

# Occurrence of cucurbit viruses on field-grown melon and watermelon in the Thrace region of Turkey

## Occurrence des virus des cucurbitacées chez le melon et le melon d'eau dans la région de Thrace en Turquie

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Résumé de l'article

De juin à juillet 2005, une enquête visant à détecter le virus de la mosaïque du concombre (CMV), la souche W du virus de la tache annulaire de la papaye (PRSV-W), le virus de la mosaïque de la courge (SqMV), le virus de la tache nécrotique du melon (MNSV), le virus de la mosaïque à marbrure verte du concombre (CGMMV), le virus de la mosaïque jaune de la courgette (ZYMV) et le virus 2 de la mosaïque de la pastèque (WMV-2) a été menée dans 17 champs de melon et 19 champs de melon d'eau des provinces de Tekirdag, d'Edirne et de Kirklareli de la Thrace turque. Un seul champ de melon parmi tous les champs testés n'était pas infecté par les virus. À l'aide d'un test ELISA utilisant un anticorps polyclonal, la présence des sept virus a été recherchée dans 502 échantillons de melon et de melon d'eau. En tout, 333 des 502 échantillons de melon et de melon d'eau examinés ont été testés positifs pour les virus de l'étude, soit 167 des 235 échantillons du Tekirdag, 103 des 187 échantillons de l'Edirne et 63 des 80 échantillons du Kirklareli. Les tests sérologiques ont révélé que six des sept virus à l'étude se trouvaient dans la région de Thrace en Turquie. L'incidence des virus à l'étude chez le melon d'eau a été établie à 45,5 % pour le ZYMV, 34,2 % pour le WMV-2, 19,9 % pour le CMV, 2,1 % pour le PRSV-W, 1,8 % pour le SqMV et 0,4 % pour le MNSV, alors qu'elle a été établie à 40,3 % pour le ZYMV, 31,2 % pour le WMV-2, 7,2 % pour le CMV, 2,3 % pour le PRSV-W, 0,5 % pour le SqMV et 1,8 % pour le MNSV chez le melon. L'infection WMV-2 + ZYMV a été le type d'infection mixte le plus répandu tant chez les échantillons de melon que chez ceux de melon d'eau, avec une présence dans respectivement 16,7 et 11,4 % des échantillons.

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# Occurrence of cucurbit viruses on field-grown melon and watermelon in the Thrace region of Turkey

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A survey for the detection of cucumber mosaic virus (CMV), papaya ringspot virus-W (PRSV-W), squash mosaic virus (SqMV), melon necrotic spot virus (MNSV), cucumber green mottle mosaic virus (CGMMV), zucchini yellow mosaic virus (ZYMV) and watermelon mosaic virus-2 (WMV-2) was carried out in June and July 2005, covering 17 melon fields and 19 watermelon fields in the Tekirdag, Edirne and Kırklareli provinces of Turkish Thrace. Among all the fields sampled, only one melon field was not virus-infected. In all, 502 melon and watermelon samples were tested for the presence of seven viruses with ELISA tests using polyclonal antisera. Overall, 333 out of 502 samples tested positive for the investigated viruses: 167 out of 235 plant samples in Tekirdag, 103 out of 187 samples in Edirne, and 63 out of 80 samples in Kırklareli were positive. Serological tests showed that six out of the seven tested viruses were present in the Thrace region of Turkey. The following rates of incidence of tested viruses on watermelon were found: ZYMV (45.5%), WMV-2 (34.2%), CMV (19.9%), PRSV-W (2.1%), SqMV (1.8%) and MNSV (0.4%), while the rates of incidence on melon were ZYMV (40.3%), WMV-2 (31.2%), CMV (7.2%), PRSV-W (2.3%), SqMV (0.5%) and MNSV (1.8%). The WMV-2+ZYMV mixed infection type was the most widespread both on melon and on watermelon samples at 16.7% and 11.4%, respectively.

Keywords: CMV, cucurbit viruses, PRSV-W, WMV-2, ZYMV.

