Acta Bot. Croat. 47, 15—19, 1988.

CODEN: ABCRA2
YU ISSN 0365—0588

UDC 576.858.8:582.951.4(497.1)=20

DETECTION OF A VARIANT OF HENBANE MOSAIC VIRUS IN *PHYSALIS ALKEKENGI* L.

*ĐORĐE MAMULA, ZLATA ŠTEFANAC, IRMTRAUD THALER** and *MANFRED GAILHOFER**

(Department of Botany, Faculty of Science, University of Zagreb, and *Institute of Plant Physiology, University of Graz)

Received January 20, 1988

In the symptom bearing specimens of *Physalis alkekengi* L. growing spontaneously in the vicinity of Zagreb (Yugoslavia), a variant of henbane mosaic virus (HMV-HZ) was detected. In parallel experiments by analysing the reaction of test plants, HMV-HZ differed significantly both from the Rothamsted type strain (HMV-R) of the virus, and the 'alkekengi' strain described from *P. alkekengi* (HMV-A) in Italy. In slide microprecipitin tests HMV-HZ reacted to the homologous titre (1:16000) of two sera prepared against HMV-R. Cylindrical inclusions and distinct crystalline structures made possible prompt virus identification.

Introduction

Henbane mosaic virus (HMV), a member of the potyvirus group, with host range limited mainly to the *Solanaceae*, was described for the first time in 1932 from henbane (*Hyoscyamus niger* L.) in England (*cf.* Govier and Plumb 1972). Later it was found in *Atropa belladonna* L. in Germany being described under the name of Atropa mild mosaic virus (Bode *et al.* 1969), in *Physalis alkekengi* L., *Datura stramonium* L. and *D. inermis* Jacq. in Italy (Lovisolo and Bartels 1970) and in *D. stramonium, Nicotiana tabacum* L. and *Solanum dulcamara* L. in Hungary (cf. Horváth et al. 1988). HMV was isolated in Britain a few times also from *A. belladonna* and *Datura sp.* (Harrison and Roberts 1971; Lovisolo and Bartels 1970).

This paper reports a recent finding of HMV in wild *P. alkekengi* plants near Zagreb and gives a comparison of the isolated virus with two HMV strains described earlier. Portion of the results has been published in short communication (Mamula *et al.* 1988).

Materials and Methods

Virus cultures. Mechanical extracts with or without addition of tap-water were taken separately from leaves of several *P. alkekengi* plants showing virus symptoms and inoculated in the usual manner to some standard solanaceous and chenopodiaceous test plants. The former became infected showing prominent and, regarding different isolates, uniform systemic symptoms. Because of the possibility of contamination with potato virus Y (Govier and Woods 1971), one of the isolates (further HMV-HZ) was passed through *D. stramonium* which is a host of HMV but not of potato virus Y (cf. Horváth et al. 1988). The Rothamsted type (HMV-R) and the *'alkekengi'* (HMV-A) strains were obtained from Dr O. Lovisolo, Istituto di Fitovirologia Applicata, Torino.

HMV-HZ, HMV-R and HMV-A were all maintained in *D. stramonium* which served as a source of the virus for inoculation of test plants and for serological reactions. In the investigation of the host range and symptoms back transmissions were not performed.

Light microscopy. Light microscopy observations were done on living tissue, mainly of the leaf lower epidermis from the mid-rib region.

Electron microscopy. Small pieces taken from the lower side of the leaf lamina basal part of infected *Datura plants* were fixed in 3% glutaraldehyde buffered at pH 7.2 with 0.06 M phosphate buffer for 90 min at room temperature, and post-fixed in 1% OsO_4 in veronal acetate for 2 hours. The tissue was dehydrated through a graded ethanol series and embedded in Spurr's medium. Ultrathin sections were stained with uranyl acetate followed by Reinold's lead citrate and examined in a Philips CM 10 electron microscope.

Serology. For serological proof of the virus two sera against HMV-R, one prepared by Dr E. Luisoni and the other by Dr E. Bartels (Institut für Viruskrankheiten der Pflanzen, Braunschweig), both of the homologous titre 1/16000, were used. Serological reactions were conducted by slide microprecipitin test in free liquid using low-speed centrifuged sap.

