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CUCUMBER MOSAIC VIRUS IN GARLIC

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Introduction

Garlic (Allium sativum L.) is obligatory propagated vegetatively and frequently shows virus-like symptoms. One of the widely distributed and economically most important diseases of garlic is garlic mosaic known to occur in many parts of the world. Recent studies of garlic mosaic have shown that it is caused by a combination of three different viruses (Delecolle and Lot 1979). Of these, two always occur together as latent infections, and severe mosaic symptoms develop when plants are additionally infected by the third, onion yellow dwarf virus (OYDV). One of the latent viruses, garlic latent virus, is a carlavirus and the other is an unnamed potyvirus. It was found earlier that garlic mosaic had a limited host range outside *Allium* spp. including *Chenopodium quinoa* (Havránek 1973, Štefanac 1976) which is now known as the host to the two latent viruses.

Two other viruses obtained from garlic are tobacco rattle virus which was occasionally found in plants in Germany and was not associated with conspicuous symptoms (Graichen 1975) and tobacco mosaic virus (TMV) in the U. S. S. R. (Vasil'eva and Mozhaeva 1977) for which symptoms were not reported.

This paper reports yet another virus obtained from garlic, cucumber mosaic virus (CMV), and describes the distinctive symptoms it causes in garlic.

Material and Methods

CMV (CMV-G hereafter) was isolated from a single garlic plant by grinding the leaves in 0.06 M-phosphate buffer (pH 7.1) containing

0.1% thioglycollic acid and inoculating the extracts to *Chenopodium quinoa*. The isolate was maintained in *Nicotiana tabacum* with inoculum originally derived from systemically infected C. *quinoa* leaves. Inoculated C. *quinoa* leaves were used as the source of virus for the host range and serological tests. Experimentally inoculated garlic and other pant species were tested for CMV-G by infectivity assays on C. *quinoa* followed by serological tests. All experimental plants were grown in an aphid-proof glasshouse.

For aphid transmissions, green peach aphids *(Myzus persicae)* were starved for 3—4 h, allowed to feed on infected *Nicotiana megalosiphon* leaves for 1—3 min and then transferred to CMV-free garlic plants which were treated with an insecticide the next day. The test-plants were examined for infection two months after the inoculation feed.

For detection of the latent viruses samples consisting of two leaves of each plant were ground with 2% (w/v) sodium phosphotungstate at pH 6.5 and examined in an electron microscope.

Serological double diffusion tests were made in gels of 0.9% Bacto agar in distilled water containing 0.3% NaN_3 with antiserum against CMV (carnation strain) and antiserum against peanut stunt virus (PSV) (clover blotch strain, Richter et al. 1979). The first was kindly provided by Dr E. Luisoni (Torino), and the second together with purified preparation of PSV by Dr M. Musil (Bratislava).

Results

Occurrence and symptomatology of CMV in garlic

Thirty-two young garlic plants grown in steam sterilized soil from bulbils which were collected from an unknown cultivar of autumn garlic *(Allium sativum* L. ssp. *sagittatum* Kuzn.) in the University Botanic Garden, Zagreb were tested twice for the presence of viruses and the virus isolate later identified as CMV was obtained from one plant one leaf of which showed a conspicuous yellow stripe. Later the stripe turned necrotic and eventually the leaf became split into two parts. Afterwards the CMV-G infected plant developed similar symptoms in another leaf and compared with other plants grew more slowly.

The remaining 31 plants showed no virus symptoms and tests failed to detect either CMV or OYDV. However, after a long period of incubation the C. *quinoa* test-plants produced chlorotic local lesions or rings; the examination of sap from the garlic plants in the electron microscope which showed filamentous virus-like particles suggested that all plants were infected with the latent viruses described by Delecolle and Lot (1979).

In tests for the transmission of CMV-G to garlic by aphids, 10 out of the 31 symptomless plants were colonized with 15 viruliferous aphids each. CMV-G was transmitted to 3 plants in which it induced disease symptoms after about two months. The initial symptoms consisted of a mild green to yellow streak and stripe mosaic pattern present in newly expanding leaves (Fig. 1A) which later turned yellow and wilted. The leaves produced during the summer looked normal. In the autumn the plants showed necrotic symptoms similar to those observed in the originally infected plant. As in the original naturally infected plant the initial chlorotic stripes were restricted to a single leaf of each plant.

Later these chlorotic stripes turned yellow, then white necrotic stripes which finally split (Fig. 1B). In the early spring of the following year new leaves developed mosaic symptoms.

During the experiment none of the 21 control plants showed any symptoms.

Reaction of test-plants

The symptoms produced by CMV-G in species infected by mechanical inoculation are given in Table 1.

