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THE INCIDENCE AND VARIATION OF CUCUMBER MOSAIC VIRUS IN FOUR VEGETABLE SPECIES IN CROATIA

ANA ŠARIĆ and *ZLATA ŠTEFANAC*

(Department of Botany, Faculty of Science, University of Zagreb)

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All isolates of CMV obtained from squash (*Cucurbita pepo* L.), peppers (*Capsicum annuum* L.), beans (*Phaseolus vulgaris* L.) and lettuce (*Lactuca sativa* L.) caused similar symptoms typical of CMV in *Cucumis sativus* Delicatess, *Datura stramonium, Nicotiana glutinosa, N. megalosiphon, N. tabacum* White Burley and *Chenopodium amaranticolor*. The reactions of other test plants showed differences existing among some isolates.

The H isolate from *C. pepo* differed from V isolate obtained from the same species by systemically infecting *C. quinoa*, and by more severe reactions of *C. pepo* and *C. maxima*. Both isolates caused local lesions in *Vigna sinensis* Midget and no reaction in *P. vulgaris* Top Crop.

All isolates from *C.annuum* except one provoked only local lesions in *P. vulgaris* and *V. sinensis*.

All isolates obtained from local selections and from imported varieties of *P. vulgaris*, including two isolates obtained from *L. sativa*, behaved similarly causing local and systemic infection of *P. vulgaris* and *V. sinensis*.

In serological tests performed in agar gel the isolates from *C. pepo* gave precipitin lines nearer to the antiserum well characteristic of degraded virus, while the precipitin lines formed by all other isolates were near the antigen wells showing that more stable antigen reacted.

The results presented show that in CMV infections of the observed vegetable species at least three different strains participated including five variants, one strain of which is common in beans and lettuce crops.

Introduction

The intensified production of vegetables on big farms in vegetable producing areas, as well as on smaller plots, especially near urban centres, has resulted in urgent and complex phytopathological problems concerning virus infections, because a number of viruses attack vegetable crops. Only one species, the pepper, for instance, is a host to more than 30 different viruses (*cf.* Edwardson and Christie 1979). Mixed infections with two or more viruses are common on these plants. The losses caused by the viruses are not manifested only in the decrease of the yield, but also in degraded quality. Different types of distortion and depigmentation diminish the value of the crops.

Conditions for the spread of the viruses in intensified cultivation of vegetables are very favourable. A dense population of different species and varieties favours their dissemination through their vectors, i. e. different kinds of aphids. Nearly all viruses attacking vegetable plants have vectors which transmit them in a nonperistent manner, and thus rapidly increase infections. Although it occurs in a low percentage, the transmission of the viruses through seed must not be overlooked because even a small number of infected plants at the start become important foci from which aphids rapidly spread infection. In the epidemiology of vegetable viruses some weed plants in which they survive when there are no vegetables are also of great importance.

One of the most widespread viruses, and particularly of vegetable plants, is cucumber mosaic virus (CMV). A large number of plant species belonging to 40 families of monocotyledones and dicotyledones are its hosts (Bos 1983). This paper present data concerning detection and some properties of CMV isolates obtained from squash (*Cucurbita pepo* L.), peppers (*Capsicum annuum* L), beans (*Phaseolus vulgaris* L.) and lettuce (*Lactuca sativa* L.) in the district of Zagreb.

Materials and Methods

Samples for investigation were gathered during three consecutive years on the experimental fields of the Faculty of Agriculture, Zagreb, where for scientific and educational purposes different species and varieties of vegetable plants are grown in a number of small plots. Young leaves of squash, peppers, beans and lettuce with well-defined symptoms were collected in order to isolate the virus. The virus was extracted from the leaves by using 0,01M potassium phosphate buffer pH 7.0 and, when required, by the addition of 0.1% thioglycollic acid or 0.2% 2-mercaptoethanol. The following species were inoculated by mechanical transmission: *Cucumis sativus* Delicatess, *Nicotiana glutinosa* and healthy specimens of the corresponding original species. They served as a source of the virus for serological reactions and inoculation of additional test plants: *Datura stramonium, N. megalosiphon, N. tabacum* White Burley, *Chenopodium amaranticolor, C. quinoa, Phaseolus vulgaris* Top Crop and *Vigna sinensis* Midget.

