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BIOLOGICAL, SEROLOGICAL AND IMMUNO-ELECTROPHORETIC STUDIES OF TOMATO ASPERMY VIRUS FROM CHRYSANTHEMUMS IN YUGOSLAVIA

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Introduction

At least two distinct viruses (tomato aspermy virus and chrysanthemum virus B) and two viroids (chrysanthemum stunt and chrysanthemum chlorotic mottle viroids) have been described from chrysanthemums which cause serious losses in the commercial chrysanthemum production (Hollings 1955, Hakkaart and Maat 1974, Horst and Lawson 1975). None of the causal agents has been reported from Yugoslavia. In Yugoslavia the hybrids of *Chrysanthemum indicum (C. morifolium)*, often called "large-flowering" or "autumn chrysanthemums", have been widely grown in open garden plots predominantly around big cities. In 1979 different chrysanthemums showing various flover abnormalities were collected in private gardens in the district of Zagreb and tested by mechanical inoculation for viruses. Many plants were found infected with tomato aspermy virus (TAV), and from a considerable number chrysanthemum virus B was also obtained. This paper reports the identification and comparative studies of some isolates of TAV.

Material and Methods

TAV was obtained by grinding distorted flower parts in rather large amounts of 0.06 M phosphate buffer (pH 7.35) containing 0.1% thioglycolic acid and by inoculating the extracts to *Chenopodium quinoa* and *Nicotiana glutinosa*. All studies were done with originally obtained isolate Y7.

In comparative tests four additional isolates were used: Y21, Y22, Y24 and Y25.

Experimental plants were grown in the glasshouse at 6—21° C in winter and at 9—23° C in spring. Tobacco (*N. tabacum*) served as propagation host, and *C. amaranticolor* and *C. quinoa* as indicator and assay hosts.

The isolates were partially purified by the method of Grogan et al. (1963) as modified by Lawson (1967). Virus pellets were resuspended in buffer containing 0.02% formaldehyde which was added for stabilization of capsid structure (cf. Francki and Habili 1972). A260/280 of these preparation varied between 1.52—1.66, and compared with the value of 1.73—1.77 for TAV (Hollings and Stone 1971) indicated the presence of normal proteins.

One of the partially purified preparations of Y7 was further purified by layering on 0.2—0.7 M sucrose gradients in 0.1 M phosphate buffer pH 7.7 and by centrifuging in a Beckman SW 25.1 rotor (60 min at 23000 rpm). Virus containing fractions were collected by puncturing the bottom of the tube, diluted in buffer and concentrated by centrifuging (150 min at 93000 g). A260/280 of final virus suspension was 1.73 suggesting well purified virus. Virus concentration was determined from its absorbancy at 260 nm by using $E_{260}^{0.1\%}$ = 5.0 (Habili and Francki 1974 b).

Approximate concentrations of partially purified virus preparations were calculated from a comparison with purified Y7 in quantitative serological tests. Partially purified virus suspensions were mixed with 20% glycerol and kept frozen at —18° C until use. For comparative tests their titres had been adjusted more or less to the same values.

Antiserum against Y7 was prepared by one intramuscular injection with partially purified virus emulsified in Freund's incomplete adjuvant. Homologous titre of antiserum was 1/64 against virus and 1/2 against soluble virus protein (= viral coat protein subunits) (cf. Habili and Francki 1975). It did not contain antibodies to normal plant components. Specific antiserum against V strain of TAV (homologous titre 1/128) and specific antiserum against Q strain of cucumber mosaic virus (CMV) (homologous titre 1/64) (Habili and Francki 1964 b) were kindly supplied by Dr. R. I. B. Francki. All serological experiments were carried out by double immunodiffusion in gels of 0.9% Bacto agar in distilled water containing 0.05% NaN_3.

Immunoelectrophoresis tests were performed in a LKB-Gelman 6800A-1 microelectrophoresis apparatus in 0,9% Special agar-noble dissolved in 0.1 M phosphate buffer pH 7.0 on groups of 6 microscope slides held in a plastic rack. Antigen wells, 1 mm diameter, were filled with partially purified virus at approximate concentrations of 1 mg/ml. Electrophoreses were done at c. 2° C, in final experiments for periods of 4 h at 250 V (about 9V/cm) and about 20 mA at the beginning and 30 mA at the end of each experiment. After electrophoresis suitably diluted antiserum (1/8) against Y7 was placed in the channels and slides were incubated for 24 h at c. 24° C. Mobilities were calculated from the average displacement from the original wall (see Hollings and Stone 1975).

Results

Reaction of test plants

The symptoms produced by isolate Y7 in species infected by mechanical inoculation are summarized in Table 1. Reaction of test plants and symptoms were similar to those reported for other isolates of TAV (cf.