## [Occurrence des virus des cucurbitacées chez le melon et le melon d'eau dans la région de Thrace en Turquie]

De juin à juillet 2005, une enquête visant à détecter le virus de la mosaïque du concombre (CMV), la souche W du virus de la tache annulaire de la papaye (PRSV-W), le virus de la mosaïque de la courge (SqMV), le virus de la tache nécrotique du melon (MNSV), le virus de la mosaïque à marbrure verte du concombre (CGMMV), le virus de la mosaïque jaune de la courgette (ZYMV) et le virus 2 de la mosaïque de la pastèque (WMV-2) a été menée dans 17 champs de melon et 19 champs de melon d'eau des provinces de Tekirdag, d'Edirne et de Kırklareli de la Thrace turque. Un seul champ de melon parmi tous les champs testés n'était pas infecté par les virus. À l'aide d'un test ELISA utilisant un anticorps polyclonal, la présence des sept virus a été recherchée dans 502 échantillons de melon et de melon d'eau. En tout, 333 des 502 échantillons de melon et de melon d'eau examinés ont été testés positifs pour les virus de l'étude, soit 167 des 235 échantillons du Tekirdag, 103 des 187 échantillons de l'Edirne et 63 des 80 échantillons du Kırklareli. Les tests sérologiques ont révélé que six des sept virus à l'étude se trouvaient dans la région de Thrace en Turquie. L'incidence des virus à l'étude chez le melon d'eau a été établie à 45,5 % pour le ZYMV, 34,2 % pour le WMV-2, 19,9 % pour le CMV, 2,1 % pour le PRSV-W, 1,8 % pour le SqMV et 0,4 % pour le MNSV, alors qu'elle a été établie à 40,3 % pour le ZYMV, 31,2 % pour le WMV-2, 7,2 % pour le CMV, 2,3 % pour le PRSV-W, 0,5 % pour le SqMV et 1,8 % pour le MNSV chez le melon. L'infection WMV-2 + ZYMV a été le type d'infection mixte le plus répandu tant chez les échantillons de melon que chez ceux de melon d'eau, avec une présence dans respectivement 16,7 et 11,4 % des échantillons.

Mots clés : CMV, PRSV-W, virus des cucurbitacées, WMV-2, ZYMV.

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## INTRODUCTION

In Turkey, in 2001, melon (*Cucumis melo* L.) and watermelon (*Citrullus lanatus* (Thunb.) Matsum. and Nakai) production reached 1 900 000 and 4 020 000 tons, respectively (Anonymous 2004). For the Thrace region alone, melon production reached 10 211 tons and that of watermelon reached 211 006 tons (Anonymous 2003).

This production can be jeopardized by various agents. For instance, cucurbits can be infected and economically affected by more than 35 viruses (Provvidenti 1996). Cucurbit viruses can infect many plants belonging to different genera and families, and only a few are restricted to cucurbits species. Cucurbit viruses cause reductions in plant vigour and in the number of flowers and induce mosaics and deformations in fruits, which result in drastic yield losses in early infected plants (Alonso-Prados *et al.* 1997; Luis-Arteaga *et al.* 1998). The most widespread viruses reported are cucumber mosaic virus (CMV), papaya ringspot virus-W (PRSV-W), watermelon mosaic virus-2 (WMV-2), and zucchini yellow mosaic virus (ZYMV) in melon and watermelon producing areas (Alonso-Prados *et al.* 1997; Davis *et al.* 2002; Luis-Arteaga *et al.* 1998; Makkouk and Lesemann 1980; Nogay and Yorgancı 1984; Rosemeyer *et al.* 1986; Sammons *et al.* 1989; Webb and Schott 1965; Yoshida *et al.* 1980; Yuki *et al.* 2000). Squash mosaic virus (SqMV), cucumber green mottle virus (CGMMV) and melon necrotic spot virus (MNSV) were also reported from cucurbits in many cucurbit growing regions (Faris-Mukhayyish and Makkouk 1983; Ryden and Persson 1986; Sammons *et al.* 1989; Tomassoli and Barba 2000; Yuki *et al.* 2000). In addition to the transmission by a vector, 26 viruses can be mechanically transmitted, and nine can be seed-transmitted (Alvarez and Campbell 1978; Faris-Mukhayyish and Makkouk 1983; Zitter *et al.* 1996).