Serological double diffusion tests were performed as described by Štefanac (1980) with antiserum against CMV (carnation strain) kindly supplied by Dr. E. Luisoni (Torino). The serum contained antibodies against the whole virus (titre 1/128) and against distintegrated (soluble) virus protein.

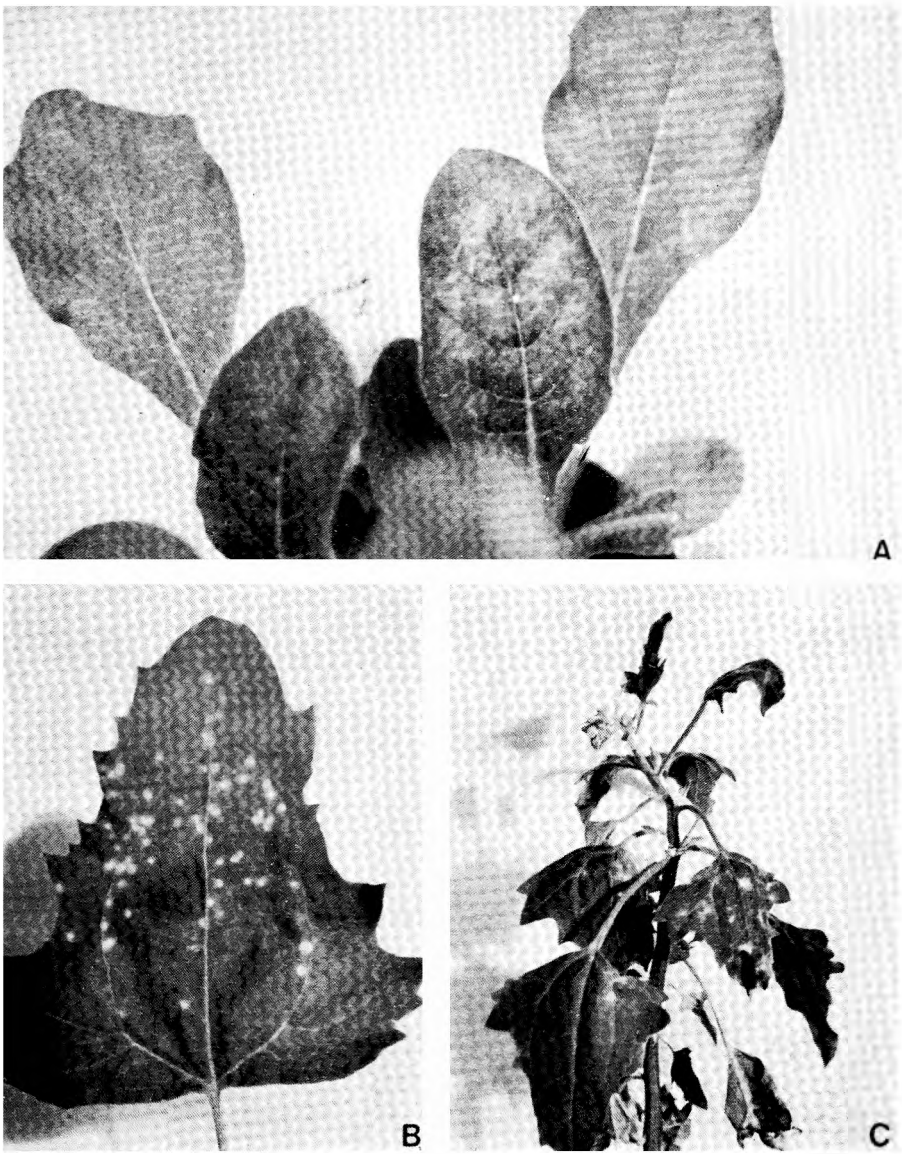

Fig. 1. **A** Systemic vein-clearing in *Nicotiana megalosiphon* typical of all CMV isolates tested. **B** Local lesions and **C** systemic symptoms in *Chenopodium quinoa* induced by H isolate from *Cucurbita pepo*.

