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# Nucleotide and amino acid sequences of a coat protein of an Ukrainian isolate of *Potato virus Y*: comparison with homologous sequences of other isolates and phylogenetic analysis

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***Aim***. *Identification of the widespread Ukrainian isolate(s) of PVY (Potato virus Y) in different potato cultivars and subsequent phylogenetic analysis of detected PVY isolates based on NA and AA sequences of coat protein.* ***Methods***. *ELISA, RT-PCR, DNA sequencing and phylogenetic analysis.* ***Results***. *PVY has been identified serologically in potato cultivars of Ukrainian selection. In this work we have optimized a method for total RNA extraction from potato samples and offered a sensitive and specific PCR-based test system of own design for diagnostics of the Ukrainian PVY isolates. Part of the CP gene of the Ukrainian PVY isolate has been sequenced and analyzed phylogenetically. It is demonstrated that the Ukrainian isolate of Potato virus Y (CP gene) has a higher percentage of homology with the recombinant isolates (strains) of this pathogen (approx. 98.8–99.8 % of homology for both nucleotide and translated amino acid sequences of the CP gene). The Ukrainian isolate of PVY is positioned in the separate cluster together with the isolates found in Syria, Japan and Iran; these isolates possibly have common origin. The Ukrainian PVY isolate is confirmed to be recombinant.* ***Conclusions***. *This work underlines the need and provides the means for accurate monitoring of Potato virus Y in the agroecosystems of Ukraine. Most importantly, the phylogenetic analysis demonstrated the recombinant nature of this PVY isolate which has been attributed to the strain group O, subclade N:O.*

*Keywords: Potato virus Y, potyvirus, PCR, phylogenetic analysis, recombinant strain.*

**Introduction**. *Potyvirus* genus is a classical example of employing computer technologies and phylogenetic analysis not only to study virus strain diversity and relationships among the representatives of this genus, but also for developing our understanding of origin and evolution of both viruses and their respective hosts.

*Potato virus Y* (PVY) is a typical member of *Potyvirus* genus, the largest genus of plant viruses. Potato growers from different parts of the world agree that today PVY is assumed to be the most economically harmful plant virus [1] with the exception of Australia where PVY has never led to serious potato crop losses. At the same time novel PVY strains arise or spread to the new geographical regions [2]. The development and use of molecular techniques for characterization of PVY allowed the appearance of many new names for its isolates and strains.

From the very beginning of plant virology as the fundamental science, visual symptoms of virus-induced diseases of cultivated plants were a major motive for

naming various viruses. At the same time, virus properties were mostly unknown and hence could not be included (and reflected) in their respective names. For instance, the most typical symptoms induced by potato viruses were divided into two groups: rugose (wrinkled) mosaics following the infection by aphid-transmitted viruses, and mottling which developed after infection with sap-transmitted pathogens. In 1931, Smith [3] isolated infectious agents and named these viruses as Y and X, in accordance with the type of transmission and the symptoms developing on infected tobacco (*Nicotiana tabacum* L.). The «X» component induced double concentric circles on tobacco and was named as the «ringspot virus» whereas the «Y» component was aphid-transmitted and led to the dark discoloration of green tissues along the leaf veins («vein streak»). These X and Y components were the prototypes of *Potato virus* X (PVX) and *Potato virus* Y (PVY) – the names which are still in use. With time these viruses became (and still remain) typical species of two respective virus genera - *Potexvirus* and *Potyvirus* [4]. During the first years of «potato virology» PVY had many names (synonymous) including: potato virus 20, potato virus C, potato acropetal necrosis virus, potato leaf streak abscission virus, potato severe mosaics virus, potato streak virus, potato striation virus, potato vein necrosis virus, Solanum virus 2, tobacco vein streak virus, tobacco vein necrosis virus.

PVY is the important pathogen infecting not only potato but also other cultures including pepper (*Capsicum* spp.), tomato *(Lycopersicum esculentum* L.) and tobaccos. Seemingly, potato and pepper serve as selective hosts for PVY strains. Despite the PVY variants isolated from these two species are specific to them, tomato and tobacco plants may be infected by the majority of both potato and pepper isolates of PVY. Phylogenetic analysis of the coat protein (CP) gene sequences suggests that some PVY variants isolated from tomatoes are closely related to PVY-C. Grouping virus isolates on the base of phylogenetic analysis of the CP gene sequences shows good correlation with phenotypic appearances (symptoms) of necrotic and mosaic strains [5–7].

