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A NEW STRAIN OF CUCUMBER GREEN MOTTLE MOSAIC VIRUS FROM IRAN[1]

H. Rahimian and K. Izadpanah[2]

ABSTRACT

A rod-shaped virus, tentatively named melon rigid-rod virus (MRRV) was isolated from mildly mottled melon plants grown in vicinity of Shiraz. The virus was confined to Cucurbitaceae in host range. It caused mottling in cantaloupe (*Cucumis melo var. reticulatus* (L.) Naud.), longmelon (*C. melo var. inodorus* (L.) Naud.), cucumber (*C. sativus* L.) and watermelon (*Citrullus vulgaris* Schrad.) and chlorotic spots in *Luffa accutangula* Roxb. but remained symptomless in *Momordica balsamina* L. Squash (*Cucurbita pepo* L.) was immune. Rigid rods, similar to tobacco mosaic virus (TMV) particles, were observed in purified as well as in leaf dip preparations when examined with the electron microscope. MRRV reacted with antisera against cucumber green mottle mosaic virus (CGMMV) and TMV.

MRRV differs from previously reported strains of CGMMV in that it produces distinct local lesions on cotyledons of cucumber and longmelon and often induces a wilting reaction in some watermelon cultivars. This is the first report on the occurrence of a rigid-rod virus in melons in Iran.

INTRODUCTION

Cucumber green mottle mosaic virus (CGMMV) or cucumber virus 3 was first re-

1. Contribution from Department of Plant Protection, College of Agriculture, Pahlavi University, Shiraz, Iran.
2. Former graduate student and professor, respectively.

ported from England in 1935 (1). The virus was found to be confined to cucurbits (1, 11), although some strains were capable of infecting plants outside the cucurbit family (6, 9, 12, 17). On the basis of similarities in morphology, chemical structure, and physical and serological properties with TMV, this and a closely related virus, cucumber virus 4, have commonly been regarded as strains of TMV (8). This paper reports isolation and identification of a strain of CGMMV in Iran.

MATERIALS AND METHODS

Specimens were collected from longmelon (*Cucumis melo var. inodorus* (L.) Naud.) plants showing mild mottling and green vein banding. The samples were maintained at -25 C until used in inoculation trials.

Test plants were grown in 10-cm plastic pots filled with a mixture of sterilized field soil and peat moss. Greenhouse temperature during the study ranged from 15 to 25C.

For mechanical transmission, the infected materials were ground in a mortar in a few drops per gram of 0.05M potassium phosphate buffer, pH 7.2, and rubbed with the forefinger on the carborundum-dusted plants. Cucurbits were inoculated at cotyledonary stage, cowpea and bean at primary leaf stage and other plants at 3-to 5-leaf stage. Controls were rubbed with the phosphate buffer alone.

For purification, the virus was propagated in cantaloupe (*Cucmis melo var reticulatus* (L.) Naud. cv. Shahd-e-Shiraz). A modification of the method of van Regenmortel *et al.* (16) for purification of watermelon mosaic virus was used. The infected leaves, harvested two weeks after inoculation, were homogenized in 1.5 ml/g cold 0.05 M sodium citrate buffer, pH 6.7, containing traces of EDTA, in a blendor. The homogenate was squeezed through cheesecloth, mixed with ¼ volume of chloroform and shaken for 20 min. After centrifugation

at 6000 g for 20 min, the aqueous phase was collected and centrifuged for 90 min at 78000 g in a Beckman Model L3-50 ultracentrifuge. The pellet was suspended in 1/40-1/20 of the original volume of 0.035 M potassium phosphate buffer, pH 7.6, and clarified by centrifugation at 5000 g for 20 min. Density gradient columns were prepared by layering 7 ml each of 40, 30, 20 and 10% (W/V) sucrose solutions in 0.035 M phosphate buffer, pH 7.6, in cellulose nitrate tubes of a Spinco SW 25.1 rotor. After 90 min centrifugation at 24000 rpm, the columns were fractionated with an ISCO density gradient fractionator (Instrumentation Specialties Co., Lincoln, Nebraska, U.S.A.). The collected fractions were dialyzed against phosphate buffer for 48 hr followed by centrifugation at 100,000 g for 60 min. The pellet from each tube was resuspended in 1/100 original volume phosphate buffer.

For electron microscopic studies a partially purified virus preparation was stained with 2% potassium phosphotungstate, pH 7, on collodion-coated grids. The dip method (3) was employed in particle size comparison with TMV. The preparation was examined in a Phillips Model 300 electron microscope.

An antiserum against CGMMV was obtained from Dr. R.J. Shepherd of the University of California, Davis. TMV antiserum was prepared locally by giving the rabbits one intramuscular and three intravenous injection with partially purified virus at weekly intervals.