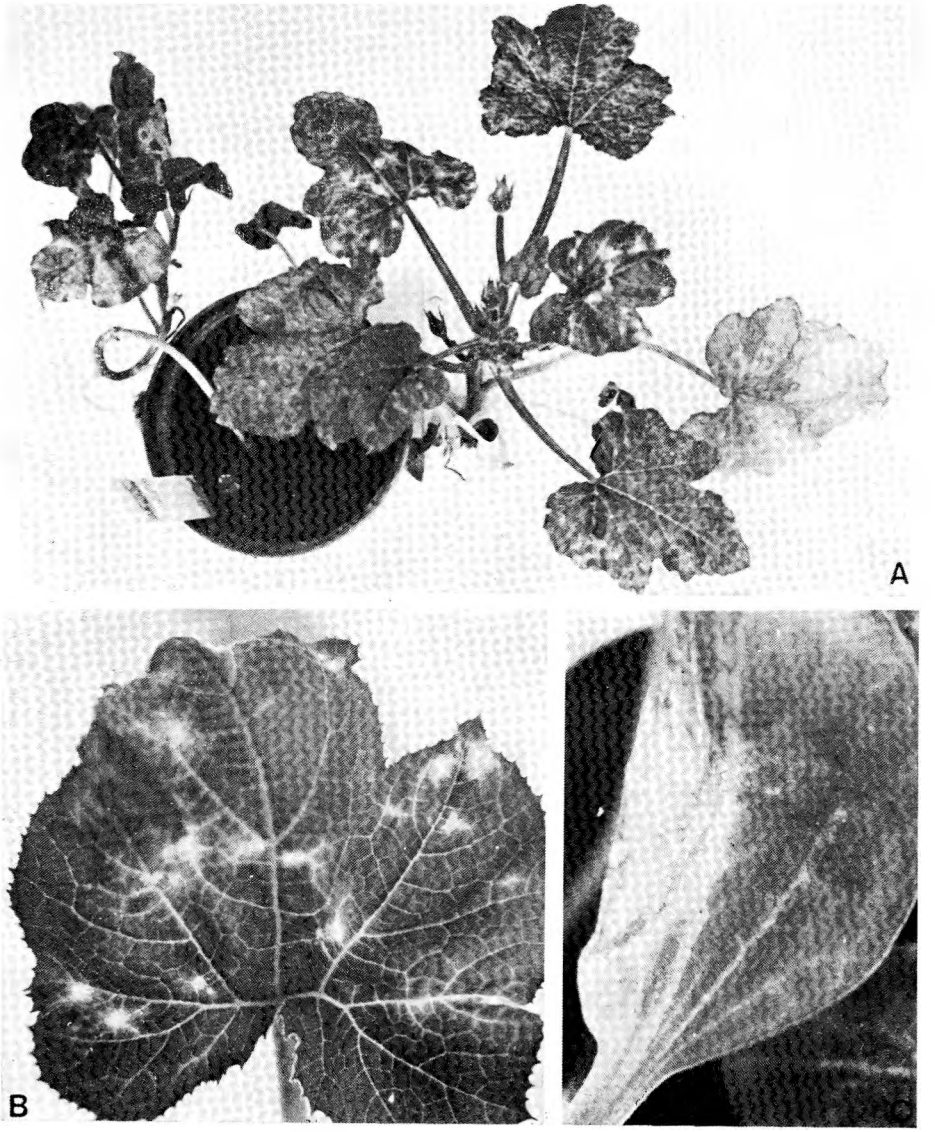

Fig. 2. Symptoms in *Cucurbita maxima* Marina di Chioggia induced by H isolate. **A** Systemically infected plant and **B** a leaf, showing severe vein clearing and star mottle accompained by distortion and necrosis. **C** Amalgamated necrotic lesions in inoculated cotyledone leaf.