Table 1. Reaction of test-plants infected with CMV—G

Species	Symptoms	
	Inoculated leaves	Uninoculated tip leaves
Allium cepa	necrotic streaks	yellowing, *retarded growth*
Chenopodium amaranticolor	necrotic pin-point lesions	not infected
C. quinoa	chlorotic lesions	occasionally chlorotic spots and leaf curl (Fig. 1C)
Cucumis sativus cv. Delicatess	chlorotic lesions	mosaic (numerous chlorotic spots), recovery
Datura stramonium	diffuse chlorotic lesions	occasionally chlorotic spots or indistinct mottling
Lycopersicum esculentum	symptomless	occasionally symtomless infection
Nicotiana glutinosa cv. Corvalis strain	chlorotic lesions	light green mosaic, chlorotic spots, light leaf distortion, recovery
N. megalosiphon	necrotic spots or rings	vein-clearing, necrosis
N. tabacum cv. Samsun NN	late necrotic lesions	intersticed vein-clearing, green mosaic, mottle and necrotic etching, recovery
N. tabacum cv. Xanthi-nc	symptomless	vein-clearing, green spots, necrotic etching, recovery
Ocimum basilicum	large necrotic rings	occasionally large necrotic rings
Petunia hybrida	diffuse chlorotic lesions	faint vein-clearing, mottle recovery
Phaseolus vulgaris cv. Perlička	scattered necrotic pin--point lesions	not infected
Pisum sativum cv. Raman	necrotic lesions	not infected
Vicia faba cv. Inovecký	necrotic lesions	not infected
Vigna sinensis cv. *Black eye*	necrotic pin-point lesions	not infected
Tetragonia expansa	diffuse chlorotic lesions	occasional chlorotic spots
Zinnia elegans	symptomless	mottling, reflexed leaves in some plants, 'broken' flowers

In most hosts the virus induced symptoms characteristic of most isolates of CMV (cp. also Tolin 1977). However, in *C. quinoa* more or less strong systemic symptoms were occasionally produced (Fig. 1C), a feature so far detected only in a few isolates of CMV (Moris-Krsinich et al. 1978). In *Solanaceae* and other hosts, symptoms were usually more severe when temperatures were lower, and in tomato, *C. quinoa* and *Datura stramonium* systemic infection occurred only during the winter.

In one experiment in which 8 onion plants (*Allium cepa* L.) were inoculated, the virus was detected in leaves of 3 but only one plant became systemically infected and showed symptoms similar to those described by Smith (1972: 294).

The following species were not infected by CMV-G: *Brassica chinensis* cv. *Michihli* and *Lactuca sativa* cv. *Nansen.*

Serological tests

In gel double diffusion tests a clear immunoprecipitin line was still produced in agar-gel between CMV-G in crude sap and antiserum to CMV (homologous titre 1/128) diluted to 1/64. A single straight line produced by CMV-G with all dilutions of antiserum was typical of a soluble CMV antigen (Fig. 1D). However, the virus failed to produce any detectable precipitin lines when tested with antiserum to PSV (homologous titre 1/256) diluted to 1/2 (dilution was obtained by adding an equal volume of healthy plant sap) (Fig. 1E).

Discussion

Until now, CMV has occasionally been reported from several monocotiledonous ornamental bulbs (cp. Scmelzer et al. 1977) in which it was often in mixed infections with aphid transmissible filamentous viruses commonly present in these species. The lack of reports on CMV in vegetable and herb plants from the genus *Allium* may suggest that these plants like monocotiledonous ornamental bulbs are seldom infected by CMV, or again it may indicate that insufficient testing has been done.

Fig. 1. **A** (left) and **B**. Symptoms in garlic experimentally inftected with garlic isolate of CMV; note the split leaf in **B**. A (right) leaf from CMV-free plant. **C**. Systemic symptoms induced by CMV-G in *Chenopodium quinoa*. **D** and **E**. Double diffusion serogical test with antiserum to CMV (SC) and antiserum to PSV (SP) diluted 1/8 and 1/2, respectively. Antigens are CMV—G (G), PSV (P) and healthy *C. quinoa* sap (H).

Sl. 1. **A** (lijevo) i **B**. Simptomi na češnjaku koji je pokusno zaražen pomoću izolata VMK iz češnjaka; u **B** vidi se rascijepani list. **A** (desno) kontrolni list. **C**. Sistemični simptomi prouzročeni istim virusom na *Chenopodium quinoa*. **D**. i **E**. *Serološki* pokus dvostruke difuzije sa serumom protiv VMK (SC) i serumom protiv virusa kržljavosti kikirikija, VKK (SP) koji su bili razrijeđeni 1/8 odn. 1/2. Antigeni su VMK—G (G), VKK (P) i sok zdravog *C. quinoa* (H).

