Chapter 4. MOLECULAR PLANT PATHOLOGY

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MOLECULAR STRUCTURE OF RUSSIAN ISOLATES OF POTATO SPINDLE TUBER VIROID

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Abstract

Nineteen Russian isolates were sequenced in 2008. Compared to the type strain (i.e., PSTVd-Intermediate, GenBank V01465), the most common distinguishing features of all Russian isolates sequenced to date include: i) the deletion of adenine 123 and ii) the substitution of uracil or cytosine for adenine at position 120. One of the new sequenced isolates had additional minor change in the right terminal domain, but a majority of isolates also contained one or more changes in the pathogenicity domain. Twenty isolates contained an A→U substitution at position 310 plus a U insertion at position 313a. Other isolates contained numerous changes distributed all of three central domains.

Key words: potato, disease of potato, *Potato spindle tuber viroid,* primary structure.

Introduction

PSTVd was the first viroid to be described /Diener, Raymer, 1967/, and known isolates contain only 341–364 nucleotides. Potato plants infected with PSTVd are stunted and exhibit an upright growth habit, leaf distortion, and a reduction in tuber size and number. Tubers from infected plants are elongated and may become small and spindly and pronounced growth cracks /Diener, 1979/. PSTVd is highly contagious and can be transmitted by mechanical contact, infected tools and farm machinery. Viroids can also be transmitted through pollen and true seeds. Yield reductions depend on the PSTVd strain, type of cultivar, and growing conditions. PSTVd was the first plant pathogen to have the complete sequence of its genome determined /Gross et al., 1978/.

The so-called “gothic” disease of potato was first described in the former USSR in the 1930’s. Based on similarities in symptoms, Leontyeva /1971/ suggested PSTVd as the likely cause of this disease. Potato gothic was most common in the Volga region of European Russia, but sporadic outbreaks of the disease were observed in other Russian

regions and in the Ukraine. During those years the disease was not wide-spread and did not have any economic importance in Russia because the small, viroid-infected tubers could be rejected as defective during harvesting. Levels of PSTVd infection remained low /Mozhaeva, Vasilyeva, 1982/.

In the 1980's and 1990's, the yield and quality of Russian seed potatoes declined dramatically – leading to an overall loss of quality for several widely planted cultivars. Initial suspicions that this decline could be due to viroid infection were later confirmed by analysis of seed potatoes collected from farms throughout Russia.

The collection of PSTVd of Russian Research Institute of Phytopathology (VNIIF) started to be formed in the early of 1990s. Since that time it has been maintained by annually reproducing potato tubers infected with PSTVd in field. Only in 2006 the first sequence analysis of Russian PSTVd isolates from VNIIF collection was done thanks to collaboration with Dr. R. Owens from Molecular Plant Pathology Laboratory of Agricultural Research Center (Beltsville, USA) and USDA/ARS funding. In this paper we would like to submit short information of the latter results on sequence analysis of Russian PSTVd isolates.

Materials and Methods

During vegetative period of 2007 potato leaves were collected from potato plants, frozen at –20°, and then RNA was isolated by Trizol-reagent method. Complementary DNA was synthesized for each PSTVd isolate, using MMuLV H-Reverse Transcriptase (Dialat, Moscow). Suitable PCR products were prepared in amount enough for automated sequence analysis. Full-size and short overlapping RT-PCR products were generated using two pairs of primers:

PSTVd180F (5'-TCACCCTTCCTTTCTTCGGGTGTC-3') +
PSTVd179R (5'-AAACCCTGTTTCGGCGGGAATTAC-3') and
PSTVd112F (5'-ACTGGCAAAAAAGGACGGTGGGGA-3') +
PSTVd359R (5'-AGGAACCAACTGCGGTTCCAAGGG-3').

Results and Discussion

Uncloned PCR products of sixteen PSTVd isolates from the VNIIF collection and several cloned cDNAs of two other isolates were submitted for automated sequence analysis in 2008. Resulting primary structures of nineteen Russian PSTVd isolates were compared with those of Intermediate PSTVd isolate (GenBank V01465). The sequence information is summarized below in a table showing the locations of all sequence differences from the Intermediate isolate. All Russian PSTVd isolates newly and earlier sequenced to date exhibit a deletion of adenine 123.

Eleven PSTVd Russian isolates sequenced in 2008 contained an A→U substitution at position 120, and eight isolates had an A→C change at the same position. Only one isolate had a substitution in the right terminal loop, a very rare occurrence among previously characterized isolates of PSTVd. Isolate VIZR-06/8L (#12 in Table) from St.-Petersburg (North-western region) had such 2 minimal distinctions as compared with Intermediate isolate. It was identical to the Russian isolate (GenBank EF 044303), which was described earlier /Kastalyeva et al., 2007/ and was shown to induce intermediate symptoms, infecting Rutgers tomato.