In 1980ies new PVY-N isolates have been described, some of which were related to the necrotic (reason for «N» in the virus name) disease of potato tubers – potato tuber necrotic ringspot disease (PTNRD) [8, 9]. These isolates (formerly named PVY-NN) were given a new acronym, PVY-NTN. Another group of PVY-N isolates characterized by the loss of virulence on potato has been registered in Poland and called PVY-N-Wi (following its description on «Wilga» cultivar of potato) [10].

Several PVY-NTN isolates have been characterized at the molecular level and shown to be recombinants of PVY-O («ordinary» strain) and PVY-N («necrotic» strain) in CP-coding region. Later on the additional recombinational events were described for HC-Pro and nuclear inclusion genes. Furthermore, PTNRD isolates lacking recombinational events in the CP gene have been found in the Northern America, Denmark, New Zealand, Germany, Poland and Japan [11].

The PVY-NTN isolates undetectable using primers developed for European PVY-NTN were named Northern American PVY-NTN, or NA-PVY-NTN. In Canada and Spain, additional variants of PVY-N were described (I-136 and I-L56 isolates in Canada and isolate 17 in Spain); these were capable of serological reaction with PVY-O-specific monoclonal antibodies, at the same time inducing veinal necrosis on tobacco (similarly to PVY-N-Wi). As was demonstrated later, these isolates were widely spread in most countries cultivating potato. These isolates were considered recombinant; another recombinational «node» has been confirmed by RT-PCR and this group was named PVY-N:O (a «cross» between the ordinary and necrotic strains) [12–13].

Some rare PVY-N-Wi isolates may have had four recombinational events. Two Spanish isolates serologically reacted with PVY-O-specific antibodies and did not induce veinal necrosis on tobacco. These viruses were put into the group PVY-ZE (variants of PVY-Z). It has been suggested to attribute them to a separate group of PVY strains, PVY-E.

Molecular analysis of the PVY genomes witnesses to an increase in the number of the recombinant PVY isolates and strains belonging to various strain groups defined on the base of host response or serological criteria. However, the serological criteria for determination of the PVY strain groups using monoclonal antibodies have not been fully elaborated. Hence, at present we have no clear understanding of molecular base for differentiation of the PVY isolates and strains. Moreover, different viewpoints are expressed and suggested by various authors.

This work has been aimed at the identification of widespread (typical) Ukrainian isolate(s) of PVY in different potato cultivars and subsequent phylogenetic analysis of the detected PVY isolates based on the gene and amino acid sequences of coat protein which was successfully used by many researchers and proved to serve as a reliable molecular marker for virus evolution.

**Materials and methods**. PVY (*Potyvirus*, *Potyviridae*) was the object of this research. Many potato cultivars of Ukrainian selection (listed further in the «Results and Discussion» section) were employed in this work and screened for PVY infection. Plant samples were collected based on visual virus-like symptoms: vein clearing, mottling leaf tips, streak necrotization along the veins on the underside of the leaves, necrotic and dry leaves. Mild virus-like symptoms typically prevailed in the field.

The virus identification has been carried out via ELISA using the PVY-specific polyclonal commercial antisera («Loewe», Germany) according to the manufacturer's recommendations. The results were measured using automated ELISA microplate reader Stat Fax 2100 («Awareness Technology», USA) at the wavelength of 405 nm. The results were considered positive if they exceeded the negative control optical density value by 3 times or more. Each sample was replicated three times. Simple average values were used [14].

In parallel to the classical method for RNA extraction [15], we have also used commercially available E.Z.N.A. Plant RNA Mini Kit (USA) was applied for comparing RNA yield and purity.

In order to select optimal primers' set, three pairs of primers of own design (widely recognized Entry and Oligo 6 software packages) were used:

1st pair:
1 (As) 5'-CAAACCATAAGCCCATTCATC-3' ;
2 (S) 5'-GCACCAAATCAGGAGATTCTACT-3';
Expected product size: 569 bp;

2nd pair:
PVY-S 5'-CACGATTGCTCAAGCAAGAA-3';
PVY-As 5'-GGCGGAGTATGCACCAAGTA-3';
Expected product size: 412 bp;

3rd pair:
PVY1-S5'-TGCACGATTGCTCAAGCAAGAAT-3';
PVY1-As 5'GGCGGAGTATGCACCAAGTA-3';
Expected product size: 480 bp.

All these three primer pairs were specific to the respective conserved regions of the CP gene of PVY (see «Results and Discussion» section for details).