Results

Detection and symptoms of HMV in naturally infected P. alkekengi plants

Infected specimens of *P. alkekengi* from which HMV-HZ was isolated were observed in 1987 on two localities about 30 km to the north of Zagreb. Some 50% of the population exhibited virus symptoms. Infected plants showed mild to moderate mosaic, interveinal chlorosis, vein-banding, slight leaf malformations (Fig. 1A) and stunting.

Test plant reactions

The HMV-HZ was transmitted by manual inoculation to 20 species of test plants which developed symptoms characteristic of HMV.

In parallel experiments, by using a reduced number of test plants, HMV-HZ was compared with HMV-R and HMV-A strains (*cf.* Table 1). In this comparison, HMV-HZ differed from both referent strains in each particular species except *C. amaranticolor* and *C. quinoa.* In all differential species, apart from *D. stramonium* which reacted to HMV-

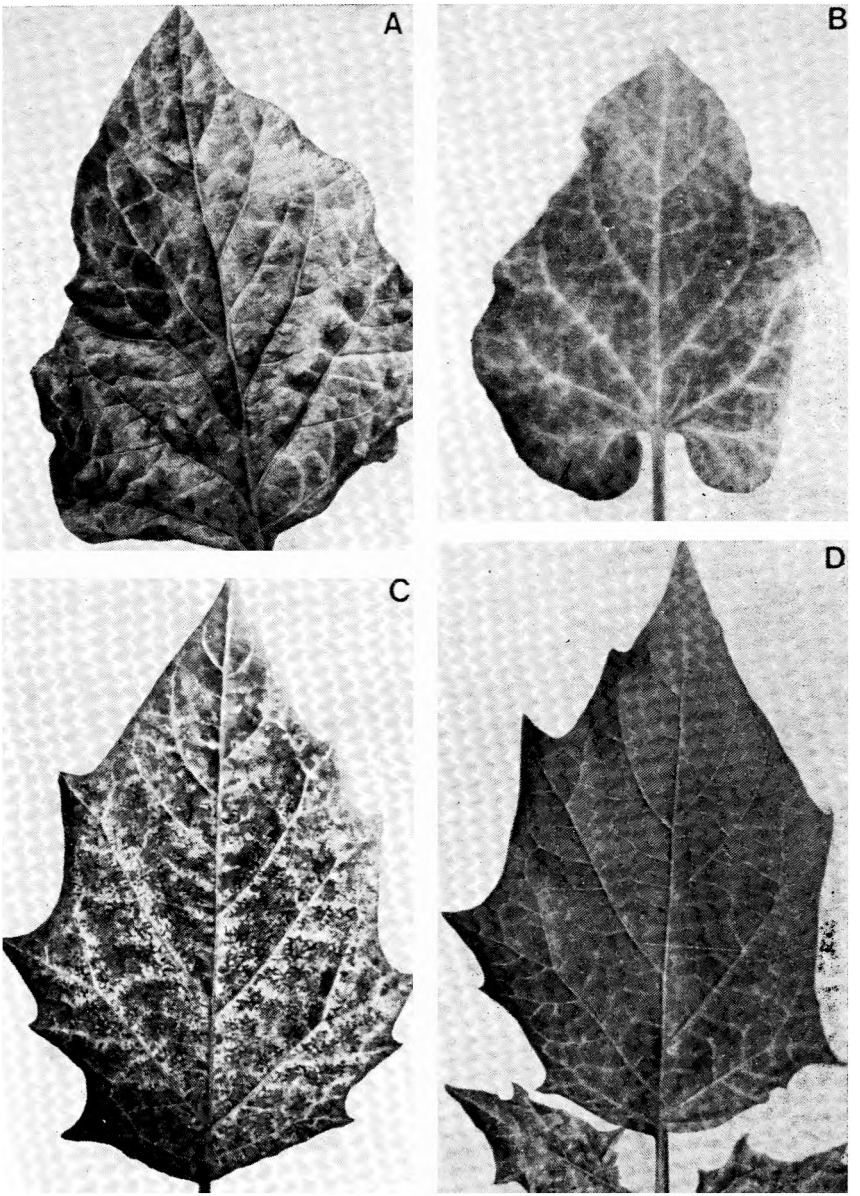

Fig. 1. **A** Leaf of naturally infected *Physalis alkekengi* showing blistering, light vein-clearing and deformation of the lamina margin. **B** Diffuse vein-clearing in *Nicotiana glutinosa* leaf systemically infected with HMV-HZ. **C** Numerous white necrotic lesions close to veins and **D** initial vein-clearing symptom in *Datura stramonium* leaves systemically infected with HMV-HZ and HMV-A, respectively (inoculation of respective plants was done simultaneously).

