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Characterization of a Novel Far-Eastern Potato Virus Y Isolates

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Abstract: Potato Virus Y (PVY) isolates differed in pathogenicity and molecular properties were found in potato and wild plants in the Far East of Russia. The results of linking research of nucleotide sequence similarity and polymorphism of P1 gene region and serological and biological assays suggested that the viruses originated from a recombination and/or host adaptation events involving the ordinary type virus (PVY^{O}) that led to development of necrotic type virus ($PVY^{N/NTN}$) properties in the Far-Eastern PVY isolates.

Key words: Potyvirus, PVY, necrosis strain, P1 protein, phylogeny

INTRODUCTION

The spreading of PVY infection characterized by occasional severe damage of crop was observed in potato fields in the Far-Eastern region of Russia. The disease rarely occurred in the Northern boundary of potato cultivation in Russia. Whereas, it was widely distributed in the Southeast (Khabarovsk krai, Primorsky krai and Amur River oblast) (Romanova *et al*., 2007). Some PVY isolates were highly variable by different pathotypic properties and frequent spontaneous differentiation. The minor differences in the variable region part of the PVY genome such as P1 coding region was found to be useful for simplification of various PVY strain groups identification and differentiation (Nie and Singh, 2003a). Clustering the PVY isolates by a high degree of polymorphism in the P1 coding region correlated with symptoms and geographical distribution (Tordo *et al*., 1995; Krishanu *et al*., 2004; Ogawa *et al*., 2007). Mosaic structure of the nucleotide sequences can cause amino acid substitutions in P1 protein that often lead to more significant changing in the virulence properties of PVY (Matousek *et al*., 2000; Nie and Singh, 2003a; Chikh Ali *et al*., 2007). The construction of chimeric genomes *in vitro* on the base of even nearly identical PVY genomes have been demonstrated to give rise to new viral strains with novel virulence and symptom phenotypes (Paalme *et al*., 2004; Tribodet *et al*., 2005). In the most cases, new viruses came into existence because of single or multiple recombination between the strain genomes (Worobey and Holmes, 1999; Glais *et al*., 2002; Fanigliulo *et al*., 2004; Almeida *et al*., 2007). However, the virus alterability can also be caused by the point mutation changes (Nie and Singh, 2003b; Chikh Ali *et al*., 2007).

The aim of this study was to characterize and compare the most pathogenic PVY isolates recently appeared in the Far-Eastern of Russia.

MATERIALS AND METHODS

Biological tests: Biological property tests of the isolates were performed with both mechanical inoculation and transmission through potato and tobacco seeds and green peach aphids. Potato fields of Primorsky krai and adjoining wild plants were surveyed during 2006 and 2008 years and virus samples were collected from symptomatic plants. Symptomless plants were screened for latent infection and used as negative controls. Thirty eight plant species were used as indicator-plants. Ten indicator-plants per species were used for inoculation. Plants were cultivated in sterile soil in greenhouse with temperature between 25-27°C for 15-18 h in the day time, 18-20°C in the night time, 60-70% relative humidity and 500-800 lx illumination intensity. Mechanical inoculation was performed at the 3-6 genuine leave stages. For vector transmission, the adult insects, *Myzus persicae* Sulz., were allowed to be starveling during 3 h and then placed to the infected tobacco plants, *N. tabacum* L. cv. Xanti nc and the different tomato cultivars. Not less than 10 insects per one indicator-plant were transferred.

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Serological tests: A necrosis strain of PVY (PVY^{NTN}), kindly given by Dr. J. Varitsev, The Russian Potato Research Institute and ordinary strain PVYcom, which was delivered with the potato from the central part of Europe in 1998 year, were used as a reference standard of antigenic properties of the isolates in ELISA tests as previously described by Koenig and Paul (1982).

RNA isolation: The viral RNAs were isolated from purified precipitate formed between PVY virions and appropriate antibodies as described by Sapotsky (Sapotsky *et al*., 2001). The sap from leaf samples (5-20 g) of tobacco, *N. tabacum* L. cv. Xanti nc, infected by the PVY isolates was pressured at 0ºC and diluted with the buffer containing 0.1 M Tris-HCl, 0.15 M NaCl, 0.002 M EDTA, 0.05% β-mercapthoethanol and 1% Triton-x 100 and then centrifuged at 13400 rpm for 20 min. The precipitate was rinsed thoroughly and dispersed in 0.1 M Tris-HCl buffer containing 0.002 M EDTA, 1% SDS and 0.5% β-mercapthoethanol and then warmed up in the water bath at 70ºC min^{-1}. RNA was extracted with phenol-chloroform and precipitated by 1 M lithium chloride.

