NEW STRAIN OF POTATO VIRUS A ISOLATED FROM HYBRIDS OF *SOLANUM MEGISTACROLOBUM*

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In 1975 a virus was isolated from several plants of hybrids between *Solanum megistacrolobum* and 24-chromosome potatoes from series Tuberosa. The virus produced severe systemic symptoms on *Datura stramonium* differing from those caused by the known potato viruses (Fig. 1). It was found that the disease was caused by a strain of potato vi-

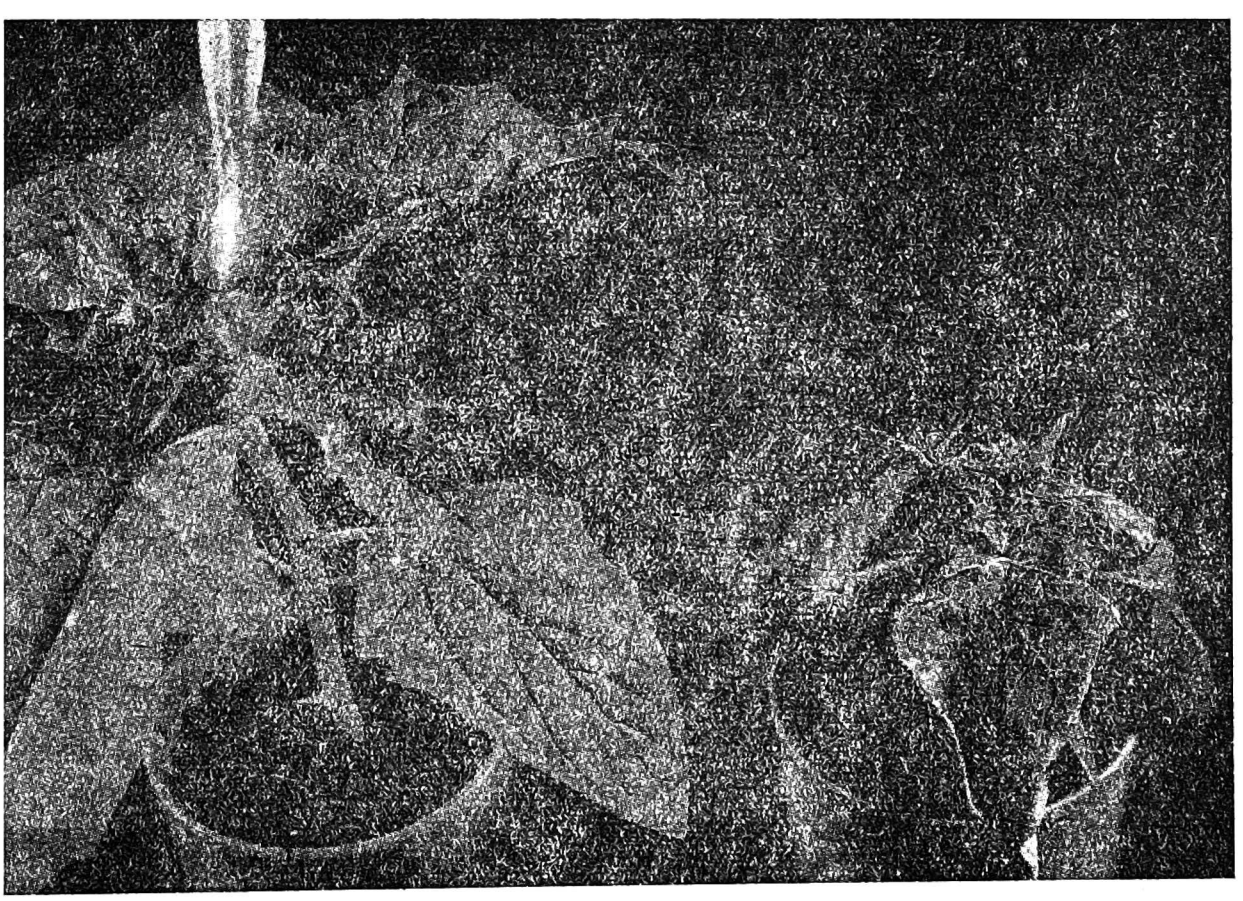

Fig. 1. Symptoms of systemic infection by strain PVA^N on *Datura stramonium* plant (22 days after inoculation) and healthy plant (Phot. A. Oraczewska)

rus A (PVA). The strain induced necrotic reaction in *D. stramonium* and therefore was designated with the symbol A^N. The research which clarified it is reported in the present paper.

MATERIAL AND METHODS

Greenhouse experiments were performed in autumn, winter and spring. In summer no experiments were carried out because of the low degree of transmission of strain A^N at this time. Plants of *D. stramonium*, *Nicotiana tabacum* cv. Samsun and *N. clevelandii* 2-3 weeks after inoculation as well as plants of potato cvs. Uran and Baca were used as sources of the virus. Moreover, in some experiments a PVA isolate from potato cv. Jara and PVY° from potato cv. Lipiński Wczesny were studied (sources of both viruses: potato, tobacco cv. Samsun, *N. clevelandii*).

