Response of Greenhouse-Grown Cucumber Cultivars to an Isolate of Zucchini Yellow Mosaic Virus (ZYMV)

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ABSTRACT

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Mechanical inoculation of 12 greenhouse-grown cucumber (*Cucumis sativus*) cultivars at the three- to four-leaf stage with an isolate of zucchini yellow mosaic virus (ZYMV) from Saudi Arabia resulted in various symptoms in 11 cultivars. Significant differences in plant height, fruit numbers, and fruit weight were observed between the inoculated and the control plants of the 11 affected cultivars. The cultivar Dina was asymptomatic, and no significant differences were observed between the inoculated and control plants for any of the parameters tested. Virus recovery test from cultivar Dina performed on the susceptible cultivar, Farol, together with lack of virus detection by enzyme-linked immunosorbent assay (ELISA) indicated that this ZYMV isolate did not infect Dina. However, inoculation of Dina at the cotyledon stage revealed minor symptoms that were confined to a few of the lower leaves, suggesting that Dina is resistant but not immune to this ZYMV isolate. Virus titer in Dina was low and the virus was restricted to the older leaves; whereas in Farol virus titer was considerably higher and the virus spread to all leaves.

Zucchini yellow mosaic potyvirus (ZYMV) threatens cucurbit production worldwide. It was first reported in Italy in 1981 (16) and since has been reported in numerous countries throughout the world (2,4,10,12,15,16,18,22).

ZYMV is still spreading to localities where it was not reported previously. It was recently detected in Canada (25). Cucumber (*Cucumis sativus* L.), like other cucurbits, suffers from ZYMV infections; and a disease incidence of 80% or more, together with severe symptoms and abnormalities on foliage and fruits, has been reported worldwide (4,25). In some countries the incidence is so high that ZYMV is limiting cucurbit production (4,25). In Saudi Arabia, the virus has been reported in three major agricultural producing regions (4,14,24), where it causes substantial damage to cucurbits, especially to cucumber. This is the most important vegetable crop in the greenhouse business in Saudi Arabia (6).

The objective of this study was to evaluate cucumber cultivars grown widely in greenhouses in the middle eastern countries for their response to ZYMV. The aim was to identify ZYMV-resistant cucumber cultivars by estimating and comparing the performance of 12 inoculated cultivars.

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MATERIALS AND METHODS

Planting and inoculation procedures. Seeds of cucumber cultivars Ambru, Arabio, Banza, Cordito, Dara, Dina, Farol, Figaro, Meta, Picobello, Sahara, and WSM-75 were germinated in petri plates on wet filter paper and directly transplanted to the soil in a commercial greenhouse. Twelve seedlings were grown for each cultivar, out of which six were inoculated with sap from ZYMV-infected cucumber plants and six were inoculated with sap from healthy plants, as controls. Cultivation (i.e., spacing, irrigation, fertilization, and all other cultural practices such as training the plants to a single stem supported by a string to an overhead wire) was done according to the standardized methods for growing cucumber in the greenhouse (3,23). The ZYMV isolate used in this experiment was detected in Saudi Arabia (4). This isolate is biologically similar to the original isolate in the host range, except that this isolate did not infect *Gomphrena globosa* L. (4). ZYMV inoculum was prepared by grinding infected symptomatic cucumber leaves harvested from previously inoculated plants in a precooled blender for 1 to 2 min with 0.02 M potassium phosphate buffer, pH 7.0 (1:5 wt/vol). The sap then was filtered through cheesecloth and maintained cool on ice. The mechanical inoculation was made at the three- to four-true leaf stage on Carborundum-dusted leaves. Daily observations were recorded throughout the 4-month duration of each experiment. In all practices, control plants were handled before the infected ones to avoid the accidental mechanical inoculation of these controls. Four parameters were used to evaluate the response of each cultivar to the virus: the type of symptom recorded 4 weeks postinoculation, plant height, fruit number, and fruit weight. The biweekly fruit picking, which started for the early cultivars 6 weeks after planting, continued through the end of the experiment. Plant height was determined by measuring the length of the remaining single shoot 14 weeks postinoculation after plants were trained to a single stem (3,23). The experiment was repeated twice.