Agar gel diffusion tests were carried out in 15 x 90 mm petri dishes containing 15 to 20 ml of 0.6% Ionagar No. 2 (Difco Laboratories, Detroit, Michigan, U.S.A.) in 0.05 M KH_2PO_4, pH 7.8, plus 0.02% sodium azide (15). A central well, 5 mm in diameter, and 8 peripheral wells, 3 mm in diameter and 7 mm apart, were cut in the agar and used for placing antisera and antigens, respectively. The plates were incubated in a humid chamber at room temperature.

For microprecipitin tests (2), 2-fold dilutions of antiserum and virus preparations were made in 0.85% NaCl and the test performed inside plastic petri dishes. Normal serum mixed with the virus dilutions and different dilutions of the virus alone were used as control. The plates were incubated at room temperature for 2 hr and at 4C overnight and examined under a dissecting microscope at 20 X magnification.

RESULTS

Host Range and Symptomatology: Melon rigid-rod virus produced systemic mosaic and distortion in cantaloupe (cvs. Shahd-e-Shiraz and Hales Best), longmelon (cvs. Mashhadi, Suski, and Tusorkh), Honey Dew melon (*C. melo var. ilnodorus),* cucumber (*C. sativus* L. cvs. National Pickling, SMR, and a local unnamed cultivar), and watermelon (*Citrullus vulgaris* Schrad. cvs. Tom Watson, Golchin and Charleston Gray). Local chlorotic lesions were produced on cotyledons of longmelon and cucumber cultivars (Fig. 1) within 2 to 3 weeks after inoculations. In watermelon cultivars, mosaic symptoms were sometimes replaced by necrosis as in 'Tom Watson' and 'Golchin'(Fig. 2) or by general yellowing as in 'Charleston Gray'. In *Luffa accutangula* Roxb. diffuse chlorotic spots appeared on leaves of some of the inoculated plants while others remained symptomless. Symptomless systemic infection occurred also in *Momoridica balsamina* L. The virus was recovered in back inoculations from these plants to cantaloupe.

The following species were found to be immune to the virus: squash (*Cucurbita pepo* L. cv. 'Table Queen' and a local cultivar), *Chenopodium album L., C. amaranticolor* Coste & Ryne, *C. quinoa* Wild., cowpea (*Vigna sinensis* Torner Savi cv. 'Early Ramshorn'), broad bean (*Vicia faba* L. local cultivar), zinnia (*Zinnia elegans* Jacq. local cultivar), pea (*Pisum sativum* L. local cultivar), *Datura stramonium* L., and *Nicotiana tabacum* L. cvs. 'Turkish' and 'Xanthi n.c.'

Attempts to transmit the virus with *Myzus persicae Sulz.* failed. Mechanical transmission occurred when healthy and infected cucumber plants were grown in close contact with each other.

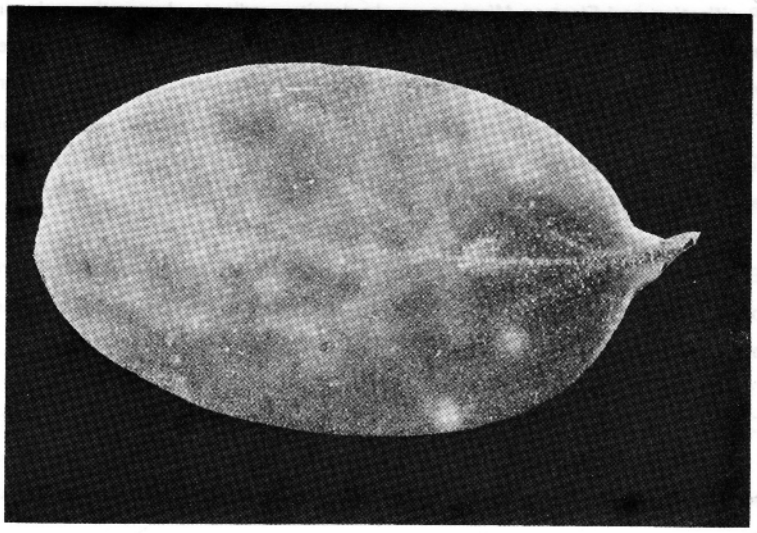

Fig. 1. Chlorotic local lesions on cotyledon of cucumber cv. 'SMR' 15 days after inoculation with CGMMV.

