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Studies on Spread and Identification of Zucchini Yellow Mosaic Virus Disease in the North-West Mediterranean Region of Turkey by Biological Indexing and Double-stranded RNA Analysis

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Abstract: Leaf and fruit samples showing symptoms of zucchini yellow mosaic virus (ZYMV) disease were collected from cucurbit fields in Isparta province in North-West Mediterranean region of Turkey. Mechanical inoculations on test plant were made by conventional leaf-inoculation method. The virus was multiplied in squash plant (*Cucurbita pepo* (L.)) and showed systemic symptom. Double-stranded RNA (dsRNA) was purified by CF-11 cellulose chromatography. Agarose gel electrophoresis indicated that dsRNA was present in infected squash plants. The disease rate of ZYMV was determined by counting the plants showing the disease symptoms in 20 cucurbit fields in Isparta. Based on our surveys the infection ZYMV was determined at the rate of 62.70% in the squash field.

Key words: Zucchini yellow mosaic virus, mechanical inoculation, survey, double-stranded RNA, agarose gel

INTRODUCTION

Cucurbitacea contribute about 40% of total vegetable production in Turkey. Cucurbits are produced with an annual yield of about 7.5 million ton mostly in Mediterranean region of Turkey. Vegetable production in Isparta is steadily increasing due to the developments of appropriate growing techniques. The total cucurbit growing area in this region is about 1500 ha with an annual production of 22.000 ton. It is one of the most important vegetables in this area[1].

Diseases are main problems in the production of cucurbit plants. More than 200 diseases on cucurbit plants cause serious losses in terms of yield and quality[2]. Viral diseases are more serious for cucurbitaceous plants compared to diseases caused by other agents. Symptoms of viral infections on Cucurbitacea are mosaic, yellowing, stunting, chlorosis, leaf and fruit deformations[3,4].

The ZYMV limitation on the production of cucurbits was first determined in France and Italy in 1981. Afterwards, the presence of the virus was reported in Algeria, Australia, Egypt, Germany, Israel, Japan, Jordan, Lebanon, Morocco, Spain, Taiwan, England, Turkey and USA[5,6].

ZYMV was isolated from squash and watermelon plants grown throughout the Mediterranean coast in Turkey[7]. The virus was isolated and partially purified from diseased squash plants collected from Adana province[5]. ZYMV on zucchini field in Ankara was determined by biological indexing, serological tests and electron microscopy and on greenhouse squash in Antalya province[8,9].

The identification and severity of the viral diseases on squash fields in Isparta province has not been studied yet. In this area, symptoms resembling to ZYMV have been commonly observed on cucurbit plants. This study aimed to identify the virus that causes mosaics, deformations and flitoform structures on leaves and severe deformations, blisters on fruits on cucurbit plants by biological indexing and dsRNA analysis.

MATERIALS AND METHODS

Survey work: The survey was conducted in the province of Isparta in the North-West Mediterranean region of Turkey from May to September for 1998-1999. In total, 2231 squash plants from 20 commercial and home gardens were visually examined. Every single plant from each selected field was observed for ZYMV.

Mechanical inoculation tests: Young leaves from the original source plant which is obtained from squash field around the Faculty of Agriculture in Atabey County in Isparta was used as the inoculums source. These leaves

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were ground in a cooled porcelain mortar in 5 parts of cold 0.05 M potassium phosphate buffer, pH 7.2, containing 0.1% 2-mercaptoethanol. The extracts were inoculated to young, tender leaves of test plants with an absorbent cotton swab by carborundum leaf–inoculation method. After inoculation, plants were kept in an air- cooled and partly shaded greenhouse at temperature below 20°C.

Double-stranded RNA analysis: The method for dsRNA extraction was an adaptation of that described by Morris and Dodds[10]. dsRNA in STE (0.1 M NaCl, 0.05 M Tris-HCl, 1.0 mM EDTA, pH 6.8), were purified from phenol/chloroform/isoamyl (25:24:1)- treated, buffered extracts from 5 g of leaf tissue by two cycles of fractionation on columns of Whatman CF-11 cellulose in the presence of 16% ethanol. dsRNAs eluted from cellulose in ethanol free STE were concentrated by precipitation with two volumes of cold 95% ethanol and 0.1 volume of 3.0 M sodium acetate, pH 5.5 and resuspanded in 30 ul TE (10 mM tris, 1.0 mM EDTA) buffer. Aliquots of 3 ul were loaded onto 1% agarose gels in a horizontal slab gel apparatus in 40 mM tris, 20 mM sodium acetate, 1 mM EDTA, pH 7.8.

Molecular weight standard included lambda DNA digested with *hind* III. Electrophoresis was at constant voltage, 70 V for 1.5 h. Gel was stained in 20 ng ml^{-1} ethidium bromide, visualized on an UV transilluminator and photographed with Polaroid type 667 black and white films.

RESULTS AND DISCUSSION

Survey work: The results of surveys conducted for ZYMV in squash fields is summarized in Table 1. ZYMV infected squash plants were found in all surveyed field. An average of 62.7% of all squash plants revealed symptoms of ZYMV.

Field symptoms observed in this study were typical of ZYMV and consisted of yellowing, mosaic on the leaves and fliform leaf forming, stunting and blistering, cracking and distortion on the fruits in squash and other Cucurbitacea plants. Studies regarding determination of diseases ratios of virus diseases of cucurbitaceous plants in Turkey are limited. The ratio of viral diseases of squash plants in Amasya province was 19.6% in 1995[11]. Additionally, ZYMV was common in squash fields in Aegean, Mediterranean and middle Anatolia region of Turkey[8,12].