ZYMV induces fruit and seed deformations in zucchini squash (*Cucurbita pepo* L.), melon, cucumber (*Cucumis sativus* L.) and watermelon (Lisa and Lecoq 1984). The virus causes local chlorotic lesions, systemic vein clearing, yellowing, mosaic, leaf deformation, stunting and occasional necrosis in melon (Campbell 1971). The virus is transmitted in a non-persistent manner by aphid species and it is transmitted mechanically (Dodds *et al.* 1984; Lecoq *et al.* 1981).

CMV, a virus distributed worldwide, affects most cucurbits but rarely watermelon. CMV causes, on cucumber and many other cucurbits, mosaics and stunting, and it reduces fruit yield since new growth is cupped downward and leaves are severely mottled with alternating light and dark green patches (Provvidenti 1996). More than 60 aphid species, including *Acyrtosiphon pisum* (Harris), *Aphis craccivora* Koch, *Myzus persicae* (Sulzer), *Macrosiphum euphorbiae* (Thomas), and *Aulacorthum solani* (Kaltenbach) [Aphididae: Homoptera] transmit the virus in a non-persistent manner (Provvidenti 1996). CMV can be transmitted by mechanical inoculation and by seed, to variable extents (Francki *et al.* 1979).

SqMV, one of several viruses causing mosaic diseases in cucurbits, causes systemic mosaic, ringspots, severe blister mottle, leaf deformation and, occasionally, enations in melon, cucumber, zucchini squash, butternut squash (*C. moschata* Poir.), and winter squash (*Cucurbita maxima* Duch.). Some isolates infect watermelon and may induce local necrotic lesions (Campbell 1971). The virus is mainly transmitted in a non-persistent manner by *Acalymma trivittatum* (Mannerheim) and *Diabrotica undecimpunctata howardi* Barber [Chrysomelidae: Coleoptera] (Provvidenti 1996). SqMV can also be transmitted by mechanical inoculation and by seeds (Alvarez and Campbell 1978; Nolan and Campbell 1984).

Two types of PRSV are characterized: PRSV-P infects papaya (*Carica papaya* L.) and PRSV-W infects watermelon. PRSV-W is transmitted mechanically and by more than 20 aphids vectors (Provvidenti 1996).

WMV-2 causes mosaic and mottle diseases in cantaloupe (*Cucumis melo* L. var. *cantalupensis* Naudin), cucumber, pumpkin (*Cucurbita pepo* L. var. *medullosa* Alef.), squash, and watermelon depending on viral strains and environmental factors. Leaf symptoms include greenish mosaic, leaf deformation, green vein banding, chlorotic spots and malformations (Purcifull *et al.* 1984a). WMV-2 is transmitted in a non-persistent manner by aphids (Castle *et al.* 1992; Provvidenti 1996; Webb and Schott 1965).

MNSV causes necrotic lesions on inoculated cotyledons of melon and induces dark brown local lesions on leaves or cotyledons of watermelon (Hibi 1996). The virus is transmitted by *Olpidium bornovanus* (Sahtiyancı) Karling (= *Olpidium radicale* Schwartz and Cook) [Olpidiaceae: Spizellomycetales] (Tomlinson and Thomas 1986).

CGMMV induces mosaic in cucumber, melon, watermelon, and bottle gourd (*Lagenaria siceraria* (Mol.)) (Francki 1996). CGMMV is found in the Eurasian region, India, Japan, and in the UK (Antignus *et al.* 1990; Avgelis and Vovlas 1986; Yoshida *et al.* 1980). CGMMV is transmitted by mechanical inoculation (Francki 1996) and by the red pumpkin beetle, *Raphidopalpa foveicollis* (Lucas) (= *Aulacophora foveicollis*) [Chrysomelidae: Coleoptera] (Rao and Varma 1984).

