Acta Bot. Croat. 32 (1973) 29—35

IDENTIFICATION OF A TOBAMOVIRUS ISOLATED FROM *RORIPA AMPHIBIA*

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Received January 21, 1973

Introduction

Although viruses belonging to the tobacco mosaic group (tobamoviruses; Harrison et al. 1971) are widely spread in nature, only few of them have been found in cruciferous plants. A tobamovirus was isolated from *Cardaria draba* and *Sisymbrium loeselii* in Czechoslovakia by Polák (1964). Almost simultaneously, Goto and Oshima (1962) found another virus in the wild crucifer *Radicula silvestris* in Japan, and Pei (1962) in *Brassica campestris* in China.

Last year we isolated a tobamovirus from the wild crucifer *Roripa amphibia* (L.) Bess. in the vicinity of Zagreb. The infected plants showed mild mottling symptoms and necrotic lines on the leaves (Fig. 1A). From these plants a virus was isolated and transmitted to a relatively large number of plants. On the basis of symptomatological, electron microscopic and serological investigations it has been established that the isolate belongs to tobamoviruses, particularly to ribgrass mosaic virus (RMV).

Material and Methods

The virus isolated from *Roripa amphibia* was marked RMV—K. Several other viruses were used for comparison with RMV—K. These viruses were: common tobacco mosaic virus (TMV), type strain of RMV that was sent us by courtesy of Professor C. Wetter (Saarbrücken) and Yugoslav strain of RMV (RMV—Y) obtained from ribgrass (Juretić et al. 1969). For serological investigation we employed the immune sera against TMV and RMV—K which were prepared after Juretić et al. (1969).

The virus investigated was passed through single-lesion culture. For this purpose leaves of *Nicotiana glutinosa* were used. After this treatment the virus was transmitted to common herbaceous plants some of which served as test plants for differentiation of tobamoviruses.

RMV—K was purified according to the method of Gooding and Hebert (1967). Serological experiments were performed by means of agar-gel double diffusion test (Dudman 1965, van Regenmortel 1966, Wetter and Luisoni 1969) and cross absorption test (van Regenmortel 1967).

Results

Investigation on herbaceous plants

The isolate RMV—K was mechanically transmitted to 12 herbaceous test plants. Symptoms caused on these plants by RMV—K are presented in Tab. 1 (comp. Fig. 1B, C, D). The reaction of some differential test species, following the inoculation with RMV—K, TMV, type strain of RMV and RMV—Y, is shown for comparison in Tab. 2. As it is visible from Tab. 2, RMV—K produced on *Datura stramonium* and *N. glutinosa* only local lesions (Fig. 1B), similar to those caused by TMV and other tobamoviruses. On the basis of these symptoms we could anticipate that RMV—K belonged to tobamoviruses.

Unlike common TMV, RMV—K caused only local lesions on *N. silvestris* and systemic symptoms on *Plantago media*, i. e. the same symptoms as the common RMV and RMV—Y (comp. Holmes 1941, Juretić et al. 1969). Moreover, the necrotic symptoms in form of rings and spots produced on tobacco by RMV—K were similar to those provoked under the influence of RMV and RMV—Y on this plant (Holmes 1941, Kovachevsky 1963, Juretić et al. 1969).

Consequently, the reactions of test plants have pointed out that RMV—K is a tobamovirus which is more closely related to RMV than to TMV.

Purification, ultraviolet absorption and electron microscopy

RMV—K was purified after the method of Gooding and Hebert (1967). The purified virus suspension had and absorption maximum in ultraviolet at 260 nm (Fig. 5). The shape of the curve indicated that the virus was well purified.

The analysis of infected sap and purified virus suspension in the electron microscope showed that the cause of disease was a rod-shaped virus about 300 nm long (Fig. 2, 4). This length is a characteristic of tobamoviruses.

Serological investigations

The purpose of these investigations was to establish whether RMV—K was more closely related to the common TMV or to the RMV. With this aim in view some experiments were made by agar-gel double diffusion and by intragel cross absorption method.

Table 1. Reactions of herbaceous plants infected with the isolate RMV-K of ribgrass mosaic virus

Explanation of signs: I *symptoms in inoculated leaves;* II *symptoms in non-inoculated top leaves;* ○ *local infection;* □ *systemic infection;* △ *unsusceptible.*

Amaranthaceae

Gomphrena globosa L. ○ local necrotic lesions surrounded with a red ring.

