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First Report of Zucchini Yellow Mosaic Virus on Cucurbits in the Central Region of Saudi Arabia

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Abstract. A greenhouse grown cucumber virus isolate from the Deriyah area induced symptoms on squash *Cucurbita pepo* L. resembling those incited by zucchini yellow mosaic virus (ZYMV). This isolate has a host range similar to that of an ZYMV-original isolate, except that it did not infect *Gomphrena globosa* L. . The complete identification of ZYMV was based on its physical properties, aphid transmission, morphology by the electron microscope, and serological properties.

This study confirms the presence of ZYMV infection of cucumber and bottlegroud plants for the first time in the central region of Saudi Arabia.

Introduction

Cucumber (*Cucmis sativus* L.) is the most economically important vegetable crop in greenhouse production in Saudi Arabia. Virus diseases are considered as a major limiting factor in the greenhouse production of this crop. The Deriyah Valley farms (located 15 km N.W. of Riyadh) are a major greenhouse crop producing area around Riyadh, the capital city of Saudi Arabia.

Greenhouse cucumber and other field cucurbit plants throughout the Deriyah Valley were infected with a virus like mosaic disease. In September, 1985 a greenhouse containing cucumber plants in that valley was visited, and many of the plants (about 85%) were severly affected with stunting and blistered and malformed leaves and fruits.

The zucchini yellow mossaic virus (ZYMV) incites a disease of cucurbits known as zucchini yellow mosaic [1]. This disease was first observed on squash in Northern Italy in 1973 [2]. Since then, the ZYMV has been reported from various geographical locations around the world [1-8].

In this paper, the identification of ZYMV for the first time in the central region of Saudi Arabia was based on its host range, physical properities, vector transmission electron microscopy, and serology.

Materials and Methods

Virus isolate and maintenance

The virus isolate used in this study was obtained from naturally infected leaves of greenhouse grown cucumber plants showing typical mosaic and leaf and fruit distortion symptoms (Fig.1) of virus infection in Deriyah valley. The virus isolate was propagated and maintained in squash (*Cucurbita pepo* L. 'Corona').

Inoculation and infectivity assay

The inoculum for host range studies was prepared by macerating infected leaf tissue of Corona squash in 0.02 M, potassium phosphate buffer, pH 7.0 (1:5, W/V) with a mortar and pestle. Inoculum was hand-rubbed onto 300-mesh carborundum dusted leaves of test plants. The bioassay plant was Corona squash unless otherwise stated.

Fig. 1. Greenhouse grown cucumber leaves and fruits naturally infected with ZYMV.

Host range and symptomatology

Test plants were selected from host ranges of cucurbit viruses. All plants were grown and tested in greenhouse under natural daylight. Greenhouse temperature averaged about 28°C. All the host range plants were grown in a sterilized soil and fertilized each week. Test plants were observed for symptom development for four weeks. Tests were replicated at least two times. Control plants were maintained and recovery tests were made on Corona squash to detect latent infections in hosts that remained symptomless after inoculation.

Virus properties in sap and virus preservation

The longevity in vitro (LIV) at room temperature (about 20°C), dilution end point (DEP), and thermal inactivation point (TIP) were determined for the cucumber isolate in sap from infected Corona squash inoculated 14 days before assays. For LIV, ten screw-capped test tubes were each filled with 2 ml of sap and test plants were inoculated at various time intervals and observed for symptom development. Sap dilutions for estimating DEP were prepared with 0.01 M, pH 7.0 potassium phosphate buffer, and sap extracts (2 ml) were exposed to various water bath temperature for 10 min. to determine the TIP. For virus preservation, infected squash leaves were diced into pieces 4-5 mm, dried over anhydrous calcium chloride and stored at -23°C for different monthly periods of time. All preparations were assayed for infectivity by mechanically inoculating "Corona" squash plants at the two cotyledonous stage.

