UDC 635,21:632.38A/2

MOLECULAR IDENTIFICATION OF EXTREME RESISTANCE GENES TO PVY AMONG BREEDING LINES AND POTATO VARIETIES OF UKRAINIAN ORIGIN

Ya. M. Hadzalo [1], S. O. Kyrychenko [2], R. O. Bondus [3], * N. O. Kozub [2]

[1] National Academy of Agrarian Sciences of Ukraine
9, Mykhaila Omelianovycha-Pavlenka Str., Kyiv, Ukraine, 01010
[2] Institute of Plant Protection the National Academy of Agrarian Sciences of Ukraine,
33, Vasylkivska Str., Kyiv, 03022, Ukraine
[3] Ustymivka Experimental Station of Plant Production of the IPP n.a. V. Ya. Yuriev
the National Academy of Agrarian Sciences of Ukraine,
15, Akademika Vavilova Str., Ustymivka, Poltava oblast, 39074, Ukraine

*E-mail: syvatoslavkirichenko@gmail.com, bondus1971@gmail.com, * natalkozub@gmail.com*

ORCID: https://orcid.org/0000-0002-5028-2048, https://orcid.org/0000-0002-4325-4022, https://orcid.org/0000-0002-2367-5225, https://orcid.org/0000-0002-3572-1786

Received March 29, 2024 / Received April 10, 2024 / Accepted April 18, 2024

Aim. Molecular identification of two genes responsible for extreme resistance (ER) to potato virus Y (PVY), viz. Ry_{adg} and Ry_{chc}. in potato lines and varieties bred in Ukraine. **Methods.** In total 78 potato samples (40 breeding lines and 38 varieties) were studied. To identify the Ry_{adg} and Ry_{chc} genes, the molecular markers RYSC3 and Ry186, respectively, were used. The silica-based technique was used to isolate DNA from potato leaves or tubers. Amplified products were analysed with agarose gel-electrophoresis. **Results.** Molecular markers were used to evaluate 40 breeding lines produced by the Polissia Experimental Department of the Institute for Potato Research, the NAAS, the harvest of 2022, and 38 cultivars, registered in the period of 1981–2015, from the collection of the Ustymivka experimental station of the Institute of Plant Production n.a. V.Ya. Yuriev, the NAAS. Five varieties, Oksamyt, Horlytsia, Lybid, Ivankivska rannia, and Ikar, and 19 lines contained the marker for $Ry_{chc.}$ The gene Ry_{adg} marker was detected in one cultivar (Obriy) and eight breeding lines. Six lines carried both genes Ry_{adg} and Ry_{chc}. Field evaluation against the natural PVY infection background indicated false-positive results of the presence of ER genes in Obriy and Lybid. The frequencies of the ER genes to PVY Ry_{adg} and Ry_{chc} were considerably higher in the group of breeding lines than in the sample of varieties, 47.5 and 10.5 % for Ry_{chc}, and 20 and 0 % for Ry_{adg}, respectively. **Conclusions**. Our study confirmed the possible presence of extreme resistance genes to PVY, Ry_{adg} and Ry_{chc}, in 4 Ukrainian potato varieties and 21 breeding lines. A higher frequency of carriers of the resistance genes was present in the new breeding lines (52.5 %) than in the varieties already in production (10.5 %). The total frequency of both genes was fairly similar to the one detected in the earlier study. If the lines and varieties where the above-mentioned resistance genes were detected are truly resistant in the field and origin of these resistance genes in the Ukrainian potato material has still to be investigated.

Key words: *Solanum tuberosum,* Ry_{adg}, Ry_{chc}, potato virus Y, molecular markers, RYSC3, Ry186, recombinant strain.

DOI: https://doi.org/10.15407/agrisp11.01.003

INTRODUCTION

Potato (*Solanum tuberosum* L.) is the most important non-cereal food crop, ranked fourth by the volume of global production, according to the FAO data for 2021 (https://www.fao.org/faostat/en/#data/QCL). Potato is unfortunately vulnerable to infection of many pests and disease agents, including viruses (Abbas et al, 2013; Kreuze et al, 2020), which diminish the yield and its quality considerably. Due to vegetative reproduction of potato, the pathogens may be transmitted

via infected seed tubers to the following generations, which leads to the decrease in the performance and the occurrence of poor-quality tubers, thus, the degradation of seed potato is an urgent problem in the production of this crop (Thomas-Sharma et al, 2016). The estimated average loss in the potato production caused by the viral infection is 20–30 %, and a severe epiphytotic may result in the reduction of the production by over 80 % (Lacomme and Jacquot, 2017; Liu et al, 2023). Among more than 50 viruses and the potato spindle tuber viroid (PSTVd), which may infect potato, only several ones are very harmful and widespread, such as potato virus Y (PVY), potato leafroll virus (PLRV), potato virus X (PVX), potato virus A (PVA), and PSTVd (Lacomme amd Jacquot, 2017; Kreuze et al, 2020). At present, the most economically important virus affecting the potato production globally, decreasing yield and its quality, is potato virus Y (PVY) (Nolte et al, 2004; Weber et al, 2021; Gao, 2020; Dupuis et al, 2023). Most damaging are recombinant strains between the classical PVY^{O} and mild PVY^{N} that appeared since the 1980s. These recombinant strains placed in the strain group PVY^{E} are insensitive to the three hypersensitivity (HR) genes that normally give resistance in potato lines and varieties (Singh et al, 2008; Fuentes et al, 2019). The increasing distribution of new recombinant strains of potato virus Y, including PVY^{NTN}, is a threat to the crop performance and its quality; it can result in the loss of the tuber harvest quality due to the fact that these strains cause the so-called tuber necrotic ringspot disease (Glais et al, 2019; Fuentes et al, 2019). The recombinant strain PVY^{NTN} was first described in Hungary (Beczner et al, 1984). Soon this strain became the prevailing PVY strain in many countries, including most European countries (Yin et al, 2012; Bellstedt et al, 2017; EFSA, 2020). Sequencing of genome fragments of some PVY isolates indicated the occurrence of recombinant strains also in Ukraine (Budzanivska et al, 2014; Dunich et al, 2020). PVY is a flexuous, rod-shaped, single-stranded RNA (ss-RNA) virus and it belongs to the genus *Potyvirus* (family *Potyviridae*). The list of host plants covers over 60 species, including representatives of the *Solanaceae, Chenopodiaceae* and *Leguminosae*. PVY is mainly transmitted by many aphid species, but can also be spread by plant contact when wounded and by seed tuber cutting (Nault 1997; Lacomme et al, 2017) and it is a widespread pest of potato plants (EFSA, 2020; Kroschel et al, 2020). Among the symptoms caused by PVY in potato are so-called rugose mosaic, leaf deformation, chlorosis with necrotic spots, leaf shedding, delayed growth of plants, early withering, and necrotic ring spot of tubers, manifested depending on the severeness of infection, virus strain, and genotype of the plant (MacKenzie et al, 2019). Therefore, the level of yield reduction is defined by the PVY strain involved, viral multiplication rate, the time when the infection takes place and the level of host resistance to the virus (Shrestha et al, 2014; MacKenzie et al, 2019). Infected seed tubers, symptomless (latently infected) or with symptoms, may easily spread the virus to other fields and regions. All the daughter tubers originating from infected maternal tubers (secondary infection) will also be infected via systemic virus translocation during growth (Hedge et al, 2020; Kumar et al, 2020). Furthermore the virus survives in volunteer potatoes for several years, which serve, together with a number of solanaceous weeds, as sources of infection (Jones et al, 1996; Coutts and Jones, 2015).