In a later experiment, Dina and Farol plants were mechanically inoculated with ZYMV at the cotyledon stage by the methods previously described. Daily observations also were recorded, and samples of the cotyledons and leaves were collected and tested by enzyme-linked immunosorbent assay (ELISA) for the presence of ZYMV.

Data analysis. Data were analyzed as a one-way analysis of variance, and differences between the control and inoculated plants were measured for each of the indicated parameters with the LSD (17). The plant height, fruit number, and weight from the inoculated plants also were expressed as percentages, using the values from the control plants as references to facilitate the comparison of the differences inherent to the cucumber cultivars. For each parameter, the analysis was done after the percentages of reduction were transformed to arcsine according to Little and Hills (17). Duncan's multiple range test then was used to separate the means in percent reductions among the different cultivars for the previously mentioned parameters following their inoculation with ZYMV.

Recovery of ZYMV from Dina. Recovery was made by two methods:

(i) Inoculation of Farol, a susceptible cultivar, with Dina sap. The uppermost younger leaves collected from Dina plants inoculated with ZYMV 4 weeks previously were ground in a mortar and pestle using 0.02 M phosphate buffer, pH 7.0 (1:5 wt/vol). Seedlings of Farol, which were grown in pots in the greenhouse, were dusted with Carborundum and mechanically inoculated with the infected leaf extract from Dina. Another set of seedlings of the same cultivar was inoculated in the same way with leaf extract prepared from symptomatic leaves of Farol (as control). These two sets of plants, along with

two other similar sets that were inoculated with extract of healthy plants of each of the two cultivars in the same buffer, were maintained in the greenhouse. Observations were recorded for 6 weeks after inoculation.

(ii) ELISA. A commercial ELISA kit was purchased from Agdia, Inc. (Elkhart, IN) for the detection of ZYMV in the two cultivars, Dina and Farol. Leaf samples from noninoculated healthy as well as inoculated cucumber plants of the two cultivars were processed as described and used in the ELISA procedure suggested by the manufacturer, which is an indirect modification of the method described by Clark and Adams (9). Sample extracts were diluted 1:10; IgG was diluted 1:100. Peroxidase conjugated IgG (diluted 1:4 in PBST buffer) together with the substrate O-phenylenediamine (OPD) were used in the test. Absorbance of each sample at 490 nm was read on a Dynatech ELISA plate reader. A test was considered positive when its absorbance was more than twice that of the healthy controls.

RESULTS

Symptoms. Typical ZYMV symptoms (4,16) were observed on 11 of the 12 inoculated cucumber cultivars (Table 1) and were recorded in the early stages of the experiment. Symptoms that started to show up on the affected cultivars 10 days postinoculation became very conspicuous 4 weeks later at the time of their final recording. On the other hand, the cultivar Dina did not show symptoms even after repeated inoculations of the same plants or other lots of Dina plants. In general, severe effects were observed on the infected cultivars, since ZYMV induced striking symptoms, including mottling, mosaic, blistering, stunting, distortion, and chlorosis on the foliage (Table 1) and mottling, mosaic, and distortion of fruits. A variety of symptoms was observed on the cultivars, indicating a variable response to this virus isolate, thus enabling the classification of the cultivars into five different groups. No foliage or fruit symptoms were observed on cultivar Dina throughout the 16 weeks of the experiment. No symptoms were observed on the control plants throughout the experiment.

Plant height. Comparing the heights of inoculated and control plants for each cultivar showed that ZYMV caused statistically significant stunting for 11 cultivars (Table 2). However, no height reduction was observed for the cultivar Dina.

The reduction in plant height of these 11 cultivars ranged between 15.7 and 54.5%. Significant differences in the percent reductions of the plant height were observed when the tested cultivars were compared. Dina height was not reduced at all. The least affected cultivar was Dara and the most affected ones were Figaro, WSM-75, Farol, and Arabio. The other cultivars performed in between.

