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NATURAL INFECTION OF *DIGITALIS CILIATA* WITH RIBGRASS MOSAIC VIRUS

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Introduction

Ribgrass mosaic virus or Holmes' ribgrass mosaic virus (HRV) represents a well characterized tobamovirus (R/1 : 2/5 : E/E : S/*) (cf. Oshima and Harrison 1975). It shares many properties with other tobamoviruses such as the size and form of virus particles physical properties, and many common antibody fractions. For a long time this virus was considered as a strain of common tobacco mosaic virus (TMV). However, the manysided investigations of HRV showed that it differed in some aspects not only from TMV but also from other tobamoviruses. For example, unlike other tobamoviruses HRV infects mostly *Plantago* spp. in nature. In contrast to other tobamoviruses which have 158 amino acids in their protein subunits, HRV contains 156 amino acids only (Wittmann-Liebold and Wittmann 1967).

According to Wittmann (1965) all tobamoviruses are originated monophyletically by means of numerous minute mutations and during their specific evolution they adapt themselves mainly to the parasitism in one plant family. For instance, TMV is adapted to *Solanaceae*, especially to tobacco, cucumber green mottle mosaic virus to *Cucurbiataceae*, sunn hemp mosaic virus to *Leguminosae*, odontoglossum ringspot virus to *Orchidaceae*, HRV to *Plantago* spp. etc.

In this paper a finding of HRV on *Digitalis ciliata* Trautv. is reported. In connection with this the natural host range of HRV will be discussed.

Materials and Methods

The subject of this paper is a virus found in *Digitalis ciliata* Trautv. specimens which were bred in the Training Field of Botanical Garden of Zagreb University (Fig. 1 A). The isolate was marked HRV-D. To

prepare the antigen for serological tests, the virus was purified by the method of Gooding and Hebert (1967). Serological tests were carried out by means of agar-gel double diffusion test using antisera to HRV-Y (Juretić et al. 1969) and TMV.

Results

1. *Investigation on test plants*

The investigated isolate HRV-D was mechanically transmitted to 10 test plants. Symptoms produced on these plants by HRV-D are shown in Table 1. On the basis of the local infection on *Datura stramonium* and *N. glutinosa* it was concluded that the HRV-D belonged to tobamoviruses. Further investigations on test plants showed that HRV-D unlike common TMV caused only local lesions on *N. silvestris* (Fig. 1 B) and systemic symptoms on *Plantago media*. It is worth mentioning that we could not transmit our isolate to *Phaseolus vulgaris* and *Lycopersicon esculentum* (comp. Holmes 1941; Juretić et al. 1973). These species react to the typical HRV in the same manner. Above data indicate that HRV-D represents a tobamovirus more similar to HRV than to TMV.

2. *Serological investigations*

To find out whether HRV-D is more closely related to TMV or to HRV some serological experiments were done. They were performed by agar-gel double diffusion test.

When HRV-D was compared with HRV-Y using HRV-Y antiserum, in the majority of tests the precipitin bands coalesced without any spur. We, certainly, could not conclude on the basis of that, that HRV-D was serologically identical to HRV-Y as the antiserum against HRV-D was not employed. Besides, the cross absorption test was not performed because only one antiserum was available. In spite of that there is no doubt that HRV-D shares many more antigenic determinants with HRV than with TMV. When HRV-D was compared with TMV using TMV antiserum a spur formation always appeared in the agar-gel diffusion test. Therefore, the serological experiments have also shown that HRV-D belongs indeed to HRV and not to TMV or any other tobamaviruses.

Fig. 1. Symptoms on plants caused by the isolate HRV-D: A — systemic symptoms on leaf of *Digitalis ciliata* (spontaneous infection), B — local lesions on *Nicotiana silvestris*, C — systemic symptoms on *Brassica rapa* var. *rapa*. D — inclusion body in hair cell of *D. ciliata* consisting of paracrystalline needles arranged irregularly. E — serological test performed in agar gel: Y Yugoslav isolate of ribgrass mosaic virus from *Plantago media* (HRV-Y), D the investigated isolate HRV-D, SY antiserum to HRV-Y.