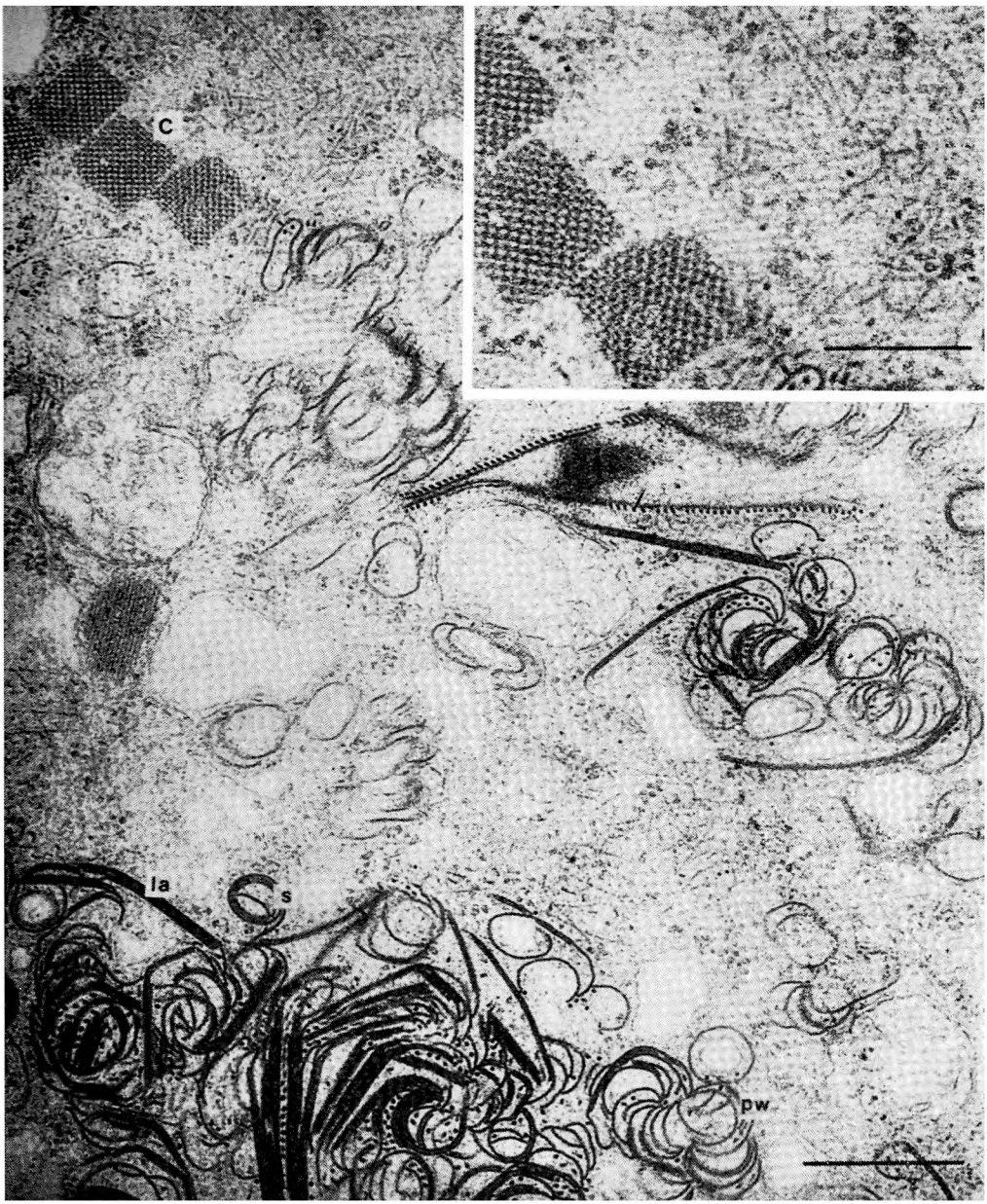

Fig. 2. Part of *Datura stramonium* mesophyll cell infected with HMV-HZ, showing different aspects of cylindrical inclusions — pinwheels (pw), laminated aggregates (la), scrolls (s), transverse section of crystalline structures (C), and virus particles. Insert shows higher magnification of the crystalline inclusions with virus particles to the right. Bars represent 0.5 μm, and 0.25 μm (insert).