Apart from the use of virus free and/or virus tested seed and control of aphid populations, the cultivation of PVY-resistant potato varieties is the most efficient and durable solution to prevent yield loss caused by the virus (Valkonen, 2015, Valkonen et al, 2017; Baebler et al, 2020). Three types of resistance genes in wild and cultivated potato are distinguished – 1) susceptibility or S-genes (no symptoms or very limited necrosis); 2) hypersensitive resistance (HR) genes (so-called *Ny* genes), with local necrotic lesions, and 3) extreme resistance (ER) genes (so-called *Ry* genes). The *Ny* genes ensure a fast defensive reaction which leads to the formation of necroses (programmed cell death) in the site of the infection, they do not impact the virus replication but hinder its movement in the plant; they are strain-specific. The dominant *Ry* genes inhibit the virus replication in the initially infected cells and are effective against a wide spectrum of strains (Valkonen 2015 and 2017; Baebler et al, 2020; Ross et al, 2021).

Breeding programs of different countries therefore preferentially use the ER genes Ry_{adg}, Ry_{sto} and Ry_{chc}. Ry_{adg} originates from the cultivated potato subspecies *S. tuberosum* subsp. *andigena* Hawkes, it is mapped on chromosome XI (Hämäläinen et al, 1997). Ry_{sto} from the wild species *S. stoloniferum* Schltdl. & Bouché is located on chromosome XII. There was a report about the introgression of another gene from *S. stoloniferum,* marked as *Ry-*f_{sto}, which was also mapped on chromosome XII (Flis et al, 2005), but it was found later that the sequences of the genes Ry_{sto} and *Ry-*f_{sto} had 100 % identity (Grech-Baran et al, 2020). Ry_{chc} originates from *S. chacoense* Bitt. and is located on the distal end of chromosome IX (Sato

et al, 2006). To identify these ER genes, a number of molecular markers were developed (Kasai et al, 2000; Song et al, 2005; Song and Schwarzfischer 2008; Valkonen et al, 2008; Mori et al, 2011; Elison et al, 2020; Caruana et al, 2021). Marker-assisted selection enhances the efficiency of creating PVY-resistant potato varieties; it is applied in the breeding programs of many countries (Rizza et al, 2006; Sagredo et al, 2009; Sharma et al, 2014; Bhardwaj et al, 2015; Fulladolsa et al, 2015; López et al, 2015; Li et al, 2017; Valkonen et al, 2017; Slater et al, 2020). The Ukrainian potato gene bank was practically not studied for the presence of ER genes. A first (preliminary) screening of breeding material was published by us in 2023 (Kyrychenko and Kozub, 2023). In those study we detected the Ry_{adg} and Ry_{chc} markers in 7 and 53 lines, respectively, and their combination in 3 lines.

The purpose of our work was to further screen the Ukrainian potato gene bank for the two extreme resistance genes Ry_{adg} and Ry_{chc} using molecular markers.

MATERIALS AND METHODS

Samples of 40 potato lines, harvest 2022, bred by the Polissia Experimental Department, the Institute for Potato Research, the National Academy of Agrarian Sciences of Ukraine (NAAS), were studied (**Table 1**). Furthermore, 38 Ukrainian potato varieties originated from the Ustymivka Experimental Station of the Institute of Plant Production named after V.Ya. Yuriev, NAAS, harvest 2022, were also analysed (**Table 2**).

Visual assessment of the degree of resistance of potato varieties to PVY was carried out in the field under the natural infection background in 2021–2023 at the Ustymivka Experimental Station according to the methodology developed in the Institute of Potato Growing of NAAS of Ukraine. The 9-score resistance scale was used to assess streak symptoms caused by PVY, where score 9 corresponded to the absence of symptoms of the disease, and score 1, to the maximum development of the disease, which causes significant inhibition of growth and development and the spread of symptoms throughout the plant (Podgayatsky and Pika,1990): 9 – plants with no signs of damage; 8 – initial symptoms of the disease; 7 – up to 25 % of leaves show signs of the disease; 5 – tissue death is observed on half of the leaves; 3 – up to 75 % of leaves show signs of the disease, lower leaves fall off or hang down along the stem;

Table 1. Results of analysis of 40 Ukrainian breeding potato lines using markers for the genes Ry_{adg} (RYSC3) and Ry_{chc} (Ry186)

