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SOME NEW DATA ON ROBINIA MOSAIC CUCUMOVIRUS

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From black locust (*Robinia pseudacacia* L.) trees with and without mosaic symptoms robinia mosaic cucumovirus (= robinia mosaic virus) which is regarded as a strain of peanut stunt cucumovirus (= peanut stunt virus) was isolated. The two isolates obtained were serologically indisinguishable from each other and reacted with serum against another previously studied isolate from black locust to its homologous titre. Certain minor differences from isolates described earlier were found in the reaction of test plants. Large virus crystals resembling those of CMV were detected in infected cells of host plants.

Introduction

Mosaic disease of black locust or robinia (*Robinia pseudacacia* L.) was detected for the first time in Bulgaria by Atanasoff (1935). Subsequently the disease was found to be present in some other countries of central and south-eastern Europe (*cf.* Schmelzer and Miličić 1965, Schmelzer 1967) including Hungary, Romania, Czechoslovakia and Yugoslavia, being particularly widespread in the Hungarian plain. Comprehensive research work on the causal virus concerning the reactions of test plants and some other classic properties was accomplished by Schmelzer (1967), who gave it the name black locust true mosaic virus or robinia mosaic virus (RoMV) (*cf.* Schmelzer 1971). Additional research into RoMV conducted by Richter et al. (1979a, b) has provided evidence that this virus belongs to the group of cucumoviruses showing closer serological relationship to the peanut stunt virus (PSV). Now the virus is listed in AAB Descriptions of Plant Vi-

ruses (Set 20) under the name of robinia mosaic cucumovirus, and is regarded as a strain of peanut stunt cucumovirus (cf. also Richter et al. 1987, Haack and Richter 1987).

In a previous work on RoMV (Schmelzer 1967) two isolates collected in Yugoslavia were investigated in a limited way together with the more throughly investigatet isolates RoK, Ro25 and RoRu originating from Hungary and Romania. This paper presents the identification and some properties of two isolates of RoMV recently obtained from black locust trees in the Zagreb area.

Materials and Methods

Both isolates of RoMV were obtained from *R. pseudacacia* plants which grew in a line of trees in Zagreb, one tree showing typical symptoms of robinia mosaic (*cf.* Schmelzer and Miličić 1965, Schmelzer 1971) while in the other no symptoms could be noticed.

The virus was isolated by the standard method of mechanical inoculation into plants of *Chenopodium quinoa.* Serological reactions were conducted by agar-gel double immunodiffusion technique (0.9% Difco-bacto agar in distilled H_2O with 0.3% NaN_3). Purification of the virus was completed according to the method used by Richter *et al.* (1979a, b). In determining the character of unusual systemic infection in *Ch. quinoa* by serology test, partially purified virus obtained by ammonium sulphate precipitation was used. Precipitin lines were equally clear as those obtained with preparations which passed the whole purification procedure. The serum against RoMV was supplied by Dr. J. Richter (Institut für Phytopathologie, Aschersleben), and a specific serum to PSV not containing antibodies to CMV by Dr. J. C. Devergne (INRA, Antibes).

Results

Reaction of test plants

The two isolates were transmitted to 12 identical species of test plants, 10 of which were earlier recognized to be hosts of RoMV (Schmelzer 1967). In these experiments both isolates provoked entirely identical symptoms as follows:

Chenopodium amaranticolor. Small chlorotic local lesions followed by systemic symptoms.

**Ch. foliosum.* Yellow-green local lesions up to 1.5 mm Ø, followed by systemic infection in the form of mosaic and vein-clearing.

Ch. murale. A few small chlorotic lesions in inoculated leaves. There were no systemic symptoms.

Ch. quinoa. Local chlorotic lesions without systemic symptoms at somewhat lower temperatures (during spring); during summer and winter also systemic mosaic.

**Datura stramonium.* Local chlorotic lesions up to 5 mm Ø later with a red ring border. Systemic symptoms were not present.

* Species not earlier tested for RoMV infection.

Gomphrena globosa. In inoculated leaves a small number of chlorotic lesions with a red ring border. Systemic symptoms were not present.

Nicotiana glutinosa. Diffuse chlorotic local lesions. Top leaves without symptoms.

N. megalosiphon. Local necrotic lesions. Systemic mosaic accompanied by necrosis.

Petunia hybrida. Local zonate chlorotic lesions 3 mm ϕ. Top leaves without symptoms.

Phaseolus vulgaris. Chlorotic lesions of medium size in inoculated leaves. Systemic symptoms in the form of irregular chlorotic spots, light mottling, and distortion of the leaf lamina showing blisters.

