domain (Keese and Symons 1985). This secondary structure is assumed to be the key for biological activity (replication, processing, transport, and pathogenesis) by being functional as such or by providing binding signals to host factors (Tabler and Tsagris 2004, Verhoeven 2010).

Viroids propagate in their plant hosts as populations of closely related variants. In the family *Pospiviroidae* one genotype usually dominates the viroid population in host plant and is called the predominant genotype or master sequence (Verhoeven and Roenhorst 2010).

Materials and methods

In the years 2006-2010 389 samples of ornamental plants were tested for PSTVd infection as described in details by Viršček Marn et al. (2013). Direct sequencing (Macrogen, Korea) of RT-PCR amplification products using primer pairs of Shamloul et al. (1997) and of Di Serio (2007) was performed to obtain whole PSTVd sequences. Additionally, amplification products from three selected samples were purified, cloned into pGEM-T easy vector (Promega, WI, USA) and sequenced (Macrogen, Korea). Obtained sequences were analysed using the computer software BioEdit version 7.0.5.3 (Hall 1999). Thermodynamic prediction of RNA secondary structure was performed using computer software mfold 2.3 in circular mode at 25°C (http://mfold.rna.albany.edu/?q=mfold/RNA-Folding-Form2.3; Zuker 2003).

Results and discussion

PSTVd infection was confirmed in 88 samples of *Solanum jasminoides*, 5 samples of *Solanum rantonnetii*, 3 samples of *Petunia* spp., 3 samples of *Solanum muricatum* and one sample of *Brugmansia cordata*. Ninety-six whole viroid sequences were obtained. Determined PSTVd variants are presented in Table 1. Some of these variants were detected in numerous samples and/or various hosts. A hotspot was found on position 65. Multiple peaks of similar height for G and U; U and A; or G and U and A were observed and/or sequences of the same sample amplified with different primers had different nucleotides (G, U or A) at position 65. The occurrence of the hotspot was confirmed by cloning tree samples showing ambiguity at this position. Apart from polymorphism at position 65, other substitutions or insertions were observed in 9 out of 33 clones (Table 1).

Slovene sequences showed differences in predicted secondary structure models, calculated at 25°C (Fig. 1). The variability was observed in three out of five structural domains: pathogenic, central and variable domain. The replacement of G with U at position 65 enlarged the loop around this site (loop 10). Loop enlargement was also observed in some other sequences. Clone 1F, which has an additional G at position 100, has an enlarged loop E (loop 16). The substitution at position 83 in clone 3M changed the secondary structure from loop 11 to loop 14. Drastically changed secondary structure was observed from loop 17 to 23 due to the substitution at position 136 in clone 2C. Other polymorphisms in the clone sequences do not affect the secondary structure. Master sequences HQ454933 and HQ454936 have the same secondary structure as the prevalent master sequence detected in Slovenia (HQ454914 – HQ454916). All of the Slovene master sequences with 360 nt have structural changes on the right side from the loop 17. These changes are the results of several mutations on positions 121, 124, 126, 127 and 238. In sequence variant HQ454937 the substitution on the position 241 contributes to the changes due to the before mentioned mutations, therefore the loop 18 is larger in this 360 nt long sequence. Differences in the position and the size of loop 10 were also observed among different 360 nt long sequence.

The study of secondary structures of genotypes detected in Slovenia on ornamental plants showed several cases where single nucleotide mutation significantly changed the predicted secondary structure of the viroid. Apart from the single-site mutations, multiple mutation events in the same domain also resulted in the secondary structure changes. Induced mutations, either of one or several nucleotides, have been used to study the role of different loops for PSTVd characteristics. It was determined that disruption of nearly every loop had an impact on either replication or systemic trafficking (Qi et al. 2004, Zhong et al. 2008). We have not tested the functional impact of variability in the secondary structure of PSTVd variants determined on ornamental plants in Slovenia, but these changes could have an impact on their characteristics.

Table 1: Characteristics of the Slovene PSTVd sequences. Differences that influence the predicted secondary structure are highlighted in bold. Mutations that influence the same domain of the secondary structure are shadowed.

Tabela 1: Lastnosti slovenskih nukleotidnih zaporedij PSTVd. Razlike, ki vplivajo na predvideno sekundarno strukturo, so označene z odebeljenim tiskom. Mutacije, ki vplivajo na isto domeno sekundarne strukture, so senčene.