For mechanical inoculation, leaves of carborundum-dusted plants were rubbed with a piece of sponge wetted with sap diluted with distilled water. For evaluation of the reaction of the test plants, 5-10 plants or detached leaves of each species were inoculated. Detached leaves were incubated in a chamber at 20°C with illumination of about 1000 lux. In case of each test plant species back-inoculation was performed on *D. stramonium:* from inoculated leaves about 1 week, and from top leaves 3-4 weeks after inoculation.

The efficiency of transmission of strain A^N by *Myzus persicae* was compared at short (3 and 10 min) and at long (4 days) duration of virus acquisition feeding. After aphid feeding on the infected plants, 10 aphids were placed on each test plant. Moreover, the efficiency of virus transmission by 1 aphid after 1 min of uninterrupted feeding on the source plant was determined. For each combination 30 plants of *D. stramonium* were inoculated.

For determination of the dilution end point of the virus, the sap from infected *D. stramonium* plants was diluted with distilled water in ratio of 10^{-1}-10^{-5}. The thermal inactivation point of the virus in sap was determined between 30-70°C (every 5°C). In studies of longevity *in vitro,* the infectivity of sap stored at room temperature was evaluated every 6-8 h. *In vitro* properties of strain A^N were determined twice. In all experiments, 10 *D. stramonium* plants were inoculated with each sample of sap.

In serological studies antisera to PVA and to PVY (obtained from Z. Mierzwa, Laboratory of Serology, Institute for Potato Research) were used. The titre of these sera was determined with the use of sap of infected plants, diluted 1 : 5. Tests were performed by the precipitation

method. Readings were taken after incubation of 2 h at 20°C and 24 h at 4°C. In three experiments determination was made of the reaction of antisera with saps of different plant species (*D. stramonium*, potato, tobacco cv. Samsun or *N. clevelandii*) infected by PVA^N, PVA-Jara and PVY.

In cytological studies of *D. stramonium* leaves infected by strain A^N, leaf slides (1 mm^2) were fixed at room temperature in a mixture of 3% glutaraldehyde and 4% paraformaldehyde, dissolved in 0.1 M cacodyl buffer, pH 7.2. After 4 h of fixation, postfixation in a 1% solution of OsO_4 for 2 h at 4°C was performed. Subsequently, the material was successively dehydrated in graded series of ethanol (10-70%) and acetone (80-100%) concentrations, and finally in propylene oxide, whereupon it was embedded in Epon 812. After cutting in an LKB ultramicrotome, the material was stained with uranyl acetate and lead citrate. Observations were taken in a JEM 100 C electron microscope.

In studies of the reaction of potato plants, virus-free plants grown from tuber eyes were inoculated mechanically, as well as by grafting and by aphids. Each method was used to inoculate 10 plants of each of following potato cultivars: Uran, Prosna, Flisak, Osa and Baca; when aphids were used, only cvs Uran, Prosna and Osa were inoculated. Mechanical inoculation of plants in the 4-6 leaves stage was repeated three times at 3-day intervals. In case of grafting, scions consisted of healthy *D. stramonium* plants which were mechanically inoculated with strain A^N after growing together into the stock (10-20 days after grafting); grafting with *D. stramonium* plants infected with PVA^N was not possible because infected scions died. In case of virus transmission by aphids, 10 wingless insects were placed on each potato plant. The duration of feeding on the infection source was 6 minutes, and the duration of remaining on inoculated plants — 24 h.

The presence of virus in inoculated potato plants was detected by biological tests. After about 4,8 and 11 weeks from inoculation of plants, inoculation of scions or application of aphids, each potato plant was tested on detached leaves of *Solanum demissum* Y, and after 4-8 weeks on *D. stramonium* plants.

Tubers of plants inoculated by the three methods were collected, and tuber indexing was performed, by planting of 1 eye each from two tubers of every plant. The presence of virus in plants was checked on *D. stramonium* 4 weeks after eyes planting.

RESULTS

HOST PLANT RANGE

Test plants inoculated with strain A^N exhibited different reactions.

D. stramonium, D. tatula. Lack of local signs or chlorotic and light--brown spots after 10 days. After 10-14 days systemic vein clearing, necroses, vein browning on the underside of the leaf, mosaic, downward rolling of the leaves, death of bottom leaves, strong growth inhibition (Fig. 1), necrotic spots on flowers, reduction of the number and size of fruits and seeds.

D. metel. Systemic mild mosaic and deformation after 15-20 days.

Lycopersicon pimpinellifolium. Symptomless systemic infection or few gray necroses after 2 weeks.

Nicandra physaloides. Very mild vein clearing and mosaic after 10-15 days.