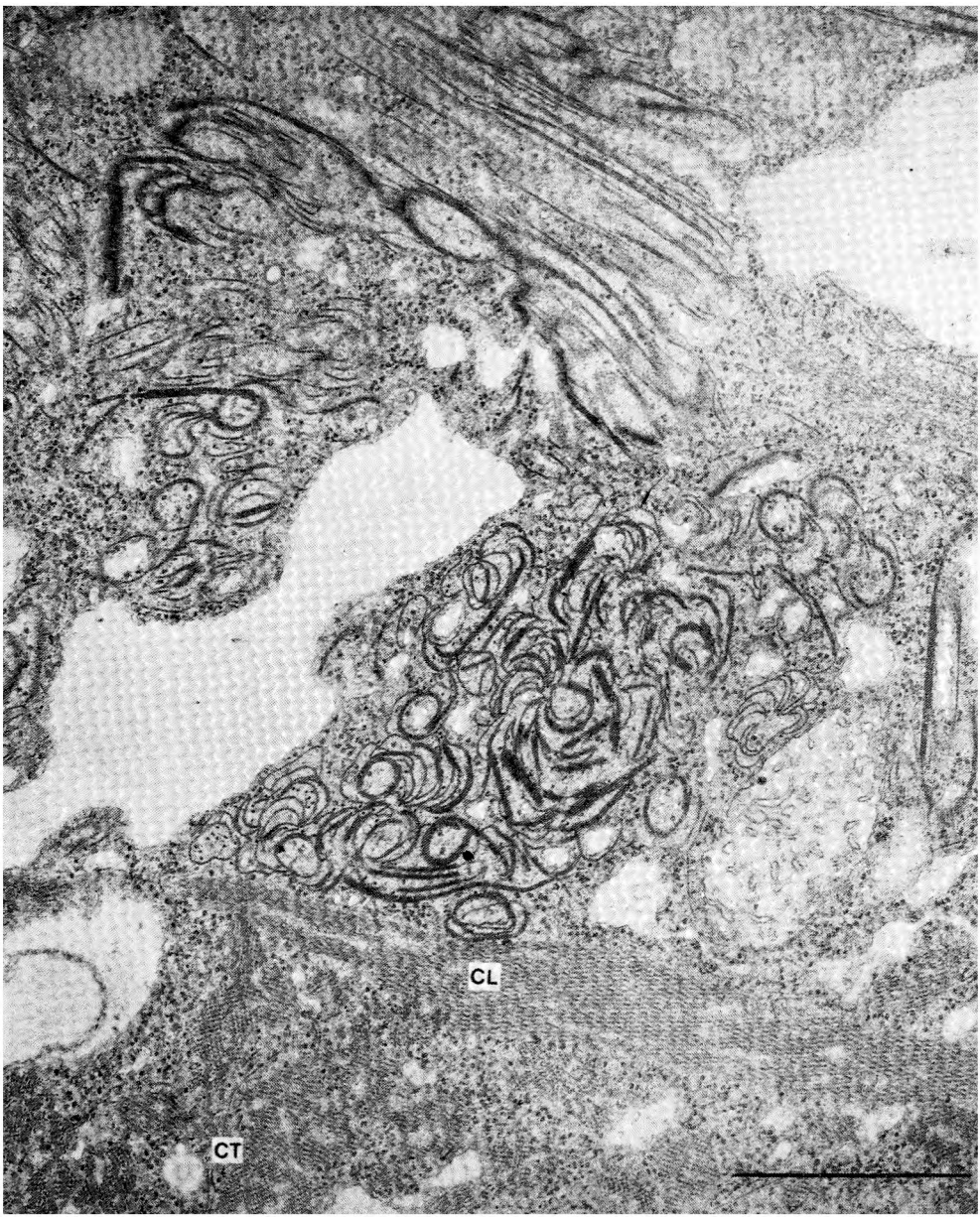

Fig. 3. Different aspects of cylindrical inclusions, and crystalline structures in almost longitudinal (CL) and transverse (CT) sections. Bar represents 1 μm.

