Survey of Virus Diseases of Cucurbits in the Batinah Region of the Sultanate of Oman

A.A. Zouba, A.J. Khan, M. Lopez and Y.M. Al-Maqbaly
Department of Plant Sciences, College of Agriculture, Sultan Qaboos University, P.O. Box 34, Al-Khob 123, Sultanate of Oman.

Abstract

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A survey of cucurbit virus diseases in the Batinah region was conducted during the 1994/95 and 1995/96 growing seasons. A total of 320 commercial cucurbit fields were visited between October and April. The disease incidence of various cucurbit species was determined by randomly examining 100 plants in each field. A total of 716 symptomatic samples were collected from 320 squash, watermelon, muskmelon, cucumber, pumpkin and bottlegourd fields. Samples were tested for the presence of viruses by enzyme-linked immunosorbent assay (ELISA). The average disease incidence at harvesting time, as assessed by symptomatology, ranged from 34.2 to 78.1% during the 1994/95 growing season and 25.6 to 75% during the 1995/96 season. Pumpkin, squash and bottlegourd showed relatively high incidence of viral diseases. Results of ELISA tests, obtained by using 8 antisera, revealed the presence of watermelon mosaic virus 2 (WMV-2), zucchini yellow mosaic virus (ZYMV), papaya ring spot virus (watermelon strain) (PRSV-W), cucumber mosaic virus (CMV), squash mosaic virus (SqMV), tomato ring spot virus (ToRSV), tobacco ring spot virus (TRSV) and tomato spotted wilt virus (TSWV). WMV-2 and ZYMV were the most frequently found viruses. They were detected in all cucurbit species throughout the Batinah region. Over the two seasons of this study, 46% of the infected samples collected harboured more than one virus.

Key words: Viruses, cucumber, squash, watermelon, pumpkin, bottlegourd.

Introduction

Cucurbit crops including watermelon (*Citrullus lunatus* (Thunb) Mansf.), cucumber (*Cucumis sativus* L.), muskmelon (*Cucumis melo* L.), squash (*Cucurbita pepo* L.), pumpkin (*Cucurbita pepo* L.) and bottlegourd (*Lagenaria siceraria* L.) are among the most popular and widely grown vegetables in the Sultanate of Oman. Losses due to virus diseases have become an important limiting factor in commercial production of cucurbits. The cultivated area has dropped by approximately 50% from 1990 to 1994 partly due to disease problems (1). The successive cropping of cucurbits, occurrence of high insect populations throughout the year and lack of efficient pest management practices favoured the build up and spread of viral diseases.

Cucurbit crops are known to be attacked by many plant viruses throughout the world (2). In the Sultanate of Oman, a survey conducted by the Ministry of Agriculture and Fisheries in 1991/92 (2) revealed the presence of cucumber mosaic virus (CMV), watermelon mosaic virus 2 (WMV-2), zucchini yellow mosaic virus (ZYMV), squash mosaic virus (SqMV), watermelon chlorotic stunt virus (WCSV) and other unspecified geminiviruses. In the United Arab Emirates, a neighboring country of Oman, a whitefly-transmitted closterolike virus appears to be the cause of widespread occurrence of yellowing and stunting disorder of cucurbit crops (4). The present study was undertaken to further assess the occurrence, incidence and distribution of cucurbit virus diseases in the Batinah region which constitutes the major production area for cucurbits in the Sultanate of Oman.

Materials and Methods

A survey of viruses infecting bottlegourd, cucumber, muskmelon, pumpkin, squash and watermelon was conducted from October to April during the 1994/95 and 1995/96 growing season. A total of 320 commercial cucurbit fields in various localities of the Batinah region were visited during both seasons. Each locality was visited at least twice per season. Field surveys were made at harvesting time by examining 100 plants randomly selected along two diagonals across in each field. The number of plants showing virus like symptoms such as mosaic, mottling, chlorosis, malformation and stunting were recorded and used to determine the disease incidence. Leaf samples of diseased plants with a wide ranged of symptoms were collected in plastic bags and taken to the laboratory for virus identification. During the 1994/95 growing season, 491 plant samples were collected from 171 cucurbit fields while during the 1995/96 growing season, 225 plant samples were collected from 149 cucurbit fields. All samples were tested for the presence of cucurbit viruses by enzyme linked immunosorbent assay (ELISA) (3).