Table. Russian PSTVd isolates sequenced in 2008

#	Isolate	Potato varieties	Region of Russia	Sequence differences from Intermediate isolate
1.	VNIIF-07/D16	Hybrid 801002	Central	120 A→U, 123 A del, 193 C→U
2.	VNIIF-07/D13	Hybrid N 800997	Central	120 A→U, 123 A del, 310 A→ U, 313a U insert
3.	VNIIKH-07/68M	Hybrid 2292-19	Central	the same
4.	VNIIKH-07/49M	Hybrid 2520-154	Central	the same
5.	Osetia-06	Volzhanin	Northern Caucasus	the same
6.	Khabarovsk-06/28B	Android	Far Eastern	the same
7.	Khabarovsk-06/29B clone_8	Udacha	Far Eastern	the same
8.	Khabarovsk-06/30B	Veteran	Far Eastern	The same
9.	Khabarovsk-06/31B clone_13	Nevskii	Far Eastern	the same
10.	Khabarovsk-06/29B clone_1	Udacha	Far Eastern	120 A→U, 123 A del, 306 U→A, 310 A→ U, 313a U insert
11.	VIR-06/5L-08	Nevskii	North-western	120 A→U, 123 A del, 126 A del, 142 A→U, 143 G→U, 219 A→G, 237 C del, 309 U →A, 311 C→U
12.	VIZR-06/8L	Petersburgskii	Northwestern	120 A→C, 123 A del
13.	VNIIF-07-K	Lugovskoi	Central	120 A→C, 123 A del, 310 A→ U, 313a U insert
14.	VNIIF-93-Lorkh	Lorkh	Central	120 A→C, 123 A del, 256 C→U, 310 A→ U, 313a U insert
15.	VNIIKH-07/1	Hybrid 2567-5	Central	120 A→C, 123 A del, 256 C→A, 310 A→ U, 313a U insert
16.	VIZR-06/6L-08	Nevskii	North-western	49 G→N, 120 A→C, 123 A del, 256 C→A
17.	VIZR-06/4L	Desnitsa	North-western	62a U ins, 120 A→C, 123 A del, 214 C→G, 216 C→G, 219 A del, 225 A →C, 294a G insert
18.	VIR-06/7L	Osen’	North-western	120 A→C, 123 A del, 212 C del, 213 G del, 214 C del, 219 A del, 223 C→N, 225 A del, 232a G insert, 249a G insert, 255a G insert, 266a G insert, 278a G insert, 294a G insert
19.	VIR-06/10L	Temp	North-western	120 A→C, 123 A del, 219 A del, 223 C→N, 225 A →C, 266a G insert, 281a G insert, 294a G insert, 314a G insert

Twelve isolates contained an A→U substitution at position 310 plus a U insertion at position 313a, changes that usually resulted in an attenuation of symptom expression in Rutgers tomato. Among them, eight Russian PSTVd isolates (isolates from

2 to #9 presented in Table) had a primary structure identical to the structure of PSTVd004 (GenBank V14814) originally reported from Germany. This type of so called "mild" PSTVd isolates was represented by isolates collected from the Northern Caucasus, Far Eastern and Central regions of Russia.

Five PSTVd isolates from the North-western region of Russia (isolate #11 and those from 16 to 19) had unique structures characterized by great variability. The most common changes were i) deletion of residue 219A and ii) insertion of one or more G residues in the lower portions of the variable, central, and pathogenicity domains.

Among presented isolates, only one, namely, VNIIF-93-Lorkh, have been existing in the VNIIF collection since its foundation. All other isolates were collected in different Russian regions in 2006 and 2007.

PSTVd isolates exhibiting A→C substitution at position 120 is characteristic exclusively for those from Russia. The changes in the following positions: 193 C→U (isolate #1), 256 C→U (isolate #14) and 306 U→A (isolate #10) characterize, perhaps, endemic Russian PSTVd isolates too.

Conclusion

Thus, among 19 Russian PSTVds sequenced in 2008, 8 isolates have primary structure identical one to others and to that earlier occurred in CenBank database (V14814). Each of the other 11 isolates has a unique primary structure which is characteristic exclusively for Russian PSTVd, one of them is identical to PSTVds isolate identified and included in GenBank database earlier /Kastalyeva et al., 2007/.

Analysis of primary structure of Russian PSTVd isolates is in progress. We hope that comparison of sequences of isolates from different Russian regions, especially those endemic in the Volga-river region shed new light on the origin of the spindle tuber disease.

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