Acta Bot. Croat. 42, 11—14, 1983.

CODEN: ABCRA2
YU ISSN 0365—0588

UDC 576.858:634.37=20

ISOLATION OF A POTYVIRUS FROM *FICUS CARICA* L.

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Received February 21, 1983.

A filamentous 750 to 800 nm long virus was isolated from a fig tree with conspicuous symptoms in Yugoslavia. The virus has a very narrow host range; it was transmitted mechanically only to some *Nicotiana* species including *N. megalosiphon* and *N. sylvestris*. It causes the formation of cylindrical cytoplasmic inclusions in infected *Nicotiana* cells. The inclusions consist of pin-wheels, scrolls and laminated aggregates. According to the form of inclusions it seems that it belongs to the third subdivision of potyviruses (Edwardson 1974). In accordance with these properties the fig potyvirus is transmissible by aphid *Myzus persicae* in a non-persistent manner.

Introduction

Fig trees with mosaic symptoms are wide-spread in the region of Herzegovina (Yugoslavia). The symptoms are caused by fig mosaic virus which is spread in all warmer regions of the world. The disease was first described by Condit and Horne (1933) in the USA. In Yugoslavia the symptoms of the disease were studied by Perišić (1952) and others.

Bradfute et al. (1970) and Plavšić and Miličić (1980) established the presence of polymorphic particles in infected fig cells by means of electron microscope. However, the fig mosaic is still a disease of unknown etiology. It is a characteristic of this disease that it cannot be transmitted mechanically but only by means of a very efficient vector, *Eriophyes ficus* Cotte (Flock and Wallace 1955, Proeseler 1969, Jeppson et al. 1975).

From mosaic diseased figs Quacquarelli (1971) mechanically isolated sowbane mosaic virus and established for the first time that the fig could be host of a true plant virus.

In this paper we bring out the data about the second mechanical virus isolation during which a potyvirus was isolated from a fig tree in Yugoslavia.

Material and Methods

In the valley of the river Dežanka (Herzegovina) numerous fig trees with well visible mosaic symptoms are present. The virus was isolated mechanically from young leaves with obvious mosaic and deformation symptoms of one tree (Fig. 1a) and transmitted to herbaceous plants. The virus isolation was performed so that young fig leaves were frozen and then homogenized in a solution of 0.067 M phosphate buffer pH 7.6 which contained 0.14% thioglycollic acid. Herbaceous plants *Nicotiana megalosiphon* Heurck and Muell. and *N. sylvestris* Spegaz. et Comes were inoculated with this mixture and the symptoms appeared after an incubation period of 8 to 10 days.

A healthy colony of *Myzus persicae* Sulz. was used in the trial which had the aim to establish the mode of virus transmission in nature. After being starved for 2 hours the aphids fed for a short time of 2–3 min on infected *N. megalosiphon* leaves. Afterwards they were transferred in groups of 20 individuals on healthy young plants. After 2 days the aphids were killed with an insecticide and plants were kept under observation.

Partial purification of the virus was performed according to a modified method of Pleše and Štefanac (1976).

The morphology of virus particles was studied by means of a JEM 100 B electron microscope using the method of negative staining with potassium phospho-tungstate. For ultrathin sectioning small pieces of leaves with symptoms were fixed in buffered glutaraldehyde and then in buffered OsO_4. The material was dehydrated in a series of alcohols and afterwards embedded in Epon 812. Leaf pieces treated in this manner were cut with a diamond knife and stained with magnesium uranyl acetate and lead citrate.

Results

Host plants of virus

The isolated virus attacked with certainty only some *Nicotiana* species. *N. megalosiphon* reacted systemically with a mild mosaic and dark green vein banding (Fig. 1b). *N. bigelovii* Wats., *N. debneyi* Domin., *N. sylvestris* Spegaz. et Comes and *N. tabacum* L. cvs. Samsun and White Burley developed a systemic infection with a gentle transient mottling.

Mild and transient symptoms which were expressed on the species mentioned made it difficult to establish the host range of the virus. The possible presence of latent infections in some *Chenopodium* species was investigated by means of electron microscope but neither virus particles nor cytoplasmic inclusions were found in inoculated plants.

External symptoms od infection were not observed on the following inoculated plants: *Caspicum annuum* L., *Chenopodium album* L., *C. amaranticolor* Coste et Reyn., *C. foetidum* Schrad., *C. murale* L., *C. quinoa* Willd., *Cucumis melo* L., *C. sativus* L. cv. Delicatess, *Datura stramonium* L., *Gomphrena globosa* L,, *Ipomoea* sp., *Lycopersicum esculentum* L., *Nicotiana glutinosa* L. cv. Corvallis strain, *Pisum sativum* L., *Phaseolus vulgaris* L. cvs. Bountiful and Top Crop, *Solanum luteum* Miller, *Tetragonia expansa* Thunb., *Vicia faba* L. and *Vigna sinensis* (L.) Savi ex Hassk. cv. Black Eye.