Breeding line	Origin	Ry186	RYSC3
G.10.9/8	N.89.721s81/Tyras	–	–
P.19.1/4	F.09.209-3/P.14.3/12	–	–
P.18.4/1	P.13.41-4/P.14.8/14	–	+
P.19.4/10	F.09.209-3/PN.09.8-14	–	–
P.19.5/14	F.09.209-3/P.10.9-3	–	–
P.19.10/1	P.09.26/2/Vyhoda	–	–
P.19.11/6	P.09.26/2/Partner	–	+
P.19.12/10	Svitana/Mezhyrichka	–	–
P.19.13/11	Vyhoda/Svitana	+	–
P.19.15/16	Radomysl/Svitana	+	–
P.19.18/5	P.10.10/35/Svitana	–	–
P.17.19-23	P.13.54-2/Vzirets	+	–
P.19.19/3	VM.193/59/Svitana	–	–
P.19.26/15	P.10.10/35/Alliance	+	–
P.19.27/5	VM.12.24-15/P.10.10/35	+	–
P.18.29-1	P.16.53/6/P.15.23-12	+	–
P.19.30/5	Mezhyrichka/Sontsedar	+	–
P.19.33/2	Svitana/Rostavytsia	+	–
P.19.33/5	Svitana/Rostavytsia	+	–
P.19.35/31	Svitana/Rostavytsia	+	–
P.19.38/1	Predslava/P.10.9-3	–	–
P.19.39/1	P.14.43-9/P.15.55/14	–	–
P.18.42-10	Levada/ Bazhana	+	+

Continuation Table 1

Breeding line	Origin	Ry186	RYSC3
P.19.53/4	unknown	–	–
Z.19.54/4	Mezhyrichka/Dorohyn	+	–
P.19.65/1	P.10.20/22/N.07.162-1	+	–
P.19.69/3	10.34/4/Poliska uvil.	+	+
P.19.70/12	10.34/4/Poliska uvil.	–	–
P.19.74/2	P.15.23/1/Slauta	–	–
P.18.75-5	Charunka/Alliance	–	–
P.19.80/2	N.09.4.g 72/Mag	–	–
G.13.55/22	05.11s108/Bellarossa	–	–
G.12.37/70	04.20s93/Bellarossa	–	–
P.19.1-19	F.09.209-3/14.3-2	–	–
P.19.42-16	12.4-3/VM 8-22	–	–
P.20.47/8	Predslava/Vyhoda	+	+
P.20.9/33	Fanatka/09.26/2	+	+
G.12.10/1	04.21s31/Santarka	+	–
P.19.11-2	09.26/2/Partner	+	+
P.20.14-1	15.43-7/09.26/2	+	+

Note: *+ positive band in gel-electrophoresis, resistance gene presumed to be present; – resistance gene not detected.

Table 2. Results of analysis of 38 Ukrainian potato varieties, using markers for the genes Ry_{adg} (RYSC3) and Ry_{chc} (Ry186) and results of field evaluation against the natural background

Variety name	Year of state registration	Ry186	RYSC3	PVY field test, score
Factor	2015	–	–	9
Bozhedar	1996	–	–	8
Hart	1990	–	–	7
Zov	1989	–	–	9
Kobza	1995	–	–	7
Kosen 95	1999	–	–	9
Molodizhna	1996	–	–	8
Mriia	2004	–	–	9
Nezabudka	1981	–	–	9
Oksamyt	2002	+	–	9
Posvit	1992	–	–	9
Prolisok	1991	–	–	5
Povin	2000	–	–	5
Sednivska rannia	1994	–	–	9
Serpanok	2001	–	–	5
Sorokadenka bila	–	–	–	9
Chernihivska rannia	1999	–	–	5
Berehynia	1992	–	–	9
Dobrochyn	1995	–	–	7
Mavka	1982	–	–	5
Malych	1988	–	–	9
Obriy	1997	–	+	5
Poliana	2002	–	–	5
Radych	1997	–	–	9
Luhovska	1987	–	–	8

Continuation Table 2

Variety name	Year of state registration	Ry186	RYSC3	PVY field test, score
Fantasia	2001	–	–	5
Horlytsia	1996	+	–	9
Dzvin	2000	–	–	7
Leleka	2002	–	–	9
Lybid	1993	+	–	5
Poliska-96	2001	–	–	9
Svaliavska	2001	–	–	9
Ukrainian Red	1989	–	–	8
Yavir	2000	–	–	9
Zarevo	1983	–	–	3
Volovetska	1985	–	–	9
Ivankivska rannia	2015	+	–	9
Ikar	1983	+	–	9

Note: *+ the marker for the resistance gene present; – the marker is absent.

1 – up to 50 % of leaves fallen off, the rest either hang down or are very affected, necrosis on the stems.

DNA was isolated from potato leaves (lines) or tubers (varieties) using the commercial silica-based kit NeoPrep_100 (Neogene LLC, Ukraine) according to the manufacturer's recommendations (http://neogene.com.ua/index.php?route=product/product&path=18_46&product_id=192.). Leaves were taken from adult greenhouse plants. The plant material was kept in a freezer. DNA was stored in a refrigerator at 4 C. For PCR, the mastermix PCR MIX 2x HOT (Neogene LLC, Ukraine) containing hot-start Taq DNA polymerase, dNTPs, $MgCl_2$, and the reaction buffer was used according to the manufacturer's recommendations (http://neogene.com.ua/index.php?route=product/product&product_id=87). PCR was conducted in a 20 µl reaction mix involving 5 µl of DNA isolated with the above kit on an Applied Biosystems 2720 Thermal Cycler. We used the varieties Kivi and Tiras as positive controls for the RYSC-3 and Ry186 markers, respectively. Asterix was used as a negative control for both the markers.

The identification of the resistance gene Ry_{chc} involved the use of molecular marker Ry186 developed by Mori et al (2011) (**Table 3**). The PCR protocol was as follows: initial denaturation – 10 min at 95 °C, 35 cycles (denaturation for 30 s at 94 °C; annealing for 30 s at 54 °C, elongation for 1 min, at 72 °C), final elongation for 7 min at 72 °C (based on the protocol of Li et al (2017) but with the annealing temperature of 54 °C). The presence of the resistance gene Ry_{chc} was presumed when the PCR resulted in obtaining the marker amplicon of 587 bp.

The presence of the resistance gene Ry_{adg} was determined using the molecular marker RYSC3 according to Kasai et al (2000) (Table 3). The following PCR protocol was used: initial denaturation – 10 min at 95 °C, 35 cycles (denaturation for 45 s at 94 °C; annealing for

Table 3. Molecular markers for detection of Ry_{adg} and Ry_{chc} and their primer sequences

Gene	Marker	Primer sequence (5′–3′)	Size, bp	Reference
Ry_{adg}	RYSC3	ATACACTCATCTAAATTTGATGG	321	Kasai et al, 2000
		AGGATATACGGCATCATTTTTCCGA		
Ry_{chc}	Ry186	TGGTAGGGATATTTTCCTTAGA	587	Mori et al, 2011
		GCAAATCCTAGGTTATCAACTCA		

45 s at 60 °C, elongation for 1 min, at 72 °C), final elongation for 7 min at 72 °C. The marker for the presence of gene Ry_{adg} was an amplicon of 321 bp.

