

UDC 576.858.8:582.951.4(497.1) = 20
Original scientific paper

POTATO VIRUS Y ON TOBACCO IN KOSOVO

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Received February 12, 1991

A very destructive virus disease of tobacco has appeared steadily during the past four growing seasons in the Province of Kosovo (southeastern part of Yugoslavia). On the basis of test plant reactions, properties *in vitro*, serological tests and analysis of the microscope and submicroscope inclusions it was stated that causal virus belongs to necrotic strain of potato virus Y (PVY). This is the first information on PVY affecting tobacco in the Province of Kosovo.

Introduction

For several years now, a severe disease of tobacco has been widely spread in the Province of Kosovo. Leaves of the diseased plants initially showed vein clearing followed by veinal necrosis, chlorotic mottling, leaf malformation and stunting. Necroses could be seen on the stem as well. According to symptoms the disease was very similar to the »necrosis severa« described by Latorre et al. (1982). Preliminary tests indicated that the causal agent of the disease was a sap and aphid transmissible virus. The disease significantly reduced tobacco yield. The first tests suggested that the virus probably belonged to potato virus Y (PVY) but not to some other viruses usually occurring in tobacco. The presence of PVY in tobacco in other parts of Yugoslavia was established earlier (Mikovski 1984, Bužančić and Devčić 1979).

The paper deals with identification and some properties of the virus which causes a pernicious disease of tobacco in the southeastern region of Yugoslavia.

Material and Methods

Four virus isolates were studied: the isolates designated T1 and T2 were found in oriental small leaf tobacco in fields near Gnjilane and Uroševac respectively, and the isolates T3 and T4 were found in the flue-cured tobacco in the vicinity of Đakovica and Prizren, respectively.

The isolates were cleaned by local lesion passages using *Chenopodium amaranticolor* as test plant. The properties *in vitro* were determined in the standard way. In serological tests carried out by SDS agar gel immunodiffusion method an antiserum to potato virus Y (PVY) was used. The antiserum (titre 1/64) was supplied by Dr. Đ. M a m u l a (Department of Botany, Zagreb). The shape of virus particle was established in the leaf sap preparations by dipping method. Light microscope observations were done on leaving tobacco hair cells. For the study of the virus submicroscope inclusions, small pieces taken from the lower side of the leaf lamina of infected tobacco were fixed in 3% phosphate-buffered glutaraldehyde at pH 7.2 with 0.06 M phosphate buffer for 90 min at room temperature and post-fixed in 1% OsO₄ in veronal acetate for 2 hours. After that the tissue was dehydrated through a graded ethanol series and embedded in Epon. Ultrathin sections were stained with uranyl acetate followed by Reinold's lead and examined in a Philips CM10 electron microscope.

Results

Test plant reactions. Each of the 4 isolates were mechanically inoculated to 9 test plants. The reactions of the plants were the same to each isolate (Table 1).

On the basis of symptoms listed in the Table it was obvious that they markedly resembled those of potato virus Y (PVY) but not some other viruses usually occurring in tobacco (De l g a d o - S a n c h e z and G r o g a n 1970). According to the symptoms in naturally and artificially infected tobacco our isolates were most similar to the tobacco vein necrosis strain of PVY [PVY-N, PVY super (N)] (V o r s t e r et al. 1990, R o s e et al. 1987).

Properties in vitro. No differences were detected among the investigated isolates in longevity *in vitro*, dilution end point or thermal inactivation point. These properties were similar to those previously reported for PVY (G o o d i n g and T o l i n 1973).

Cytopathology. Our isolates provoked amorphous cytoplasmic inclusion bodies visible by light microscope. These inclusions could be seen in infected tobacco hair cells of systemically infected tobacco plants about 25 days after inoculation.

Electron microscope analysis of the cytoplasm of infected tobacco leaf cells revealed that all 4 isolates induced cylindrical inclusions. These structures were seen in transverse sections as a pinwheel and a scroll, and in longitudinal section as laminated aggregates (Fig. 2). The inclusions were completely similar to ones caused by PVY (comp. E d w a r d s o n 1974). In addition to the cylindrical inclusions, crystalline structures were observed as well (Fig. 2C).