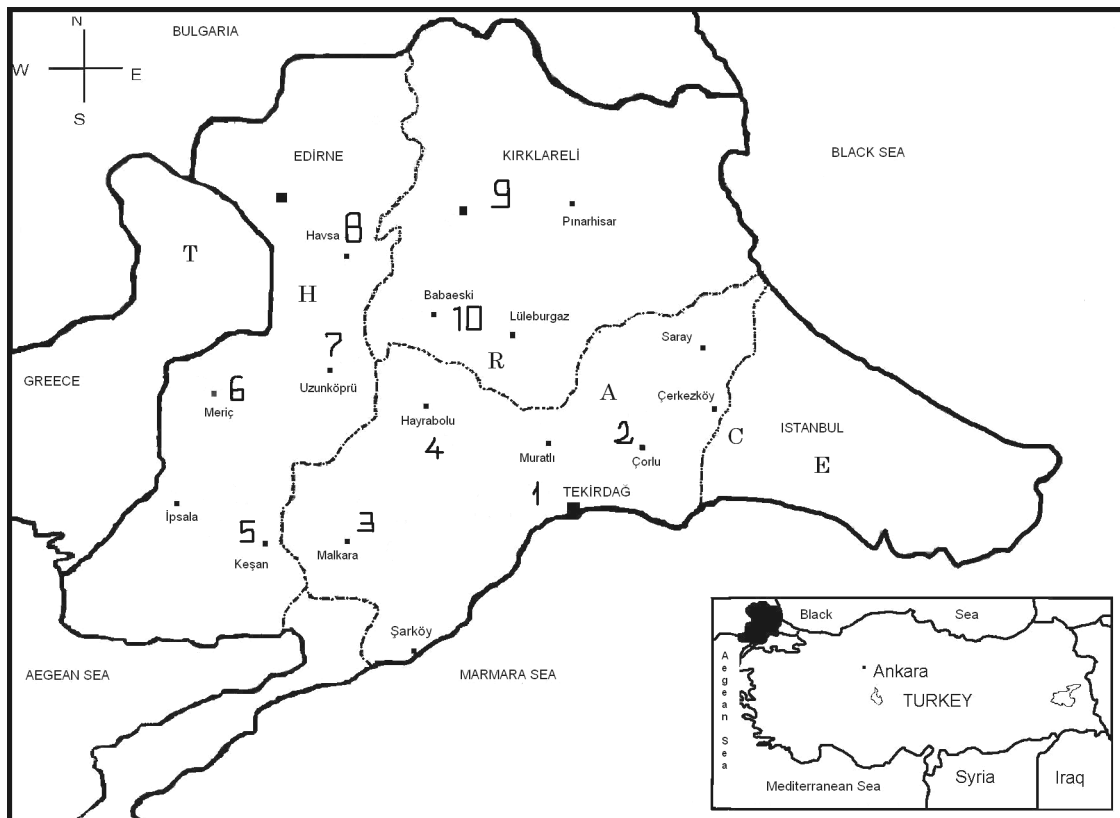
Several virus diseases inducing mosaic symptoms have previously been reported from Turkey, including CMV, CVYV, ZYMV, SqMV, PRSV-W, and WMV-2 (Erdiller and Ertunç 1987; Lisa and Lecoq 1984; Nogay and Yorgancı 1984; Sevik and Arlı-Sökmen 2003; Yılmaz and Davis 1985). Sevik and Arlı-Sökmen (2003) determined that the incidence of WMV-2, ZYMV and CMV on cucurbits was 53.9%, 38.8% and 20.6%, respectively. Nogay and Yorgancı (1984) also reported, after studying various samples, that disease occurrence for CMV, WMV-2 and CMV+WMV-2 was 52.8%, 43.9% and 0.03%, respectively.

The occurrence and incidence of CMV, ZYMV, SqMV, PRSV-W, WMV-2, MNSV, and CGMMV on field-grown melon and watermelon in the Thrace region of Turkey are investigated in this study for the first time.