The electrophoresis was conducted in 1.5% agarose gel with 1 μg/ml ethidium bromide using 1 × TBE (Tris Borate EDTA) buffer. The gels were photographed using the gel-documentation system VISION Gel (Scie-Plas Ltd, UK).

RESULTS

The investigated potato samples included 40 new breeding lines representing a new breeding stage and 38 Ukrainian varieties registered within 1981–2015 (Tables 1 and 2). A few examples of the results of genotyping using markers Ry186 and RYSC3 are shown in **Fig. 1** and **2**, where the presence of an amplicon of 587 bp demonstrates the possible presence of the gene Ry_{chc} (Mori et al, 2011), and an amplicon of 321 bp, the possible presence of the gene Ry_{adg} (Kasai et al, 2000).

Among the Ukrainian potato varieties, the marker amplicon for the resistance gene Ry_{adg} was detected only in the variety Obriy. The presence of the gene Ry_{chc} was determined in five varieties, viz. Oksamyt, Lybid, Horlytsia, Ivankivska rannia, and Ikar. In the breeding material, Ry_{adg} was presumably present in 8 lines, and Ry_{chc} in 19 lines.

Simultaneous presence of the markers for Ry_{adg} and Ry_{chc} was not found in the varieties tested, but both were present in six breeding lines (P.18.42-10, P.19.69/3, P.20.47/8, P.20.9/33, P.19.11-2, and P.20.14-1) (Table 1).

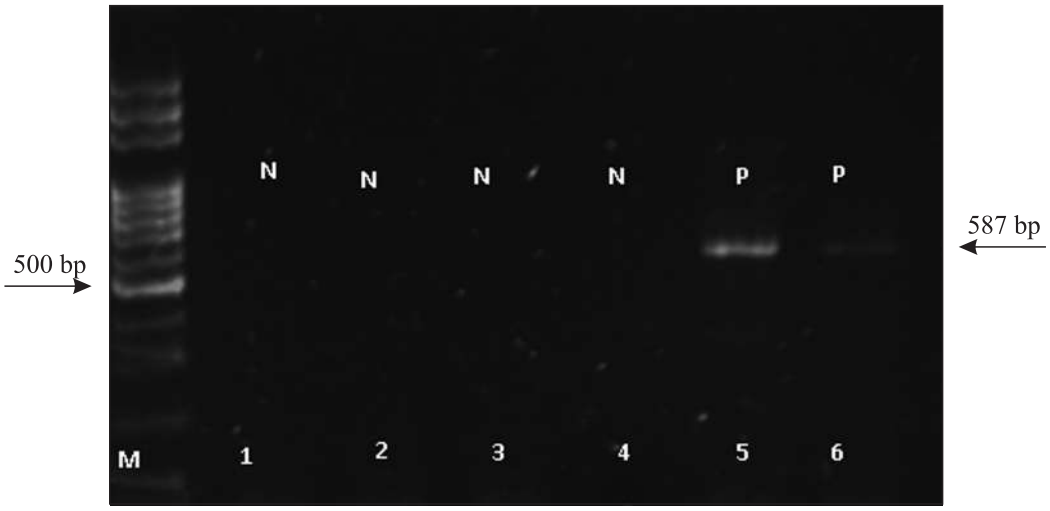

Fig. 1. PCR results with marker Ry186 for detection of the gene Ry_{chc} in potato samples: 1 – line P.19.5/14, 2 – P.19.10/1, 3 – P.19.11/6, 4 – P.19.12/10, 5 – P.19.13/11, 6 – P.19.15/16. M – marker (100 bp DNA Ladder). N = negative; P = positive

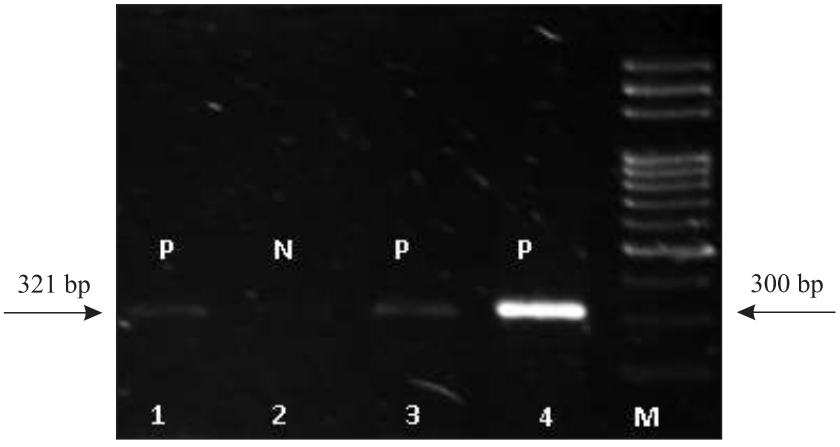

Fig. 2. PCR results with marker RYSC3 for detection of the Ry_{adg} gene in potato samples: 1 – P.20.9/33, 2 – G.12.10/1, 3 – P.19.11-2, 4 – P.20.14-1. M – marker (100 bp DNA Ladder). N = negative; P= positive

Thus, the frequency of the samples having the marker RYSC3 for the resistance gene Ry_{adg} is 20 % in the sample of 40 breeding lines and 2.6 % among 38 Ukrainian varieties tested. The frequency of the marker Ry186 for the Ry_{chc} gene was 47.5 % in the sample of breeding lines and 13 % in the sample of varieties.

The comparison of the results of molecular identification of the extreme resistance genes with the results of field evaluation against the natural infection background indicated that in two cases there were false-positive results. The varieties Obriy and Lybid showed score 5, which indicates that they are susceptible to PVY (Table 2). The other carriers of the marker amplicons for Ry_{chc} (Oksamyt, Horlytsia, Ivankivska rannia, and Ikar) proved to be resistant (with score 9). Thus, the frequency of possible carriers of the Ry_{chc} gene in the sample of Ukrainian varieties is 10.5 %, and none of the varieties has the Ry_{adg} gene.