Aphid transmission

Aphid colonies of *Aphis gossypii* Glover were raised on disease free squash plants. After a starvation period of 2-3hr, apterous aphids were placed for a 5- or 10-min. acquisition access period (AAP) on detached infected or healthy squash leaves. The exposed aphids were then transferred to 8 healthy "Clarita" squash and 8 healthy "Hybrid Amera-11" cucumber plants (5 aphids per plant) for a 24hr inoculation access period (IAP) before the plants were sprayed with insecticide. Small pieces of wax paper were placed on the healthy plant leaf surface, and aphids were placed on them using a fine artist hair brush to avoid the possibility of mechanical inoculation. The test plants were observed for symptom development after 2-3 weeks in the greenhouse.

Electron microscopy

Electron microscopic observations were made with partially purified leaf extract of "Corona" squash. Formvar coated copper grids (300 mesh) were allowed to float on the leaf extract for 5 min at room temperature, and the excess was removed by absorption with the tip of a wedge of filter paper. When the grid was dry, 2-3 drops of 2% potassium phosphotungstate (KPT) solution, pH 6.5, were placed on the dried grids for 1.5 min as a negative stain. The grids were examined with the GOEL-100 electron microscope at 60 kv.

Serological assay

The ZYMV antiserum, kindley supplied by Dr. K. M. Makkouk (ICARDA, Aleppo, Syria) was used in the agar gel double diffusion assay. The leaf tissue was prepared for this test using the methods described by Hill [9]. The gel medium for immunodiffusion test consisted of 0.6% agarose, 0.85% NaCl and 0.02 sodium azide. All wells were cut at 4 mm in diameter, and each of the four peripheral wells was 4 mm from the center well. A 20-uL volume of reactant was added to each well, and then the plates were incubated for 24-48 hr in a humid chamber at 6°C before results were recorded. One of the peripheral wells contained healthy plant sap and another one contained phosphate buffer as control. The other two wells contained infected plant sap. One of the infected plant sap wells contained 0.015 g sodium dodecyl sulphate (SDS).

Infected "Corona" squash leaves were diced into small pieces 4-5 mm, dried over anhydrous calcium chloride and sent (with other samples including one sample from infected bottle gourds (*Lagenaria siceraria* L.) to Dr. K.M. Makkouk. The samples were tested against bean common mosaic virus (BCMV), cucumber mosaic virus (CMV), watermelon mosaic virus 1 (WMV-1), watermelon mosaic virus 2 (WMV-2), zucchini yellow fleck virus (ZYFV) and zucchini yellow mosaic virus (ZYMV) antisera. Dr. Makkouk used the enzyme-linked immunosorbent assay (EIISA) to check the identity of the virus in the submitted samples.

Results

Host range and symptomatology

The symptoms that developed in different indicator and host range plant species are shown in Table 1. The central region cucumber virus isolate was easily sap transmitted to many plant species in the cucurbit family, Squash, *C. pepo* (any cultivar) was very usefull as a diagnostic and propagation host developing distinct symptoms that included systemic mosaic, leaf blistering, shoestring and leaf deformation (Fig. 2). The systemic mosaic symptoms usually appeared on squash plants within 10-14 days after inoculation. In the cucurbit family, only two cultivars ("Byblos F1 hybrid" and "Pepino concombre") of *C. sativus* L. and *Luffa egyptiaca* Mill that were not infected by this isolate. *Chenopodium amaranticolor* Coste and Reyn (Table 1) was the only test species that did not belong to Cucurbitaceae but developed symptoms. It developed chlorotic (then necrotic) local lesions on inoculated leaves, however virus failed to infect *Gomphrena globosa* L. No symptoms were observed, and the back inoculation tests were negative with the rest of plant species (Table 1).

Virus properties

The virus remained infective after storage of sap from infected squash for 7 but not 8 days at room temperature, and remained infective after dilution of this sap to 10^{-3} but not 10^{-4}. It was infective after being heated for 10 min at 55°C but not 60°C. Infections virus was also recovered from *C. pepo* samples stored for 33 mo.

Table 1. Partial host range for the central region cucumber isolate of zucchini yellow mosaic virus (ZYMV-C).