Fruit numbers. ZYMV also caused a reduction in the number of fruits produced by each of the inoculated cucumber cultivars, except for Dina, which did not show any reduction (Table 3). Significant differences were observed between inoculated and control plants of each cultivar. The percentages of reduction in fruit number exceeded 50% in all the affected cultivars, but no significant differences were found among cultivars.

Fruit weight. Inoculation of cucumber cultivars with ZYMV significantly reduced the average fruit weight per plant in all cultivars except Dina (Table 4). The average fruit weight reduction for the affected cultivars ranged between 64 and 85.3%. The cultivar Dina was not affected; the cultivar Meta showed the least loss; and the cultivar Farol was the most affected. Significant differences in percent reduction of fruit weight were found among cultivars (Table 4).

Virus recovery tests. *(i) Inoculation of Farol, a susceptible cultivar, with Dina sap.* None of the Farol seedlings inoculated with extract prepared from the uppermost leaves of ZYMV-inoculated Dina plants showed any symptoms. However, the set of plants inoculated with the leaf extract of ZYMV-inoculated Farol plants showed mosaic symptoms within 2 weeks. The other sets of Farol seedlings, which were inoculated with leaf extract of healthy cucumber plants of each of the two cultivars separately, did not show any symptoms.

(ii) ELISA. Negative results were obtained when extract from the upper leaves of Dina plants (inoculated at the three- to four-leaf stage with ZYMV) were tested by ELISA. OD_{490} values of samples from healthy and inoculated Dina plants were identical (0.01) and much lower than the OD_{490} values from the cultivar Farol (0.24), indicating lack of multiplication of this virus in Dina.

Inoculation of Dina and Farol at the cotyledonous stages. Inoculation of the cotyledons and the first true leaves of cultivars Farol and Dina revealed striking symptoms on all leaves of Farol but sparse, mottlelike symptoms on only the first and

Table 1. Symptoms developed by 12 cucumber cultivars inoculated with zucchini yellow mosaic virus (ZYMV) at the three- to four-leaf stage

Group[y]	Cultivar	Leaf symptoms[z]
1	Dina	No symptoms
2	Meta	Mosaic, blistering, distortion
3	Dara Sahara	Mottle, mosaic, stunting, chlorosis
4	Picobello Cordito Arabio	Mottle, mosaic, blistering, stunting, distortion
5	Ambru Banza Figaro Farol WSM-75	Mottle, mosaic, blistering, stunting, distortion, chlorosis

[y] The 12 cultivars were classified into five groups according to the five different symptom patterns observed.
[z] Leaf symptoms were recorded 4 weeks postinoculation.

Table 2. Means of plant heights (cm) and comparison of the percent reduction of 12 greenhouse-grown cucumber cultivars inoculated with zucchini yellow mosaic virus (ZYMV)

Cultivar[x]	Control[y]	Inoc.[y]	Reduc. (%)[z]
Dina	314.5 a	338.3 a	00.0 a
Dara	265.3 a	198.3 b	15.7 b
Ambru	282.3 a	212.9 b	23.6 bc
Cordito	304.6 a	192.0 b	35.2 bc
Picobello	271.1 a	151.8 b	36.3 bcd
Meta	306.3 a	171.8 b	42.2 bcd
Sahara	320.1 a	168.4 b	43.2 cd
Banza	310.1 a	164.6 b	43.9 cd
Figaro	248.7 a	127.0 b	48.3 d
WSM-75	269.2 a	126.0 b	50.9 d
Farol	253.0 a	112.1 b	52.7 d
Arabio	259.9 a	118.1 b	54.5 d

[x] For each treatment, six plants were used per cultivar, and data were recorded 14 weeks postinoculation.
[y] For each cultivar, means followed by the same letter in the control and inoculated columns are not significantly different ($P = 0.05$) according to LSD test.
[z] Average percent reduction for the cultivars followed by the same letter in the percent reduction column are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