ELISA test: During the 1994/95 season, samples were assayed for the presence of cucumber mosaic virus (CMV), squash mosaic virus (SqMV), watermelon strain of papaya ring spot virus (PRSV-W), zucchini yellow mosaic virus (ZYMV) and watermelon mosaic virus 2 (WMV-2). During 1995/96 season, in addition to the above viruses samples were also tested for the presence of tobacco ring spot virus (TRSV), tomato ring spot virus (ToRSV) and tomato spotted wilt virus (TSWV). The diagnostic kit of ELISA against these viruses were obtained from Agdia Inc., (Elkhart, IN, USA). CMV, SqMV, TRSV, ToRSV and TSWV were assayed by using the direct ELISA procedure (3). Plates were coated with 100 ul per well of prepared antibody and incubated at 4°C overnight, washed with wash buffer. 100 µl of prepared plant samples per well was dispensed followed by incubation overnight at 4°C. Plates were washed thoroughly with wash buffer then coated with 100 µl/well of prepared enzyme conjugate and incubated for 2 hr at room temperature. After washing, 100 µl/well of prepared substrate (1 mg/ml p-Nitrophenyl phosphate in

substrate buffer) was dispensed. Plates were incubated for 60 min at room temperature and optical density was recorded using a Bio-Tek Ceres UV900C microplate reader at 405 nm and 630 nm as reference filters. For ZYMV and WMV-2, samples were assayed by indirect ELISA method (5, 6). Plates were coated with 100 µl/well of prepared plant sap, incubated for 1 hr at room temperature, washed and dispensed with 100 µl/well of prepared antibody and incubated overnight at 4°C. After washing, plates were coated with 100 µl/well of prepared enzyme conjugate, incubated for 1 hr at room temperature followed by washing. It was dispensed with 100 µl/well of prepared enzyme substrate as described above. Plates were incubated for 1 hr at room temperature and optical density was recorded on a microplate reader at 405 nm.

Results

Disease incidence and field symptoms: The average percentage of plants showing symptoms in cucurbit fields at harvesting stage ranged from 34.2 to 78.1% and 25.6 to 75% in the 1994/95 and 1995/96 growing seasons, respectively (Table 1). During the first season, squash showed the highest disease incidence (78.1%) followed by pumpkin, bottlegourd, cucumber, watermelon and muskmelon whereas during the second season, pumpkin showed the highest disease incidence (75.1%) followed by bottlegourd, squash, cucumber, muskmelon and watermelon. Field symptoms are most severe on squash and watermelon. Affected squash plants usually become stunted, develop chlorotic deformed and rugose leaves with severe vein distortion, consequently producing less and smaller fruits. On the other hand, affected watermelon plants produce chlorotic symptoms, severely stunted, developing shoots with small leaf curling downward, reduced fruit set, and malformed developing fruits. Bottlegourd and pumpkin plants develop essentially mosaic and mottle symptoms. Affected cucumber and muskmelon are stunted, and generally develop chlorotic mottle symptoms with occasionally diffused chlorotic spots scattered on the lamina.

Virus identification: During the 1994/95 season viruses were detected in 77.3% of the symptomatic samples collected form the Batinah region. ELISA tests revealed the presence of ZYMV, WMV-2, PRSV-W, CMV and SqMV (Table 2). ZYMV was the most frequently detected virus, with WMV-2, PRSV-W, CMV and SMV occurring in frequencies in descending order. Of the 389 virus-detected samples, 145 were infected with a single virus, 168 with a mixture of two viruses and 76 with a mixture of three viruses or more. A mixture of ZYMV and WMV-2 was found in 85% of the samples infected with more than one virus. The frequency of detection of the 5 viruses was variable with the cucurbit species. ZYMV was most frequently found on muskmelon, WMV-2 and CMV on squash, PRSV-W and SqMV on watermelon. During the 1995/96 season, viruses were detected in 72% of the collected symptomatic samples. In addition to the five previously detected viruses, three more were identified, namely: TRSV, ToRSV and TSWV. WMV-2 was found to be the most frequent virus with SqMV, CMV, ZYMV, PRSV, TRSV, ToRSV and TSWV occurring in frequencies in descending order (Table 3). of the 163 samples found to be virus infected, 104 were infected with a single virus, 26 with a mixture of two viruses and 23 with a mixture of 3 viruses or more. The highest incidence of CMV, SqMV, TRSV and TSWV was found on bottlegourd, PRSV-W and ZYMV on pumpkin, ToRSV on squash and WMV-2 on watermelon.

Table 1. Incidence of viral diseases, at harvesting stage in various cucurbit species as assessed by symptomatology (1994/95 and 1995/96 growing seasons).