Nicotiana clevelandii. Distinct systemic mosaic, deformation and death of bottom leaves after 11-21 days (Fig. 2).

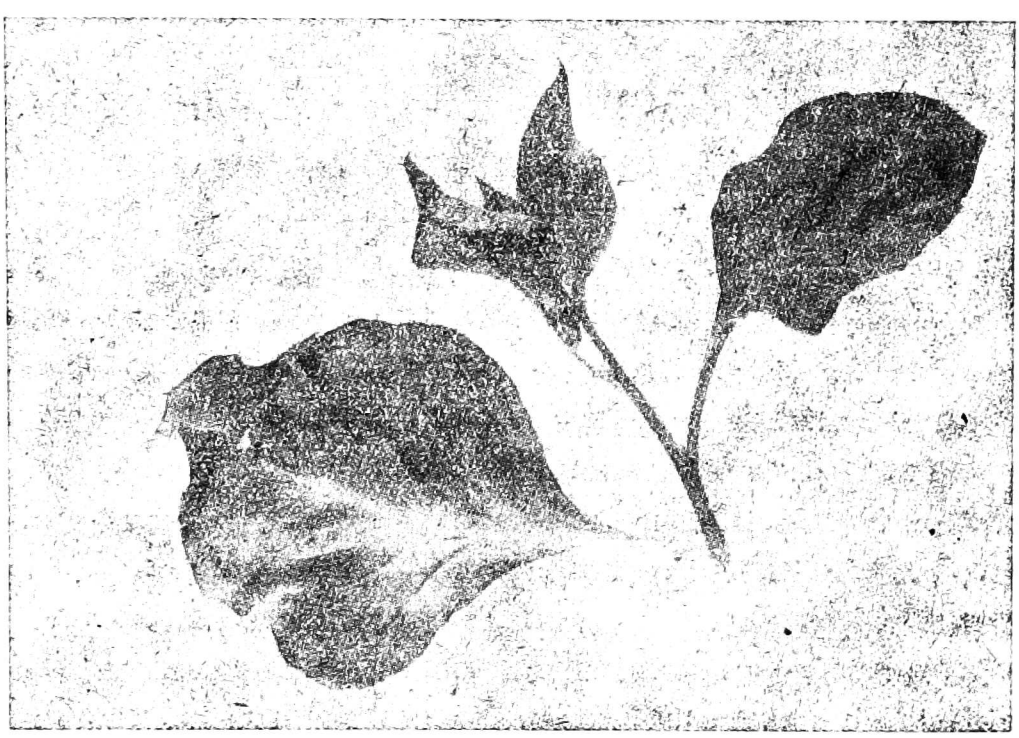

Fig. 2. Mosaic and deformation of *Nicotiana clevelandii* leaves infected by strain PVA^N (27 days after inoculation)

N. debneyi. Very mild vein clearing after 14-20 days.

N. rustica. Systemic mild vein clearing, mosaic and curling of leaf blades after 10-21 days.

N. tabacum cv. Samsun. Symptomless systemic infection or very mild vein clearing and mosaic after 11-15 days.

On detached leaves of *Solanum demissum* Y (SdY), *S. demissum* A

(SdA) and hybrid A-6, strain A^N induced necrotic spots if the source of the virus were: potato, *N. clevelandii* or *N. tabacum*. After inoculation of leaves with sap from infected *D. stramonium* plants, no symptoms occurred. Symptomless systemic infection of plants by strain A^N was found on *Hyoscyamus albus*, *Physalis floridana* and *Solanum stoloniferum* EBS 26/30.

The following plant species did not become infected by strain A^N: *Capsicum annuum*, *Celosia argentea*, *C. cristata*, *Chenopodium amaranticolor*, *C. murale*, *C. quinoa*, *Chrysanthemum maximum*, *Datura fastuosa*, *D. giganthea*, *D. Leichhardii*, *D. meteloides*, *D. sanguinea*, *Dianthus barbatus*, *Gomphrena globosa*, *Lycopersicum chilense*, *L. esculentum* cv. Najwcześniejszy, *Nicotiana glutinosa*, *Ocimum basilicum*, *Phaseolus vulgaris* cv. Red Kidney and Saxa Złota.

The reaction of several plant species and detached leaves inoculated with PVA^N and PVA-Jara was compared. A similar reaction to both virus isolates was found in following plant species: *N. clevelandii* (distinct symptoms), *L. pimpinellifolium*, *N. physaloides*, *N. rustica*, *N. tabacum* cv. Samsun (mild symptoms), *C. annuum* (no infection), detached leaves of SdY, SdA and A-6 (necroses). Differences between the reaction to PVA^N and PVA-Jara were found only in *D. stramonium* and *D. tatula*, which did not become infected by strain PVA-Jara.

EFFECT OF EXTERNAL CONDITIONS ON INFECTION OF *D. STRAMONIUM* BY THE STRAIN A^N

During one year, at about 1 month intervals, 20—100 *D. stramonium* plants were mechanically inoculated with strain A^N. From October till February 60-100% plants became infected and from March till September only 0-44% became infected.