For the virus detection via RT-PCR we employed one-step-RT-PCR kit («Qiagen», UK). Software packages Entry and Oligo 6 were used for design of own primers; optimal reaction conditions were selected.

The amplicons were analyzed using 1 % agarose gel electrophoresis with markers Gene Ruller 100 bp DNA Ladder plus («Fermentas Inc.», USA).

The purified amplicons were sequenced using Applied Biosystems 3730x1 DNA Analyzer with Big Dye terminators, version 3.1 («Applied Biosystems», USA).

The identification and comparison of obtained sequences were carried out with BLAST analysis (http://www.ncbi.nlm.him.gov). For phylogenetic analysis the software package MEGA 5 was used [16]. To check the validity of constructed trees, the boostrap test was employed with 1000 replications [17]. Phylogenenic trees were constructed using neighbour-joining (NJ) [18] and maximum likelihood (ML) [19] approaches. Statistic evaluation of results was done with the help of Microsoft Excel 2003 using Student's test.

**Results and discussion**. Numerous potato cultivars of Ukrainian selection kindly provided by the Institute of potato research of NAASU have been screened for PVY detection: Povin, Ariel, Dovira, Promin, Mandrivnytsya, Serpanok, Bylyna, Presto, Zelenyy Gay, Okolytsya, Broza, Fantaziya, Podolyanka, Maxam rosa. These are popular cultivars selected and bred at the Institute facilities and then marketed in virtually every region of Ukraine for commercial production. This fact allows suggesting that, in general, the fields of this Institute may serve as a major «hotbed» of PVY origination/microevolution/recombination/spread and hence may represent typical Ukrainian population mix for this virus.

Further diagnostics using DAS-ELISA with the PVY-specific commercial test system («Loewe») demonstrated high content of PVY in the cultivars Povin, Dovira, Promin, Mandrivnytsya, Serpanok, Bylyna, Zelenyy Gay, Okolytsya (ELISA data not shown). These PVY-positive samples were then used for total RNA extraction and subsequent amplification.

The comparison of two different techniques for total RNA extraction proved that commercial E.Z.N.A. Plant RNA Mini Kit (USA) was by far more preferable

for molecular work and provided a higher RNA yield (data not shown). The classical method was also suitable, but less reliable.

Further on, we went to select optimal primers' set for the PVY detection in Ukrainian potato cultivars and to optimize the PCR conditions (annealing temperature and $MgCl_2$ concentration in the reaction mix). We have demonstrated that only one pair of primers (the first one from the three tested, see «Materials and Methods» section) worked well in PCR with total RNA extracted from collected potato samples (*i. e.*, with «Ukrainian» isolates of PVY) yielding the amplicon of 569 bp:

1 (As) 5'-CAAACCATAAGCCCATTCATC-3';

2 (S) 5'-GCACCAAATCAGGAGATTCTACT-3'.

These sets of primers were of such design that theoretically allowed the PCR detection of each PVY isolate (*i. e.*, every set of primers could be used for the detection of both ordinary virus strain and necrotic or C strains). In general we should add that the use of the coat protein gene sequence for primers' design and subsequent virus detection and its phylogenetic analysis is the common practice in plant virology [11].

According to the obtained results, the second and the third pairs of primers (based on the sequences (Gen Bank: DQ000989.1 and GenBank: DQ000990.1), were not capable of detecting PVY in collected plant samples (data not shown).

However, using the first set of primers we have successfully amplified the PVY cDNA from the initial potato leaf samples. This primers' set was designed against a part of CP gene of the ordinary strain of PVY (Gen Bank: DQ000987.1).

Most probably, the ordinary strain of PVY dominates in Ukraine which is indirectly confirmed by the absence of PVY-invoked epidemics and by the relatively mild virus-like symptoms prevailing in the field (see «Materials and Methods» section). Hence, it is logical to assume that the primers designed using the CP gene sequence of the ordinary strain of PVY would be most suitable for the virus detection. However, as it was pointed above, all three sets of primers were of universal design allowing detection of all three PVY strains. As such, we may propose several reasons for only one pair of primers allowing PVY detection in the Ukrainian potato samples. These include tentative mutations in the specific regions of the PVY CP gene preventing the annealing of primers' sets N 2 and 3, and possibly non-optimal RT-PCR conditions.