Host[a]	Symptoms[b]
Capsicum annuum L.	
California wonder	NR
Chenopodium amaranticolor Coste & Reyn	LL
Citrullus vulgaris Schrad	
Black diamond	SM
Charleston 76	SM,Y
Charleston gray	sm,Y
Chilean black	Sm,Y
Cucumis melo L.	
Cantaloupe	SM,S,LD
Casaba golden beauty	SM,S.LD
Honey dew green	SM,S,LD
Cuclumis sativus L.	
Bybles F1 hybrid	NR
Hybrid amera -II	SM,LD,S
Hybrid niagra green	SM LD,S
Pepino concombre	NR
Perfecto formula mix	SM LD
Tender green	SM LD,B
Cucurbita pepo L.	
Calabasa	SM,B,LD,SS
Caserta	SM,B,LD,SS
Clarita	SM,B,LD,S,SS
Sqash ghado	SM,B,LD
Zucchini	SM,B, LD S,SS
Gomphrena globosa L.	NR
Glycine max (L.) Merr	NR
Lagenaria siceraria L.	SM,B
Luffa acutangula L.	SM
L. aegyptiaca Mill	NR
Lycopersicon esculantum Mill.	NR
Nicotiana clevelandii Gray	NR
N. glutinosa L.	NR
Vicia faba L.	
Long Poc	NR
Vigna unguiculata (L.) Walp.	
California Blackeye	NR

[a]Plants were mechanically inoculated with sap from infected tissue ground in 0.02M $K_2 HPO_4$ buffer, pH 7.0 and kept under greenhouse conditions.

[b]LL = Local lesions, SM= systemic mosaic, and NR= no reaction and the back inoculation test was negative. LD= Leaf deformation, Y= Yellowing, SS = shoestring, S= Blisteris on leaves.

Aphid transmission

A. gossypii transmitted the virus from ZYMV infected 'Clarita' squash to all 'Clarita' squash and 'Hybrid amera-11' cucumber test plants exposed to aphids after a 5 and 10-min AAP. Plants inoculated with this isolate by aphids developed systemic mosaic after 10-14 days, followed by leaf distortion and blistering. No symptoms were observed on control plants.

Fig. 2. Symptoms induced in *C. pepo* 'Corona' 5 weeks after mechanical inoculation with sap from *C. pepo* inoculated with sap from infected greenhouse grown cucumber (left) and healthy squash control (right).

Electron microscopy

Flexuous rod-shaped particles were observed in a partially purifed extract of leaves from infected squash plants showing mosaic symptoms (Fig. 3). The virus - like particles were typical of those associated with potyvirus [10, pp. 731-807] infections.

Serological assays

The known ZYMV antiserum (supplied by Dr. Makkouk) reacted specifically to exracts of infected tissues to form a single precipitin line in the agar-gel double diffusion test. No reactions were observed between the ZYMV antiserum and the healthy plant extract or phosphate buffer.