Table 1. Symptoms in test plants induced by HMV-HZ, HMV-A and HMV-R in parallel experiments

Test plants	Symptoms					
	HMV-HZ		HMV-A		HMV-R	
	local	systemic	local	systemic	local	systemic
Chenopodium amaranticolor	lcl/lnl	0	lcl/lnl	0	lcl/lnl	0
C. quinoa	lcl/lnl	0	lcl/lnl	0	lcl/lnl	0
Datura stramonium	lcl/lnl	severe chlorosis, severe necrosis (Fig. 1C)	0	severe mosaic	ll	severe mosaic (necrotic flecking)
Nicandra physaloides	0	mild mosaic	(lnl)	(mild mosaic)	lnl	necrotic flecking
Nicotiana bigelovii	0	(mild mosaic)	0	mild mosaic	lcl/lnl	severe mosaic (necrosis)
N. glutinosa	lcl	diffuse mosaic (Fig. 1B)	lcl	mosaic (necrotic netting)	lcl/lnl	severe mosaic (necrotic netting)
N. tabacum Hicks	lcl	mild mosaic (necrosis, recovery)	(lnl)	mild mosaic	lnl	severe mosaic, severe stunting (necrosis)
Samsun	lcl	necrotic pattern, recovery	lnl	mild mosaic	lnl	severe chlorosis, severe stunting (necrosis)
Solanum dulcamara	0	0	0	(necrotic netting)	(lnl)	(necrotic netting)

ll = local lesions, rapidly enlarging into necrotic flecks, lcl = local chlorotic lesions, lnl = = local necrotic lesions, ( ) = the brackets indicate the symptoms appearing only occasionally.

-HZ by the most severe symptoms (cf. also Fig. 1C, D), our isolate provoked milder reaction and/or growth inhibition not only than the more aggressive HMV-R, but also than the HMV-A strain from *P. alkekengi* from Italy.

The HMV-HZ provoked various systemic symptoms also in the following species: *Atropa belladonna, Capsicum annuum, Hyoscyamus niger, Lycopersicum esculentum cv.* Rutgers, *Nicotiana clevelandii, N. megalosiphon, Petunia hybrida, Physalis alkekengi, Solanum luteum, S. nigrum* and *S. rostratum.* At moderate temperatures, the specimens of the tomato cv. used developed severe necrosis on the leaves and stem followed by plant wilting. Somewhat lower temperatures generally were more appropriate for development of more severe reactions following inoculation with HMV-HZ. In conditions particularly favourable to infection some species (*D. stramonium, N. clevelandii, N. glutinosa, P. hybrida*) did not produce seed.

Detection of diagnostic inclusion bodies

In *Datura* epidermal cells HMV-HZ induced voluminous light microscopic inclusion bodies resembling accumulations of randomly oriented cylindrical inclusions provoked by different potyviruses.

The ultrastructure of infected cells was essentially similar to that influenced by two HMV strains investigated earlier (Harrison and Roberts 1971, Kitajima and Lovisolo 1972). In addition to the flexuous virions, the cytoplasm of the mesophyll cells contained cylindrical inclusions and HMV-specific crystalline structures cut at various angles (Figs. 2,3).

Serology

In slide microprecipitin tests HMV-HZ reacted with both antisera to HMV-R to their homologous titre. Control reactions with sap from healthy plants were negative.

Discussion

First described in 1932 HMV was reported to the best of our knowledge from five European countries, i. e. Britain, Germany, Italy (cf. Govier and Plumb 1972), Bulgaria and Hungary (cf. Horváth et al. 1988). From the literature data (Lovisolo and Bartels 1970, Govier and Plumb 1972) it follows that various HMV isolates are closely related serologically but showing certain variability in respect to the reaction of test plants. For that reason also Lovisolo and Bartels (1970) considered their HMV isolates from *Physalis* alkekengi and *Datura* species to be a distinct *'alkekengi' strain.* In this respect, however, HMV-HZ we obtained from *P. alkekengi* plants near Zagreb differed both from the Rothamsted type and the *'alkekengi'* strains, which we think to be a consequence of adaptation to the specific climatic conditions.

Though first isolated from biennial henbane (*Hyoscyamus niger*), the permanent reservoirs of HMV are also perennials *Atropa belladonna* and *P. alkekengi,* and perhaps some other solanaceous weed species. In which degree and how efficiently in natural conditions the HMV can be transmitted from these plants by its aphid vectors to some vegetable species is not known. As known from the literature data (Lovisolo and Bartels 1970) and from results of our experiments, under the experimental conditions HMV can induce severe changes in red pepper (*Capsicum annuum*) and tomatoes.