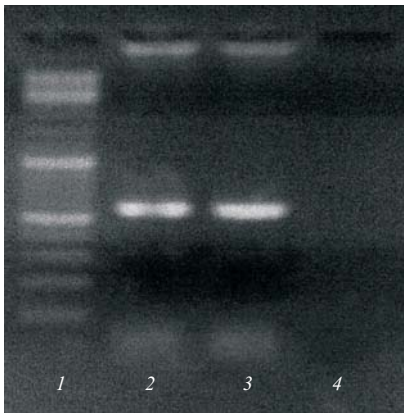


Fig. 1. Results of RT-PCR for PVY diagnostics: *1* – markers (Gene Ruler DNA Ladder Mix, Fermentas); *2* – PCR product generated on the insertion of a fragment of the capsid gene in plasmid pJET1.2. (positive control); *3* – RNA of PVY-positive sample; *4* – negative control

The first set of primers (capable of amplifying PCY cDNA) was further employed for optimization of the PCR parameters (determination of optimal $MgCl_2$ content and annealing temperature).

In short, we have found that optimal primer annealing temperature was 62 °C and optimal content of $MgCl_2$ equaled 1.75 mM, providing more specific product.

The amplicon obtained using primers' set N 1 has been cloned for generation of a positive control for PCR. The product has been detected in bacteria samples via PCR using specific primers (Fig. 1).

Thus, we have optimized the PCR-based diagnostics of Ukrainian isolate(s) of PVY consisting of: (1) selected specific primers with yield up to 569 bp product and the positive reaction control; (2) optimal parameters of RT-PCR: annealing temperature and $MgCl_2$ concentration were 62 °C and 1.75 mM, respectively. This forms the base for future design of the test system for routine PVY screening.

The RT-PCR yielded cDNA corresponding to the part of CP gene of Ukrainian isolate of PVY. This cDNA has been further sequenced and compared with some of the known sequences of PVY isolates and strains published in the GenBank. Though more than 600 of such sequences are available in public databases, we have used only 18 published PVY sequences showing the highest percent identity to our RT-PCR product as determined by BLAST feature (NCBI). Additionally, we have also compared corresponding ami-

*Evolutionary homology of Ukrainian isolate of PVY with selected PVY sequences available in the GenBank (%)*

| GenBank reference number | Country | Strain | Homology of nucleotide sequences, % | Homology of AA sequences, % |
|---|---|---|---|---|
| PVY_gi325053317_ | Syria | Rec | 99.8 | 99.8 |
| PVY_gi264160858_ | Japan | PVYNTN-NW | 99.8 | 99.8 |
| PVY_gi264160830_ | Japan | PVYNTN-NW | 99.8 | 99.8 |
| PVY_gi264160900_ | Japan | PVYNTN-NW | 99.5 | 99.5 |
| PVY_gi264160917_ | Japan | PVYNTN-NW | 99.3 | 99.3 |
| PVY_gi192763859_ | Iran | N:O | 99.3 | 99.3 |
| PVY_gi336318904_ | USA | N:O | 99 | 99 |
| PVY_gi347514591_ | Poland | N-Wi | 98.8 | 98.8 |
| PVY_gi336318948_ | USA | O | 98.8 | 98.8 |
| PVY_gi307094821_ | France | NW | 98.8 | 98.8 |
| PVY_strain_Wilga_gi90968475_ | Germany | Wigna | 98.8 | 98.8 |
| PVY_gi336318902_ | USA | O | 98.6 | 98.6 |
| PVY_strain_O_gi90968473_ | Germany | O | 98.3 | 98.3 |
| PVY_gi2808577_ | Switzerland | – | 97.6 | 97.6 |
| PVY_gi12005812_ | Brazil | O | 97.4 | 97.4 |
| PVY_gi2808576_ | Switzerland | O | 97.4 | 97.4 |
| PVY_gi156766622 | China | Isolate = «Laiwu1» | 97.1 | 97.1 |
| PVY_gi31338457_ | China | Isolate = «Hangzhou» | 97.1 | 97.1 |

no acid sequences. It follows that the CP gene of Ukrainian isolate of PVY is more homologous to the recombinant isolates (strains) – for instance, Syria Rec, Japan PVYNTN-NW, Iran N:O, USA N:O, Poland N-Wi (98.8–99.8 % of homology for both nucleotide and amino acid sequences) (Table).

These relationships are even more obvious when analyzed using phylogenetic trees constructed for nucleotide and amino acid sequences of CP employing the Neighbor-Joining (NJ) method (Fig. 2, 3).