DISCUSSION

In 2021, Ukraine was ranked the third largest producer of potato in the world, after China and India, (https://www.fao.org/faostat/en/#data/QCL). Viral diseases are among the main detrimental phytopathogens in the agrocenosis of potato in Ukraine (Boroday and Parfenyuk, 2018; Volkova et al, 2021).

The annual losses of the European Union due to PVY are estimated as EUR 187 million, mainly caused by the expenditures on the chemical treatment applied while cultivating seed potato and yield losses in seed, industrial and consumption potatoes (Dupuis et al., 2023). An older study conducted in the USA demonstrated that harvest losses due to PVY amounted to 0.18 t/ha per each per cent of the increase in the virus incidence (Nolte et al. 2004). In a long-term study over the years 1996–2009, Kostiw (2011), in a field/greenhouse inoculation experiment, determined that 32.5% of the daughter tubers were infected after a 10 days exposure to several viruses, and the infection percentage of virus Y greatly exceeded that of potato virus M (18.2 %), potato virus S (22.1 %), and potato leaf roll virus (15.3 %).

The results of our investigation of Ukrainian potato varieties, which cover almost 30 years of the breeding, using markers to the ER genes Ry_{adg} and Ry_{chc}, demonstrated a low frequency of the marker for the gene Ry_{adg} (2.6 %) and somewhat higher incidence of the marker of gene Ry_{chc} (13 %). Moreover, field evaluation of the varieties indicated false positive results in two varieties showing the marker amplicons: these are Obriy with the positive result of amplification with the marker RYSC3 and Lybid with the Ry186 marker. So the sample analysed does not contain any varieties with the gene Ry_{adg} and its frequency of Ry_{chc} carriers is 10.5 %. Such cases with the presence of marker amplicons in PVY susceptible genotypes of potato were previously described, for example, the variety Emma, which showed two Ry_{adg} markers (RYSC3 and M45) along with the susceptible phenotype (Slater et al, 2020, Caruana et al, 2021), and the susceptible accessions TARN187 (with RYSC3 and M45) and A6 (with the RYSC3 marker) (Herrera et al, 2018). However, among the new breeding lines, the incidence of the two ER markers was found to be considerably higher for both genes (20 and 47.5 % respectively), with a prevalence of the incidence of the marker for the Ry_{chc} gene. In addition, in six lines (15 %) the markers for the two resistance genes were detected, which makes them a promising material in terms of breeding for extreme resistance to PVY.

The marker RYSC3, used by us and developed by Kasai et al, 2000, is most frequently used in the analysis of the potato samples for the presence of Ry_{adg} (Rizza et al, 2006; Sagredo et al, 2009; Sharma et al, 2014; Bhardwaj et al, 2015; Fulladolsa et al, 2015; López et al, 2015; Li et al, 2017; Slater et al, 2020). The geographical occurrence of Ry_{adg} is quite different. The highest percentage of Ry_{adg} carriers was identified in samples of the Uruguay collection (68 %) (Rizza et al, 2006), in the Chili potato collection of the Chilota Potato Genebank (36.5 %) (López et al, 2015), and among Chinese varieties (22.4 %) (Li et al, 2017). The combination of different Ry_{adg} markers proved to be more efficient for detecting Ry_{adg} carriers. For example, in the Australian collection of potato samples, the RYSC3 marker was detected in three varieties, but the M45 marker was amplified by the nine additional varieties (Slater et al, 2020). Thus, the recent detection and description of novel markers, such as M45 and M6, and the SNP marker (SNP37279) validated as a Kompetitive Allele-specific PCR (KASP) marker for more precise and extensive identification of Ry_{adg} may lead to a more accurate identification of the respective ER gene (Herrera et al, 2018; Caruana et al, 2021).

There is much less information about the occurrence of the gene Ry_{chc} among potato varieties and lines of different origin. Using marker Ry186, in 6.6 % potato varieties from China, Ry_{chc} was detected (Li et al, 2017). The presumed frequency of this gene in the investigated sample of Ukrainian varieties was 10,5 %, but it was

found considerably higher (47.5 %) in the sample of breeding lines. The markers, used by us, are not direct, so there is a possibility of recombination between the marker and the resistance gene, and thus, false positive results (Kinghorn, 2010) due to the distance between the diagnostic marker and the PVY resistance gene. After sequencing the gene Ry_{chc}, it was found to be the resistance gene of the Toll/interleukin-1 receptor-nucleotide-binding site-leucine rich repeat (TIR-NBS-LRR) type (Li et al, 2022; Akai et al, 2023). From this sequence new direct markers to identify gene Ry_{chc} were designed (Li et al, 2022; Akai et al, 2023).

Our research demonstrates the likely presence of the genes Ry_{adg} and Ry_{chc} in potato material bred in Ukraine present in our gene bank. In the future we will continue similar studies on more breeding material that could lead to detection of further resistance genes and eventually to more resistant Ukrainian varieties. Furthermore, we will check the actual presence of resistance in the lines and varieties by inoculation experiments

CONCLUSIONS

The study using molecular markers demonstrated the presumed presence of the extreme resistance genes to PVY Ry_{adg} and Ry_{chc} in a number of Ukrainian potato varieties and breeding lines. The frequency of carriers of these resistance genes was considerably higher among the breeding lines than in the sample of varieties, namely 52.5, and 10.5 %, respectively. The identification of sources of extreme resistance of potato in the breeding material is an efficient way to reduce yield losses due to virus Y when introducing new varieties.

Adherence to ethical principles. This article does not contain any studies with human participants and animals performed by any of the authors.

Conflict of interests. The authors declare the absence of any conflicts of interests.

Financing. This work was financed by the NAAS according to task No. DR 0121U000082 ASR Plant Protection.