Sl. 1. Simptomi na biljkama koje uzrokuje izolat HRV-D: A — sistemični simptomi na listu vrste *Digitalis ciliata* (spontana infekcija), B — lokalne lezije na vrsti *Nicotiana silvestris*, C — sistemični simptomi na vrsti *Brassica rapa* var. *rapa*. D — virusna inkluzija sastavljena od nepravilno skupljenih parakristaliničnih iglica u dlačnoj stanici vrste *D. ciliata*. E — serološki pokus izveden u gelu agara: Y jugoslavenski izolat virusa mozaika trpuca (ribgrass mosaic virus) iz vrste *Plantago media* (HRV-Y), D istraživani izolat HRV-D, SY imuni serum od HRV-Y.

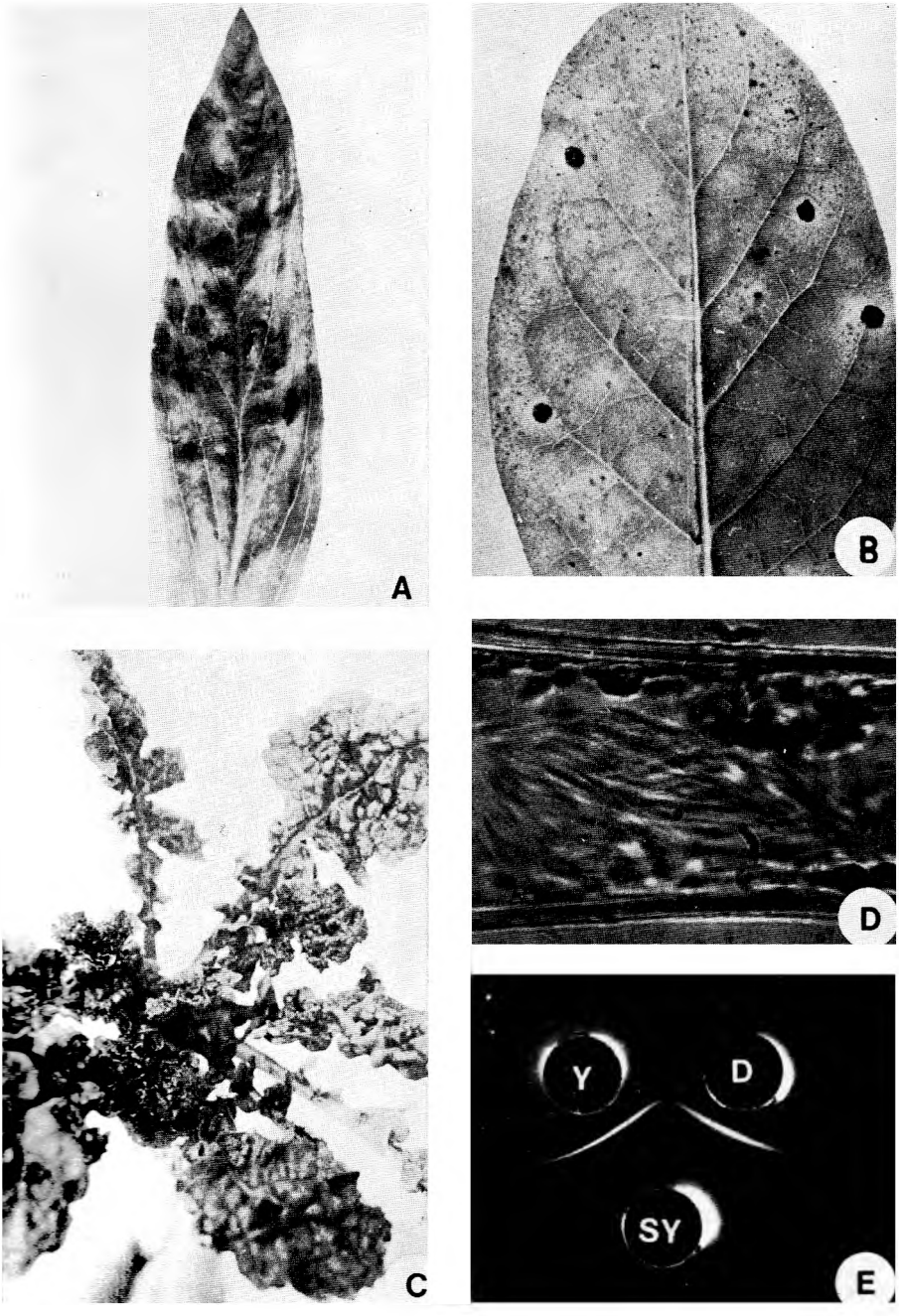

Fig. 1. — Sl. 1.

Table 1. Symptoms on some test plants caused by HRV-D

Test plant	Symptoms*
CHENOPODIACEAE	
Chenopodium amaranticolor Coste & Reyn.	L — necrotic local lesions; S — chlorotic zones, necrotic spots on top leaves, curling, blistering
Chenopodium quinoa Willd.	L — chlorotic lesions
CRUCIFERAE	
Brassica rapa L. var. *rapa*	L — necrotic lesions; S — mosaic, fine blistering, curving, growth is checked (Fig. 1c)
SOLANACEAE	
Datura stramonium L.	L — necrotic lesions
Nicotiana glutinosa L.	L — necrotic lesions
Nicotiana silvestris Speg. et Comes	L — necrotic lesions (Fig. 1B)
Nicotiana megalosiphon Heurck & Muell. Arg.	S — necrotic spots, mosaic
Nicotiana tabacum L. cv. Samsun	L — necrotic spots; S — necrotic spots, mild mosaic
Petunia hybrida hort. ex Vilm.	S — mild mosaic, mild deformation of leaves
PLANTAGINACEAE	
Plantago media L.	S — mild mottling which disappears

L — local infection; S — systemic infection

3. *Observations concerning virus cell inclusions*

Light microscope analyses of living cells of infected tissue of *Digitalis ciliata* and *Brassica rapa* var. *rapa* showed that HRV-D produced paracrystalline needles. They were usually crowded as can be seen in Fig. 1d, and were arranged irregularly. Moreover, transitive crystalline forms from hexagonal prisms to rounded plates were sometimes observed (cf. Miličić and Štefanac 1971). Therefore, the HRV-D isolate described here differs in respect of inclusion bodies from the typical HRV (Miličić et al. 1968) and from RMV-K found in Yugoslavia earlier (Juretić et al. 1973).