Chenopodiaceae

Chenopodium amaranticolor Coste & Reyn. ○ necrotic lesions.
Ch. murale L. ○ necrotic lesions.
Ch. quinoa Willd. ○ necrotic lesions.

Cruciferae

Brassica rapa L. var. *rapa* □ I necrotic lesions; II vein clearing, mottling.

Cucurbitaceae

Cucumis sativus L. cv. Delicatesse △.

Leguminosae

Phaseolus vulgaris L. cv. Pinto △.

Plantaginaceae

Plantago media L. □ II mottling.

Solanaceae

Datura stramonium L. ○ necrotic lesions.
Lycopersicum esculentum L. △.
Nicotiana glutinosa L. ○ necrotic lesions (Fig. 1B).
N. megalosiphon Heurck & Muell. Arg. ○ necrotic lesions.
N. tabacum L. cv. Samsun □ I sporadic necrotic lesions; II necrotic rings, line pattern and oak leaf pattern, mottling, mosaic (Fig. 1C, D).
N. silvestris Speg. and Comes ○ necrotic lesions
Petunia hybrida hort. ex Vilm. ○ necrotic lesions.

Table 2. Symptoms in some test plants infected by RMV-K isolate and three other tobamoviruses

Test plants	TMV	RMV	RMV-Y	RMV-K
Phaseolus vulgaris	L	O	O	O
Plantago media	L	S	S	S
Datura stramonium	L	L	L	L
Nicotiana glutinosa	L	L	L	L
N. silvestris	S	L	L	L
N. tabacum cv. Samsun	S	S (necrotic rings)	S (necrotic spots)	S **(necrotic rings and spots)**

L *local symptoms,* S *systemic symptoms,* O *not infected*

First, experiments in agar-gel were performed to examine the serological relationship between RMV—K and common TMV. As Fig. 3A shows, the precipitin bands of TMV and RMV—K made a spur indicating that RMV—K differs from TMV, i. e. that these two viruses are not closely related.

Afterwards, we carried out an experiment which was to answer the question of whether RMV—K is more closely related to the common TMV or to the RMV. As can be seen from Fig. 3B the immune serum against TMV was absorbed by the type strain of RMV. The absorbed serum could react neither with RMV nor with RMV—K but it reacted with the homologous virus. On the basis of this experiment we could conclude that RMV—K is more closely related to the type strain of RMV than to the common TMV, i. e. that RMV—K shares more common determinants with the type strain of RMV than with the common TMV.

Discussion

So far, RMV has been isolated from a large number of wild and cultivated plants belonging to various families (Holmes 1941; Goldin 1953; Harrison 1956; Schumann 1963a, b; Silva and Pop 1965; Kovachevsky 1963; Juretić et al. 1969). It is interesting that the tobamoviruses isolated so far from crucifers were more closely related to RMV than to common TMV (Polák 1964, Goto and Oshima 1962, Pei 1962). It seems that the crucifers are suitable natural hosts to RMV. It should be noted that the agent isolated by Polák (1964) from crucifers was a mixture of two tobamoviruses; only one component was a strain of RMV, the second component being another virus (Juretić and Wetter 1971).

The investigations of RMV have shown that this virus is widespread in nature. It would be interesting to know whether this virus has in nature a larger number of hosts than the common TMV (comp. Kovachevsky 1963).

The detailed serological investigations of RMV—K will be reported elsewhere.

Summary

On the basis of the investigations presented it has been established that RMV—K isolate from *Roripa amphibia* belongs to tobamoviruses. This virus is symptomatologically and serologically more closely related to the type strain of ribgrass mosaic virus (RMV) than to the common tobacco mosaic virus. Consequently, the virus which was isolated and investigated is a strain of RMV.

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We wish to thank Dr N. Ljubešić (Ruder Bošković Institute, and Institute of Biology, University of Zagreb, Zagreb) for the electron microscopic examination and the micrograph presented.