The effect of constant temperature of 16, 22 and 28°C on infection of *D. stramonium* plants by strain A^N in controlled-temperature chambers was investigated. Out of 5 inoculated plants, infection symptoms occurred at 16°C — on 5 plants, and at 22°C — on 4 plants. At 28°C none of the *D. stramonium* plants exhibited pathologic symptoms.

TRANSMISSION BY APHIDS

High efficiency of transmission of strain A^N by *M. persicae* was observed after acquisition feeding period of several minutes and placing 10 aphids per test-plant. After 1 min of uninterrupted feeding on the source-plant, single individuals transmitted the virus to 23% of the test-plants. On the other hand, the degree of infection of the test-plants

Table 1

Efficiency of transmission of strain PVA^N by *M. persicae* (source plant and test plant *D. stramonium*)

Duration of		No. of aphids per plant	No. of infected plants per 30 inoculated	% of infected plants
acquisition feeding	inoculation feeding			
1 min	24h	1	7	23
3 min	24h	10	22	73
10 min	24h	10	28	93
4 days	4 days	10	2	7

was low, when the aphids were kept for 4 days both on the source-plants and on the test-plants (Table 1).

Lack of a period of virus incubation in the insect, short duration of the acquisition feeding and high efficiency of strain A^N transmission by *M. persicae* indicate that aphids transmit this virus in a non-persistent manner.

PROPERTIES *IN VITRO*

Dilution end point of the virus was 10^{-4}, thermal inactivation occurred between 45-50°C, and longevity *in vitro* amounted to 32-48 h.

SEROLOGICAL STUDIES

In some tests antiserum to PVA reacted with the sap of plants infected by strain A^N, and in the others it did not. Inconsistent results were obtained also in tests of plants infected by PVA-Jara. The titre of antiserum to PVA varied from 1 : 2 to 1 : 64 irrespective of PVA strain used.

Thus no differences in the reaction with antiserum to PVA between strains were demonstrated by the method used. In no experiment the sap from plants infected by PVA^N and PVA-Jara reacted with antiserum to PVY.

CYTOLOGICAL STUDIES

In the cytoplasm of parenchymal cells of *D. stramonium* leaves infected by strain PVA^N, long filamentous virus-like particles forming characteristic aggregates were found. These particles were situated in parallel to the tonoplast in the cytoplasmatic bridges formed between the large central vacuole of the cell and small vacuoles, and around the

tonoplast of the small vacuoles. Thus, monolayers of virions arranged parallel were formed (Figs. 3 and 4).

Moreover, parenchymal cells of the infected plants contained pinwheel inclusions characteristic of plant infection by viruses of potyvirus group; [6, 14]. Furthermore, unidentified structures closely connected with the pinwheel inclusions, in many cases forming their centre or adjoining them, were present (Figs. 3, 5). Sometimes chloroplast protrusions, whose structure resembled that of the unidentified forms, were visible. From the side of cytoplasm, plates of the pinwheel inclusions adjoined these protrusions (Fig. 6).

REACTION OF POTATO PLANTS

Inoculation of five potato cultivars with the strain A^N was successful (Table 2). Only in case of cultivar Uran mechanical inoculation was of low efficiency. In most plants, which became infected, strain A^N was detected already in the year of inoculation.

Plants of potato cvs. Prosna and Flisak, infected by strain A^N, exhibited no symptoms. On potatoes cv. Osa mild mosaic or no symptoms were observed. Relatively most pronounced symptoms occurred on cvs. Uran and Baca. The first symptoms on potato plants were observed 6 weeks after inoculation.

DISCUSSION

Transmission by aphids in a non-persistent manner, several days'longevity *in vitro,* thermal inactivation point amounting to 50-60°C, narrow range of host plants, the occurrence of pinwheel inclusions in the cytoplasm of cells of the infected plants [6, 14]. The virus investigated in this study exhibited similar properties. Its thermal inactivation point amounting to 45-50°C approached the value accepted for PVA [2].

Infection by strain A^N was associated with formation of two types of structures. Pinwheel inclusions have already been observed in cells of plant infected by PVA [3]. On the other hand, structures designated here as „unidentified" have not so far been described for other viruses, including PVA [3].

