NEW STRAIN OF POTATO VIRUS Y ISOLATED FROM POTATO CV. EPOKA PLANTS

Czesław Śniegowski, Anna Kowalska, Izabella Cieślewicz, Mirosława Chrzanowska

Institute for Potato Research, Department of Genetics and Parental Lines Breeding, Młochów

In 1966 the isolation of a non-identified virus from potato cv. Epoka plants has been described [13]. The best test plant for distinguishing this virus from other potato viruses was *Nicotiana debneyi* reacting locally 4 days and systemically 12 days after inoculation (Fig. 1). The first results pointed that this virus was a new strain of potato virus Y (PVY). In the present investigation the results of further studies on

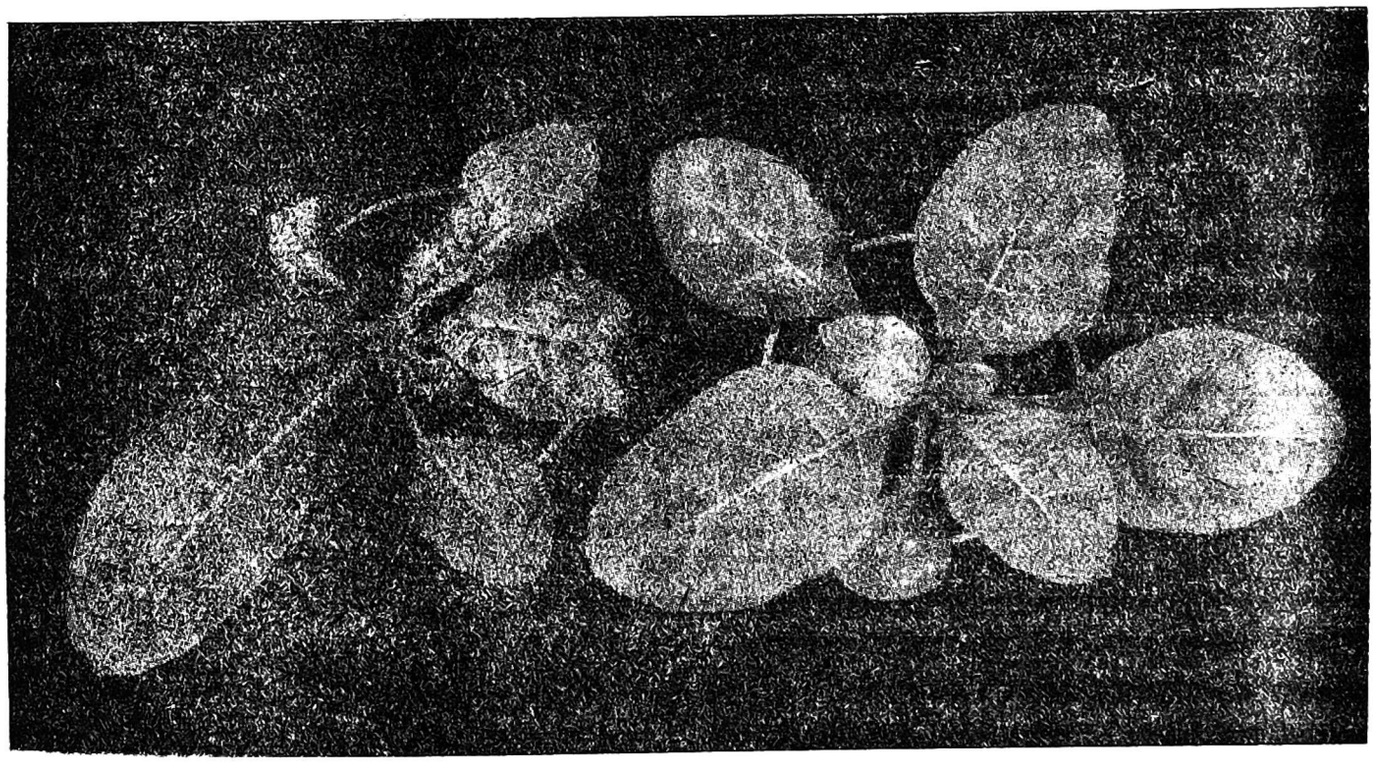

Fig. 1. Local necroses and systemic infection symptoms (mosaic, necroses, deformations, vein necrosis) on *N. debneyi* plants infected with PVY^{E}.

identification of this virus are reported. This investigation confirmed previous assumption that the virus was a new PVY strain. It was designated Y^E.