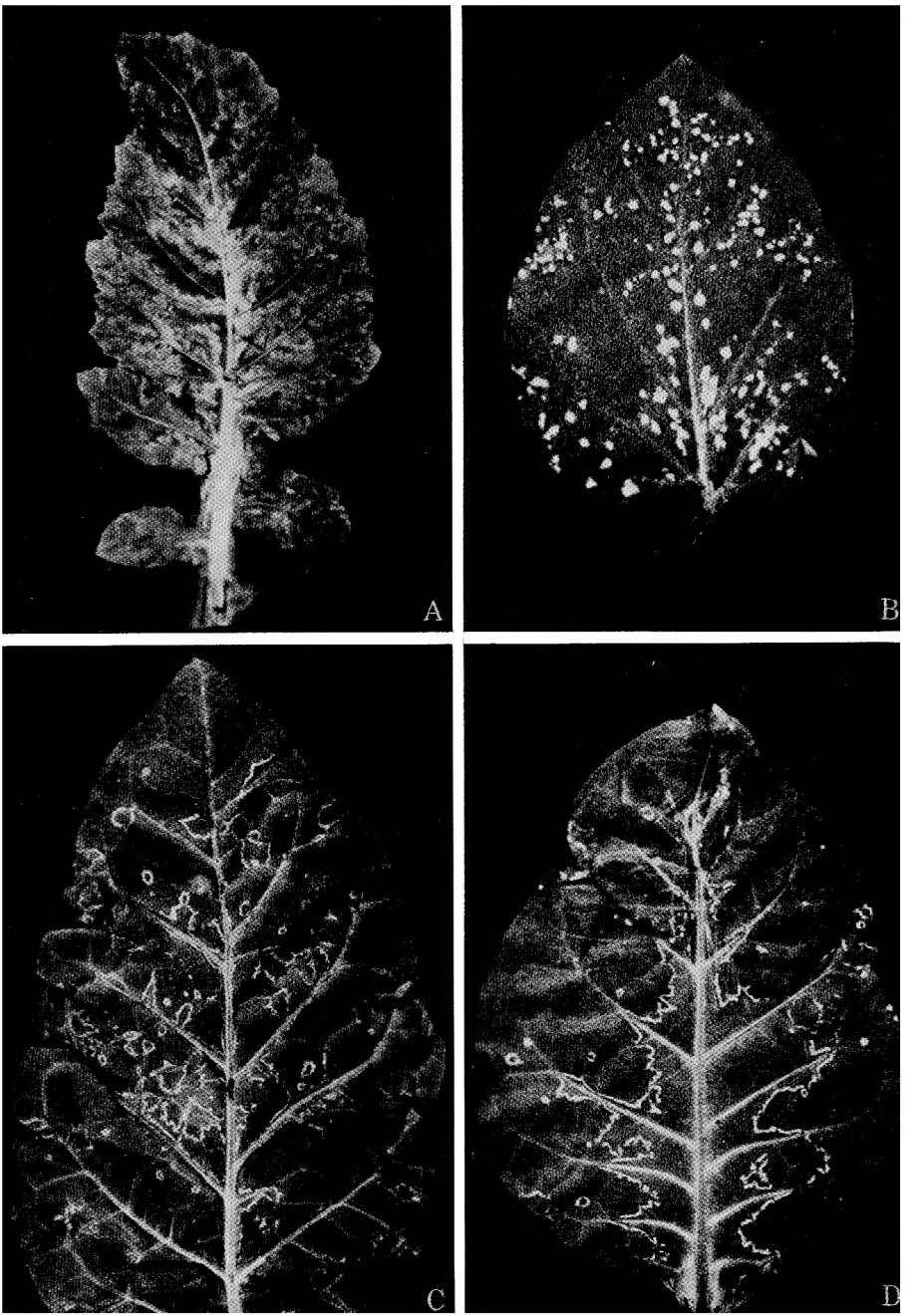
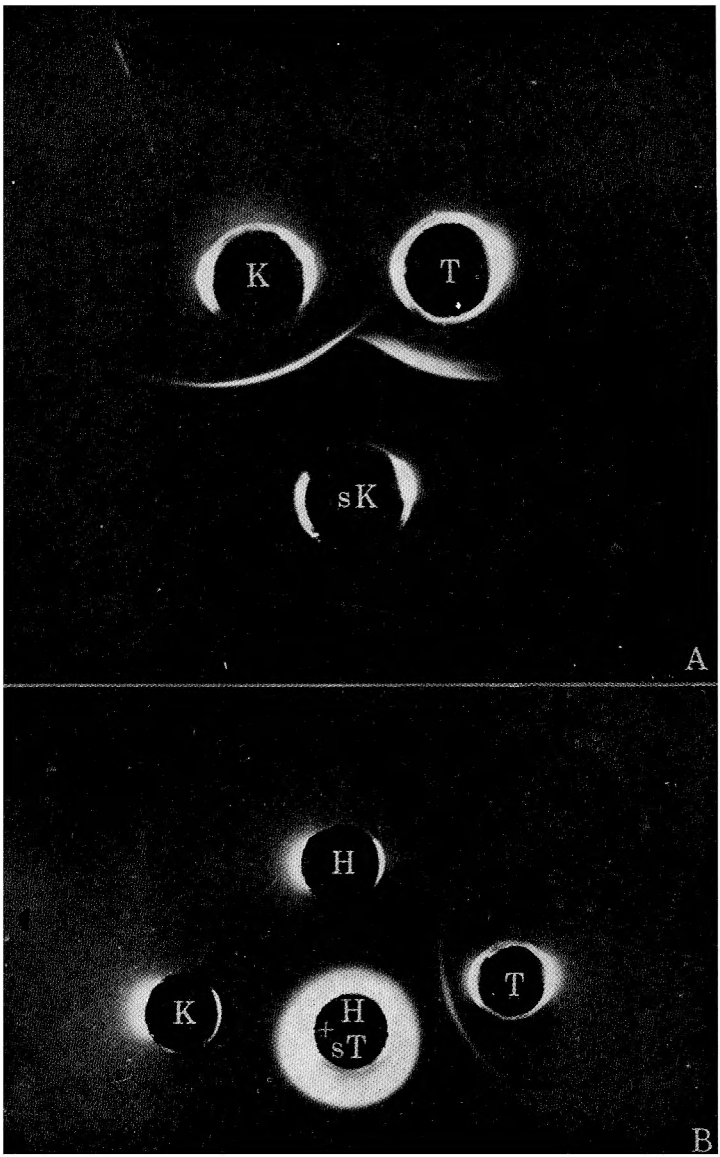