In cells of infected plants, particles of potyviruses are arranged in aggregates or distributed singly within the cytoplasm [15]. Monolayer arrangement of virions, similar to that found in case of strain A^N, has been observed in cells of plants infected by some strains of pea seedbor-

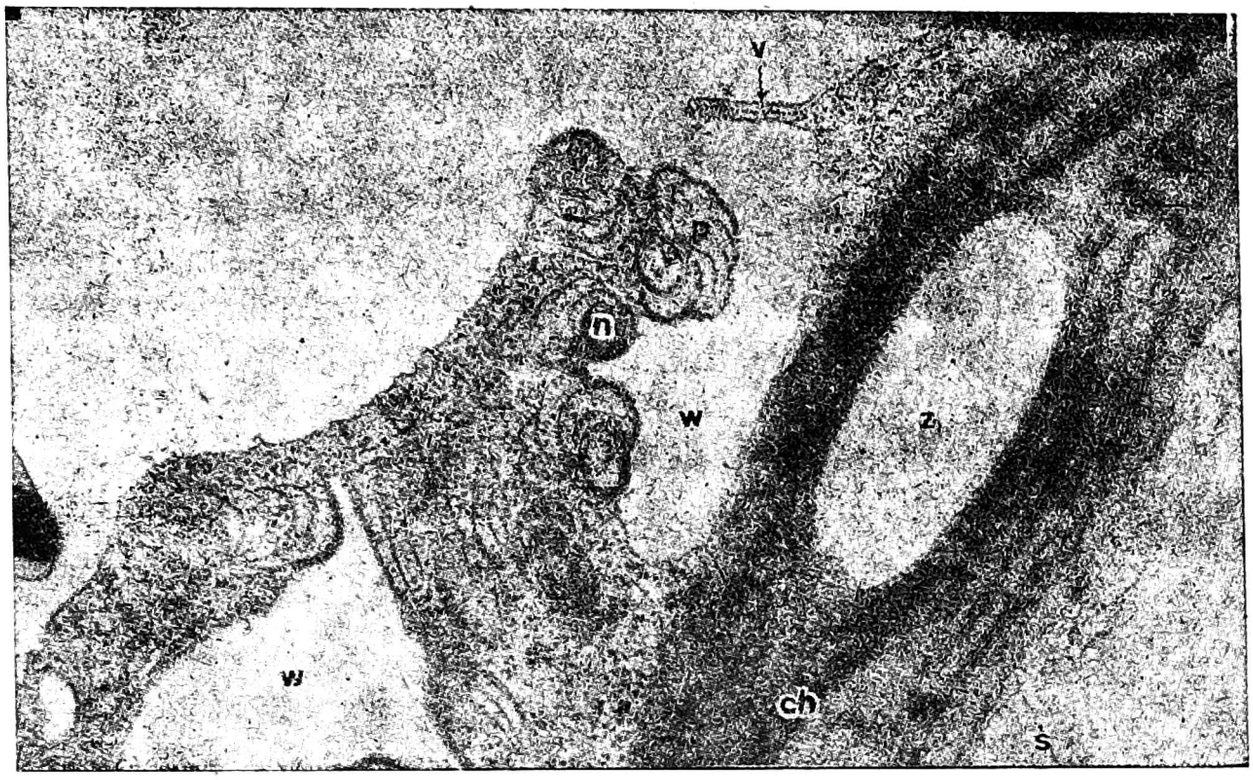

Fig. 3. Cross-section through fragment of parenchymal cell of *Datura stramonium* leaf infected by PVA^N.

Virus particles cut transversely. Monolayer arrangement of virus particles in cytoplasmatic bridge surrounded from both sides by tonoplast, and aggregates of pinwheels and unidentified structures distributed between them;

ch — chloroplast, z — grain of assimilated starch, w — vacuole, v — virus particles in cytoplasmatic bridges, p — pinwheels, n — unidentified structures, ś — cell wall (magn. × 35 000)