Transmission by Myzus persicae

The virus was efficiently transmitted from diseased to healthy *N. megalosiphon* plants by means of *M. persicae* as a vector. The transmission was performed in the nonperistent manner.

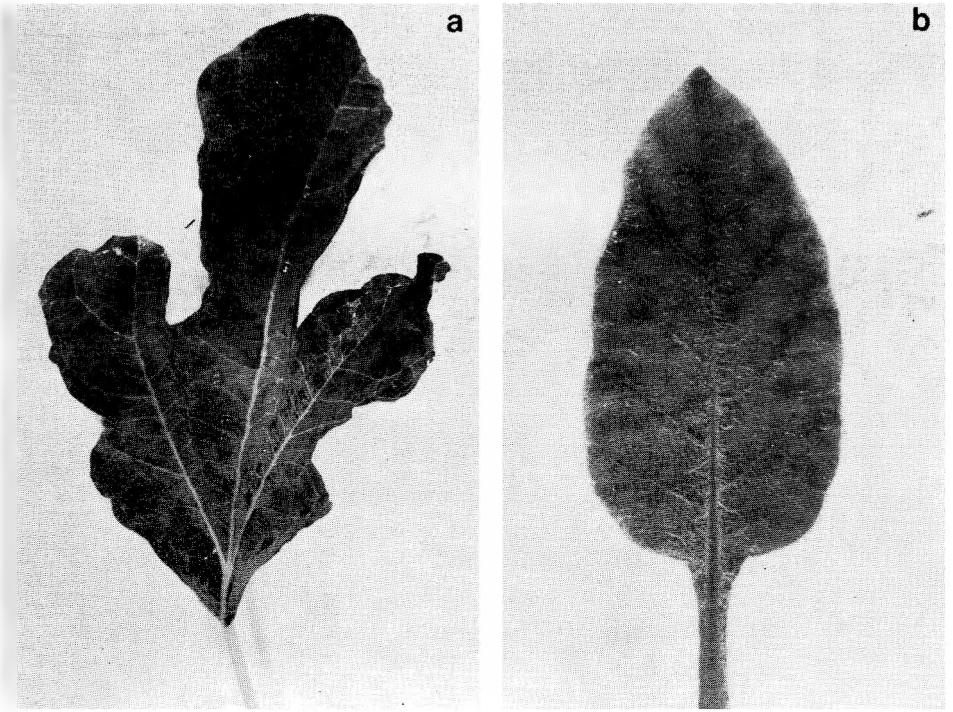

Fig. 1. Fig leaf with mosaic symptoms and with dark green areas containing shallow blisters (a). *Nicotiana megalosiphon* with systemic symptoms in the form of dark green bands along the veins (b).