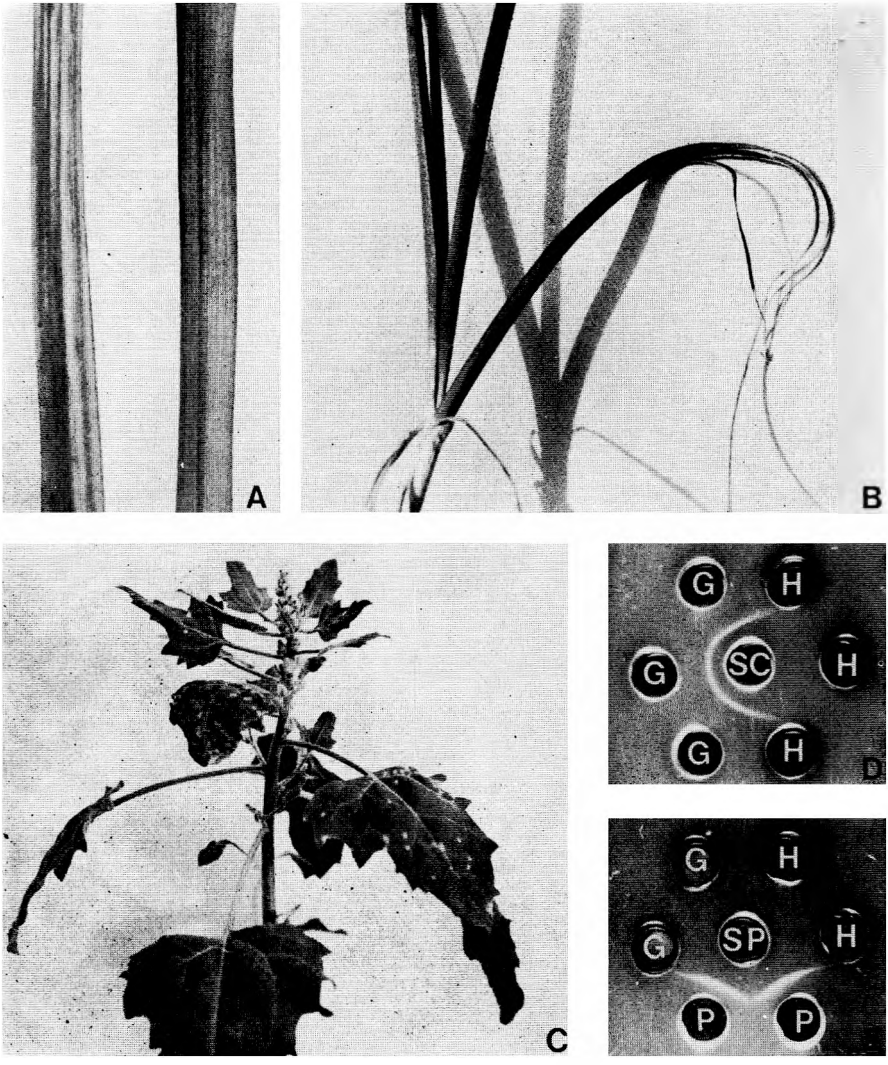

Fig. 1. — Sl. 1.

The present isolate CMV-G produced several kinds of disease symptoms in garlic plants infected by latent viruses of garlic (Delecolle and Lot 1979). A rather characteristic symptom among them was the frayed appearance of some leaves (Fig. 1B) originating from splitting of the necrotic stripes. The mosaic symptoms and especially the necrosis which developed in the garlic infected by CMV had similarities with lily necrotic fleck disease, which is caused by the combination of CMV and lily symptomless virus (Smith 1972, Mowat and Štefanac 1974). It is very likely that the symptoms described in garlic are also the result of a synergetic action of CMV with one or both latent viruses, a supposition which has yet to be examined.

It follows that in addition to OYDV (Delecolle and Lot 1979) CMV can also induce disease symptoms in garlic, although the disease may occur only rarely. It is possible, however, that in conditions when sources of CMV are present, disease outbreaks could occur especially in plantations of autumn garlic because lower temperatures favour multiplication of the CMV isolate studied.

Summary

Cucumber mosaic virus (CMV) was isolated from a stunted garlic plant showing a conspicuous yellow stripe in one of its leaves. The virus was transmitted by *Myzus persicae* to symptomless garlic plants naturally infected with a carlavirus and a potyvirus. CMV infected plants produced successively streaks and mosaic in leaves or a few chlorotic stripes, which later became necrotic and split giving the leaves a distinctive appearance. It is suggested that the cause of these symptoms was the interaction of CMV with one or both latent viruses.

The isolate of CMV obtained from garlic differed from most described isolates of this virus by its ability to induce occasional systemic infection in *C. quinoa*.

This is the first report of CMV in garlic.

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SAŽETAK

VIRUS MOZAIKA KRASTAVCA U ČEŠNJAKU

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Iz zakržljalog češnjaka (*Allium sativum* L.) s izrazitom klorotičnom prugom na listu izdvojen je virus mozaika krastavca (VMK). S pomoću lisne uši *Myzus persicae* virus je prenesen na primjerke češnjaka koji su bili prirodno zaraženi latentnim virusima iz karla-skupine i poti-skupine i nisu pokazivali nikakve vanjske promjene. U navedenim biljkama VMK izazivao je crtičasti odnosno prugasti mozaik, te malobrojne klorotične pruge koje su nekrotizirale i kidale se davajući listovima karakterističan rascijepani izgled (sl. 1B). Vjerojatno je uzrok navedenih promjena bilo zajedničko djelovanje VMK s jednim ili s oba latentna virusa.

VMK izdvojen iz češnjaka razlikovao se od većine izolata toga virusa po tome što je na važnoj pokusnoj vrsti *Chenopodium quinoa* povremeno izazivao sistemičnu zarazu (sl. 1C).

Ovo je prvi izvještaj o nalazu VMK na češnjaku.

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