Host	Acc. No.	No. of sequences	Sequence name	No. of nt	Difference in comparison with HQ454914 on position of the relevant sequence
Master sequences					
Solanum jasminoides	HQ454914	52	G-type	357	–
S. rantonnetii	HQ454915	2	G-type	357	–
Petunia sp.	HQ454916	1	G-type	357	–
S. jasminoides	HQ454917	10	U-type	357	**G → U on 65**
S. rantonnetii	HQ454918	2	U-type	357	G → U on 65
S. jasminoides	HQ454919	6	K-type	357	G → K on 65
S. jasminoides	HQ454920	3	W-type	357	G → W on 65
S. jasminoides	HQ454921	12	D-type	357	G → D on 65
S. jasminoides	HQ454933	1	B29, B140	357	A → U on 221
Petunia sp.	HQ454936	1	B141	357	A → U on 221
S. jasminoides	HQ454937	2	B14,B82	360	**U → A ob 121, G → A on 124, insertion of GA on 126-127, insertion of C on 238, U → C on 241**
S. jasminoides	HQ454934	1	B90	360	**U → A ob 121, G → A on 124, insertion of GA on 126-127, insertion of C on 238, C → A on 311, A → U on 63, U → A on 64**
S. muricatum	HQ454935	2	B313, B314	360	**U → A ob 121, G → A on 124, insertion of GA on 126-127, insertion of C on 238**
S. muricatum	HQ454932	1	B315	360	**U → A ob 121, G → A on 124, insertion of GA on 126-127, insertion of C on 238, G → U on 65**
Clones					
S. jasminoides	HQ454914	7	G-type	357	–
S. jasminoides	HQ454917	10	U-type	357	–
S. jasminoides	HQ454922	7	clone A	357	**G → A on 65**
S. jasminoides	HQ454923	1	clone 1J	357	A → G on 30
S. jasminoides	HQ454924	1	clone 1D	357	A → G on 90, U → C on 258
S. jasminoides	HQ454925	1	clone 1F	358	G → A on 65, **insertion of G on 100**
S. jasminoides	HQ454926	1	clone 1G	357	U → C on 35
S. jasminoides	HQ454927	1	clone 2C	357	**G → U on 65, U → C on 136**, U → C on 302
S. jasminoides	HQ454928	1	clone 2J	357	U → C on 331
S. jasminoides	HQ454929	1	clone 3M	357	**G → U on 65, U → C on 83**
S. jasminoides	HQ454930	1	clone 3J	357	**G → U on 65**, A → G on 122
S. jasminoides	HQ454931	1	clone 3L	357	**G → U on 65**, A → G on 100