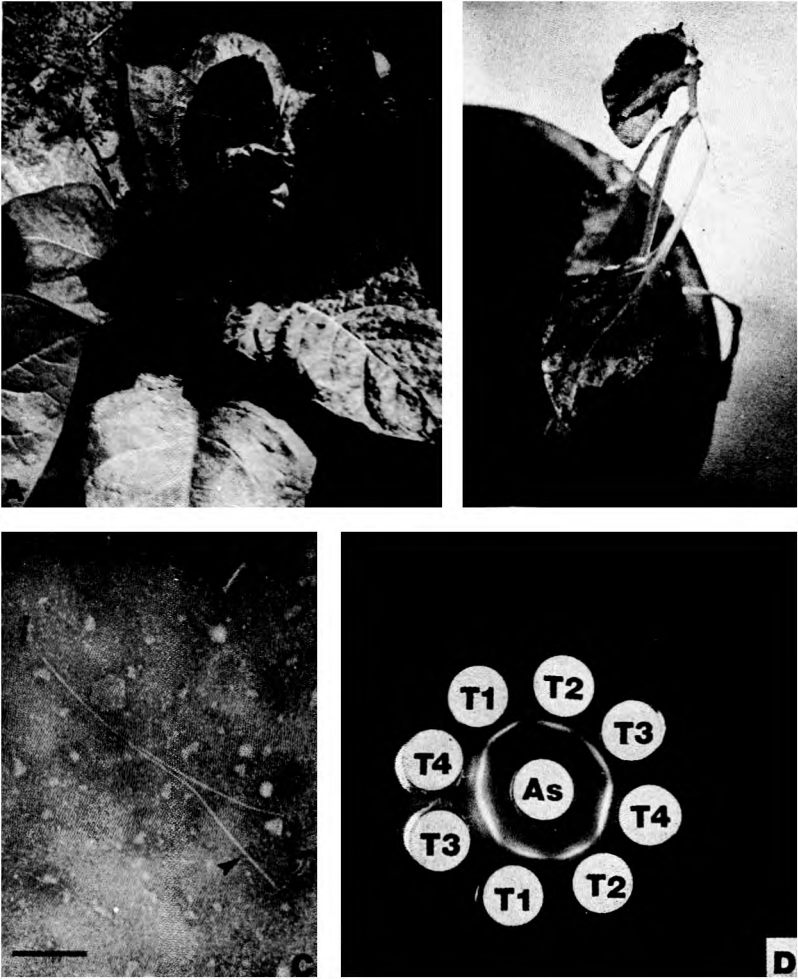


Fig. 1. A — naturally infected tobacco (cv. Jaka) with isolate T1; B — artificially infected tobacco (cv. Samsun) with isolate T2; C — virus particles of isolate T1 in leaf dip preparation of infected tobacco (bar represents 175 nm); D — agar gel diffusion serological test with SDS: peripheral wells contained 4 virus isolates (T1 — T4) and central well antiserum to potato virus Y.



Fig. 2. Ultrathin section of *Nicotiana tabacum* leaf cell infected with T1 isolate; cylindrical inclusions in transverse section appearing as a pinwheel (Pw) and in longitudinal section as a laminated aggregates (La); C — transverse section of a crystalline structure. Bar represents 100 nm.

Table 1. The reactions of test plants to infection by isolate T1, T2, T3 and T4

Test plant	Symptoms	
<i>Chenopodiaceae</i>		
<i>Chenopodium amaranticolor</i>	+L: local lesions	S: 0
<i>Ch. quinoa</i>	L: local lesions	S: 0
<i>Fabaceae</i>		
<i>Phaseolus vulgaris</i> cv. Trešnjevac	L: 0	S: 0
<i>Solanaceae</i>		
<i>Capsicum annuum</i>	L: 0	S: vein clearing, mild mottle
<i>Datura stramonium</i>	L: 0	S: 0
<i>Lycopersicum esculentum</i>	L: 0	S: mosaic
<i>Nicotiana glutinosa</i>	L: 0	S: severe mottling
<i>N. tabacum</i> cv. Jaka	L: 0	S: vein clearing, veinal necrosis (Fig. 1A)
<i>N. tabacum</i> cv. Samsun	L: 0	S: vein clearing, severe mottling, veinal necrosis, stunting (Fig. 1B)