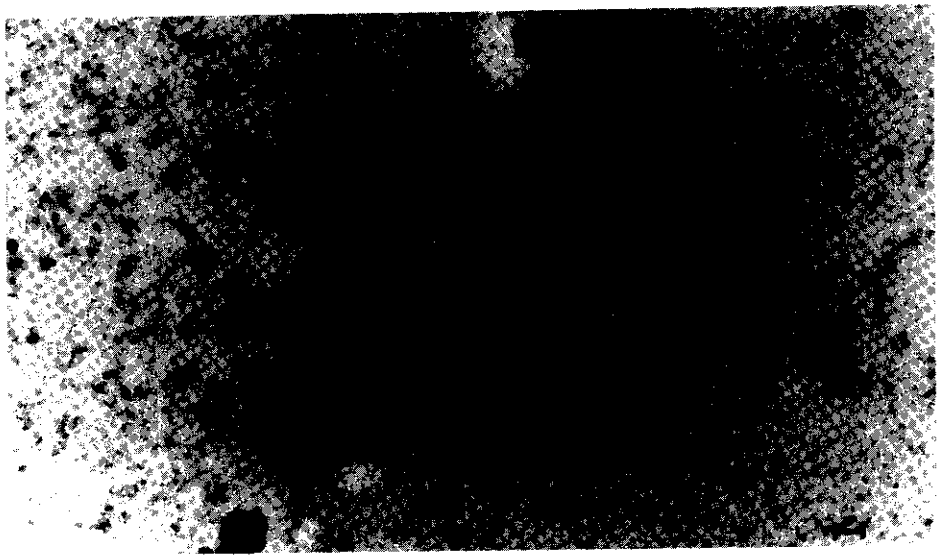

Fig. 3. Electron micrograph of a partially purified preparation from infected squash plants negatively stained with phosphotugastate. Bar represent 100 nm.

The results of ELISA test showed that this sample and another sample from naturally infected bottlegourd (*L siceraria*) plants gave strong positive reaction against ZYMV antiserum only and negative reactions with all other tested antisera.

Discussion

It can be concluded that the virus disease infecting greenhouse grown cucumber in Deriyah valley in the central region of Saudi Arabia was incited by a potyvirus [10, pp. 731-807, 11, p. 292]. This conclusion was based on the following reasons, particle morphology and length are similar to those of potyviruses. It was transmitted by aphids in a non-persistant manner, and it was easily transmitted by mechanical inoculation.

The host range, symptomatology and physical properities in sap of this isolate are very similar to those of the ZYMV isolates studied by Lisa *et al.* [2] and Lisa and Lecoq [1], in the original description of ZYMV except that this cucumber isolate did not incite local infection in inoculated leaves of *G. globosa* as does the Italian isolate [2]. The presence of ZYMV was further confirmed by the positive reaction of the infected plant sap with known ZYMV antiserum.

Since the results of the ELISA test showed that this cucumber isolate and another isolate from infected bottle gourd (also found in another farm in the same

valley) strongly reacted only against ZYMV antiserum and were negative to all other tested antisera, the virus in these two samples was ZYMV.

The virus isolate from cucumbers in the central region was ZYMV, and will be designated as ZYMV-C. Recently, Khan and Alamy, [12] and Salama *et al.* [13] have reported in their abstracts (presented in the annual meeting of Saudi Biological Society, held in Jaddah 1987) that the ZYMV causes a new problem in cucurbit production in the Eastern and Western regions of Saudi Arabia. This is the first report of ZYMV infecting cucurbits in the central region of Saudi Arabia and causing an important disease in greenhouse grown cucumber crop. Work on the effect of this virus on yield losses of greenhouse cucumber cultivars and virus distribution in the central region are currently in prograss.

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أول تقـريـر عن وجـود فيروس التبرقش الأصفر على الكوسة الخضراء على نباتات العائلة القرعية في المنطقة الوسطى من المملكة العربية السعودية

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ملخص البحث. سببت السلالة الفيروسية المعزولة من نباتات خيار مزروع في البيوت المحمية بمنطقة الـدرعية والمعـداه على نباتات الكوسة *Cucurbita pepo* L. أعراضًا مشابهة لتلك الأعراض الناتجة عن فيروس التـبرقش الأصفـر على الكوسة الخضراء (ZYMV). لقد كان المدى العائلي لهذه السلالة مشابهًا للمدى العائلي الخاص بالسلالة الأصلية لهذا الفيروس عدا أن هذه السلالة لاتصيب نباتات الـ *Gomehrena globosa* L.. لقد تم التعرف التام على هذا الفيروس عن طريق معرفة خصائصه الفيزيائية وطريقة انتقاله بحشرة ألمن وشكل الفيروس تحت المجهر الألكتروني بالإضافة إلى الخصائص المصلية له.

تعتبر هذه الدراسة أول دراسة موثّقة عن إصابة هذا الفيروس (ZYMV) لكل من نباتات الخيار واليقطين (القرع الرقيبي) وللمرة الأولى في المنطقة الوسطى من المملكة العربية السعودية.