Both nucleotide sequence- and amino acid sequence-based phylogenetic trees for PVY CP demonstrate higly similar (though not 100 % identical) position of Ukrainian isolate in relation to others. In both cases we can see a separate cluster including recombinant isolates (strains) from Syria, Iran and Japan; PVY N-Wi Poland isolate is one of the most closely related to the cluster with Ukrainian isolate of PVY.

To make a conclusion on the evolutionary history of these isolates, the maximum likelihood (ML) approach has been used on the base of Tamura-Nei model. A tree with high logarithm of likelihood has been constructed for 1000 bootstrap replications. The branch length corresponds to the number of changes per site.

As shown in Fig. 4, the phylogenetic tree constructed using ML method underlines that the main tendencies remain the same. We can say that Ukrainian PVY isolates and PVY isolates from Syria, Iran and Japan have descended from a single ancestor.

Study of state-of-the-art (2006–2012 yy) and previous (1992–2005 yy) literature sources confirms the absence of common opinion of the authors regarding unified nomenclature for strains and isolates of PVY (in particular, those found in potato). The comparison of phylogenetic trees published by different researchers is not an easy task as (most often) different «source» virus

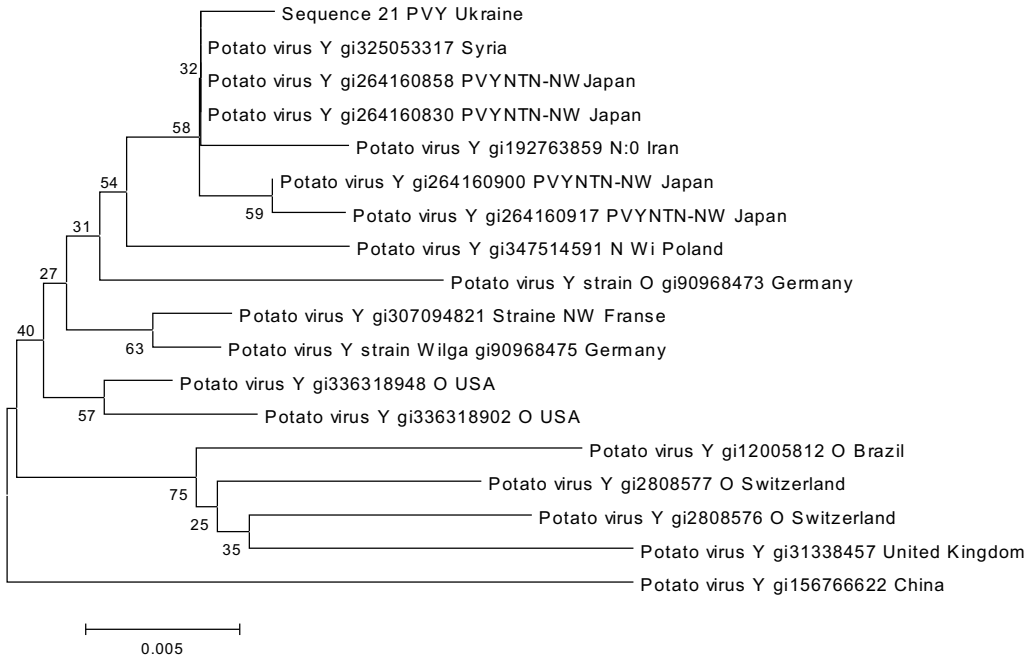


Fig. 2. Evolutionary relationships of Ukrainian PVY isolates with PVY isolates/ strains deposited in the Gen Bank. Evolutionary history has been reconstructed using NJ method [17] for nucleotide sequence of the CP gene. Evolutionary distances were calculated using p-distance method [15] for 1000 bootstrap replications (number of base changes per site). MEGA5 has been employed for evolutionary analysis