MATERIAL AND METHODS

The following PVY strains were compared with the non-identified virus from potato cv. Epoka: two isolates of Y^o (from cv. Epoka and Lipiński Wczesny), Y^N (from cv. Epoka) and Y^{NZ} (obtained from dr Z. Gajos, Central Laboratory of Tobacco Industry in Kraków). The most comparison were made with the strain Y^o from cv. Lipiński Wczesny.

Some experiments were performed in a greenhouse with uncontrolled temperature, and some (virus isolation from single necroses, determination of the properties *in vitro*) — in temperature controlled chamber at 22°C. Tobacco var. Samsun plants about 2 weeks after inoculation served as virus source. Leaves of mechanically inoculated plants were dusted with carborundum and after inoculation rinsed with water.

Detached leaves of *Solanum demissum* Y (SdY), hybrid of *S. demissum* × Aquilla (A-6) and *S. chacoense* (TE-1) plants were after inoculation incubated at 20°C and illumination of about 1000 lux.

Since the investigated virus could possibly occur in complex with strain Y^o, strain Y^E was passaged through single local necroses from a leaf of hybrid A-6 by two methods:

1) from a leaf of A-6 infected by a primary source of strain Y^E a disc of the tissue with necrosis was excised. The disc was ground with one drop of distilled water, and a leaf of *N. debneyi* was rubbed with so prepared inoculum;

2) another method of virus isolation from necroses consisted of puncturing, with a needle, at first of the center of necrosis and then of a leaf of the test plant (*N. debneyi*) near the main vein. Necrosis which developed around the puncture site was excised and served as the source of virus.

In most experiments strain Y^E obtained from a single necrosis by the second method was studied. In studies of the host plants range, each PVY strain was used to inoculate 5-10 plants or detached leaves of each species. From plants free from pathologic symptoms back inoculation was performed (using *N. debneyi* and *N. tabacum* var. Samsun) to reveal a possible symptomless infection.

Plants (obtained from tuber eyes) of potato cv. Epoka and clone PW 80/65 with extreme resistance to PVY (received from M. Dziewońska, Institute of Potato Research, Młochów) at 4-6 leaves stage were inoculated with sap or grafted with scions of tobacco var. Samsun infected by

virus Y^E or Y^o. For each combination 6-8 plants were used. All tubers of the inoculated plants were collected and planted. The presence of virus in the infected plants was chacked by the biological test (on *N. debneyi, N. tabacum* var. Samsun, leaves of SdY and A-6) 20, 30 and 40 days after mechanical inoculation or grafting, and 6 and 8 weeks after tuber planting. In studies of the dilution end point of strains Y^E and Y^o, the sap from the infected tobacco plants was diluted with distilled water, for the determination of the thermal inactivation point the sap was heated for 10 min at 40-70°C. For estimation of longevity *in vtiro* the sap was stored at room temperature.

In the cross protection test, plants of *N. debneyi* were inoculated with strain Y^o, whereas a second batch of plants remained non-inoculated. After 14 days, the detached leaves of Y^o-infected plants were inoculated for the second time with strain Y^E, using various sap dilutions (1 : 1, 1 : 10, 1 : 50, 1 : 100). Leaves of *N. debneyi* inoculated only with strain Y^E on the second date served as control. After 8 days from inoculation with strain Y^E, necroses were counted.

In serological studies a series of dilutions of centrifuged sap from tobacco plants infected by strains Y^O and Y^E, as well as a series of dilutions of antiserum to PVY were run. The reaction of the different sap samples with antiserum samples was examined. Bonitation was taken after 2 h of inocubation at room temperature and after 14 h of incubation at 4°C.

Transmission of strain Y^E by aphids (*Myzus persicae*) to plants of potato cv. Epoka and Wyszoborski was attempted. Prior to being placed on the infection source (tobacco var. Samsun), aphids were starved for 3 h at 5°C. After 15 min or 3 h of feeding on the infected tobacco plants, on each potato plant 10 aphids were placed. For each potato cultivar 3-7 young plants were inoculated. After 24 h the aphids were destroyed. Potato plants were tested by inoculation of *N. debneyi* plants 20 and 40 days after inoculation.

RESULTS

Reaction of test plants. With respect to the host plant range and duration of the incubation period on different plant species, strain Y^E resambled the other PVY strains. On tobacco var. Samsun and White Burley as well as on *Chenopodium amaranticolor* the virus induced symptoms similar to those observed after inoculation with strain Y^o (Table 1). On *N. debneyi* plants strain Y^o, Y^N, Y^{NZ} induced only chlorotic symptoms, whereas strain Y^E caused distinct necrotic reaction.