Table 1. Reaction of test plants infected with isolate Y7

Species	Symptoms	
	Inoculated leaves	Uninoculated tip leaves
Capsicum annuum	necrotic lesions	chlorosis, mottling, leaf distortions, wilting at low temperatures
Chenopodium amaranticolor	chlorotic pin-point lesions	not infected
C. murale	necrotic pin-point lesions	not infected
C. quinoa	yellow-green lesions (Fig. 1)	not infected
C. urbicum	chlorotic, later red-brown dots	not infected
Datura stramonium	diffuse chlorotic lesions, sporadic necrotic lesions	diffuse chlorotic lesions, mottling, leaf distortions (Fig. 1B) including narrowed lamina, reflexed leaves and enations
Lycopersicum esculentum	symptomless	vein-clearing, mottling, leaf distortion, inhibition of apical growth (Fig. 1C), bushy appearance
Nicotiana bigelovii	symptomless	vein-clearing
N. clevelandii	symptomless	vein-clearing, narrowed lamina
N. glutinosa cv. Corvallis strain	sporadic diffuse chlorotic lesions	vein-clearing, mosaic, mottle, leaf distortions including blisters, thickened veins, enations, tendril-like and filiform leaves, dwarfing and bushy appearance (Fig. 1D)
N. megalosiphon	necrotic lesions	vein-clearing, top necrosis
N. tabacum cv. Samsun NN	chlorotic blotches	vein-clearing, mottling, narrowed lamina and other distortions, enations (Fig. 2A, B, C)
N. tabacum cv. Hicks resistant	sometimes diffuse chlorotic blotches	occasionally at low temperatures vein-clearing and mosaic, otherwise not infected
N. tabacum cv. Xanthi-nc	chlorotic blotches, white necrotic lesions	the same as in cv. Samsun NN with addition of necrotic etching (Fig. 2D)
Ocimum basilicum	necrotic lesions	large diffuse chlorotic lesions later turning necrotic
Petunia hybrida	necrotic lesions	vein-clearing, blister mottle, thickened veins, enations, tendril-like and reflexed leaves, bushy appearance
Spinacia oleracea cv. Matador	symptomless	vein-clearing, chlorotic mosaic, blisters
Tetragonia expansa	chlorotic lesions about 2 mm diam.	not infected
Vicia faba cv. Inovecký	necrotic lesions	not infected
Vigna sinensis cv. Black eye	reddish dots	not infected
Zinnia elegans	diffuse chlorotic lesions	vein-clearing, mottling, necrosis, reflexed leaves, broken flowers (Fig. 2E), bleaching

Brierley et al. 1955, Hollings 1955, Hollings and Stone 1971, Procter 1975, Schmelzer et al. 1977, Horváth et al. 1980), although small differences from each of the isolates described earlier could be found. In repeated experiments Y7 did not infect *Brassica rapa* var. *rapa, Cichorium endivia, Cucumis sativus* cv. Delicatess, *Lactuca sativa* cv. Vanguard, *Pisum sativum* cv. Telephone and *Phaseolus vulgaris* cv. Top Crop which were not hosts for a considerable number of other TAV isolates.

Isolates Y21, Y22, Y24 and Y25 all produced enations in tobacco and solanaceous species in which isolate Y7 did, and did not differ in their virulence towards these hosts. Inoculations to *C. sativus* cv. Delicatess have not been successful either.

Aphid transmission test

A test for transmission of Y7 by aphids was done with *Myzus persicae* using *Tetragonia expansa* as source plant and *Nicotiana megalosiphon* as test plant. The aphids were given 1—2 min acquisition feed followed by a 1-day test feed of 10 aphids on each of 6 test plants. Systemic symptoms occurred in 5 plants.

Stability in sap

In *Nicotiana tabacum* sap, infectivity of Y7 was lost after 10 min at 60—65° C, 52 h at room temperature (20° C) or after dilution (0.06 M phosphate buffer pH 7.3) between 10^{-3} and 10^{-4}, which coincides with the values obtained for TAV by other authors (Hollings and Stone 1971).

Electron microscopy

Numerous spherical particles about 27 nm in diameter were observed in electron micrographs of partially purified preparations stained with 2% sodium phosphotungstate (Fig. 3A).

Serological tests

Serological identity of the virus. Gel double diffusion tests showed that isolate Y7 was serologically closely related to V strain of TAV: it reacted with antiserum to V strain up to the homologous titre of the serum of 1/128. No reaction was observed between the Yugoslav isolate and the antiserum to Q strain of CMV, which shows that our TAV is a typical representative of this virus (cf. Habili and Francki 1974 a).