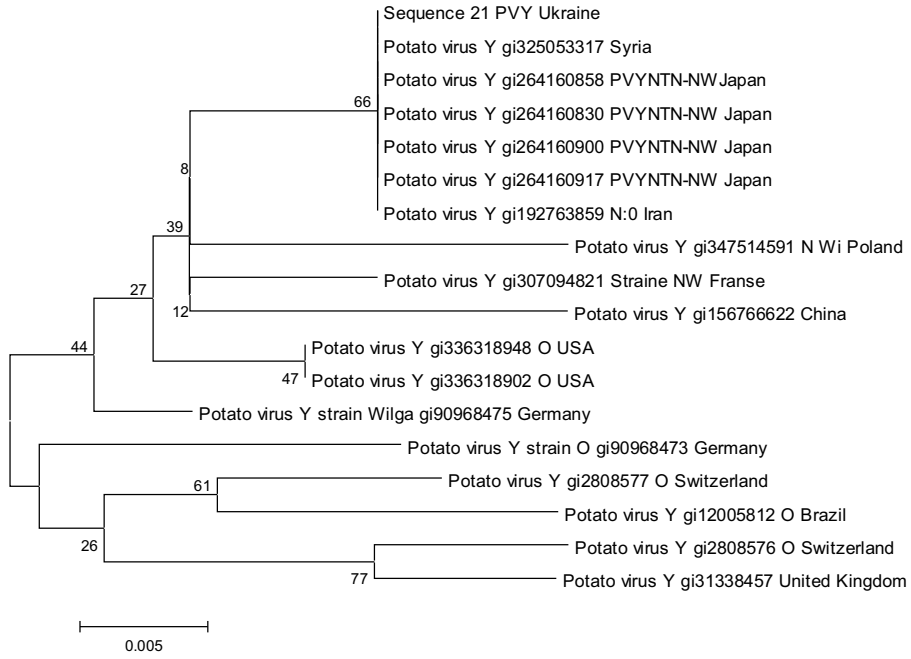


Fig. 3. Evolutionary relationships of Ukrainian PVY isolates with PVY isolates/ strains deposited in the Gen Bank. Evolutionary history has been reconstructed using NJ method [17] for amino acid sequence of the CP gene. Evolutionary distances were calculated using Poisson correction method [15] for 1000 bootstrap replications (number of base changes per site). MEGA5 has been employed for evolutionary analysis

sequences (both nucleotide and amino acid) have been used for the analysis. Apparently, the recombination among the PVY genomes may lead to the development of novel variants of the virus which may have differing phenotype (without any significant correlation with coding sequences). In turn, phenotypic appearances will depend on the specific species and cultivar of the virus-infected plant.

As soon as our work has not been aimed at studying phenotypic variations of Ukrainian isolates of PVY, our conclusions are based exclusively on the comparative analysis of nucleotide sequences in the part of virus genome (the coat protein gene of PVY). The phylogenetic relationships based on the *CP* gene of PVY and explored using ML method may be an indirect index of the relationships for full-size genomes of potyviruses

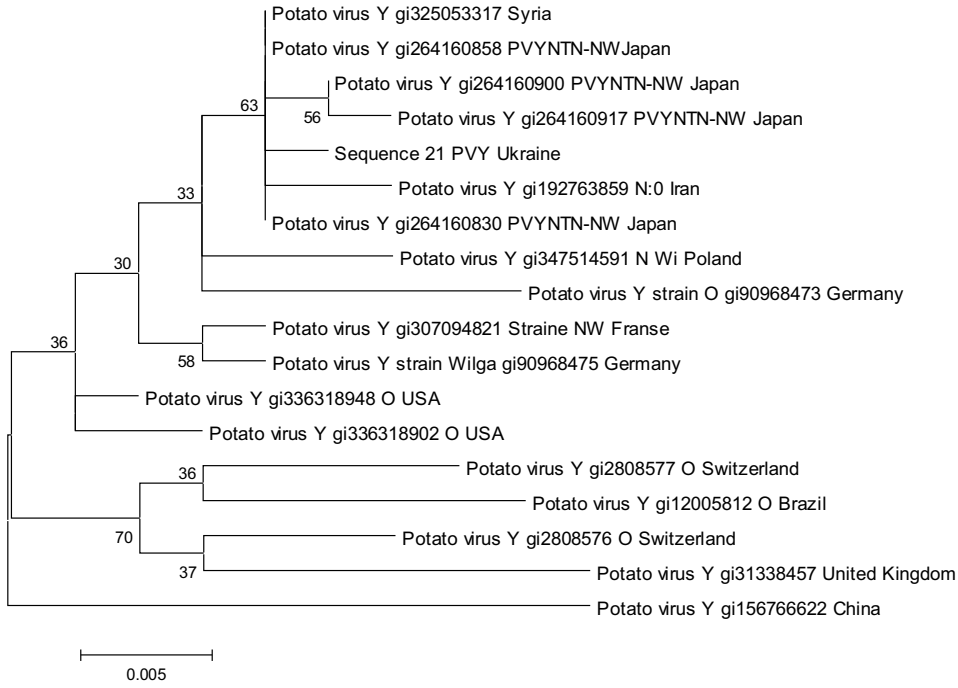


Fig. 4. Molecular phylogenetic analysis of nucleotide sequences of PVY CP gene using maximum likelihood (ML) method [16]

[10] and for establishing the species of viruses, for which only the *CP* gene sequences are available (which prevail in the GenBank).