Table 1

Comparison of the reaction of test plants inoculated with various PVY strains

Plant species	PVY strain				
	Y^o		Y^N	Y^{NZ}	Y^E
	Epoka	Lipiński Wczesny			
Chenopodium amaranticolor	L SpC	L SpC	0	0	L SpC
C. quinoa	L SpC	0	0	0	0
Nicotiana debneyi	S VcM	S VcM	S Vc	S Vc	L SpN S MVn
N. tabacum var. Samsun and White Burley	S Vc	S Vc	S VcVn	S VcVn	S Vc

L — local, S — systemic, Sp — spots, C — chlorotic, N — necrotic, M — mosaic, Vc — vein clearing, Vn — vein necrosis, O — no symptoms

All investigated strains produced necroses on detached leaves of SdY, A-6, TE-1. *Amaranthus caudatus, Datura ferox, D. meteloides* and *D. stramonium* plants did not become infected by either strain Y^E or other PVY strains.

The reaction of tobacco var. Samsun and *N. debneyi* as well as of detached leaves of A-6 inoculated with a primary source of Y^E from potato cv. Epoka and, on the other hand, with virus obtained from single necroses from A-6 leaves (by the method of necrosis excision and puncture) was compared. Plants reacted similarly to the primary isolate of strain Y^E and to the two new ones.

Reaction of potato plants. On mechanically inoculated plants of potato cv. Epoka strains Y^o and Y^N induced local necroses and systemic mosaic, necrotic spots and vein necrosis. In case of strain Y^E local necroses occurred several days later, and systemic symptoms were milder (less intense mosaic, fewer and brighter necroses). Similar differences in the intensity of systemic symptoms between virus strains were observed on the grafted plants. Plants of clone PW 80/65 with extreme resistance to PVY did not become infected by any investigated virus strains neither by mechanical inoculation nor by grafting.

Properties *in vitro*. Sap from tobacco plants infected by strain Y^E lost infectivity after dilution above 10^{-4}, after heating above 50°C and after storage exceeding 11 days. Similar properties in vitro were exhibited by strain Y^o (Tables 2 and 3).

Cross protection test. Strain Y^E induced fewer necroses on

Table 2

Dilution end point of strains Y^E and Y°

Dilution of sap	No. of necroses on halves of A-6 leaves (mean of 10 halves)		No. of infected plants (per 4 inoculated ones)			
			— Y^E —		Y°	
	Y^E	Y°	*N. debneyi*	Samsun	*N. debneyi*	Samsun
Undiluted	48	47	4	4	4	4
10^{-1}	34	46	4	4	4	4
10^{-2}	5	4	4	4	4	4
10^{-3}	2	1	1	3	2	4
10^{-4}	3	2	0	1	2	2
10^{-5}	—	—	0	0	0	0
10^{-6}	—	—	0	0	0	0

Table 3

Thermal inactivation point and longevity *in vitro* of strains Y^E and Y° (number of infected plants per 4 inoculated ones)

Temperature °C	Thermal inactivation point				No. of days	Longevity *in vitro*			
	Y^E		Y°			Y^E		Y°	
	N. debneyi	Samsun	*N. debneyi*	Samsun		*N. debneyi*	Samsun	*N. debneyi*	Samsun
40	4	4	4	4	7	4	4	4	4
45	4	4	4	4	8	2	4	4	4
50	4	4	4	3	9	4	4	2	4
55	0	0	0	0	10	4	2	2	4
60	0	0	0	0	11	2	2	1	0
65	0	0	0	0	12	0	0	1	0
70	0	0	0	0	13	0	0	0	0

leaves of *N. debneyi* plants earlier inoculated with strain Y°, as compared with leaves of healthy plants. The reduction of the number of necroses was more pronounced at higher dilutions of the sap used for inoculation (Table 4).

Serological tests. Strain Y^E reacted with antiserum to PVY. Dilution end point of sap from tobacco plants infected by strain Y^E and strain Y° was similar, amounting to 1 : 32-1 : 64. The titer of antiserum to PVY was 1 : 32, irrespective of the PVY strain used.

Transmission by aphids. Attempts of transmission of

Table 4

Cross protection test — number of necroses caused by strain Y^E on *N. debneyi* leaves inoculated 14 days earlier with strain Y°, and on healthy leaves

Sap dilution	Mean no. of necroses from 24 leaves		% of reduction of no. of necroses
	infected by Y°	control	
Undiluted	154	286	46
1:10	168	286	41
1:50	35	153	77
1:100	13	58	77

Table 5

Transmission of strain Y^E by *Myzus persicae*

Potato cultivar	Duration of vira contraction feeding	No. of plants	
		inoculated	infected
Epoka	15 min	5	3
	3 h	5	2
Wyszoborski	15 min	7	2
	3 h	6	5

strain Y^E to potato plants by aphids were successful, though not all inoculated plants became infected (Table 5). The virus isolated from the infected potato plants induced characteristic necrotic symptoms on *N. debneyi* plants.