	1994/95 season			1995/96 season		
Cucurbit species	Number of fields surveyed	Range of % plant showing symptoms	Average of % plants showing symptoms	Number of fields surveyed	Range of % plant showing symptoms	Average of % plants showing symptoms
Watermelon (*Citrullus lunatus*)	13	0 - 70	35.5	10	3 - 100	25.6
Cucumber (*Cucumis sativus*)	12	7 - 100	44.0	18	8 - 100	60.4
Muskmelon (*Cucumis melo*)	12	1 - 65	34.2	3	5 - 100	53.7
Squash (*Cucurbita pepo*)	14	26 - 100	78.1	22	4 - 100	62.5
Pumpkin (*Cucurbita pepo*)	7	9 - 100	62.2	10	10 - 100	75.1
Bottle gourd (*Lagenaria siceraria*)	27	0 - 100	52.2	38	3 - 100	69.1

Table 2. Viruses detected in various cucurbit species from samples collected during 1994/95 season as determined by ELISA

Cucurbit species	No. of samples infected/ No. of samples tested	Virus detected				
		CMV	SqMV	PRSV-W	ZYMV	WMV-2
Watermelon (*Citrullus lunatus*)	101/120	35	6	52	97	68
Cucumber (*Cucumis sativus*)	50/56	14	2	16	45	23
Muskmelon (*Cucumis melo*)	83/100	13	2	19	81	36
Squash (*Cucurbita pepo*)	35/48	16	0	13	31	27
Pumpkin (*Cucurbita pepo*)	30/48	7	2	7	26	10
Bottle gourd (*Lagenaria siceraria*)	90/119	24	2	29	69	50

Table 3. Virus detected in various cucurbit species from samples collected during 1995/96 season as determined by ELISA

Cucurbit species	No. of samples infected/ No. of samples tested	Virus detected							
		CMV	SqMV	PRSV-W	TRSV	ToRSV	TSWV	ZYMV	WMV-2
Watermelon (*Citrullus lunatus*)	42/48	3	11	1	2	1	0	6	40
Cucumber (*Cucumis sativus*)	26/32	0	0	4	2	3	0	2	19
Muskmelon (*Cucumis melo*)	6/14	0	0	0	0	0	0	0	5
Squash (*Cucurbita pepo*)	22/41	6	4	1	3	6	0	6	15
Pumpkin (*Cucurbita pepo*)	24/34	6	0	5	0	0	0	12	17
Bottle gourd (*Lagenaria siceraria*)	43/56	19	20	8	8	4	5	6	36

Virus distribution: The occurrence and distribution of cucurbit viruses in the Batinah region varied with the locality and the growing season (Tables 4 & 5). During the 1994/95 season, ZYMV, WMV-2, PRSV-W and CMV were detected in all localities. SqMV was detected in three localities namely Suweiq, Khaboura and Liwa. ZYMV and WMV-2 were the predominant viruses in all localities. Suweiq showed the highest incidence of CMV, PRSV-W, ZYMV and WMV-2 (Table 4). During the 1995/96 season, WMV-2 and SqMV showed the largest distribution with WMV-2 being the most prevalent virus. ToRSV and TSWV showed a limited distribution since they were detected in only 4 and 2 localities, respectively (Table 5).

Table 4. Occurrence of cucurbit viruses in the major agricultural localities of the Batinah region during 1994/95 season.

Locality	No. of samples infected / No. tested	Virus detected				
		CMV	SqMV	PRSV -W	ZYMV	WMV-2
Barka	43/46	8	0	17	36	36
Musana	26/52	1	0	5	22	5
Suweiq	72/79	65	4	55	72	71
Khaboura	62/72	9	4	2	57	43
Saham	66/77	11	0	13	56	17
Sohar	48/48	3	0	4	48	34
Liwa	28/28	1	0	10	28	11
Shinas	39/40	4	1	14	39	7

Discussion

This study showed that cucurbit viral disease are widely distributed throughout the Batinah region. High disease incidence reaching 100% has been commonly observed in commercial cucurbit fields. Although the use of the crop cover (Agril) has become a common practice to protect squash, melon, cucumber and watermelon crops from insect vectors (37% of the visited fields have been covered for an average period of 33 days), the incidence of viral disease remains high, ranging between 35.4 and 78% during the 1994/95 season and between 25.6 and 71.1% during the following season. During both seasons squash, pumpkin and bottlegourd showed relatively high disease incidence (Table 1). Watermelon consistently had the lowest disease incidence but inspite of it, yield of infected plants was highly reduced due to severity of symptoms.