Mechanical inoculation: The symptoms obtained by using infected squash leaves and mechanical inoculations are given in Table 2. Tobacco (*Nicotiana tabacum* var. White Burley, *N. tabacum* var. Xanti, *N. glutinosa* (L.),

Table 1: Diseases ratio of zucchini yellow mosaic virus determined in squash (*Cucurbita pepo* (L.)) fields in Isparta province of Turkey in 1998-1999.

Field number	Number of total plants	Number of infected plants	Disease ratio %
1	108	101	93.51
2	100	97	97.00
3	126	122	96.82
4	85	30	35.29
5	98	42	42.85
6	27	22	81.48
7	46	23	50.00
8	48	31	64.58
9	39	34	87.17
10	59	44	74.57
11	78	73	93.58
12	354	122	34.46
13	63	60	95.23
14	52	52	100.00
15	291	122	41.92
16	46	14	30.43
17	60	40	66.66
18	50	25	50.00
19	309	182	58.89
20	192	163	84.89
Total	2231	1399	62.70

Table 2: Symptoms of zucchini yellow mosaic virus on the test plants

Test plants	Symptoms
Nicotiana tabacum var. White Burley	No symptom
N. tabacum var. Xanthii	No symptom
N. tabacum var. Xanthii	No symptom
N. glutinosa L.	No symptoms
N. rustica L.	No symptom
Chenepodium amaranticolor Coste & Reyn,	CLL
Chenepodium quinoa Wild.	CLL
Datura citramonium L.	No symptoms
Cucurbita maxima L.	Sev. Mos., Drying, Def.
C. pepo L.	Sev. Mos., Drying, Def.
Citrullus lanatus var. Paladin	St.
Cucumis mello var Hasanbey	Sev. Mos., Drying, Def.
C. melo var. Flexious	Mo., NLL, Drying
C. sativus L.	Mo., Drying, Def.
Phaseolus vulgaris var Sofia	Tip leaf def.
P. vulgaris var. Atlanta	Tip leaf def.
Vigna unguiculata L.	No symptoms
Pisum sativum L.	No symptoms

CLL: Chlorotic Local Lesion, Sev. Mos.: Severe Mosaic, Def.: Deformation, Mo.:Mosaic, St.:Stunting, NLL: Necrotic Local Lesion

N. rustica (L.) plants, *Pisum sativum* (L.), *Vigna unquiculata* (L.) and *Datura citramonium* (L.) did not show any symptoms. The virus caused symptoms on Cucurbitacea including *Cucurbita maxima* (L.), *Cucurbita melo* (L.), *Cucumis melo* (L.), *Cucumis melo* var. flexious, *Cucumis sativus* (L.), *Cucurbita pepo* (L.) and *Citrullus lanatus* var. Paladin and *Phaseolus vulgaris* var. Sofia, *Phaseolus vulgaris* var. Atlanta, *Vigna unguiculata* (L.) and *Chenopodium amaranticolor* Coste and Reyn, *Chenepodium quinoa* Wild. Chlorotic local lesions on *C. amaranticolor* and *C. quinoa* were observed 8-10 days after inoculation.

Severe mosaic symptoms, immediate leaf wilting and drying on the host plants including *Cucurbita maxima* (L.), *Cucurbita pepo* (L.), *Cucumis melo* var flexious,

Fig. 1: Symptoms of zucchini yellow mosaic virus on the leaves of *Cucurbita pepo* L., 10-12 days after inoculations

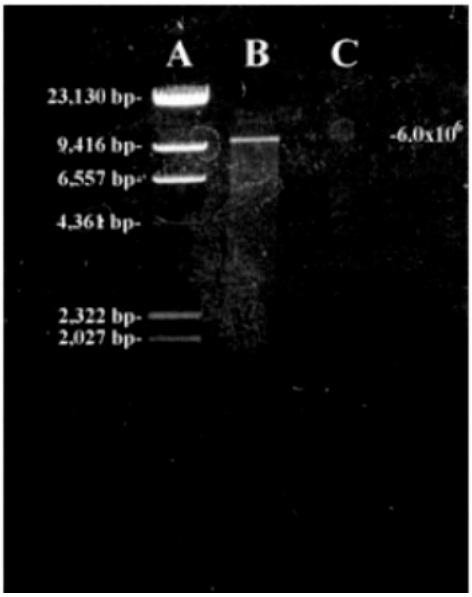

Fig. 2: Agarose gel electrophoresis (1%) of dsRNA from squash plant infected with zucchini yellow mosaic virus. Lanes: A, molecular weight standard lambda DNA hind III; B, zucchini yellow mosaic virus; C, healthy squash. Samples were analyzed by electrophoresis for 1.5 h at 70 V.

Cucumis sativus (L.) were observed in 10-12 days after inoculation (Fig. 1). The symptoms observed on the Cucurbitaceous plants were typical for those reported for ZYMV[6,13].

Since ZYMV is transmitted by aphids and vector aphids are common and widespread on vegetable fields in Isparta[14] may account for the high infection rate.

dsRNA analysis: Profile of dsRNA preparation is shown in Fig. 2. The dsRNA profiles of preparations from squash plants showed one virus specific dsRNA band of $6x10^6$ Da. MW, respectively, which is corresponded to the full length of replicative form of ZYMV[4]. However, healthy squash plant did not show any dsRNA band onto agarose gel. Agarose gel electophoresis of dsRNA aliquots equivalent to one-tenth of extracted dsRNA normally resulted in detectable dsRNA band.

Single-stranded RNA viruses compose approximately 90% of all known viruses. During their replication in plant cells, dsRNA is produced as an intermediate product. This dsRNA is called the replicative form (RF) and is consistently present when a plant is infected with an ssRNA virus, regardless of the host[15].

This technique is simple and relatively inexpensive. Results are obtained in a relatively short time. Technique detects mix infections, which often go undetected with other methods and result in inadequate diagnosis[16].

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