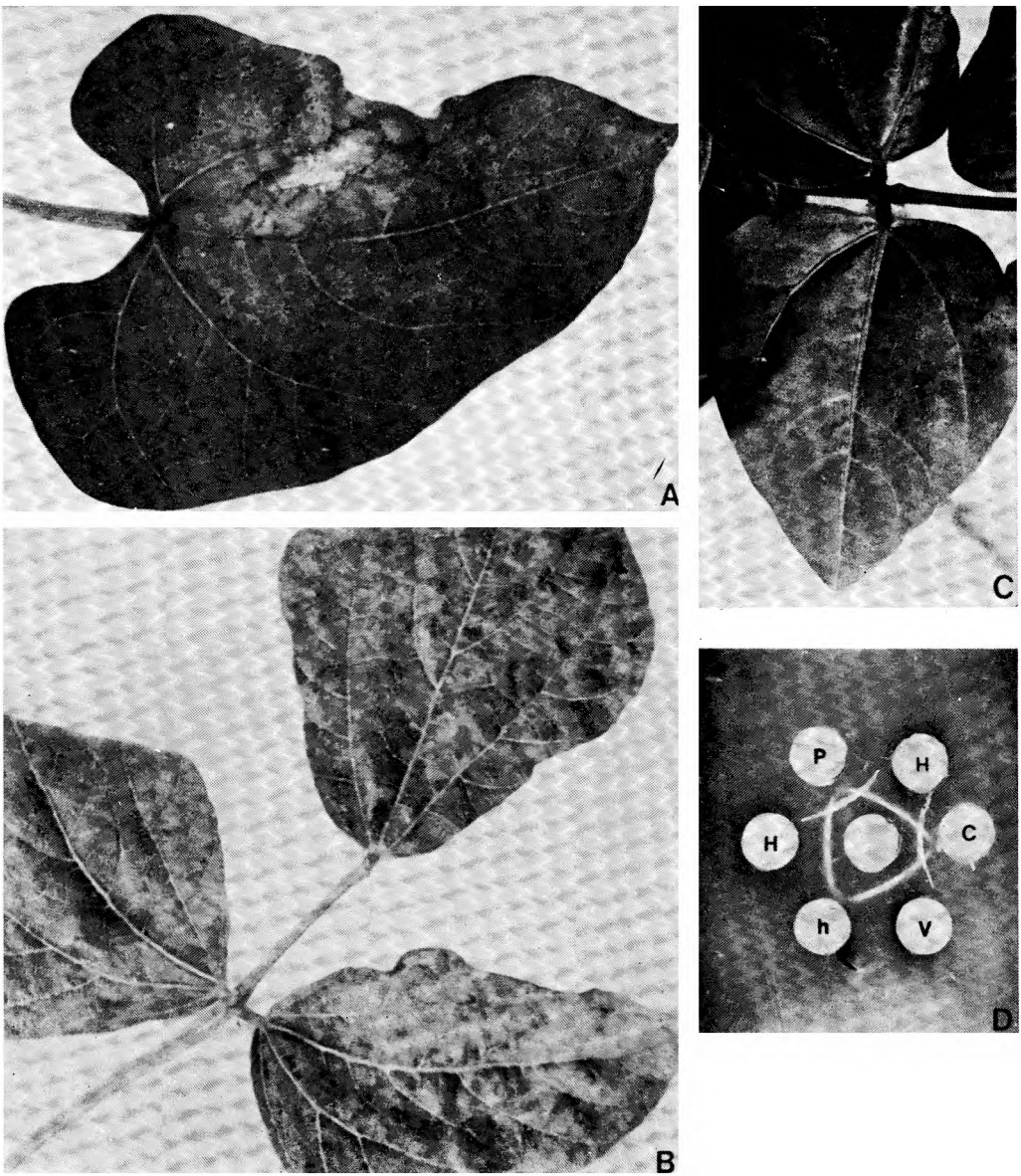

Fig. 3. **A** Local lesions in primary leaf and **B** systemic symptoms in trifoliate leaf of *Phaseolus vulgaris cv.* Top Crop infected with an isolate from *P. vulgaris*. **C** Pin-point necrotic lesions in *Vigna sinensis* Midget leaf inoculated with an isolate from *Capsicum annuum*. **D** Immunodiffusion test, showing differences in stability among CMV isolates obtained from different sources [H and V isolates from C. pepo, an isolate from *P. vulgaris* (P), an isolate from *C. annuum* (C), healthy *C. quinoa* sap (h)]. Centre well was charged with antiserum to CMV (carnation strain). Results identical to those with P and C isolates were obtained with the isolates from *Lactuca sativa*.