Table 3. Means of fruit numbers per plant and comparison of the percent reductions of 12 greenhouse-grown cucumber cultivars inoculated with zucchini yellow mosaic virus (ZYMV)

Cultivar[x]	Control[y]	Inoc.[y]	Reduc. (%)[z]
Dina	37.8 a	39.3 a	00.0 a
Cordito	51.3 a	22.7 b	52.8 b
Meta	38.6 a	15.0 b	54.4 b
Dara	35.6 a	15.1 b	56.4 b
Ambru	28.7 a	10.6 b	57.7 b
Picobello	48.8 a	16.6 b	60.2 b
Banza	45.6 a	15.4 b	61.6 b
Arabio	40.6 a	13.4 b	68.4 b
Figaro	43.9 a	11.9 b	69.7 b
Sahara	43.8 a	07.3 b	71.5 b
WSM-75	41.1 a	10.9 b	74.0 b
Farol	50.3 a	12.1 b	79.4 b

[x] For each treatment, six plants were used per cultivar, and fruit picking was performed twice a week throughout the experiment.
[y] Means followed by the same letter in the control and inoculated columns for each cultivar are not significantly different ($P = 0.05$) according to LSD test.
[z] Average percent reduction for the cultivars followed by the same letter in the percent reduction column are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

second expanded leaves of Dina. ELISAs of samples collected from these two cultivars indicated that virus concentration was higher in Farol than in Dina and that, whereas the virus was detected in the cotyledons and all leaves of Farol, it was detected only in the cotyledons and the first three expanded leaves of Dina (Table 5). It also was noted that virus concentration decreased upward in Dina, and no virus was detected by ELISA beyond the third expanded leaf. No such pattern of decrease in virus concentration was observed in Farol.

DISCUSSION

This study shows that the isolate of ZYMV from Saudi Arabia infected all tested cucumber cultivars except Dina when inoculation took place at the three- to four-leaf stage. This ZYMV isolate also caused severe foliage and fruit symptoms, significant plant stunting, and reductions in fruit number and weight. These data indicated that 11 tested cultivars are susceptible to this ZYMV isolate, whereas Dina is the only resistant one.

Most of the previous investigations on resistance to ZYMV were carried out with cucurbits other than cucumber. Nameth et al. (19) tested the response of different cultivars of cantaloupe, mixed melon, and watermelon to a California isolate of ZYMV. Wickizer et al. (26) and Dolores and Valdez (12) tested the response of different squash cultivars to isolates of the same virus. Our study was conducted to provide information on the response of greenhouse-grown cucumber cultivars to ZYMV.

Table 4. Means of fruit weight (g/plant) and comparison of the percent reductions of 12 greenhouse-grown cucumber cultivars inoculated with zucchini yellow mosaic virus (ZYMV)

Cultivar[x]	Control[y]	Inoc.[y]	Reduc. (%)[z]
Dina	4096.4 a	4388.3 a	00.0 a
Meta	4046.8 a	1179.0 b	64.0 b
Dara	3308.0 a	1111.9 b	65.4 bc
Picobello	4725.6 a	1234.4 b	65.9 bc
Cordito	6221.5 a	1903.1 b	66.9 bc
Ambru	2690.5 a	0702.7 b	71.6 bc
Banza	4403.3 a	1165.9 b	71.8 bc
Sahara	4609.3 a	0937.4 b	72.5 bc
Arabio	4362.8 a	1050.5 b	72.5 bc
Figaro	4291.5 a	0775.3 b	79.4 bc
WSM-75	4184.3 a	0819.2 b	80.4 bc
Farol	3631.9 a	0545.0 b	85.3 c

[x] For each treatment, six plants were used per cultivar.
[y] Means followed by the same letter in the control and inoculated columns for each cultivar are not significantly different ($P = 0.05$) according to LSD test.
[z] Average percent reduction for the cultivars followed by the same letter in the percent reduction column are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

The effect of virus diseases on cucurbits has been of interest for decades, and several studies were conducted on quantitative losses inflicted by viruses on cucurbits prior to the discovery of ZYMV (7,11,13). Subsequent to ZYMV discovery, numerous studies indicated the importance of ZYMV and its threat to the cucurbit industry (4,20,22,25). However, all of these studies were qualitative except one. In the one quantitative study, ZYMV was reported to cause as much as 94% reduction of marketable cantaloupe (8).