DISCUSSION

Results of the experiments described in this and in previous [13] paper lead to the conclusion that the virus isolated from potato cv. Epoka is a so far unknown PVY strain. This is proved by all its properties, and in particular by induction of characteristic symptoms on tobacco and on leaves of SdY, A-6, and TE-1, failure to infect plants of *D. stramonium* and potato with extreme resistance to PVY, transmission by aphids, and reaction with antiserum to PVY. The claim that no mixture of an unknown virus with strain Y° is concerned was proved, among others, by the fact that the virus retained its characteristic properties after passage through single necroses.

Properties *in vitro* of strain Y^E resembled those assumed for PVY [5], only longevity *in vitro* was somewhat more prolonged (48-72 h for PVY, 11 days for strain Y^E). However, when tested in parallel, strain Y° exhibited similar longevity, and in the experiments of de Bokx et al. [2] four PVY strains have remained infective in tobacco sap for 35-50 days.

Characteristic reaction of one plant species already afforded a basis for distinction of several PVY strains: well known strain Y^N, strain Y^{NZ} (braeking the resistance of some tobacco varieties [6] (and Y 264) causing no necroses on A-6 leaves [2]. Likewise strain Y^E is characterised by the ability to induce necrotic symptoms on *N. debneyi*.

In contrast to other above mentioned PVY strains, there are no grounds to fear that strain Y^E could be of economic importance. It was isolated from potato plants only once, though we often use *N. debneyi* for PVY detection in field materials. Also the strains and isolates of PVY investigated by other authors have caused only chlorotic symptoms on *N. debneyi* [7, 8].

Low spread of strain Y^E could result from its genetic unstability. However, this is contradicted by the fact that strain Y^E kept in virus collection [12] failed to lose its properties after 15 years of plant reproduction from tubers (Waś, Skrzeczkowska, unpublished data). On the other hand, it seems that Y^E is a mild strain which could not stand competition with other PVY strains under field conditions. This property of Y^E has been confirmed by Chrzanowska [3, 4] who compared the reaction of potato plants to different PVY strains. In case of Y^E, on plants of clone 01 20931 (Granit) local necroses have occurred on the inoculated leaves several days later, and systemic symptoms were milder than after inoculation with Y^o and Y^N strains. Systemic infection by strain Y^E has affected a smaller number of plants (40%), as compared with the remaining strains (60-80%) [3]. Also in studies of the reaction of cv. Uran strain Y^E has been found to be milder than eight isolates of strains Y^o, Y^N and Y^C, because it induced the milder symptoms and infected the lowest number of plants. When used for inoculation of 11 potato varieties with a different degree of resistance to PVY, strain Y^E has exhibited infectivity similar to that of a mild isolate of Y^o from potato cv. Pionier and induced slight symptoms [4].

Owing to formation of local necroses on *N. debneyi* leaves, strain Y^E is a convenient model for studies of the degree of cross protection between PVY strains. The present results are consistent with the previous studies [1, 2, 6, 9-11] which indicate that infection of plants by one PVY strain often fails to protect them from infection by another PVY strain.

REFERENCES

1. Bawden F. C., Kassanis B.: Serologically related strains of potato virus Y that are not mutually antagonistic in plants. Ann. appl. Biol., 38: 402-410, 1951.
2. Bokx J. A. de: Some properties of deviating strain of potato virus Y. Potato Res., 18: 38-51. 1975.