Молекулярна ідентифікація генів екстремальної стійкості до PVY серед ліній і сортів картоплі української селекції

Я. М. Гадзало [1], С. О. Кириченко [2], Р. О. Бондус [3], * Н. О. Козуб [2]

[1] Національна академія аграрних наук України, вул. Михайла Омеляновича-Павленка, 9, м. Київ, Україна, 01010

[2] Інститут захисту рослин Національної академії аграрних наук України, вул. Васильківська, 33, м. Київ, Україна, 03022

[3] Устимівська дослідна станція рослинництва Інституту рослинництва ім. В. Я. Юр'єва Національної академії аграрних наук України, вул. Академіка Вавілова 15, Устимівка, Полтавська обл., Україна, 39074

E-mail: syvatoslavkirichenko@gmail.com, bondus1971@gmail.com, * natalkozub@gmail.com

orcid: https://orcid.org/0000-0002-5028-2048, https://orcid.org/0000-0002-4325-4022, https://orcid.org/0000-0002-2367-5225, https://orcid.org/0000-0002-3572-1786

Мета. Молекулярна ідентифікація двох генів, що визначають екстремальну стійкість (ER) до вірусу Y картоплі (PVY), а саме Ry_{adg} та Ry_{chc}, у лініях та сортах картоплі української селекції. **Методи.** Всього було досліджено 78 зразків картоплі (40 селекційних ліній та 38 сортів). Для ідентифікації генів Ry_{adg} та Ry_{chc} використовували молекулярні маркери RYSC3 та Ry186, відповідно. Для виділення ДНК з листків або бульб картоплі застосовували методику на основі силіки. Ампліфіковані продукти аналізували за допомогою електрофорезу в агарозному гелі. **Результати.** За допомогою молекулярних маркерів оцінено 40 селекційних ліній, створених Поліським дослідним відділенням Інституту картоплярства НААН України врожаю 2022 р., та 38 сортів, зареєстрованих у період 1981–2015 рр., з колекції Устимівської дослідної станції Інституту рослинництва ім. В. Я. Юр'єва НААН України. П'ять сортів – Оксамит, Горлиця, Либідь, Іванківська рання та Ікар, а також 19 ліній, мали маркер гена Ry_{chc}. Маркер гена Ry_{adg} виявлено у одного сорту (Обрій) та восьми селекційних ліній. Шість ліній несли обидва гени Ry_{adg} і Ry_{chc}. Польова оцінка на природному інфекційному фоні PVY показала хибнопозитивні результати наявності генів ER у сортів Обрій та Либідь. Частоти генів Ry_{adg} і Ry_{chc} екстремальної стійкості до PVY були значно вищими в групі селекційних ліній, ніж серед досліджуваних сортів: 47,5 і 10,5 % для Ry_{chc} і 20 і 0 % для $Ry_{adg,}$ відповідно. **Висновки.** Наше дослідження підтвердило можливу наявність генів екстремальної стійкості до PVY Ry_{adg} і Ry_{chc} у 4 українських сортів картоплі та 21 селекційної лінії. Вища частота носіїв генів стійкості спостерігалась у нових селекційних ліній (52,5 %), ніж у сортів, що вже використовуються у виробництві (10,5 %). Загальна частота обох генів була досить подібною до тієї, що була виявлена в попередньому дослідженні. Чи є лінії та сорти, в яких були виявлені вищезгадані гени стійкості, дійсно стійкими в польових умовах, та походження цих генів стійкості в українському картопляному матеріалі, ще належить дослідити.

Ключові слова: *Solanum tuberosum,* Ry_{adg}, Ry_{chc}, вірус картоплі Y, молекулярні маркери, RYSC3, Ry186, рекомбінантний штам.

REFERENCES

Abbas M, Naz F, Irshad G (2013) Important fungal diseases of potato and their management-a brief review. Mycopath 11:45–50

Akai K, Asano K, Suzuki C et al (2023) *De novo* genome assembly of the partial homozygous dihaploid potato identified PVY resistance gene (*Rychc*) derived from *Solanum chacoense*. Breed Sci 73(2):168–179. https://doi.org/10.1270/jsbbs.22078

Baebler Š, Coll A, Gruden K (2020) Plant molecular responses to *Potato Virus Y*: A continuum of outcomes from sensitivity and tolerance to resistance. Viruses 12 (2):217. https://doi.org/10.3390/v12020217

Beczner L, Horváth J, Romhányi I et al (1984) Studies on the etiology of tuber necrotic ringspot disease in potato. Potato Res 27:339–352. https://doi.org/10.1007/BF02357646

Bellstedt DU, Glais L, Davie K, Lacomme C (2017) Evolution and origin of PVY. In: Lacomme C., Glais L, Bellstedt D, Dupuis B, Karasev A, Jacquot E (eds) *Potato virus* Y: biodiversity, pathogenicity, epidemiology and management. Springer, Cham. https://doi.org/10.1007/978-3-319-58860-5_4

Bhardwaj V, Sharma R, Dalamu SAK (2015) Molecular characterization of potato virus Y resistance in potato (*Solanum tuberosum* L.). INDIAN J GENET PL BR 5(03):389–392. https://doi.org/10.5958/0975-6906.2015.00062.0

Boroday VV, Parfenyuk A (2018) The prevalence and development of the main potato diseases in Ukraine. Ahroekolohichnyi zhurnal: Agroecological Journal 4:82–87 (in Ukrainian).

Budzanivska IG, Ovcharenko LP, Kharina et al (2014) 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. Biopolymers and Cell 30(2):141–148. https://doi.org/ 10.7124/bc.00088D

Caruana BM, Rodoni BC, Constable F et al (2021) Genome enhanced marker improvement for *Potato Virus Y* disease resistance in potato. Agronomy 11(5):832. https://doi.org/10.3390/agronomy11050832

Coutts BA, Jones RAC (2015) Potato virus Y: Contact transmission, stability, inactivation, and infection sources. Plant Dis 99(3):387–94. https://doi.org/10.1094/PDIS-07-14-0674-RE

Devaux A, Goffart J-P, Petsakos A, Kromann P et al (2020) Global food security, contributions from sustainable potato agri-food systems. In: Campos H, Ortiz O (eds) The Potato Crop Springer, Cham. https://doi.org/10.1007/978-3-030-28683-5_1

Dunich AA, Mishchenko LT, Dashchenko AV (2020) PVYN-Wi isolate from tomato plants in Ukraine. Archives of Phytopathology and Plant Protection, 53(3–4):112–126. https://doi.org/10.1080/03235408.2020.1730021

Dupuis B, Nkuriyingoma P, Ballmer T (2023) Economic impact of Potato virus Y (PVY) in Europe. Potato Res https://doi.org/10.1007/s11540-023-09623-x