+L = symptoms in inoculated leaves; S = symptoms in non-inoculated leaves;
0 = insusceptible

In the plant sap obtained from infected tobacco plants virus particles in the form of flexuous filaments about 700 nm in length were seen (Fig. 1C).

Serology. In serological tests carried out in agar gel using SDS, all 4 isolates reacted with antiserum to PVY. When the isolates were compared with each other by means of the same antiserum they were serologically identical (Fig. 1D).

Discussion

Investigations of virus diseases of tobacco in the Province of Kosovo began only a few years ago. The first data of a virus affecting tobacco in this region were reported by Taraku (1983). So far from tobacco plants grown in the Province of Kosovo only tobacco mosaic virus and tomato spotted wilt viruses were isolated. Of course, it does not mean that some other viruses do not affect this plant in that region; future research will probably confirm it.

In our serological tests polyclonal antibodies were used. Since these antibodies are unreliable in differentiating PVY super (N) strain from the ordinary strain (Rose et al. 1987), our strain identification was based only on symptoms on Samsun tobacco. Anyway, our isolates belong to severe PVY strains (see Gooding and Tolin 1973).

Because PVY is transmissible by aphid species it is probable that its explosive occurrence in the last 4—5 years in the Province of Kosovo may be related to the presence in large numbers of various aphid species, especially *Myzus persicae* (green peach aphid) (Singh and Khurana 1985). The occurrence of this virus is also connected with other PVY susceptible crops and weeds (comp. Latorre et al. 1982). The main sources of tobacco infections with PVY in this region are likely to be potato crop (Gray and Lampert 1988) and various weed plants. Until now, ecology of *M. persicae* has not been studied in the Province of Kosovo. However, it has been observed that the green peach aphid in spring builds up on weed hosts (*Brassica* sp., *Malva* sp., *Rumex* sp., *Chenopodium* sp. etc.) and on some woody plants (plum, cherry, rose), and in summer and autumn they build up on cultivated crops such as potato and tobacco. In connection with this tobacco disease in the Province of Kosovo it will be useful to investigate resistance of tobacco breeding lines used to inoculation with PVY by aphids (Gooding and Kennedy 1985, Marte et al. 1987). Also, the significance of cross-protection in the epidemiology of PVY in this region should be clarified by additional research (Latorre and Florrs 1985).

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We are grateful to Dr. Manfred Gailhofer (Institute of Plant Physiology, University of Graz) for the electron microscope analysis of cylindrical inclusions.

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SAŽETAK

Y-VIRUS KRUMPIRA NA DUHANU NA KOSOVU

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U prošlih nekoliko godina javlja se na duhanu na području Kosova vrlo agresivna virusna bolest zbog koje nastaju znatne gospodarske štete. Na oboljelim biljkama virus uzrokuje nekrotične promjene na listu, osobito nekroze žila, te mozaik. U ovom su radu istražena 4 virusna izolata (T1, T2, T3, T4) nađena na duhanu u okolini Gnjilana, Uroševca, Đakovice i Prizrena. Na osnovi istraživanja kruga domaćina, osobina *in vitro*, seroloških svojstava te studija mikroskopskih i submikroskopskih inkluzija u citoplazmi inficiranih stanica, ustanovili smo da se 4 virusna izolata međusobno ne razlikuju i da svi oni pripadaju Y-virusu krumpira (potato virus Y, PVY). Prema nekrotičnim promjenama na listu duhana, izolati vjerojatno pripadaju nekrotičnom soju PVY-a. Ovo je prvo priopćenje o dolaženju PVY na duhanu na području Kosova.

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