Our conclusions regarding close relationships between Ukrainian PVY isolate and isolates from Syria, Iran and Japan, as well as their putative common origin are strongly supported by the fact that these isolates are closely positioned at the other phylogenetic trees. Following this lead and according to the latest trends in classification of PVY isolates/strains, it is viable to suggest that Ukrainian isolate of PVY belongs to the strain group O, subclade N:O (or to the subclade O:N-Wilga N:O) [11, 12, 20].

Based on these comparative data we may conclude that Ukrainian isolate of PVY is a recombinant virus. Considering the experience of other research groups [1, 12, 13, 20], we would assume that such recombinant isolate of PVY might be capable of phenotypic appearances typical for both ordinary (O) and necrotic (N) strains, depending on the specific conditions of the plant virus infection development.

The fact that the recombinations more likely occur in the limits of the strain is soothing; however, PVY strain diversity in wild nature (which may serve as a rich additional source of recombination material) is completely obscure and requires accurate monitoring for forecasting the appearance of new highly pathogenic virus variants and prevention of the disease de v-elopment.

This has been brightly examplified in 1980ies in Europe by the appearance of necrotic strain invading potato tubers, PVY-NTN.

**Conclusions**. This work underlines the need and provides means for accurate monitoring of *Potato virus Y* in the agroecosystems (and preferably – in wild-growing plants) of Ukraine where potato remains one of the most commercially important crop.

Aiming at designing proprietary PCR-based test system for PVY, we have isolated PVY isolate, designed own specific primers and optimized the RT-PCR conditions.

Most importantly, phylogenetic analysis demonstrated the recombinant nature of this PVY isolate which has been attributed to strain group O, subclade N:O.

Further work will focus on search of other widespread PVY isolates and their molecular characterization with subsequent progress with the evaluation of sensitivity and specificity of the test system.

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Нуклеотидні і амінокислотні послідовності білка оболонки українського ізоляту Y вірусу картоплі:порівняння з гомологічними послідовностями інших ізолятів та філогенетичний аналіз

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Резюме

***Мета***. *Виявлення українських ізолятів Y вірусу картоплі (PVY) у різних сортах картоплі і їхній наступний філогенетичного аналіз на основі нуклеотидних і амінокислотних послідовностей білка оболонки.* ***Методи***. *ІФА, ЗТ-ПЛР, секвенування ДНК і філогенетичний аналіз.* ***Результати***. *У зразках картоплі вітчизняної селекції методом ІФА ідентифіковано PVY. Оптимізовано процес виділення РНК та розроблено тест-систему на базі ПЛР для діагностики українських ізолятів PVY. Секвеновано ділянку гена капсидного білка українського ізоляту та здійснено філогенетичний аналіз. Встановлено, що зазначений ген демонструє вищий ступінь гомології з генами капсидних білків рекомбінантних ізолятів (штамів) (98,8–99,8 % гомології для нуклеотидних і амінокислотних послідовностей). Український ізолят перебуває в окремому кластері разом з рекомбінантними ізолятами із Сирії, Ірану та Японії і має з ними спільне походження.* ***Висновки***. *В результаті роботи визначено засоби для точного моніторингу вірусу картоплі в агроекосистемах України. Філогенетичний аналіз продемонстрував рекомбінантну природу досліджуваного ізоляту PVY, який раніше був приписаний до групи штамів O, субклади N:O.*

*Ключові слова: Y вірус картоплі, потівірус, ПЛР, філогенетичний аналіз, рекомбінантний штам.*

Нуклеотидные и аминокислотные последовательности белка оболочки украинского изолята Y вируса картофеля: сравнение с гомологичными последовательностями других изолятов и филогенетический анализ