Vigna sinensis. Vein clearing accompanied by chlorotic mosaic. In inoculated leaves subsequently a small number of chlorotic lesions along the veins.

Zinnia elegans. Elongated yellow lesions with necrotic centre along some veins of inoculated leaves. Top leaves without symptoms.

Brassica rapa, Cucumis sativus, N. tabacum Hicks resistant and White Burley did not show any symptoms.

Serology

In gel diffusion tests purified preparations of two of our isolates reacted with serum against RoMV to its homologous titre (1/4). When isolates were placed in adjacent wells their precipitin lines joined indicating identity. Purified virus preparations reacted also with specific serum against PSV forming typical virus arches.

Virus inclusion bodies

Large virus crystals with hollow centres resembling those often found within the central vacuoles of CMV infections (Christe and Edwardson 1979, Francki *et al.* 1987), were present in some epidermal and mesophyll cells of infected *Ch. quinoa* (Fig. 1) and *N. megalosiphon.* Their light and electron microscopy and detection of some other cytophatic structures in RoMV infected cells are the subject of another paper (Štefanac *et al.* 1988).

Discussion

On the basis of the test plant reactions, serological experiments and appearance of crystalline virus inclusion bodies we conclude that the virus we isolated from *R. pseudacacia* belongs to RoMV. We also found this virus to be serologically related to PSV, as earlier established by Richter *et al.* (1979b). However, the small differences from the previously analysed RoMV isolates (Schmelzer 1967) were observed in the reaction of test plants. Our two isolates, similarly to the earlier analysed isolate RoK, did not cause systemic symptoms in *Ch. quinoa* during a part of year, whereas the reaction of other tested species were more like those of the previously analysed isolate RoRu.

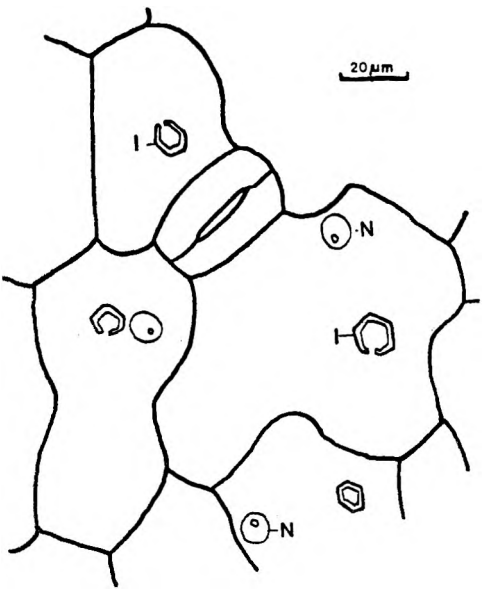

Fig. 1. A sketch of RoMV intracellular inclusions (I) in epidermal cells of *Chenopodium quinoa*. Nucleus (N).

As far it is known, the isolates of RoMV obtained earlier originated from black locust trees that showed mosaic symptoms at least in some branches of a tree (Schmelzer 1971). Here we obtained the virus from the locust tree showing symptoms and from the tree without any symptoms. Whether the reason for the lack of symptoms in the latter plant was a relatively new infection, involvement of a special variant of the virus or something else, is not known. According to the data from the literature (Schmelzer and Miličić 1965) the growth of a few years old mosaic diseased black locust trees can be reduced up to 50%, showing robinia mosaic to be an important problem in black locust cultivation, especially in south-east Europe where the disease commonly occurs (Schmelzer 1971).

Intracellular inclusions of RoMV have not been reported before. Since apart from RoMV mosaic symptoms in black locust are also provoked by tomato black ring and strawberry latent ringspot viruses (Schmelzer 1971), RoMV can be distinguished in herbatious test plants from these nepoviruses by the presence of specific virus crystals as well.

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SAŽETAK

NEKI NOVI PODACI O VIRUSU MOZAIKA BAGREMA

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Iz primjerka bagrema (*Robinia pseudacacia* L.) s virusnim simptomima i iz primjerka bez simptoma izdvojen je virus mozaika bagrema (= robinia mosaic cucumovirus, RoMV) koji se smatra sojem virusa kržljavosti orašca (= peanut stunt cucumovirus). Dva izdvojena izolata nisu se mogla međusobno razlikovati u serološkim pokusima sa serumom protiv jednog ranije analiziranog izolata RoMV s kojim su reagirali do njegovog homolognog titra. Uočene su izvjesne manje razlike prema ranije opisanim izolatima RoMV u vezi sa simptomom na pokusnim biljkama. U inficiranim stanicama domaćina zapaženi su veliki virusni kristali koji su nalikovali onima virusa mozaika krastavca.

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