Fig. 1. — Sl. 1.

Fig. 2. — Sl. 2.



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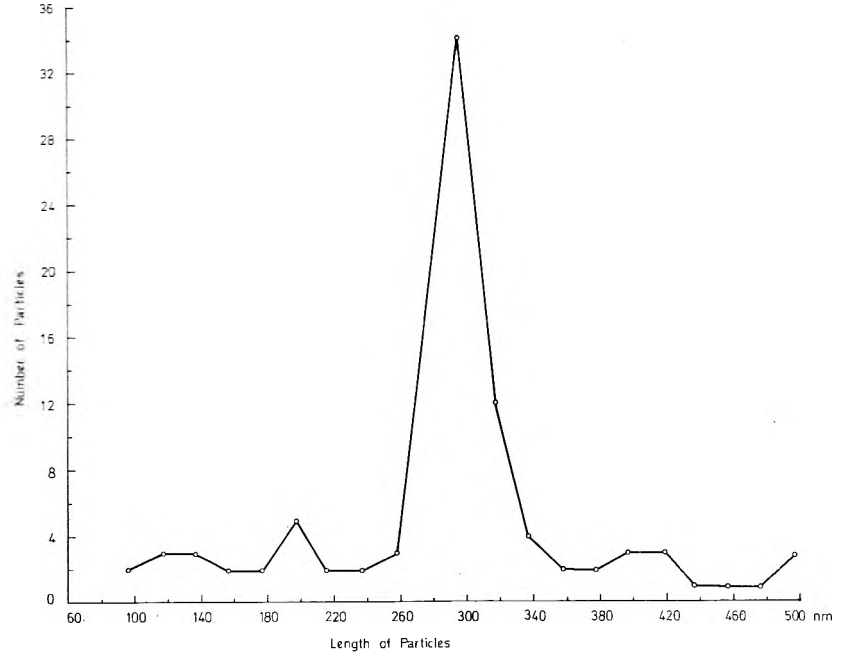

Fig. 4. — Sl. 4.

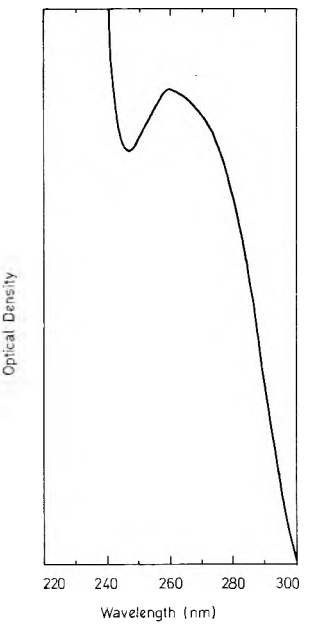

Fig. 5. — Sl. 5.