PCR and sequence analysis: For reverse transcription, 2 µL of total RNA diluted with 30 µL distilled water up to 200 ng μL^{-1} was incubated at 65°C for 8 min and chilled on ice for 3 min. RT mixture was added to provide a final concentration 20 ng μL^{-1} of the reverse primer A (5'- CATTTGTGCCCAATTGCC-3'), 50 mM Tris-HCl, pH 8.3, 50 mM KCl, 10 mM DTT, 4 mM $MgCl_2$, 1.5 mM of each dNTPs and 200 U Revert Aid^{tm} Minus M-MuLV Reverse Transcriptase (Fermentas). For amplification, samples were incubated for 1 h at 42°C and subsequently incubated for 3 min at 95°C. Primers were synthesized according to the virus-specific oligonucleotide database (Onodera and Melcher, 2002). Universal for both PVY^{0} and $PVY^{N/NTN}$ sense primer S2 (5'-AAACTTCCATACTCACCCGC-3') to 230-249 nucleotide target site and reverse pimer A (5'-CATTTGTGCCCAATTGCC-3') to 1056-1039 nucleotide target site of PVY genome were taken to amplify the P1 coding region as a molecular marker for PVY strain groups differentiation as previously described by Nie and Singh (2003a). Samples were amplified for 30 cycles using an Eppendorf Thermal Cycler using Smart Taq DNA polymerase (GosNIIgenetika, Moscow) at concentration of 1 U 20 μL^{-1}. The PCR conditions were the following: (92ºC 30 sec-60ºC 30 sec-72ºC 90 sec) for 5 cycles; (92ºC 30 sec>58ºC 30 sec>72ºC 90 sec) for 5 cycles; (92ºC 30 sec>55ºC 30 sec>72ºC 90 sec) for 10 cycles; 72ºC for 10 min. The PCR products were cloned and sequenced in each direction using a PE/ABI 310 DNA sequencer and the PE/ABI-ABI PRISM Big Dye terminator cycle sequencing ready reaction kit (PE Applied Biosystem). Data from raw sequencing were edited using Chromas software. Phylogenetic relationship of the PVY isolates was inferred by comparing their P1 gene sequences with 150 closest homologies from different geographical regions found by NCBI-BLAST2 software package (http://www.ebi.ac.uk/blastall/). Phylogeny construction and evaluation was performed using the neighbour-joining method implemented in the PHYLIP software version 3.6 package (Felsenstein, 2005). Bootstrap analysis was applied using 1000 bootstrap replications.

RESULTS AND DISCUSSION

It has been found that the most PVY isolates recently appeared in the Far-Eastern potato fields can be reserved in sow thistle *Cirsium cetosum*, patience-dock *Rumex patientia*, dandelian *Taraxacum officinale*, sow-thistle *Sonchus oleraceus*, wild radish *Raphanus raphanistrum*, black nightshade *Solanum nigrum*, frost-blite *Chenopodium album* and field bindweed *Convolvulus arvensis*. It is evident that the PVY infections in the wild plants were related with hotbeds of the disease in the potato fields. The PVY isolates from potato and wild plants differed in biological and molecular properties as well as antigenic affinity. Particularly, the isolates PVYagr and PVYhum collected from *Agrimonia pilosa* and *Humulopsis japonicus*, respectively, at the edge of potato fields in the South of Primorsky krai were the causal agents of new diseases involved light necrosis symptoms. The isolate PVYsp from the regional potato cultivar Filatovsky occasionally induced severe tuber necrosis. However, the leaf chlorosis was more frequently appeared as large yellow spots, including the leaf curling symptoms without visible inhibition of the plant growth. It has been shown that the symptoms were associated with both the PVYsp and spindle tuber viroid (PSTV) infections that led to suppress development of the necrotic properties of the virus (Romanova *et al*., 2007).