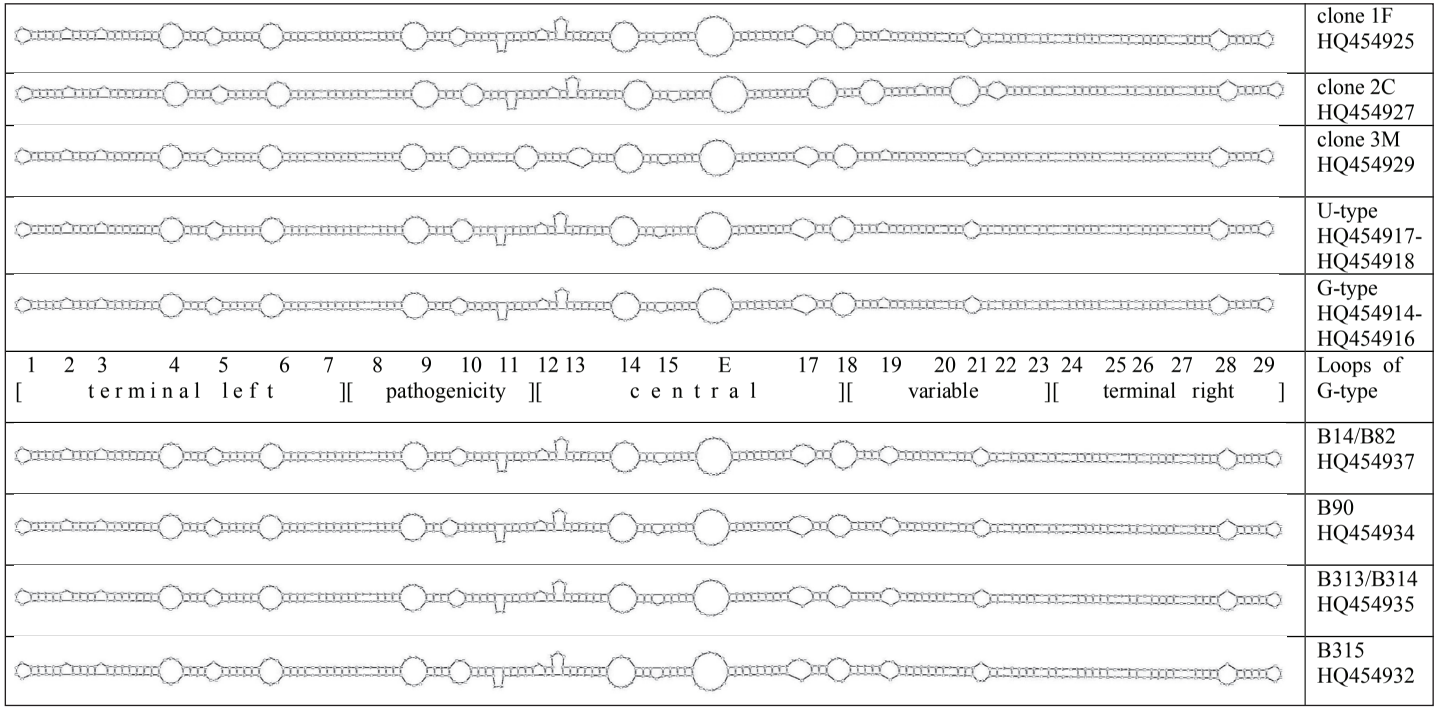

Figure 1: Thermodynamic predictions of secondary structure for PSTVd sequences detected on ornamental plants. Changed secondary structures in comparison with the most prevalent PSTVd sequence (G-type, HQ454914- HQ454916) are shown above and below G-type. The term loop is used for both loops and bulges.

Slika 1: Termodinamične napovedi sekundarnih struktur nukleotidnih zaporedij PSTVd potrjenih na okrasnih rastlinah v Sloveniji. Spremenjene sekundarne strukture v primerjavi z v Sloveniji najbolj pogosto najdenim nukleotidnim zaporedjem PSTVd (G-type, HQ454914- HQ454916) so prikazane nad in pod G-type nukleotidnim zaporedjem.

Conclusions

Predicted secondary structure of PSTVd sequences detected on ornamental host plants in Slovenia showed considerable variation. The variability was observed in the pathogenic, central and variable domains.

Povzetek

Leta 2006 smo v Sloveniji uvedli posebni nadzor viroida vretenatosti krompirjevih gomoljev (*Potato spindle tuber viroid*, PSTVd). Do konca leta 2010 smo analizirali 398 vzorcev večinoma okrasnih rastlin in okužbo potrdili v 88 vzorcih *Solanum jasminoides*, 5 vzorcih *Solanum rantonnetii*, 3 vzorcih *Petunia* spp., 3 vzorcih *Solanum muricatum* in enem vzorcu *Brugmansia cordata.* Določili in analizirali smo 96 celotnih prevladujočih nukleotidnih zaporedij. Nekatera od teh nukleotidnih zaporedij smo našli v številnih vzorcih in/ali v vzorcih iz različnih gostiteljskih rastlin. Na mestu 65 smo potrdili visoko pogostnost mutacij. Za termodinamično napoved sekundarne strukture RNA smo uporabili program mfold 2.3 pri 25 °C. Odkrili smo nove genotipe in znatno variabilnost sekundarnih struktur in sicer v patogeni, osrednji in variabilni domeni viroida. V določenih primerih je že mutacija enega samega nukleotida povzročila spremembo sekundarne strukture. Glede na objave drugih avtorjev o pomenu zank v strukturi PSTVd imajo lahko spremembe sekundarne strukture v Sloveniji najdenih nukleotidnih zaporedij PSTVd pomemben vpliv na njihove lastnosti.

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