Although a large number of viruses is known to infect different species of the genus *Physalis (cf.* also Horváth 1983), the HMV-HZ was easily identified by analysing thin sections of infected cells. Beyond the cylindrical inclusions of the appearance characteristic of HMV, HMV-specific elongated crystalline structures (Harison and Roberts 1971, Kitajima and Lovisolo 1972) were also present in infected cells. According to our unpublished results, the latter structures are of a protein nature.

*

This research was partially supported by the Research Council of the SR Croatia (SIZ—IV).

We thank Dr O. Lovisolo for sending us HMV-R and HMV-A strains including antiserum to HMV-R, and Dr R. Koenig for HMV-R antiserum prepared by Dr Bartels.

References

Bode, O., J. Brandes, H. L. Paul, 1969: Untersuchungen über ein neues, langgestrecktes Virus aus *Atropa belladonna.* Biologische Bundesanstalt für Land- und Forstwirtschaft in Berlin und Braunschweig, Jahresbericht 1986, p. A61.

Govier, D. A., R. T. Plumb, 1972: Henbane mosaic virus. C. M. I./A. A. B. Descriptions of Plant Viruses, No 95.

Govier, D. A., R. D. Woods, 1971: Changes induced by magnesium ions in the morphology of some plant viruses with filamentous particles. J. gen. Virol. *13,* 127—132.

Harrison, B. D., I. M. Roberts, 1971: Pinwheels and crystalline structures induced by Atropa mild mosaic virus, a plant virus with particles 925 nm long. J. gen. Virol. *10,* 71—78.

Horváth, J., 1983: Reaction of *Physalis* species to plant viruses. VII. Additional data on the virus susceptibility of *Physalis minima* L. Acta Phytopath. Hung. *10,* 247—256.

Horváth, J., P. Salamon, I. Wolf, M. Kölber, 1988: Henbane mosaic potyvirus pathogenic to wild and cultivated potato. Potato Research *31,* 311—320.

Kitajima, E. W., O. Lovisolo, 1972: Mitochondrial aggregates in *Datura* leaf cells infected with henbane mosaic virus. J. gen. Virol. *16,* 265—271.

Lovisolo, O., R. Bartels, 1970: On a new strain of henbane mosaic virus from *Physalis alkekengi.* Phytopath. Z. *69,* 189—201.

Mamula, Đ., Z. Štefanac, I. Thaler, M. Gailhofer, 1988: Karakteristike virusa henbane mosaic iz vrste *Physalis alkekengi.* Zbornik radova naučnog sastanka virusologa Jugoslavije, pp. 220—222. Vojnomedicinska akademija, Beograd.

SAŽETAK

NALAZ POSEBNE ODLIKE VIRUSA MOZAIKA BUNIKE (HENBANE MOSAIC VIRUS) U VRSTI *PHYSALIS ALKEKENGI* L.

Đorđe Mamula, Zlata Štefanac, Irmtraud Thaler i *Manfred Gailhofer*

(Botanički zavod Prirodoslovno-matematičkog fakulteta Sveučilišta u Zagrebu i Institut za biljnu fiziologiju Sveučilišta u Grazu)

U primjercima biljke *Physalis alkekengi* L. sa simptomima virusne infekcije koji su spontano rasli u blizini Zagreba, nađena je posebna odlika virusa mozaika bunike (henbane mosaic virus, HMV-HZ). Na osnovi reakcije pokusnih biljaka u paralelnim pokusima, HMV-HZ izrazito se je razlikovao i od Rothamstedskog tipičnog soja (HMV-R) i od soja izdvojenog iz vrste *P. alkekengi* (HMV-A) u Italiji. U kapljičnom pokusu izolat HMV-HZ je reagirao s dva antiseruma od soja HMV-R do njihovog homolognog titra (1:16000). Cilindrične uklopine i osebujne kristalične strukture omogućili su brzu identifikaciju virusa.

Dr. Đorđe Mamula
Prof. dr. Zlata Štefanac
Botanički zavod
Prirodoslovno-matematički fakultet
Marulićev trg 20/II
YU-41000 Zagreb (Jugoslavija)

Prof. Dr. Irmtraud Thaler
Doz. Dr. Manfred Gailhofer
Institut für Pflanzenphysiologie
Schubert-Strasse 51
A-8010 Graz (Österreich)