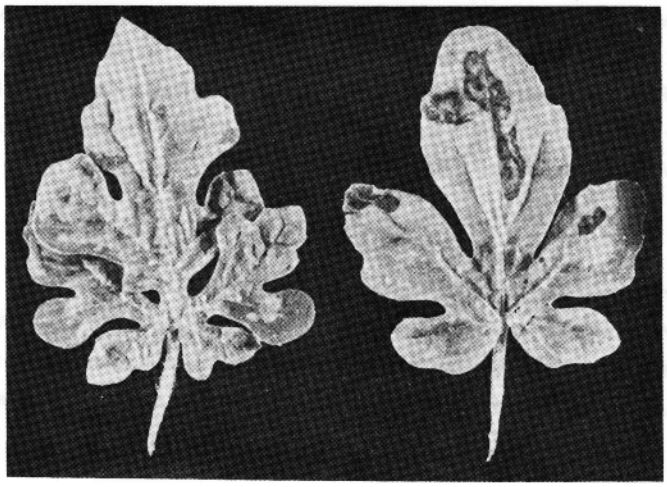

Fig. 2. Leaves of watermelon cv. 'Golchin' showing mottling (left) and necrotic spots (right) 25 days after inoculation with CGMMV.

Purification and Electron Microscopy: In density gradient columns the main virus band was formed 25 mm below the meniscus, occasionally two upper (minor) bands were also present. Ultraviolet absorption spectra of the three bands were typical of nucleoproteins. Absorption was maximum at 258-260 nm and minimum at 248-250 nm. The uncorrected A 260: 280 ratio was 1.42 for the major component, slightly lower values were obtained for the two upper bands.

Examination of the purified preparations by electron microscope revealed the presence of numerous rigid-rods in the two minor zones. However, the bottom zone was not completely devoid of the shorter rods.

Over 500 particles of MRRV were measured in electron micrographs of dip preparations. Electron micrographs of TMV prepared similarly were used for comparison. Measurements indicated that normal length of MRRV was approximately equal to that of TMV.

Serology: In agar gel diffusion tests, partially purified MRRV produced 2 precipitin lines with CGMMV antiserum, one close to the antigen well and the other near the antiserum well, TMV apparently did not react with this antiserum. When the two viruses were tested against TMV antiserum, the precipitin line of TMV produced a spur beyond that of MRRV. Crude leaf extracts of infected plants and purified preparations of both viruses gave similar results. In microprecipitin tests, TMV antiserum gave titer of 1/128 with MRRV while its homologous titer was 1/2048.

DISCUSSION

Regarding host range, morphology and serology, MRRV seems to be closely similar to CGMMV. It was confined in host range to Cucurbitaceae and similar to CGMMV of Ainsworth (1) and the isolate reported from Finalnd (11) in causing mosaic symptoms in cantaloupe, cucumber and watermelon and inability to infect squash. Three Japanese

strains of CGMMV have been reported which differ from each other as well as from the type strain. The strain of Inouye *et al.* (6) infected a number of solanaceous species; the watermelon strain produced local lesions on *C. amaranticolor* but no symptoms on *D. stramonium* while the cucumber strain formed local lesions on *D. stramonium* but not on *C. amaranticolor* (9, 12). The strain of Vasudeva *et al.* (17) produced visible symptoms only in cucumber and *Cucurbita moschata* Duchesne but caused symptomless infection of watermelon, *M. charantia L., L. accutangula, and D. stramonium.* MRRV is thus similar to, though not identical with, the type strain of CGMMV (1, 11). The type strain produces mottling without necrosis in watermelon and no local lesions in cucumber and melon whereas MRRV induces mottling and necrosis in some cultivars of watermelon and local lesions as well as systemic mottling in cucumber and longmelon.

In our particle size measurements, the length of MRRV particles were almost equal to TMV. The modal length of CGMMV has been reported to be 292 ± 11 nm for the isolates resembling the type strain (11) and 300 nm for the strain of Inouye *et al.* (6). In Russia the particles of cucumis virus 2 were found to be 280-300 nm (14). The significance of shorter particles constantly present in the upper zones in density gradients was not studied although such particles could be regarded as the breakage products of longer particles (13).

The results of serological tests indicate that MRRV is a strain of CGMMV and related to TMV. Serological relationship of TMV and CGMMV has been worked out by several investigators. For instance Inouye *et al.* (6) found a distant serological relationship between CGMMV and TMV in precipitin tests. Different degrees of serological relationship were observed between the watermelon (CGMMV-W) and the cucumber (CGMMV-C) strains of CGMMV and the common (TMV-OM) and tomato (TMV-T) strains of TMV (12). CGMMV-W was more closely related to TMV-OM than to CGMMV-C and TMV-T; antiserum to CGMMV-C gave only a faint reaction with other strains. Failure of TMV to

give a distinct reaction with CGMMV antiserum in agar gel diffusion tests has also been noticed by Inouy *et al.* (6). Presence of two precipitin bands in agar gel diffusion tests has also been reported for another isolate of CGMMV (10).

MRRV is considered a new strain of CGMMV, similar to the type strain in host range but different from it in producing local lesions in cucumber and longmelon plants. However, no reports are available on the infection of *L. accutangula* and *M. balsamina* by the type strain.

Occurrence of cucumber mosaic virus and watermelon mosaic virus in cucurbits in Iran has already been reported (4, 5, 7). This is the first report on the occurrence of CGMMV in this country.

ACKNOWLEDGEMENT

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