3. Chrzanowska M.: Ocena odporności ziemniaka na wirus Y na podstawie sztucznego zakażania roślin w szklarni. Praca doktorska wyk. w Inst. Ziemniaka, Młochów, 1973.
4. Chrzanowska M.: Reakcja odmian ziemniaka na różne izolaty wirusa Y. IX Sesja Nauk. Nasiennictwo ziemniaka. Koszalin 11-12 marca 7-9: 1976.
5. Delgado-Sanchez S., Grogan R. G.: Potato virus Y. C. M. I./A. A. B. Descriptions of Plant Viruses No. 37, 1970.
6. Gajos Z.: Silnie wirulentny szczep wirusa Y na tytoniu w Polsce, jego występowanie i właściwości w porównaniu ze szczepami nekrotycznym i zwykłym. Zesz. Probl. Post. Nauk rol. 115: 87-98, 1971.
7. Horwáth J.: Symptoms evoked by different strains of potato virus Y on *Nicotiana* species resistant to *Peronospora tabacina* Adam. Acta phytopath. acad. Scient. hung., 18: 55-66, 1969.
8. MacKinnon J. P., Bagnall R. H.: Use of *Nicotiana debneyi* to detect viruses S, X and Y in potato seed stocks an relative susceptibility of six common varieties to potato virus S. Potato Res., 15: 81-85. 1972.
9. Miczyński K. A.: Z badań nad rasami wirusa ziemniaczanego „Y", występującymi na terenie Podgórza Karpackiego w uprawach ziemniaków odmian Dar i Epoka. Zesz. Probl. Post. Nauk rol., 70: 15-68. 1967.
10. Ramirez W., Gamez R., Gonzalez L. G.: Cross-protection tests with potato virus Y and potato veinal necrosis virus. Phytopathology, 54: 500. 1964.
11. Richardson D. E.: Some observations on the tobacco veinal necrosis strain of potato virus Y. Plant Path., 7: 133-135 1958.
12. Skrzeczkowska S., Kowalska A.: Utrzymywanie kolekcji wirusów w bulwach ziemniaka i zliofilizowanym soku. Zesz. Probl. Post. Nauk rol., 174: 49-54. 1976.
13. Śniegowski C.: Z badań nad wykrytym nie rozpoznanym dotychczas wirusem w ziemniakach odmiany Epoka. Biul. Inst. Hod. Aklim. Rośl., 5: 55-60. 1966.

Чеслав Снеговски, Анна Ковальска, Изабелла Цесьлевич, Мирослава Хшановска

НОВЫЙ ШТАММ Y ВИРУСА, ИЗОЛИРОВАННЫЙ ИЗ РАСТЕНИЙ КАРТОФЕЛЯ СОРТА ЭПОКА

Резюме

Из растений картофеля сорта Эпока изолирован новый штамм PVY, который обозначен символом $У^{Е}$. Вирус вызывал характерные симптомы на *N. debneyi*: локальные некрозы и системную мозаику, некрозы, побурение жилок, замирание верхушки и затем всего растения. На других видах тестрастений (в том числе на табаке Самсун) штамм $У^{Е}$ вызвал реакцию, подобную к вызванной $У^{О}$. Растения картофеля, крайне устойчивые к PVY, не подвергаются поражению $У^{Е}$ как после механической инокуляции, так и после прививки. На растениях картофеля сорта Эпока штамм $У^{Е}$ вызывал симптомы, несколько более слабые от вызываемых штаммом $У^{О}$. Предельное разбавление штамма $У^{Е}$ составляло 10^{-4}, термическая инактивация происходила при температуре 50-55°C, а стойкость *in vitro* составляла 11 дней. Вирус переносился через *Myzus persicae*. Сок из растений, пораженных $У^{Е}$, реагировал сывороткой

против PVY. В тестах перекрестной защиты показано, что на растениях, ранее пораженных штамм Y^o, штамм Y^E вызвал на 46-77% меньшее количество некрозов, чем на растениях здоровых.

Czesław Śniegowski, Anna Kowalska, Izabella Cieślewicz, Mirosława Chrzanowska

NOWY SZCZEP WIRUSA Y WYIZOLOWANY Z ROŚLIN ZIEMNIAKA ODM. EPOKA

Streszczenie

Z roślin ziemniaka odm. Epoka wyizolowano nowy szczep PVY, który oznaczono symbolem Y^E. Wirus wywoływał charakterystyczne objawy na *N. debneyi*: lokalne nekrozy, oraz systemiczną mozaikę, nekrozy, brązowienie nerwów, zamieranie wierzchołka i następnie całej rośliny. Na innych gatunkach roślin testowych (w tym na tytoniu Samsun) szczep Y^E wywoływał reakcję podobną do powodowanej przez Y^o. Rośliny ziemniaka skrajnie odporne na PVY nie podlegały porażeniu przez Y^E ani po inokulacji mechanicznej ani po szczepieniu. Na roślinach ziemniaka odm. Epoka szczep Y^E wywoływał objawy nieco słabsze od powodowanych przez szczep Y^o. Graniczne rozcieńczenie szczepu Y^E wynosiło 10^{-4}, termiczna inaktywacja następowała w temperaturze 50-55°C, a trwałość in vitro wynosiła 11 dni. Wirus przenoszony był przez *Myzus persicae*. Sok z roślin porażonych Y^E reagował z surowicą przeciw PVY. W testach ochrony krzyżowej wykazano, że na roślinach porażonych wcześniej szczepem Y^o, szczep Y^E spowodował o 46-77% mniejszą liczbę nekroz niż na roślinach zdrowych.

Accepted 15 03 79