To our knowledge, the present study is one of the few that present quantitative data on the effect of ZYMV on cucurbits. The crop losses caused by ZYMV in cucumber in this study ranged between 64 and 85.3% for the affected cultivars. It is worth mentioning that the data were recorded whether fruits were marketable or not. If the nonmarketable fruits were excluded, the losses would have been much greater, since more than 95% of the fruits harvested from the infected plants were nonmarketable. It is clear from the control plants in Table 4 that some cultivars, such as Cordito, Picobello, and Farol, performed better than Dina. However, after ZYMV infection, Dina was the only cultivar producing marketable fruits.

Our study shows the different responses to ZYMV infection of 12 widely grown cucumber cultivars in the Middle East and reports on the resistance of the cucumber cultivar Dina to this isolate of ZYMV. Our initial bioassays and ELISA showed that there was no latent infection in the cultivar Dina, suggesting a lack of multiplication of this ZYMV isolate in the top leaves of cultivar Dina that were tested by ELISA. These results also lead us to assume that Dina could probably be immune to the ZYMV isolate from Saudi Arabia.

When inoculation took place on the cotyledons or the first two expanded leaves, sparse symptoms that were limited to only a few of the lower leaves were observed, and no virus detection by ELISA was achieved beyond the third true leaf. These results suggest that Dina, which appeared to be immune when inoculated at the three- to four-leaf stage, is infectible at the second expanded leaf stage or earlier and hence is considered resistant rather than immune. The results of this investigation are therefore similar to those obtained by Provvidenti et al. (22) in their efforts to obtain sources of resistance for isolates of the same virus. In this study, cultivar Dina performed similarly to the Chinese cucumber cultivar TMG-1, which initially appeared to be free of symptoms but later developed a sparse, systemic veinal chlorosis that was limited to a few basal leaves. Also, based on the preliminary results of the reaction of these cucumber cultivars to ZYMV (5), Abul Hayja and Al-Shahwan conducted further investigations (1), in which they indicated that the resistance in Dina is of a recessive nature, similar to that in TMG-1, which was reported by Provvidenti (21) in his crosses between TMG-1 and domestic susceptible cucumber cultivars.

Since the results of inoculation of Dina with the Saudi isolate of ZYMV at an early stage of growth resembled those of the inoculation of TMG-1 with the American isolates of the same virus (21), it will be interesting to compare the response of each of the two resistant cultivars, Dina and TMG-1, to the Saudi as well as to the American isolates of this virus. It also will be interesting to determine the mechanism in cultivar Dina that prevents detection of ZYMV by ELISA beyond the third expanded leaf. It is possible that virus multiplication is reduced or the upward translocation of the virus is curtailed. Future studies on the serological comparison of this isolate with the well-characterized isolates together with the comparison of the pathological impact of these isolates on cucumber and other cucurbits will be useful.

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Table 5. Symptoms and average enzyme-linked immunosorbent assay (ELISA) absorbances of different leaf samples of cultivars Farol and Dina inoculated with zucchini yellow mosaic virus (ZYMV) at the cotyledon stage

Leaf age at sampling	Farol		Dina	
	Symptoms[y]	490 nm value[z]	Symptoms[y]	490 nm value[z]
Cotyledons	+	>2.25	+	0.68
First true leaf	+	1.30	+	0.51
Second true leaf	+	1.23	+	0.52
Third true leaf	+	1.63	–	0.22
Fourth true leaf	+	0.96	–	0.00

[y] + = symptoms, – = no symptoms.
[z] Absorbance for control plants of each cultivar averaged (0.00) and the absorbance for the control as well as the inoculated plants of each leaf age were averages of eight ELISA plate well readings.

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