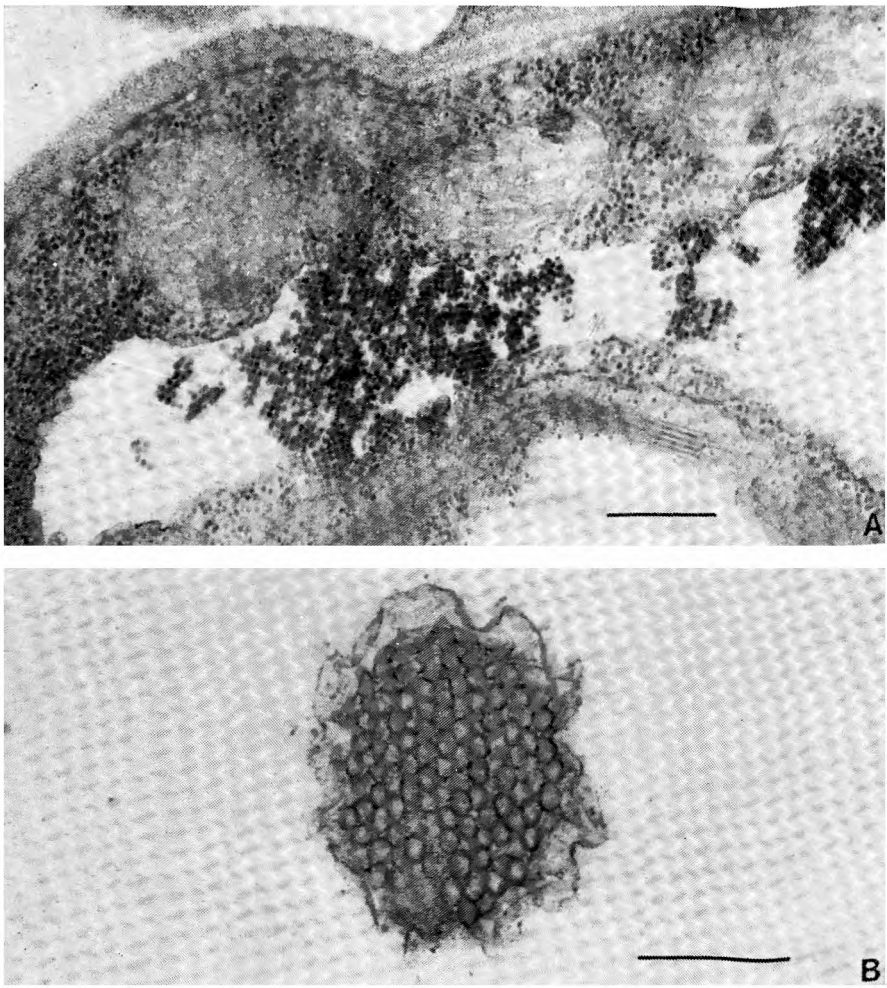

Fig. 4. Thin sections of *C. quinoa* leaf cells infected with H isolate of CMV. **A** The accumulation of virus particles in the cytoplasm and vacuole, inside the vacuole in crystalline arrangement. **B** Membrane bound aggregation of vesicles. Bars = 300 nm (A), 500 nm (B).

Light microscopy observations were carried out as described earlier (Šarić *et al.* 1984) using upper leaf epidermis of infected C. *quinoa* from the place of local lesions.

Thin sections for electron microscopy were prepared from systemically infected *C. quinoa* leaves. Small pieces of tissue were processed using the procedure described earlier (Šarić *et al.* 1984) and examined with a Siemens Elmiskop I.

Results

Reactions of test plants

All isolates caused similar response in *Cucumis sativus* Delicatess, i.e. appearance of local necrotic lesions in cotyledones and pronounced systemic mosaic in true leaves. The symptoms in *Nicotiana glutinosa, N. megalosiphon* (Fig. 1A), *N. tabacum* White Burley and *Datura stramonium* were typical of CMV, i.e. local necrotic lesions and systemic vein-clearing and mosaic, more or less uniform with all isolates. Reactions of other test plants, however, showed reliable differences between certain isolates (*cf.* Table 1).

Table 1. Types of reactions in differential hosts following inoculation with cucumber mosaic virus

Differential host	Virus isolates obtained from			
	Cucurbita pepo	*Capsicum annuum*	*Phaseolus vulgaris*	*Lactuca sativa*
Chenopodium quinoa	L or L, S	L	L	L
Phaseolus vulgaris Top Crop	—	L or L, S (1)	L, S	L, S
Vigna sinensis Midget	L	L	L, S	L, S

L local symptoms, S systemic symptoms, S (1) systemic latent infection, — no infection.