Fig. 1. Symptoms caused by the isolate RMV-K of ribgrass mosaic virus. *A* — Leaf of *Roripa amphibia* with vein banding and necrotic spots. *B* — Leaf of *Nicotiana glutinosa* with necrotic local lesions. Leaves of *N. tabacum* 'Samsun' with systemic symptoms in the form of necrotic rings, lines (*C*) and of necrotic oak leaf pattern (*D*).

Sl. 1. Simptomi oboljenja koje uzrokuje izolat RMV-K virusa mozaika trpuca. *A* — list vrste *Roripa amphibia* s vrpcama uz nerve i nekrotičnim pjegama. *B* — list vrste *Nicotiana glutinosa* s nekrotičnim lokalnim lezijama. Listovi vrste *N. tabacum*, tip Samsun, sa sistemičnim simptomima u obliku nekrotičnih prstenova i linija (*C*) i simptoma poput hrastova lista (*D*).

Fig. 2. Particles of ribgrass mosaic virus (RMV-K) in a purified preparation treated with phosphotungstic acid. Magn. 50 000 X. Photogr. in Ruđer Bošković Institute in Zagreb.

Sl. 2. Elementarne čestice virusa mozaika trpuca (RMV-K) u purificiranom preparatu obrađenom fosforno-volframskom kiselinom. Snimljeno u Institutu Ruđer Bošković u Zagrebu.

Fig. 3. Precipitin reactions in immuno-diffusion tests in agar. *A* — Reaction of RMV-K (K) and TMV (T) with the serum against RMV-K (sK). *B* — Result of the absorption test in agar: The middle well was first filled with the suspension of the type strain of ribgrass mosaic virus (H) and two hours later with the serum against TMV (sT). The absorbed sT still reacted with TMV (T) but not any more with RMV-K (K) or the type strain of ribgrass mosaic virus (H).

Sl. 3. Precipitacijske reakcije u imunodifuzijskim pokusima u agaru: *A* — reakcija RMV-K (K) i virusa mozaika duhana (T) s antiserumom od RMV-K (sK). *B* — rezultat pokusa zasićavanja u agaru: središnji bazen bio je početno napunjen suspenzijom tipičnog virusa mozaika trpuca (H), a 2 sata kasnije antiserumom od virusa mozaika duhana (sT). Zasićeni sT još je reagirao s virusom mozaika duhana (T), ali ne i s RMV-K (K) i tipičnim sojem virusa mozaika trpuca (H).

Fig. 4. Size distribution of rods of ribgrass mosaic virus (RMV-K).

Sl. 4. Raspodjela štapićastih čestica virusa mozaika trpuca (RMV-K).

Fig. 5. Ultraviolet absorption of purified isolate RMV-K of ribgrass mosaic virus.

Sl. 5. Ultravioletna apsorpcija purificiranog izolata RMV-K virusa mozaika trpuca.

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SADRŽAJ

IDENTIFIKACIJA TOBAMOVIRUSA IZOLIRANOG IZ VRSTE *RORIPA AMPHIBIA*

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Prošle godine izolirali smo virus iz divlje krucifere *Roripa amphibia* (L.) Bess. u blizini Zagreba i dali mu oznaku RMV—K. Inficirane biljke imale su simptome u obliku blage išaranosti i nekrotičnih linija na listovima (sl. 1A).

Elektronsko-mikroskopska analiza purificirane virusne suspenzije pokazala je da RMV—K ima čestice približno 300 nm duge (sl. 4). Apsorpcija virusne suspenzije dosizala je maksimum u ultravioletnom dijelu spektra kod 260 nm (sl. 5).

Izolirani virus prenijeli smo na 12 zeljastih biljaka (tablica 1). Virus je prouzrokovao lokalne lezije na listovima vrsta *Nicotiana silvestris, N. glutinosa, D. stramonium* i vrlo jake sistemične simptome na vrsti *N. tabacum,* tip Samsun (sl. 1). Na osnovi simptoma na tim vrstama bilo je očito da je RMV—K vrlo sličan običnom virusu mozaika trpuca.

Serološki pokusi vršeni su metodom dvostruke difuzije u agaru te unakrsnim zasićavanjem u agaru. Ti su pokusi pokazali da je istraživani virus srodniji virusu mozaika trpuca nego virusu mozaika duhana (sl. 3).

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