Serological comparison between the isolates. A serological comparison among isolates Y7, Y21, Y22, Y24 and Y25 was done by using antiserum to isolate Y7 and antiserum to V strain of TAV. The first was diluted 1/4 to avoid reactions with soluble protein, and the second was concentrated. In these experiments all isolates were compared with each other. The result of double diffusion tests was the formation of precipitin lines which always joined without spur formation (Fig. 3 B, C). In absorption tests the absorbed sera did not additionally react with any isolate, either (Fig. 3 D).

Accordingly, all isolates appeared serologically identical to isolate Y7 and with each other.

Immunoelectrophoresis

In 0.1 M phosphate buffer pH 7.0 all isolates migrated rather slowly towards the cathode as a single moving antigen component.

Electrophoresis for 2 h at 200 V with 3 plastic racks did not enable us to differentiate the isolates. However, when it was carried out for 4 h at 250 V by using a single rack, all isolates could be clearly differentiated from each other. Under these conditions the electrophoretic movements of Y21, Y7, Y22, Y25 and Y24 were calculated to be 1.1, 1.8, 2.9, 3.1 and 3.6 mm/h, respectively. The effects of possible unequal virus concentrations were investigated by placing pairs of isolates in the same walls. The result was either the formation of a single line with two arches (Fig. 3E) or of two precipitin lines (Fig. 3F), depending on the differences in the mobility of two isolates in combination. The rate of migration of each isolate in mixture was in approximate accordance with the values obtained in previous experiments, i. e. with the rate of migration in a single isolate sample (Fig. 3E, F). The possible differences in the rate of electrophoretic movement of the virus in various preparations of the same isolate were also investigated. A comparison between four partially purified preparations of Y7 gave closely similar values, and in no case the indication of differences in electrophoretic mobility could be observed.

Discussion

Soon after the initial finding of TAV on tomatoes Blencowe and Caldwell (1949) established that chrysanthemums served as the primary source of infection for tomatoes. Not long after, it was found that TAV was widespread in chrysanthemums and influenced their quality and yield. This virus is prevalent in most countries where chrysanthemums are extensively grown (Hollings and Stone 1971) and has been until now detected in a number of European countries. During this work we identified TAV on chrysanthemus in Yugoslavia.

According to earlier literature data most isolates of TAV obtained from chrysanthemums exhibited close serological relationship (Lawson 1967, Hollings and Stone 1971) although they usually differed in the reaction of some test plants (Hollings 1955, Lawson 1967, Hollings and Stone 1971, Procter 1975). In this respect our Y isolates are similar to most of the others. They strongly crossreacted with antiserum to V strain of TAV described from chrysanthemums from Australia (Habili and Francki 1974a) and were serologically identical with each other; in the reaction of test plants Y7 also slightly differed from individual isolates described earlier. In comparative tests our isolates induced identical reactions in each particular species examined which shows that they are very closely related. However, immunoelectrophoresis was a suitable method for distinguishing these isolates because it showed significant, although not big, differences in their electrophoretic run (Fig. 3E, F). In combination with serology the method should be useful in establishing relationships between various isolates of this relatively stable and homogeneous cucumvirus.

Summary

Tomato aspermy virus (TAV), the cause of flower deformities and reduction of the yield in cultivated chrysanthemums (*Chrysanthemum*

spp.), was detected in these species in Yugoslavia. The TAV isolates possessed properties similar to those of isolates obtained from chrysanthemums in other countries. Biological and serological tests were not adequate to differentiate between five Yugoslav isolates of the virus. However, in immunoelectrophoresis in agar gel clear and constant, although not big, differences in their electrophoretic movement have been found.

This is the first report of TAV in Yugoslavia.

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SAŽETAK

BIOLOŠKA, SEROLOŠKA I IMUNOELEKTROFORETSKA ISTRAŽIVANJA VIRUSA BESJEMENOSTI RAJČICE IZ KRIZANTEMA U JUGOSLAVIJI

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Virus besjemenosti rajčice (VBR; tomato aspermy virus), uzročnik deformacija cvijeta i smanjivanja priroda kultiviranih hibrida roda *Chrysanthemum* utvrđen je na krizantemama u Jugoslaviji. Virusni izolati pokazivali su svojstva slična izolatima VBR, koji su bili opisani na krizantemama u drugim zemljama. Izvršeni biološki i serološki pokusi nisu bili prikladni za razlikovanje pet jugoslavenskih izolata VBR. Međutim, u imunoelektroforetskim pokusima ustanovljene su jasne i specifične razlike u elektroforetskoj pokretljivosti izolata.

Ovo je prvi nalaz VBR u Jugoslaviji.

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