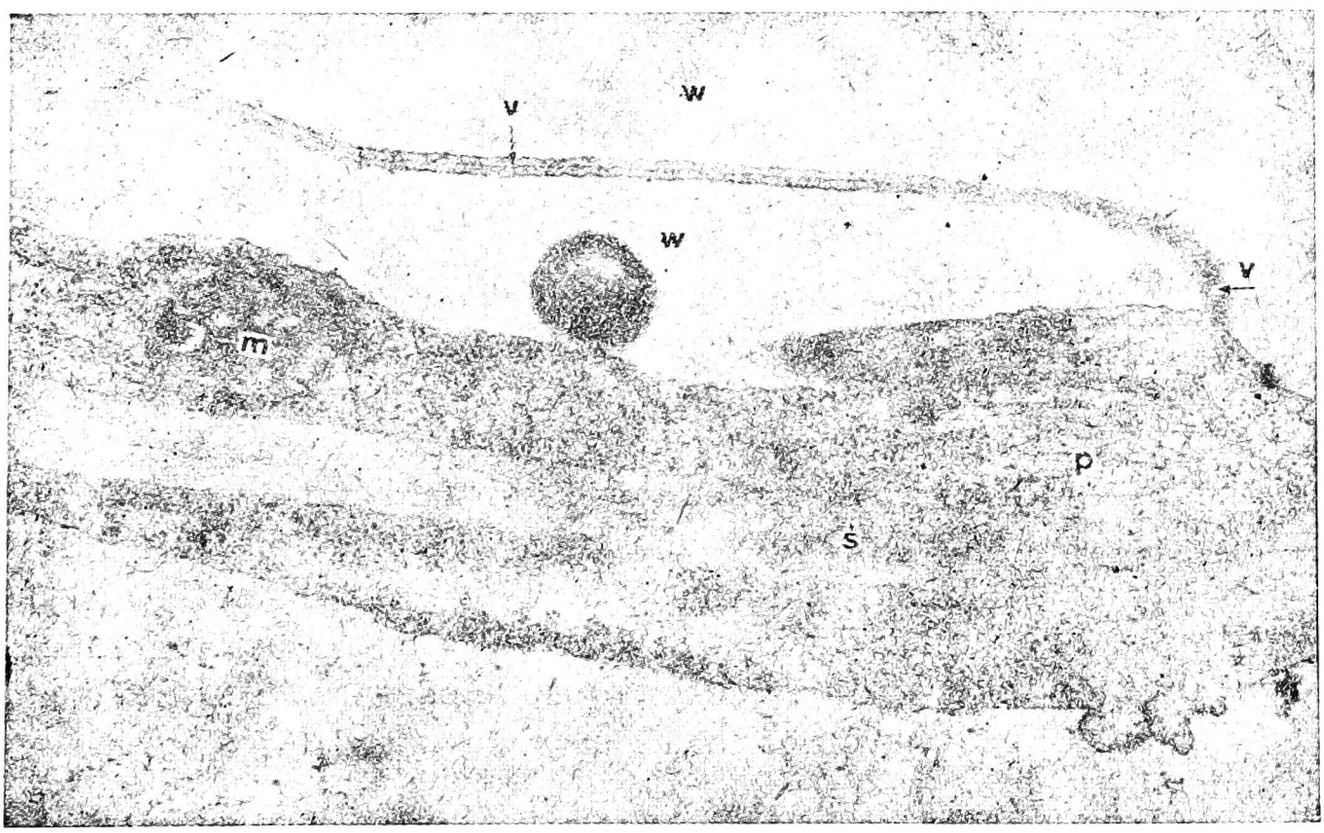

Fig. 4. Cross-section through fragment of parenchymal cell of *Datura stramonium* leaf infected by PVA^N;

Virus particles in cytoplasmatic bridges situated longitudinally (v at the left side) and obliquely (v at the right side);

ś — cell wall, w — vacuole, m — mitochondria, v — virus particles in cytoplasmatic bridges, p — pinwheels (magn. × 35 000)

Fig. 5. Cross-section through fragment of parenchymal cell of *Datura stramonium* leaf infected by PVA^N; two unidentified structures(n), one of them is adjoined by plates of a pinwheel (p); m — mitochondria, ch — chloroplast (magn. × 35 000)