Cucurbita pepo isolates. Two isolates, one obtained from a local variety of Zucchini-type squash (H isolate) and the other of Vegetable marrow (V isolate), distinguished by the reaction of *C. quinoa* in which one (H isolate) besides the characteristic local lesions (Fig. 1B) caused also systemic symptoms (Fig. 1C). The H isolate was found to be more virulent both in *C. maxima* Marin di Chioggia, causing a severe necrotic reaction (Fig. 2A,B) accompanied by wilting of the cotyledones (Fig. 2C), and in *C. pepo* Spagetti provoking a severe reaction on the leaves and even wilting of young plants. In *Vigna sinensis* Midget both isolates caused a local reaction in the form of single small brown spots. In *Phaseolus vulgaris* Top Crop there was no infection.

Capsicum annuum isolates. All 8 isolates obtained from peppers provoked similar local and systemic reactions in inoculated *C. annuum* plants. *P. vulgaris* responded with the appearance of small necrotic local lesions in primary leaves, and after inoculation with isolates obtained from *cv.* Kalinkova also with latent systemic infection. Symptoms in *C. quinoa* and *V. sinensis* (Fig. 3C) were local, in the latter similar to those caused by both isolates obtained from squash.

Phaseolus vulgaris isolates. Eight isolates from different local selections and four from introduced varieties (Blue lake, Groffy, Niagara, Harvest) caused small pin-point necrosis in inoculated primary leaves of *P. vulgaris* which were later surrounded by chlorotic rings (Fig. 3A). Systemic chlorotic variegation and blistering appeared in trifoliate leaves (Fig. 3B). The symptoms in *C. quinoa* were typical local symptoms, and in V. *sinensis* pin-point brown lesions were followed by systemic symptoms.

In local bean varieties infections with other viruses of beans were also found including bean yellow mosaic and bean common mosaic potyviruses. These viruses were found either alone or in mixed infections with CMV, which will be reported on in a separate paper.

Lactuca sativa isolates. Two isolates obtained from lettuce appeared to be similar. They caused local lesions in *P. vulgargs* in the form of numerous small chlorotic rings, and systemic infection with mosaic and blistering in trifoliate leaves. In addition to chlorotic local reaction in *V. sinensis* there also appeared systemic chlorotic rings and blisters. Transmission of the isolates to lettuce was not successful either with extracts from the original lettuce leaves or by using infected *C. quinoa* leaves.

Serology

In gel double diffusion tests the isolates obtained from *C. pepo* reacted in crude sap with antiserum to CMV. Preciptin lines were straight and closer to the antiserum well (Fig. 3D), which suggested that these isolates belonged to labile CMV strains. In contrast, preciptin lines of all other isolates were characteristic of intact virus, being closer to the antigen well (Fig. 3D). All isolates, including those from *C. pepo* after being stabilised with low molarity citrate buffre (Devergne et al. 1972), reacted with the antiserum to its homologous titre: in that case preciptin lines of all isolates were typical of intact virus.

Light and electron microscopy

Light microscopic survey for inclusion bodies was done with H and V isolates from *C. pepo.* In naturally infected leaves of Zuchini and Vegetable marrow no intracellular changes were noticed. However, in experimentally infected *C. quinoa* roundish or hexagonal crystals with clear centre resembling those of other CMV strains (cf. Christie and Edwardson 1977, Edwardson and Christie 1979) were visible in some cells.

In thin sections which were prepared from tissue infected with H isolate, virus particles were observed in the cell cytoplasm and vacuole (Fig. 4A). Inside the vacuole they were in the form of numerous small crystalline aggregations (Fig. 4A) or a single large crystal, both characteristic of CMV infections. Membrane bound aggregation of vesicles was foud in one place (Fig. 4B).

Discussion

CMV is a dangerous pathogen of many vegetable species. The characteristic of this virus is great variability with respect to the host range, expression of symptoms, vectors and temperature stability. Its genome divided among three species of RNA makes possible new combinations of properties which increase its variability (cf. Edwards *et al.* 1983). Judging by the reaction of test plants the variability in investigated isolates was expressed through the occurrence of three different strains including five variants. Unexpected reactions of some test plants were also present. They were latent systemic infection of *Phaseolus vulgaris* Top Crop by certain isolates from pepper, and the appearance of systemic infection in *C. quinoa* by H isolate from squash. However, all the isolates investigated certainly belog to the (common strains of) CMV which was proved by serological tests. We also noticed that the properties of the isolates we obtained from beans generally agree with those of CMV earlier obtained from this species in Yugoslavia (Babović *et. al.* 1978, Taraku 1982).