In almost all cases, the isolates PVYagr, PVYhum and PVYsp were easily transmitted. Twenty indicator-plants from Chenopodiaceae, Amaranthaceae, Asteraceae and Solanaceae species showed the most typical symptoms associating with necrotic pathotype of PVY (Table 1). In plants of family Chenopodiaceae, these isolates developed only local necrotic lesions, whereas more of Solanaceae species were affected with system diseases. Transmission from the diseased potato seeds was not

Table 1: Symptoms induced by PVY isolates in indicator-plants

Species and cultivars	Symptoms emergence (days)	Symptoms induced by PVYcom	PVYagr	PVYhum	PVYsp
Chenopodiaceae					
Chenopodium amaranticolor Coste et Reyn.	10-15	L:N		L:N	
C. quinoa Willd.	8-10	L:N		L:N	
C. murale L.	8-10	L:N		L:N	
Solanaceae					
Capsicum annum L.	9-11	S:Vc,clM		S:Vc, clM	
Datura metel L.	3-05	S:Vc		S:Vc, Dis	
Nicandra physaloides (L.) Gaerth.	8-10	L:N		L:N	
Nicotiana clevelandii Gray	6-08	S:Vc		S:Vc, Vn	
N. glutinosa L.	6-08	S:Vc		S:Vc, Dis	
N. debneyi Domin.	6-08	S:Vc		S:Vc	
N. rustica L.	8-10	S:clM		S:clM	
N. longiflora Weinm.	6-10	S:Vc		S:Vc Vn	
***N. tabacum* L. cvs**					
Samsun	6-10	S:Vc		S:Vc Vn	
Xanthi	6-08	S:Vc		S:Vc Vn	
Petunia hybrida Vilm.	8-12	S:N		S:N, LeA	
Physalis floridana Rydb.	10-15	S:M		S:M, N	
Solanum demissum	5-06	L: brNSp		L: brNSp	
S. demissum x *Aquilum* (A-6 hybrid)	5-06	L: brN		L: brN	
S. chacoense Bitt. (TE-1)	5-08	S:Vc		S:Vc	
S. nigrum L.		S:O		S:O	
Lycopersicon esculentum Mill., cv Khabarovsk-308 (regional)	10-12	S:Vc		S:N	
Amaranthaceae Juss.					
Celosia argentea L.	8-10	S:Sp		S:nSp	

S: System reaction, M: Mosaic, Vc: Vein clearing, Br: Brown, LeAb: Leave Abscission, Vn : Vein necrosis, L: Local reaction, N: Tuber necrosis, ClM: Chlorotic mosaic, Dis: Distortion, O: Symptomless, Sp: Spots, NSp: Necrotic spots

registered. However, when seeds from diseased tobacco, *N. tabacum* cv. Xanti nc, were sown, emerged plantlets showed the symptoms.

All novel PVY isolates were determined to have a thermal inactivation point between 55-60°C, dilution end point of 10^{-2}-10^{-3} and longevity of 48-72 h.

ELISA results obtained in the PVY^{NTN} system demonstrated that the isolates were distinguished by the quantity of their antigenic determinants. The PVYsp isolate suggested to belong to PVY^{NTN} type because of a highest level of affinity with the type strain. The PVYagr isolate showed an intermediate level of affinity with the PVY^{NTN} strain. The lowest affinity with PVY^{NTN} was observed in the PVYhum isolate. The PVYsp isolate demonstrated, respectively the lowest affinity in the system of the ordinary PVYcom strain.

Comparative analysis of the partial sequences of the isolates showed that their P1 coding region had identity with the published ordinary strains of PVY (PVY^{0}) as well as necrotic strains including PVY^{N} and PVY^{NTN}. PVYsp (DQ458958) showed a high level of the nucleotide sequence identity (up to 98%) with $PVY^{N/NTN}$ strains. PVYagr (DQ458960) and PVYhum (DQ458959) nucleotide sequences showed the levels of identity ranging from 82 to 89% among both PVY^{0} and $PVY^{N/NTN}$ strains simultaneously. As shown in Fig. 1, nucleotide insertions of PVY^{O} and $PVY^{N/NTN}$ are combined in various orders along the P1 gene sequences of every isolate.

Taken together, the analysis of the isolates suggested that we have identified some genome combinations originated from a recombination or host adaptation events involving the ordinary strain PVYcom delivered from Europe in 1998 year (Glais *et al*., 2002; Fanigliulo *et al*., 2004; Schubert *et al*., 2006). Considering the similarity of the level of the isolate nucleotide identities with both PVY^{0} and $PVY^{N/NTN}$ strains, the phylogenetic analysis can develop their positions in the context of the molecular ancestry and evolution (Moury *et al*., 2002; Chare and Holmes, 2005). As shown in Fig. 2, the isolates PVYagr, PVYhum, PVYsp, PVYcom occupy separated subclusters of PVY family. The Far-Eastern isolates are evidently in the intermediate position between PVY^{0} and $PVY^{N/NTN}$. The ordinary PVYcom strain is closer to PVY^{0} from China (accession n. AY095170) and Finland (accession n. AJ315742). This may be explained by common geographic percentage with the Chinese strain, which was also introduced into China from Europe (Krishanu *et al*., 2004). It may as well get there through the Russian border for the first time. The probability is that further alteration of the initial ordinary strain into the current necrosis PVY strain variations occurred in the Far-Eastern region of Russia. Moreover, hotbeds of a new