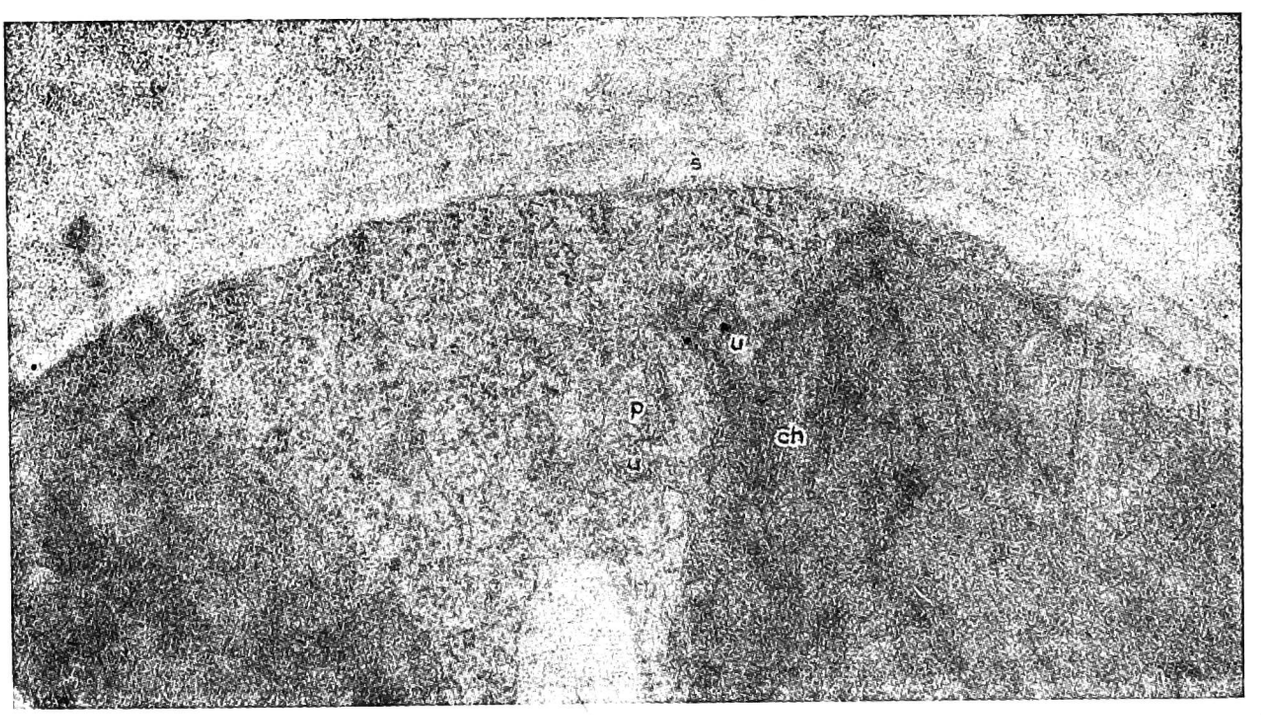

Fig. 6. Cross-section through fragment of parenchymal cell of *Datura stramonium* leaf infected by PVA^N; Chloroplast protrusions (u) surrounded from the side of cytoplasm by a pinwheel (p); ch — chloroplast, ś — cell wall (magn. × 51 000)

ne mosaic virus [5]. Distinction between individual representatives of the group of potato virus Y is based on: the range of host plants, type of symptoms, degree of serological relationship, and amino acid composition of coat protein [11, 14].

Table 2

Reaction of 5 potato cultivars inoculated with strain PVA^N mechanically, by grafting and by aphids

Potato cultivar	No. of infected plants per 10 inoculated						Symptoms
	mechanical inoculation		grafting		transmission by aphids		
	a	b	a	b	a	b	
Uran	0	3	10	10	4	7	mosaic, deformation
Prosna	7	10	8	8	4	5	no symptoms
Osa	10	10	5	6	8	10	mild mosaic or no symptoms
Flisak	8	9	6	7	—	—	no symptoms
Baca	9	10	8	9	—	—	mosaic, deformation

a — No. of plants in which virus was detected in the year of inoculation.
b — No. of plants in which the virus was detected in tuber indexing.

Beside of PVY and PVA, the following viruses infecting plants from family *Solanaceae* belong to the discussed group: henbane mosaic virus, Columbian *Datura* virus and tobacco etch virus [6]. Distant serological relationship between these viruses has been found [1, 7, 8]. Titres of antisera with heterologous antigens are much lower than those with homologous, and low titre antisera do not react at all with heterologous viruses [1]. In studies of virus in crude sap, it happens that antiserum does not react even with homologus virus [9]. The reaction of antiserum to PVA with non-purified and non-concentrated strain A^N, as observed in the present studies, indicates that the investigated virus in fact belongs to the PVA group.

PVA^N infected the test plants and potato plants symptomlessly or induced mild symptoms. This reaction is typical of PVA infection [2, 10 13], and distinguishes this virus from HMV, TEV and CDV [4, 7, 12]. Only on *D. stramonium* plants strain A^N induced very distinct symptoms, contrary to other PVA strains which either do not infect this plant or cause only slight pathologic signs (similarly to strain PVA-Jara, compared in this study). Type of symptoms induced by PVA^N on *D. stramonium* is different from that produced by HMV, TEV or CDV. Moreover, in

contrast to strain A^N, these viruses induce distinct symptoms in other plant species, e.g. in tobacco [4, 7, 8, 12].

PVA^N did not cause distinct symptoms on N. physaloides and *L. pimpinellifolium*, similarly as in the studies of Zaklukiewicz and Kaczmarek [16], who have found symptoms of PVA only if this virus occurred in complex with potato virus X.

According to other communications, *L. pimpinellifolium* develops distinct symptoms upon infection by all PVA strains [2, 10], whereas the intensity of the reaction of *N. physaloides* plants depends on the strain, and therefore the latter species serves for differentiation of PVA strains [10].

PVA strains investigated in the present study and this of Zaklukiewicz and Kaczmarek [16] may belong to the strain group inducing mild symptoms on *N. physaloides*. On the other hand, the mild reaction of *L. pimpinellifolium* plants to our PVA strains can result from genetic specificity of the representatives of these species applied in the presented studies. Different reaction of test plants derived from various sources, caused by genetic heterogeneity, has already been found for several species [15].