EFSA PLH Panel (EFSA Panel on Plant Health) (2020). Scientific Opinion on the pest categorisation of potato virus Y (non-EU isolates). EFSA J 18(1):5938, 38 pp. https://doi.org/10.2903/j.efsa.2020.5938

Elison GL, Hall DG, Novy RG et al (2020) Development and application of a multiplex marker assay to detect PVY resistance genes in *Solanum tuberosum*. Am J Potato Res 97:289–296. https://doi.org/10.1007/s12230-020-09777-1

Flis B, Hennig J, Strzelczyk-Żyta D et al (2005) The *Ry-f*$_{sto}$ gene from *Solanum stoloniferum* for extreme resistance to *Potato virus Y* maps to potato chromosome XII and is diagnosed by PCR marker GP122718 in PVY resistant potato cultivars. Mol Breed 15:95–101. http://dx.doi.org/10.1007/s11032-004-2736-3

Fuentes S, Jones RAC, Matsuoka H et al (2019) Potato virus Y; the Andean connection. Virus evolution 5(2), vez037. https://doi.org/10.1093/ve/vez037

Fulladolsa AC, Navarro FM, Kota R et al (2015) Application of marker assisted selection for *Potato Virus Y* resistance in the University of Wisconsin potato breeding program. Am J Potato Res 92:444–450. https://doi.org/10.1007/s12230-015-9431-2

Gao F, Kawakubo S, Ho SYW, Ohshima K (2020) The evolutionary history and global spatio-temporal dynamics of potato virus Y. Virus Evol 21:6(2):veaa056. https://doi.org/10.1093/ve/veaa056

Glais L, Bellstedt DU, Lacomme C (2017) Diversity, characterisation and classification of PVY. In: Lacomme C, Glais, L, Bellstedt D, Dupuis B, Karasev A, Jacquot E (eds). Potato virus Y: biodiversity, pathogenicity, epidemiology, and management. Springer, Cham. https://doi.org/10.1007/978-3-319-58860-5_3

Grech-Baran M, Witek K, Szajko K et al (2020) Extreme resistance to potato virus Y in potato carrying the *Ry*$_{sto}$ gene is mediated by a TIR-NLR immune receptor. Plant Biotechnol J 18:655-67. https://doi.org/10.1111/pbi.13230

Hämäläinen JH, Watanabe KN, Valkonen JP, Arihara A, Plaisted RL, Pehu E, Miller L, Slack SA (1997) Mapping and marker-assisted selection for a gene of extreme resistance to potato virus Y. Theor Appl Genet 94:192–197. https://doi.org/10.1007/s001220050399

Hegde K, Kalleshwaraswamy C M, Venkataravanappa V (2020) Role of virus infection in seed tubers, secondary spread and insecticidal spray on the yield of potato in Deccan Plateau, India. Potato Res 64(3):339–351. https://doi.org/10.1007/s11540-020-09480-y

Herrera MDR, Vidalon LJ, Montenegro JD et al (2018) Molecular and genetic characterization of the *Ry*$_{adg}$ locus on chromosome XI from *Andigena* potatoes conferring extreme resistance to *potato virus Y*. Theor Appl Genet 131 (9):1925–1938. https://doi.org/10.1007/s00122-018-3123-5.

Jones DAC, Woodford JAT, Main SC et al (1996) The role of volunteer potatoes in the spread of potato virus YN in ware crops of cv. Record. Ann Appl Biol 129:471–478. https://doi.org/10.1111/j.1744-7348.1996.tb05769.x

Kasai K, Morikawa YV, Sorri A, Valkonen JP et al (2000) Development of SCAR markers to the PVY resistance gene Ry_{adg} based on a common feature of plant disease resistance genes. Genome (1):1–8. https://doi.org/10.1139/GEN-43-1-1

Kinghorn BP (2010) A method to detect recombination between a genetic marker and the QTL that it marks. J Anim Breed Genet 127:199–206. https://doi.org/10.1111/j.1439-0388.2009.00836.x

Kostiw M (2011) The occurrence of major potato viruses in Poland. J Plant Prot Res, 51(3):204-209. https://doi.org/10.2478/v10045-011-0035-7

Kreuze JF, Souza-Dias JAC, Jeevalatha A, Figueira AR, Valkonen JPT, Jones RAC (2020) Viral diseases in potato. In: Campos H, Ortiz O (eds) The Potato Crop. Springer, Cham. https://doi.org/10.1007/978-3-030-28683-5_11

Kroschel J, Mujica N, Okonya J, Alyokhin A (2020) Insect pests affecting potatoes in tropical, subtropical, and temperate regions. In: Campos H, Ortiz O (eds) The Potato Crop. Springer, Cham. https://doi.org/10.1007/978-3-030-28683-5_8

Kumar RR, Ansar M, Rajan K et al (2020) First report on molecular basis of potato leaf roll virus (PLRV) aggravation by combined effect of tuber and prevailing aphid. BMC Res Notes 13:523 https://doi.org/10.1186/s13104-020-05370-1

Kyrychenko S, Kozub N (2023) Screening of potato breeding lines for the potato virus Y resistance genes Ry_{chc} and Ry_{adg}. Quarantine and Plant Protection (2):9–13. https://doi.org/10.36495/2312-0614.2023.2.9-13

Lacomme C, Jacquot E (2017) General characteristics of potato virus Y (PVY) and its impact on potato production: An Overview. In: Lacomme C, Glais L, Bellstedt, D et al (eds) *Potato virus Y*: biodiversity, pathogenicity, epidemiology, and management. Springer, Cham, 1–19. https://doi.org/10.1007/978-3-319-58860-5_1

Lacomme C, Pickup J, Fox A et al (2017) Transmission and epidemiology of potato virus Y, In: Lacomme C, Glais L, Bellstedt D, Dupuis B, Karasev A, Jacquot E (eds) Potato virus Y: biodiversity, pathogenicity, epidemiology, and management. Springer, Cham, 141–176. https://doi.org/10.1007/978-3-319-58860-5_6

Li WL, Guo W, Xiao JP, Bai L, Gui HC (2017) Germplasm evaluation and molecular selection of potato (*Solanum tuberosum* L.) cultivars with disease resistance in China. Czech J. Genet. Plant Breed 53(3):114–121. https://doi.org/10.17221/101/2016-CJGPB.