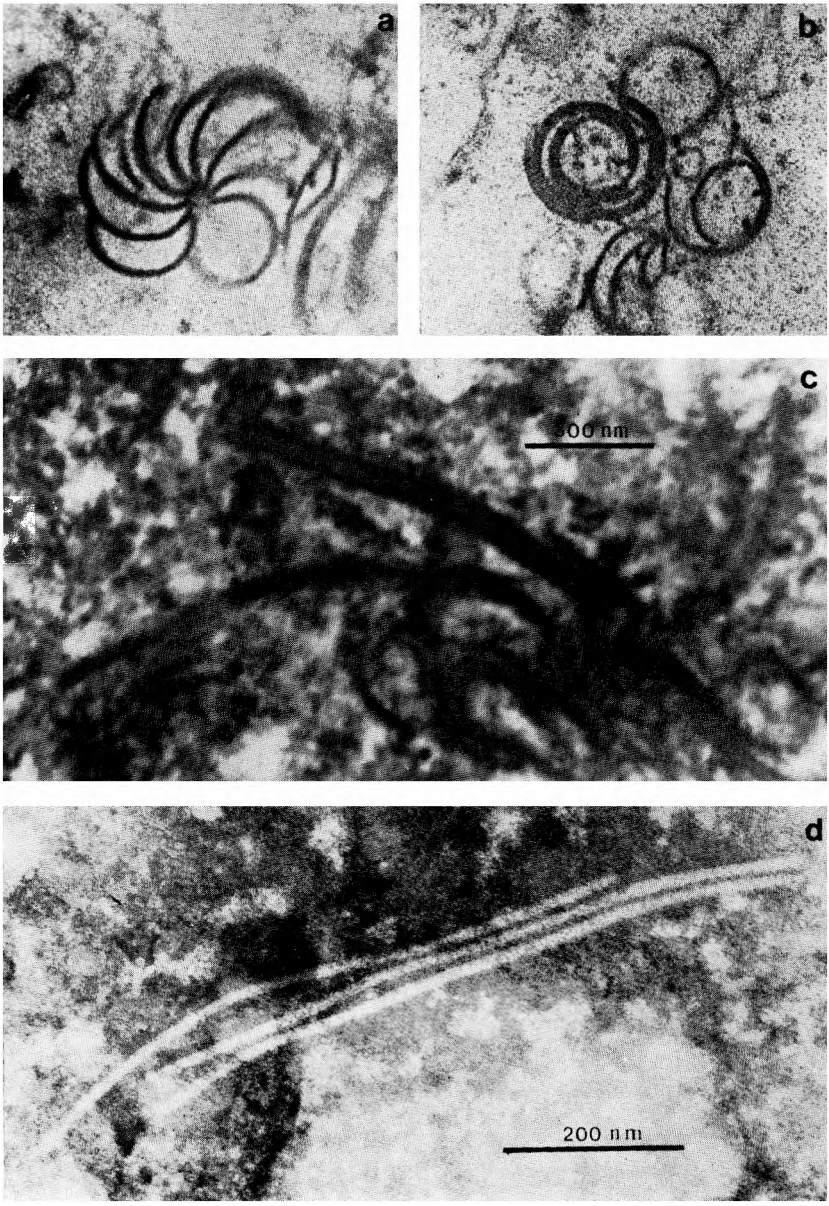

Fig. 2. Cylindrical cytoplasmic inclusions from leaf cells of *Nicotiana megalosiphon:* pin-wheel (a), scrolls (b), and laminated aggregates (c). Virus particles (d).

Virus particles and cell inclusions

The partially purified virus was examined in electron microscope and filamentous particles from 750 to 800 nm long were found (Fig. 2d). Some particles were broken and therefore shorter, and some others longitudinally aggregated and therefore very long.

Virus particles were observed also in unpurified infective sap of *N. megalosiphon* plants.

In ultrathin sections, cylindrical cytoplasmic inclusions were observed (Fig. 2a—c). They had the form of pin-wheels, scrolls and laminated aggregates.

The length and the form of virus particles, the presence of cylindrical cytoplasmic inclusions and transmission with aphids in nonpersistent manner indicate that this virus belongs to the group of potyviruses (Edwardson 1974, Edwardson and Christie 1978, Hollings and Brunt 1981).

According to the presence of three thypes of cylindrical cytoplasmic inclusions, i. e. of pin-wheels, scrolls and laminated aggregates, it can be considered that this virus appertains to the third subdivision of potyviruses (Edwardson 1974).

We tried to find elongated virus particles and cylindrical cytoplasmic inclusions in the leaf cells of the infected *F. carica* tree, from which the virus was isolated, but neither virus particles nor inclusions were detected.

Discussion

As we did not find either virus particles or cylindrical cytoplasmic inclusions in the infected fig tree, we repeated the mechanical isolation of the virus several times during 1980 and 1981. The isolation experiments were always positive so that we are sure that a potyvirus is present in the diseased fig tree. *N. megalosiphon* and *N. sylvestris* plants served in all cases as test plants for isolation of the virus.

As known, only one virus has been till now isolated mechanically from the fig, i. e. sowbane mosaic virus (Quacquarelli 1971). Therefore, the potyvirus described here is the second virus which has been mechanically isolated from fig leaves.

According to the property of mechanical transmission, sowbane mosaic virus and the potyvirus differ sharply from the fig mosaic which cannot be transmitted mechanically but only by the mite *Eriophyes ficus* (Flock and Wallace 1955, Proeseler 1969). This mite is very common on fig trees along the Yugoslav coastline. It is possible that the mosaic symptoms on the fig tree investigated (Fig. 1a) derive from this mite transmissible virus.

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SAŽETAK

IZOLACIJA POTYVIRUSA IZ VRSTE *FICUS CARICA* L.

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Iz jednog stabla smokve (*Ficus carica* L.) s izraženim mozaičnim simptomima koje je raslo u dolini Dražanke (Hercegovina) izoliran je mehaničkim putem filamentozni virus dužine 750—800 nm. Virusom smo uspjeli inficirati izvjestan broj biljnih vrsta roda *Nicotiana.* Domaćini su reagirali sistemično u vidu blagog mozaika, tamnozelenih vrpci duž nerava ili su nakon prolaznog šarenila ostajali besimptomni.

Na osnovi oblika i dužine virusnih čestica i prisutnih cilindričnih inkluzija te neperzistentnog prenošenja lisnom uši *Myzus persicae* Sulz. može se zaključiti da virus izoliran iz smokve pripada skupini potyvirusa (Hollings i Brunt 1981).

Prisutnost cilindričnih inkluzija »pinwheel«, smotaka i laminatnih agregata upućuje da virus izoliran iz smokve pripada III. podskupini potyvirusa (Edwardson 1974, Edwardson i Christie 1978).

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Rad je financiran sredstvima Republičke zajednice za naučni rad Bosne i Hercegovine.