Discussion

Wittmann (1965) considered that HRV occurred in nature predominantly on *Plantago* spp. However, recent data concerning the occurrence of HRV in natural hosts show that this virus is found on plants belonging to different plant families (see Table 2).

As can be seen from Table 2, HRV has been isolated so far from 15 different plants which belong to 7 plant families. It is an established fact that HRV was isolated from genus *Plantago* in various parts of the world. However, HRV was also found in 6 plants from *Cruciferae* which grow in distant areas of the world. In addition, this virus often affects *Digitalis lanata* (Schumann 1963a; Silva and Pop 1965). Therefore,

Table 2. Natural hosts of HRV

Plant host	Author	Country
BORAGINACEAE		
Anchusa officinalis	Polák (1964)	Czechoslovakia
CARYOPHYLLACEAE		
Melandrium album	Kovachevsky (1963/64)	Bulgaria
(Lychnis alba)	Chessin et al. (1967)	U.S.A.
CRUCIFERAE		
Brassica campestris	Pei (1962)	China
Cardaria draba	Polák (1964)	Czechoslovakia
Sisymbrium loeselii	Polák (1964)	Czechoslovakia
Radicula sylvestris	Goto and Oshima (1962)	Japan
Eutrema wasabii	Tochihara et al. (1964)	Japan
Roripa amphibia	Juretić et al. (1973)	Yugoslavia
PLANTAGINACEAE		
Plantago major	Holmes (1941), Goldin (1953), Harrison (1956)	U.S.A., U.S.S.R., Great Britain
Plantago media	Juretić et al. (1969)	Yugoslavia
Plantago lanceolata	Holmes (1941), Harrison (1956), Schumann (1963b)	U.S.A., G. Britain, Germany
PRIMULACEAE		
Primula obconica	Kovachevsky (1969)	Bulgaria
SCROPHULARIACEAE		
Digitalis lanata	Schumann (1963a), Silva and Pop (1965)	Germany, Rumania
SOLANACEAE		
Lycopersicon esculentum	Kovachevsky (1963/4)	Bulgaria
Nicotiana tabacum	Kovachevsky (1963/4)	Bulgaria