## MATERIALS AND METHODS

In June and July 2005, a survey was carried out to determine the incidence rates of CMV, PRSV-W, WMV-2, ZYMV, SqMV, MNSV, and CGMMV in the Tekirdag (Center, Çorlu, Malkara, and Hayrabolu counties), Edirne (Uzunköprü, Kesan, Meric, and Havsa counties) and Kırklareli (Center and Babaeski counties) provinces of Turkey (Fig. 1). About 10 plant samples exhibiting yellowing, dwarfing, leaf streaking and mosaic-like symptoms were collected from each of the 36 selected fields (19 watermelon and 17 melon fields). About 3-5 young leaves were collected from different shoots of selected plants. A total of 502 samples (235 from Tekirdag, 187 from Edirne and 80 from Kırklareli) were kept in nylon bags in ice boxes during the survey, and later at 4°C in a refrigerator for 1-2 d before being ELISA-tested. Virus diagnosis was done using DAS-ELISA tests as described by Clark and Adams (1977). The antisera and conjugates were purchased from BIOREBA AG (Reinach, Switzerland) (SqMV, CMV, ZYMV, WMV-2 and PRSV-W), and LOEWE Biochemica GmbH (Sauerlach, Germany) (MNSV and CGMMV). Lyophilized positive controls were obtained from the above given sources except for CMV, which was obtained from fresh leaves of infected watermelon plants available in our laboratory.

The samples were tested in the Plant Pathology Laboratory, Department of Plant Protection, Tekirdag Faculty of Agriculture, Tekirdag, Turkey. The melon and watermelon samples were homogenized using a mortar and pestle with the addition of the sample extraction phosphate buffer solution (8.0 g NaCl, 0.2 g  $\text{KH}_2\text{PO}_4$ , 2.9 g  $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ , 0.2 g KCl, 0.2 g  $\text{NaN}_3$ , 20 g polyvinylpyrrolidone-25 per L, pH 7.4) at a ratio of 1:5. Plates were precoated with virus antisera that were diluted in carbonate buffer (1.59 g  $\text{Na}_2\text{CO}_3$ , 2.93 g  $\text{NaHCO}_3$ , 0.2 g  $\text{NaN}_3$  per L, pH 9.6), and incubated for 2 h at 37°C. After washing the plates with PBST buffer (8.0 g NaCl, 0.2 g  $\text{KH}_2\text{PO}_4$ , 2.9 g  $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ , 0.2 g KCl, 0.2 g  $\text{NaN}_3$ , 0.5 mL Tween-20 per L), samples were added to wells and incubated overnight at 4°C. Alkaline phosphatase conjugated antibody diluted in conjugate buffer (PBST + 2% polyvinylpyrrolidone-25 + 0.2% BSA (bovine serum albumin, Sigma A-4503) pH 7.4) was added after washing the plates, and incubated for 2 h at 37°C. P-nitrophenylphosphate in substrate buffer (97 mL diethanolamine, 0.2 g  $\text{NaN}_3$  L<sup>-1</sup>, pH 9.8) was added to each well and incubated for 2 h at room temperature. Absorbance values were measured at 405 nm using an ELx800 Universal Microplate Reader (Bio-Tek Instruments Inc., Vermont, USA) at the Department of Food Engineering. A sample was considered positive when the mean absorbance value of the two wells used for each tested sample was greater than twice that of the healthy or buffer control.



**Figure 1. Location of collected melon and watermelon samples in the Thrace region of Turkey.** Tekirdag Center (1), Çorlu (2), Malkara (3), Hayrabolu (4), Kesan (5), Meric (6), Uzunköprü (7), Havsa (8), Kırklareli Center (9), Babaeski (10). Surveyed area indicated in black on inset map.