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Резюме

***Цель***. *Выявление украинских изолятов Y вируса картофеля (PVY) в различных сортах картофеля и их последующий филогенетический анализ на основе нуклеотидных и аминокислотных последовательностей белка оболочки.* ***Методы***. *ИФА, ОТ-ПЦР, секвенирование ДНК и филогенетический анализ.* ***Результаты***. *В образцах картофеля отечественной селекции методом ИФА идентифицирован PVY. Оптимизирован процесс выделения РНК и разработана тест-система на базе ПЦР для диагностики украинских изолятов PVY. Секвенирован участок гена капсидного белка украинского изолята и проведен филогенетический анализ. Показано, что указанный ген демонстрирует высокую степень гомологии с генами капсидных белков рекомбинантных изолятов (штаммов) (98,8–99,8 % гомологии для нуклеотидных и аминокислотных последовательностей). Украинский изолят находится в отдельном кластере вместе с рекомбинантными изолятами из Сирии, Ирана и Японии и имеет с ними общее происхождение.* ***Выводы***. *В результате работы определены средства для точного мониторинга вирусов картофеля в агроэкосистемах Украины. Филогенетический анализ продемонстрировал рекомбинантную природу исследуемого изолята PVY, который ранее был приписан к группе штаммов O, субклады N:O.*

*Ключевые слова: Y вирус картофеля, потивирус, ПЦР, филогенетический анализ, рекомбинантный штамм.*

REFERENCES

1. *Moury B, Morel C, Johansen E, Jacquemond M.* Evidence for diversifying selection in *Potato virus Y* and in the coat protein of other potyviruses. *J Gen Virol*. 2002; **83**(Pt 10):2563–73.
2. *Singh RP, Valkonen JP, Gray SM, Boonham N, Jones RA, Kerlan C, Schubert J.* Discussion paper: the naming of *Potato virus Y* strains infecting potato. *Arch Virol*. 2008; **153**(1):1–13.
3. *Valkonen JPT. Potato* viruses: economical losses and biotechnological potential. *Potato biology and biotechnology*. San Diego: Elsevier, 2007; 619–641.
4. *Virus taxonomy. Ninth report of the International Committee on Taxonomy of Viruses* / Eds AMQ King, E Lefkowitz, MJ Adams, EB Carstens. Wien: Springer, 2012. 1327 p.
5. *Smith KM.* Composite nature of certain potato viruses of the mosaic group. *Nature*. 1931; **127**:702.
6. *Bawden FC, Kassanis B.* Varietal differences in susceptibility to potato virus Y. *Ann Appl Biol*. 1946; **33**(1):46–50.
7. *Cockerham G.* The reactions of the potato varieties to viruses X, A, B, and C. *Ann Appl Biol*. 1943; **30**(4):338–344.
8. *Kus M*. The epidemic of the tuber necrotic ringspot strain of potato virus Y (PVYNTN) and its effect on potato crops in Slovenia. *9th EAPR Virology Section Meeting Bled* (18–22 June, 1995): 159–160.
9. *Kerlan C, Le Romancer M*. Potato tuber necrotic ringspot disease. *Proc. EAPR Meeting, Virology section.* Vitoria-Gasteiz (Spain), 1992:77–79.
10. *Roossinck MJ*. Mechanisms of plant virus evolution. *Annu Rev Phytopathol*. 1997; **35**:191–209.
11. *Schubert J, Fomitcheva V, Sztangret-Wisniewska J.* Differentiation of *Potato virus Y* strains using improved sets of diagnostic PCR-primers. *J Virol Methods*. 2007; **140**(1–2):66–74.
12. *Nie X, Singh RP*. Specific differentiation of recombinant PVYN: O and PVYNTN isolates by multiplex RT-PCR. *J Virol Methods*. 2003; **113**(2):69–77.
13. *Nie X, Singh RP, Singh M.* Molecular and pathological characterization of N:O isolates of the *Potato virus Y* from Manitoba, Canada. *Can J Plant Pathol*. 2004; **26**(4):573–83.
14. *Lakin GF*. Biometrics: studies: manual [for biological. specials. universities]. M.: Higher School, 1980; 293 p.
15. *Dzheyper J, Scott RF*. Genetic engineering of plants. Moscow: Mir, 1991; 236–269.
16. *Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S.* MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol*. 2011; **28**(10):2731–9.
17. *Felsenstein J.* Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol*. 1981; **17**(6):368–376.
18. *Saitou N, Nei M.* The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol.* 1987; **4**(4): 406–425.
19. *Huelsenbeck JP, Crandall KA.* Phylogeny estimation and hypothesis testing using maximum likelihood. *Annu Rev Ecol Syst*. 1997; **28**:437–66.
20. *Chikh Ali M, Maoka T, Natsuaki KT.* The occurrence and characterization of new recombinant Isolates of PVY displaying shared properties of $PVY^{N}W$ and $PVY^{NTN}$. *J Phytopathol*. 2007; **155** (7–8):409

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