the data from Table 2 show that HRV is adapted to defferent families in nature and not only to *Plantaginaceae*. It seems therefore that HRV has a wider spectrum of natural hosts than it was considered earlier. Our finding of HRV in *Digitalis ciliata* also supports this opinion.

It must be stressed that HRV has a large number of variants. Juretić and Wetter (1973) established that 4 variants of HRV which were found in distant regions differed serologically from the type HRV, and some of them differed mutually from one other. Amino acid composition was investigated with the following HRV variants: the type HRV, *Lichnis* isolate (Chessin et al. 1967), TMV-C (Oshima and Kanazawa 1968), and Wasabi strain (W; Oshima et al. 1974) only. It is interesting that in respect of amino acid composition *Lychnis* isolate differs from the type HRV in 7 amino acid exchanges, TMV-C in at least 9, and W strain in at least 7 exchanges. In contrast to HRV variants, 13 strains of tomato mosaic virus which were also found in widely distant geographic regions were serologically indistinguishable. In addition, five tomato mosaic strains had amino acid composition identical to that of the type tomato mosaic virus; seven showed one exchange and one showed two exchanges only (cf. Wang and Knight 1967). These data indicate that HRV has a greater variability of protein

content and serological properties than tomato mosaic virus. Although all four HR variants have a similar content of amino acids His and Met, acording to the content of other amino acids they differ greatly from one other. In connection with this it is a matter of discussion whether all "strains" of HRV known up to now really are strains of HRV or are maybe separate tobamoviruses which could be adapted to different plant families in nature. Further investigations HVR variants, especially in respect of their chemical properties, could probably help clarify these problems.

Summary

A virus isolate (HRV-D) from *Digitalis ciliata* Trautv. collected in the Botanical Garden of Zagreb University was investigated in respect of its relation with some other viruses. The virus was compared on basis of its symptoms on differential test plants, serological properties and inclusion bodies with the Yugoslav ribgrass mosaic virus (HRV-Y) and with the common tobacco mosaic virus (TMV). It has been established that the investigated HRV-D belongs to ribgrass mosaic virus (HRV). The rounded plates which are characteristic of the type HRV were very rare, but groups of paracrystalline needles were frequent.

The natural host range of HRV isolates is also discussed.

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SADRŽAJ

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Iz vrste *Digitalis ciliata* Trautv., koja je skupljena na školskom polju Botaničkog vrta Prirodoslovno-matematičkog fakulteta Sveučilišta u Zagrebu, izoliran je virus za koji smo ustanovili da pripada virusu mozaika trpuca (ribgrass mosaic virus; H o l m e s' ribgrass mosaic virus; HRV). Do toga smo došli na osnovi istraživanja virusa s pomoću diferencijalnih domaćina, te seroloških pokusa izvedenih u gelu agara. Izolirani virus je uspoređivan s ranije nađenim izolatom HRV u Jugoslaviji (HRV-Y) kao i s običnim virusom mozaika duhana (TMV). Za razliku od tipičnog HRV i još nekih drugih tobamovirusa istraživani izolat stvarao je vrlo rijetko u inficiranim stanicama inkluzije u obliku tzv. okruglastih pločica, a često nakupine kristaliničnih iglica.

U radu je dan pregled dosadašnjih nalaza HRV u prirodi i s time u vezi razmatrani su srodstveni odnosi među dosad poznatim izolatima HRV.

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