REFERENCES

1. Bartels R.: Untersuchungen über serologische Beziehungen zwischen Viren der "tobacco-etch-Virus-Gruppe". Phytopath. Z., 49: 257-265, 1963/64.
2. Bartels R.: Potato virus A. C. M. I./A. A. B. Descriptions of Plant Viruses, 54, 1971.
3. Bokx J. A. de, Waterreus H. A. J. I.: Electron microscopy of plant tissues infected with potato viruses A and S. Neth. J. Pl. Path. 77: 106-112, 1971.
4. Govier D. A., Plumb R. T.: Henbane mosaic virus. C. M. I./A. A. B. Descriptions of Plant Viruses. 95, 1972.
5. Hampton R. O., Sandra Phillips, Knesek J. E., Mink G. I.: Ultrastructural cytology of pea leaves and roots infected by pea seedborne mosaic virus. Archiv ges. Virusforsch. 42: 242-253, 1973.
6. Harrison B. D., Finch J. T., Gibbs A. J., Hollings M., Shepherd R. J., Valenta V., Wetter C.: Sixteen groups of plant viruses. Virology. 45: 356-363. 1971.
7. Kahn R. P., Bartels R.: The Columbian Datura virus — a new virus in the potato virus Y group. Phytopathology. 58: 587-592. 1968.
8. Lovisolo O., Bartels R.: On a new strain of henbane mosaic virus from *Physalis alkekengi*. Phytopath. Z. 69: 189-201. 1970.
9. Maat D. Z., Mierzwa Z.: Potato virus A: purification and antiserum preparation. Potato Res. 18: 126-129. 1975.
10. MacLachlan D. S., Larson R. H., Walker J. C.: Strain interrelationships in potato virus A. Res. Bull. Agric. Exp. Stn. Univ. Wisconsin 180: 1-36, 1953.
11. Moghal S. M., Francki R. I. B.: Towards a system for the identification and

classification of potyviruses. I. Serology and amino acid composition of six distinct viruses. Virology 73: 350-362, 1976.
12. Shepherd R. J., Purcifull D. E.: Tobacco etch virus. C. M. I./A. A. B. Descriptions of Plant Viruses. 55: 1971.
13. Spire D., Férault A. C., Bertrandy J.: Extraction, purification et caractérisation du virus A de la pomme de terre. Ann. Phytopath. 1: 541-552, 1969.
14. Teakle D. S., Pares R. D.: Potyvirus (potato virus Y) group. In: The atlas of insect and plant viruses. Ed. K. Maramorosch, Academic Press.: 311-321, 1977.
15. Want van der J. P. H., Boerjan M. L., Peters D.: Variability of some plant species from different origins and their suitability for virus work. Neth. J. Pl. Path., 81: 205-216, 1975.
16. Zaklukiewicz K., Kaczmarek U.: Z badań nad występowaniem wirusa A w odmianach ziemniaka. Pr I. Ziem., 6: 8-13, 1974.

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НОВЫЙ ШТАММ А ВИРУСА, ИЗОЛИРОВАННЫЙ ИЗ ГИБРИДОВ ПРОИСХОДЯЩИХ ОТ *SOLANUM MEGISTACROLOBUM*

Резюме

Из гибридов, происходящих от *Solanum megistacrolobum*, изолирован штамм PVA, который был обозначен симболом A^N. Вирус вызвал сильные системные симптомы на *Datura stramonium*: прояснение жилок, мозаику, подвертывание листьев вниз, сильное торможение роста через 10-14 дней от инокуляции. Вирус имел узкий диапазон растений-хозяев. На большинстве пораженных тест-растений, на растениях картофеля PVA^N не вызывал никаких симптомов или вызывал только слабые симптомы. Предельное разбавление штамма A^N составляло 10^{-4}, термическая инактивация наступала при 45-50°C, а стойкость *in vitro* составляла 32-48 часов. Вирус переносился *Myzus persicae* неустойчивым способом. Сок из растений, пораженных PVA^N, реагировал с сывороткой против PVA, но не реагировал с сывороткой PVY. В клетках *D. stramonium*, пораженных вирусом, была констатирована однорядная установка вирионов. Кроме того в клетках наблюдались розеточные структуры и связанные с ними неидентифицированные структуры.

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NOWY SZCZEP WIRUSA A WYIZOLOWANY Z MIESZAŃCÓW POCHODZĄCYCH OD *SOLANUM MEGISTACROLOBUM*

Streszczenie

Z mieszańców pochodzących od *Solanum megistacrolobum* wyizolowano szczep PVA, który oznaczono symbolem A^N. Wirus wywoływał silne objawy systemiczne

na *Datura stramonium*: przejaśnienie nerwów, mozaikę, podwijanie liści w dół, nekrozę nerwów, zamieranie liści, silne zahamowanie wzrostu po 10-14 dniach od inokulacji. Wirus miał wąski zakres roślin-gospodarzy. Na większości roślin testowych, które podległy porażeniu oraz na roślinach ziemniaka PVA^N nie wywoływał żadnych objawów lub powodował objawy słabe. Graniczne rozcieńczenie szczepu A^N wynosiło 10^{-4}, termiczna inaktywacja następowała w 45-50°C, a trwałość *in vitro* wynosiła 32-48 godz. Wirus przenoszony był przez *Myzus persicae* w sposób nietrwały. Sok z roślin porażonych PVA^N reagował z surowicą przeciw PVA, a nie reagował z surowicą przeciw PVY. W komórkach *D. stramonium* porażonych wirusem stwierdzono jednorzędowe ustawienie wirionów. Ponadto w komórkach obserwowano struktury rozetkowate oraz związane z nimi struktury niezidentyfikowane.

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