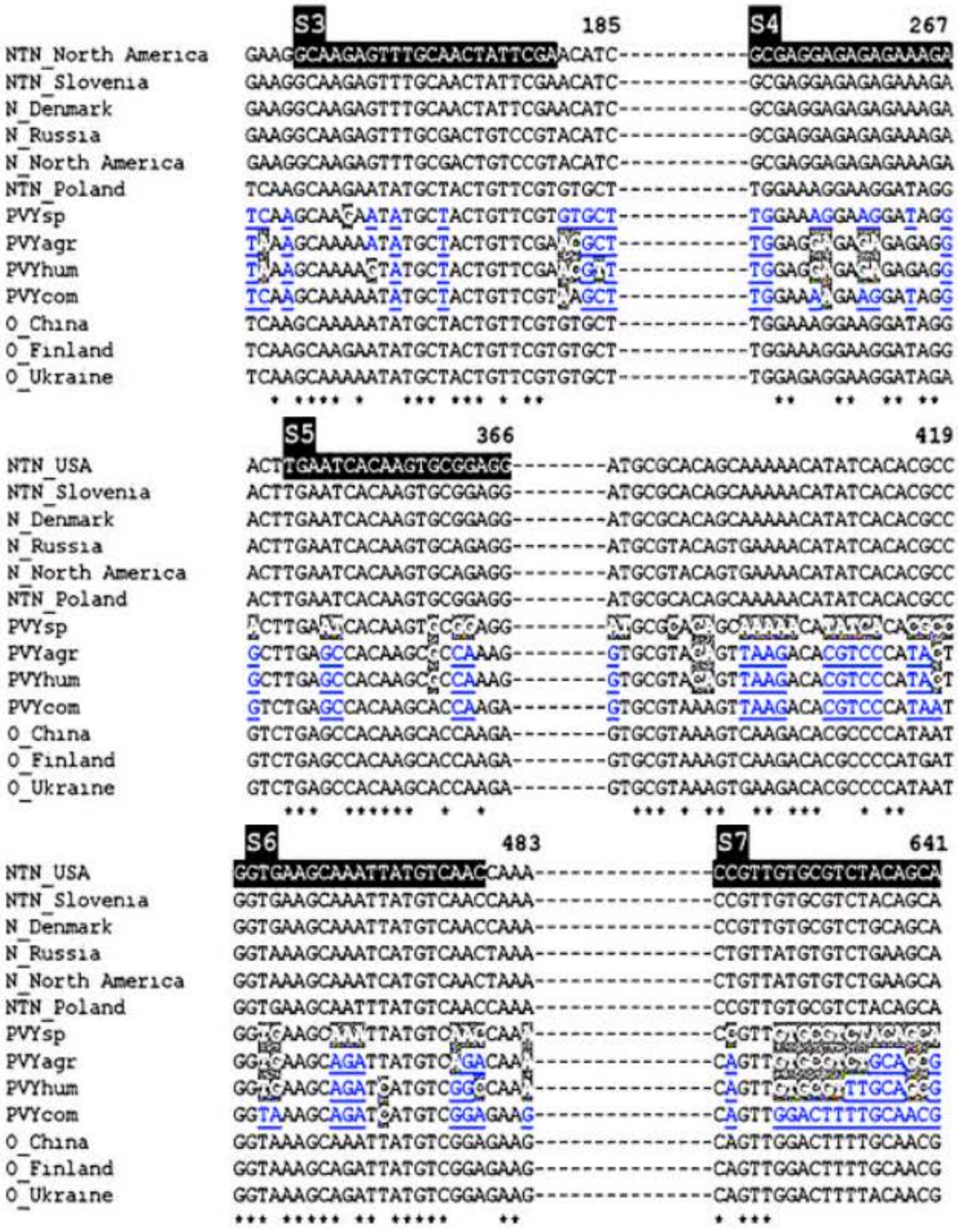

Fig. 1: Partial alignment of nucleotide sequences of the most variable P1 gene region of some Russian Far-Eastern isolates: PVYsp (DQ458958), PVYcom (DQ458782), PVYhum (DQ458959), PVYagr (DQ458960) with the different PVY strains: PVY^{NTN}_North America (isolate Tu 648, European type, AF401610); PVY^{NTN}_Slovenia (AJ585342); PVY^{N}_Denmark (AJ315738); PVY^{N}_Russia (AJ315746); PVY^{N}_North America, isolate N27 (AF401606); PVY^{0}_Finland (AJ245554); PVY^{0}_China (AY095170); PVY^{0}_Ukraine (AJ315740). Diagnostic sequence sites (S3, S4, S5, S6, S7) are black boxed according to Nie and Singh (2003b). Nucleotides of P1 gene region are numbered above the sequences. Nucleotide sequences colored grey indicate $PVY^{N/NTN}$ type. Nucleotide sequences underlined indicate PVY^{0} type

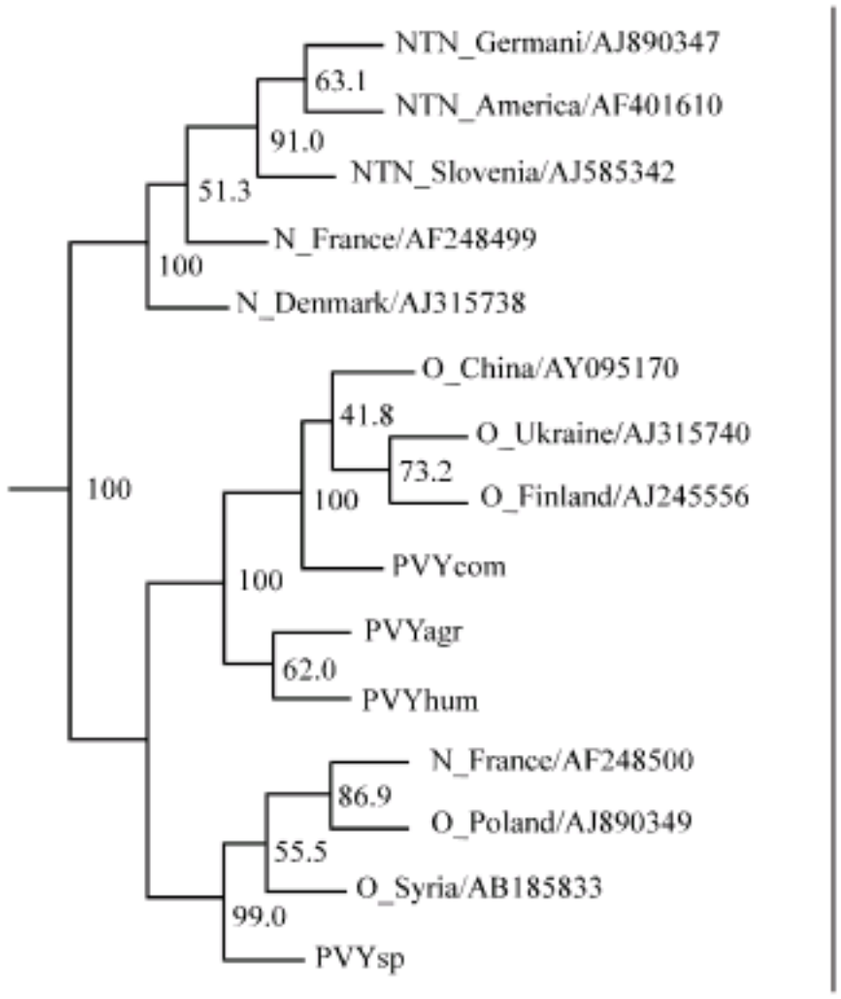

Fig. 2: Partial neighbor-joining unrooted tree constructed on the base of the nucleotide sequence alignments of P1 coding regions of the Russian Far-Eastern isolates and homologies. Nodes with bootstrap probabilities are indicated by %

emerged PVY infection can consume agrocenoses as well as the remote biocenosis. The results of nucleotide recombination or resorting among strains in the period of coexistence in the same host-plants have been suggested to play a role in their adaptation and acquiring of necrotic characteristics of the Far-Eastern PVY isolates. The role of allopatric geographical features as well as the host adaptation in the evolutionary divergence and selection of particular virus isolates is also within the realm of possibility (Bousalem *et al*., 2000; Romero *et al*., 2001; Ohshima *et al*., 2002; Ogawa *et al*., 2007). It is evidently that phylogenetic analysis of viral genomics can be effectively used in the study of pathogenesis, epidemiology and evolution of PVY.

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