In addition to ZYMV, CMV, WMV-2 and SqMV which have been previously reported by the Ministry of Agriculture and Fisheries (7), four other viruses were serologically identified for the first time in Oman namely: PRSV-W, ToRSV, TRSV and TSWV. WMV-2 and ZYMV were the most frequently found viruses, which were detected at high frequencies in all cucurbit species and localities. Season-wise, ZYMV was more frequently found during the first season of this study since it was detected in 89.7% of the infected samples against 19.4% during the second season. CMV and PRSV-W were detected throughout the Batinah region but were less frequently isolated than WMV-2 (Tables 2 & 3). SqMV showed a wider distribution during the second season of study, and its detection in diseased watermelon plants suggests its similarity to the Arizona strain of SqMV (8). The occurrence and distribution of ToRSV, TRSV and TSWV are still limited as the percent detection in infected samples was 9, 8, 9 and 3%, respectively. TSWV was detected from bottlegourd and watermelon (preserved watermelon samples collected during 1994/95). Systemic infection of cucurbits by TSWV was reported to be caused by a specific watermelon strain of TSWV (9). Over the two seasons of the study, 46% of infected samples were harbouring more than one virus. This situation, coupled with the existence of several cucurbit viruses, make field symptoms of little diagnostic value.

Viruses were not detected in 20.8 and 27.5% of the symptomatic cucurbit samples collected in 1st and 2nd year of the study, respectively. This may indicate the presence of other unidentified viruses. Preliminary transmission tests showed that the whitefly (*Bemisia tabaci*) was able to transmit the disease from some of the watermelon, squash and cucumber field collected samples to healthy plants of the same species. Whitefly-transmitted virus has been previously recorded in Oman by the ministry of Agriculture and Fisheries (7). Electron microscopic investigations of some selected specimens revealed the presence of virus particles of about 300-320 nm long in association with diseased bottlegourd plants. Further investigation of these viruses is in progress.

Table 5. Occurrence of cucurbit viruses in the major agricultural localities of the Batinah region during 1995/96 season.

Locality	No. of samples infected/ No. of samples tested	Virus detected							
		CMV	SqMV	PRSV-W	TRSV	ToRSV	TSWV	ZYMV	WMV-2
Barka	24/30	3	8	6	5	7	0	9	17
Musana	12/20	8	3	2	0	2	0	3	6
Suweiq	23/29	7	2	0	0	0	0	4	17
Khaboura	19/22	0	3	3	0	0	0	2	18
Saham	19/26	4	4	1	1	4	0	4	15
Sohar	21/30	2	6	2	4	2	2	2	18
Liwa	11/16	4	6	2	2	0	3	3	6
Shinas	15/20	0	6	0	1	0	0	0	15

الملخص

زوبا، علي، أ. ج. خان، م. لوبيز، و ي. م. المقبلي. 1997. حصر للأمراض الفيروسية التي تصيب القرعيات في منطقة الباطنة بسلطة عمان. مجلة وقاية النبات العربية. 15 (1): 43-46.

نفذ حصر لأهم الأمراض الفيروسية التي تصيب القرعيات في منطقة الباطنة بسلطنة عمان وذلك في الموسيمن الزراعيين 95/1994 و 96/1995. تمت زيارة 320 حقل قرعيات خلال الفترة الممتدة من تشرين الأول/ أكتوبر إلى نيسان/ أبريل. قدرت نسبة الإصابة في كل حقل من خلال فحص 100 نبتة أختيرت بطريقة عشوائية. جمعت خلال الحصر 716 عينة، شملت محاصيل الكوسة، الجح، الشمام، الخيار، اليقطين والقرع القناني، تبدي أعراضاً فيروسية. تم إختبار العينات بطريقة إليزا (ELISA) باستعمال ثمانية أمصال مناعية. تراوح متوسط نسبة الإصابة، خلال فترة جني الثمار، ما بين 34.2 و 78.1% خلال الموسم الزراعي 95/1994 وما بين 25.6 و 75% خلال الموسم الزراعي 96/1995. ظهرت أكبر نسبة إصابة على محاصيل اليقطين، الكوسة والقرع القناني. أظهرت نتائج اختبار اليزا وجود فيروس موزاييك الجح 2 (WMV-2)، فيروس الموزاييك الأصفر للكوسة (ZYMV)، فيروس التبقع الحلقي للفافيا سلالة الجح (PRSV-W)، فيروس موزاييك الخيار (CMV)، فيروس موزاييك الكوسة (SqMV)، فيروس التبقع الحلقي للطماطم/ البندورة (ToRSV)، فيروس التبقع الحلقي للتبغ (TRSV) وفيروس تبقع الذبول للطماطم/ البندورة (TSWV). كان فيروس موزاييك الجح 2 والموزاييك الأصفر للكوسة من أكثر الفيروسات انتشاراً حيث وقع التعرف عليها في جميع أجزاء الباطنة وعلى كل محاصيل القرعيات. وشكلت العينات التي احتوت على أكثر من فيروس نسبة 46%.

كلمات مفتاحية: فيروسات، الكوسة، الجح، الشمام، الخيار، اليقطين، القرع.

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