Li G, Shao J, Wang Y, et al (2022) Ry_{chc} confers extreme resistance to potato virus Y in potato. Cells 11(16):2577. https://doi.org/10.3390/cells11162577

Liu J, Yue J, Wang, H et al (2023) Strategies for engineering virus resistance in potato. Plants (Basel, Switzerland), 12(9):1736. https://doi.org/10.3390/plants12091736

López M, Riegel R, Lizana C, & Behn A (2015) Identification of virus and nematode resistance genes in the Chilota Potato Genebank of the Universidad Austral de Chile. Chil J Agric Res 75(3):320–327. https://dx.doi.org/10.4067/S0718-58392015000400008

MacKenzie TDB, Nie X, Bisht V, Singh M (2019) Proliferation of recombinant PVY strains in two potato-producing regions of Canada, and symptom expression in 30 important potato varieties with different PVY strains. Plant Dis 103(9):2221–2230. https://doi.org/10.1094/PDIS-09-18-1564-RE

Mori K, Sakamoto Y, Mukojima N et al (2011) Development of a multiplex PCR method for simultaneous detection of diagnostic DNA markers of five disease and pest resistance genes in potato. Euphytica 180:347–355. https://doi.org/10.1007/s10681-011-0381-6

Nault L (1997) Arthropod transmission of plant viruses: A new synthesis. Ann Entomolog Soc America. 90:521–541. https://doi.org/10.1093/aesa/90.5.521

Nolte P, Whitworth JL, Thornton MK, McIntosh CS (2004) Effect of seedborne potato virus Y on performance of Russet Burbank, Russet Norkotah, and Shepody potato. Plant Dis Mar 88(3):248–252. https://doi.org/10.1094/PDIS.2004.88.3.248

Podgayetsky AA, Pika MA (1990) Isolation of virus-resistant interspecific potato hybrids. Potato Growing 21:27–32 (in Ukrainian)

Rizza MD, Vilaró FL, Torres DG et al (2006) Detection of PVY extreme resistance genes in potato germplasm from the Uruguayan breeding program. Am J Pot Res 83: 297–304. https://doi.org/10.1007/BF02871590

Ross BT, Zidack NK, Flenniken ML (2021) Extreme resistance to viruses in potato and soybean. Front Plant Sci 12:658981. http://dx.doi.org/ 10.3389/fpls.2021.658981

Sagredo B, Mathias M, Barrientos P et al (2009) Evaluation of a SCAR RYSC3 marker of the Ry_{adg} gene to select resistant genotypes to potato virus Y (PVY) in the INIA potato breeding program. Chil J Agric Res 69:305–315.

Sato M, Nishikawa K, Komura K, Hosaka K (2006) Potato virus Y resistance gene, Ry_{chc}, mapped to the distal end of potato chromosome 9. Euphytica 149:367–372. https://doi.org/10.1007/s10681-006-9090-y

Sharma R, Bhardwaj V, Dalamu D et al (2014) Identification of elite potato genotypes possessing multiple disease resistance genes through molecular approaches. Scientia Horticulturae 179:204–211. https://doi.org/10.1016/j.scienta.2014.09.018

Shrestha D, Wenninger EJ, Hutchinson PJ et al (2014) Interactions among potato genotypes, growth stages, virus strains, and inoculation methods in the potato virus Y and green peach aphid pathosystem. Environ Entomol 43(3):662–671. https://doi.org/10.1603/EN13323

Singh R, Valkonen J, Gray S et al. (2008) Discussion paper:

The naming of *Potato virus Y* strains infecting potato. Arch Virol 153:1–13. https://doi.org/10.1007/s00705-007-1059-1

Slater AT, Schultz L, Lombardi M (2020) Screening for resistance to PVY in Australian potato germplasm. Genes (Basel). 11(4):429. https://doi.org/10.3390/genes11040429

Song, YS, Schwarzfischer A (2008) Development of STS markers for selection of extreme resistance (Ry_{sto}) to PVY and maternal pedigree analysis of extremely resistant cultivars. Am J Pot Res 85:159–170. https://doi.org/10.1007/s12230-008-9012-8

Song YS, Hepting L, Schweizer G et al (2005) Mapping of extreme resistance to PVY (Ry_{sto}) on chromosome XII using anther-culture-derived primary dihaploid potato lines. Theor Appl Genet 111:879–887. https://doi.org/10.1007/s00122-005-0010-7

Thomas-Sharma S, Abdurahman A, Ali S et al (2016) Seed degeneration in potato: the need for an integrated seed health strategy to mitigate the problem in developing countries. Plant Pathol 65:3–16. https://doi.org/10.1111/ppa.12439

Valkonen JP (2015) Elucidation of virus-host interactions to enhance resistance breeding for control of virus diseases in potato. Breed Sci 65(1):69–76. https://doi.org/10.1270/jsbbs.65.69

Valkonen JPT, Wiegmann K, Hämäläinen JH et al (2008) Evidence for utility of the same PCR-based markers for selection of extreme resistance to potato virus Y controlled by Ry_{sto} of *Solanum stoloniferum* derived from different sources. Ann Appl Biol 152:121–130. https://doi.org/10.1111/j.1744-7348.2007.00194.x

Valkonen JPT, Gebhardt C, Zimnoch-Guzowska E, Watanabe KN (2017) Resistance to potato virus Y in potato. In: Lacomme C, Glais L, Bellstedt D, Dupuis B, Karasev A, Jacquot E (eds) *Potato virus Y*: biodiversity, pathogenicity, epidemiology and management. Springer, Cham. https://doi.org/10.1007/978-3-319-58860-5_8

Volkova IV, Reshotko LM, Dmytruk OO (2021) Spread of potato viral disease pathogens in culture cultivation zones. Agric Microbiol 32:67–73. https://doi.org/10.35868/1997-3004.32.67-73 (in Ukrainian)

Weber EL, Busse JS, Bethke PC (2021) Current-season infection with ordinary and recombinant strains of potato virus Y has detrimental effects on chipping potato yield and tuber quality. Plant Dis 105(2):450–455. https://doi.org/10.1094/PDIS-06-20-1281-RE

Yin Z, Chrzanowska M, Michalak K et al (2012) Recombinants of PVY strains predominate among isolates from potato crop in Poland. J Plant Protect Res 52(2):214–219. https://doi.org/10.2478/v10045-012-0033-4