CMV is not seed-transmitted in the seed of CMV-susceptible vegetable crops such as celery, cucurbits, lettuce and spinach (Tomlinson 1987), unless perhaps in a very low percentage. However, it can be transmitted in the seed of certain bean varieties (Bos and Maat 1974, Babović *et. al.* 1979) and very probably some other *Fabaceae.* Since certain strains are seed-borne they could easily have been brought from other countries. In the spread of CMV to vegetable plants, weed-plants have an important role. In France, CMV was detected in 40 wild species growing near an experimental field (Quiot *et al.* 1979), including some in which it was seed-born. In a preliminary survey conducted in the area of central Dalmatia (Štefanac 1988) CMV was also detected in some weed species. Important reservoirs of the CMV were found to be such species as *Matricaria spp., Senecio vulgaris, Capsella bursa-pastoris* and particularly *Stellaria media* (Tomlinson 1987). In the last two species the virus was almost symptomless and in the last one found to be transmitted by the seed. These and other weed species were frequently present in uncultivated soil near the margins of experimental plots from which samples for this study were collected. Therefore, we believe that the initial infections mostly derived from such plants. In the protection of vegetables from CMV it is necessary to know its ecology and epidemiology in the specific area in order to undertake adequate protective measures.

We would like to draw attention to the fact that it was not possible to transmit the isolates from lettuce to the lettuce plants under the experimental conditions by using mechanical inoculation. The failure of a mechanical transmission of CMV to certain original species has already been recorder in literature (Wolf 1968, Horvath 1969).

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SAŽETAK

UČESTALOST I VARIJABILNOST VIRUSA MOZAIKA KRASTAVACA U ČETIRI VRSTE POVRĆA U HRVATSKOJ

Ana Šarić i *Zlata Štefanac*

(Botanički zavod Prirodoslovno-matematičkog fakulteta Sveučilišta u Zagrebu)

Svi izolati virusa mozaika krastavca izdvojeni iz tikvica (*Cucurbita pepo* L.), paprika (*Capsicum annuum* L.), graha (*Phaseolus vulgaris* L.) i salate (*Lactuca sativa* L.) uzrokovali su slične simptome i tipične za ovaj virus na vrstama *Cucumis sativus* Delicatess, *Datura stramonium*, *Nicotiana glutinosa*, *N. megalosiphon*, *N. tabacum* White Burley i *Chenopodium amaranticolor*. Reagiranja drugih pokusnih biljaka ukazala su na razlike koje su postojale između nekih izolata.

Izolat H iz tikvice razlikovao se od izolata V iz iste vrste po sistemičnoj reakciji na *C. quinoa* te jačem reagiranju vrsta *C. pepo* i *C. maxima*. Oba izolata uzrokovala su lokalne lezije na *Vigna sinensis* Midget te nisu zaražavala vrstu *P. vulgaris* Top Crop.

Svi izolati iz paprike, izuzev jednog, uzrokovali su isključivo lokalne lezije na vrstama *P. vulgaris* i *V. sinensis*.

Svi izolati izdvojeni iz domaćih selekcija i uvezenih varijeteta graha, uključujući oba izolata iz salate, bili su slični dovodeći do lokalne i sistemične infekcije vrsta *P. vulgaris* i *V. sinensis* uz pojavu simptoma.

U serološkim pokusima koji su izvršeni u agrarskom gelu izolati iz tikvica dali su linije precipitacije bliže bazenu s antiserumom koje su karakteristične za raspadnuti virus, dok su linije precipitacije svih drugih izolata bile bliže bazenima s antigenom ukazujući na to da je reagirao stabilniji antigen.

Izneseni rezultati pokazuju da pri zarazama istraživanih povrtnih vrsta virusom mozaika krastavca sudjeluju najmanje tri virusna soja, uključujući pet varijanti, od kojih je jedan soj raširen na grahu i salati.

Prof. dr. Ana Šarić
Prof. dr. Zlata Štefanac
Prirodoslovno-matematički fakultet
Maruličev trg 20/II
YU-41000 Zagreb (Jugoslavija)