**Table 1. Number of tested and virus-infected watermelon fields in the Thrace region of Turkey**

Location of collected samples	Number of watermelon fields infected by a given virus							No. of tested fields	No. of infected fields
	CMV	SqMV	WMV2	CGMMV	PRSV-W	MNSV	ZYMV		
Tekirdag-Center	3	1	4	0	1	0	4	4	4
Tekirdag-Çorlu	1	0	1	0	1	0	1	1	1
Tekirdag-Malkara	1	1	2	0	0	1	2	2	2
Tekirdag-Hayrabolu	1	0	1	0	0	0	1	1	1
Edirne-Kesan	1	0	3	0	0	0	2	3	3
Edirne-Meric	0	0	1	0	0	0	1	1	1
Edirne-Uzunköprü	0	0	2	0	0	0	2	2	2
Edirne-Havsa	0	1	2	0	1	0	2	2	2
Kırklareli-Center	1	0	1	0	0	0	1	1	1
Kırklareli-Babaeski	1	0	2	0	0	0	2	2	2
<b>Total</b>	<b>9</b>	<b>3</b>	<b>19</b>	<b>0</b>	<b>3</b>	<b>1</b>	<b>18</b>	<b>19</b>	<b>19</b>

## RESULTS

Surveys were carried out in field-grown melon and watermelon in the Tekirdag, Kırklareli and Edirne provinces of Turkey (Fig. 1). A total of 19 watermelon and 17 melon fields at 17 locations were surveyed. Symptoms of virus infection were observed in 34 fields but the incidence of symptomatic plants varied among the fields surveyed. The incidence of virus infection symptoms was the highest in the Babaeski county of Kırklareli, followed by the Meric county and Kesan county of Edirne (results not shown). The most common symptoms were mosaic and leaf deformation both on new shoots and leaves of melon and watermelon. Aphid populations observed on the abaxial side of leaves exhibiting symptoms were not identified.

The main symptoms observed in melon and watermelon grown in the Merkez county of Tekirdag were yellowing, deformation, mosaic and yellow mottle; however, reduced growth was rarely observed. Necrotic spots were observed on melon leaves in a

few fields, but more frequently in the fields of one village (location No. 1; Fig. 1). Disease symptoms were observed only in some parts of the fields. Mosaic symptoms on young leaves and stunting were rarely observed on watermelon plants in the Malkara county, but leaf deformation, necrotic spots and yellow mosaic were frequently found on melon leaves (location No. 3; Fig. 1). Mosaic and yellowing symptoms were observed on melon and watermelon samples in the Corlu and Hayrabolu counties of Tekirdag (locations No. 2 and 4; Fig. 1) and in the Meric and Havsa counties of Edirne (locations No. 6 and 8; Fig. 1). Mosaic lesions, but rarely necrotic lesions, were observed on watermelon leaves, while severe yellowing symptoms were observed on older leaves of melon in the Kesan county of Edirne (location No. 5; Fig. 1).

Discoloration and leaf deformation were observed in melon and watermelon fields in the Uzunköprü county (location No. 7; Fig. 1). Almost all plants in tested melon fields were affected, and typical mosaic and stunting were observed on melon plants.

**Table 2. Number of virus-infected melon fields in the Thrace region of Turkey**

Location of collected samples	Number of melon fields infected by a given virus							No. of tested fields	No. of infected fields
	CMV	SqMV	WMV2	CGMMV	PRSV-W	MNSV	ZYMV		
Tekirdag-Center	2	1	2	0	1	0	2	3	2
Tekirdag-Çorlu	1	0	1	0	0	0	1	1	1
Tekirdag-Malkara	3	0	5	0	2	1	5	5	5
Tekirdag-Hayrabolu	1	0	1	0	0	0	1	1	1
Edirne-Kesan	1	0	1	0	0	0	2	3	3
Edirne-Meric	0	0	0	0	0	0	1	1	1
Edirne-Uzunköprü	1	0	2	0	0	0	1	2	2
Kırklareli-Babaeski	0	0	1	0	0	0	1	1	1
<b>Total</b>	<b>9</b>	<b>1</b>	<b>13</b>	<b>0</b>	<b>3</b>	<b>1</b>	<b>14</b>	<b>17</b>	<b>16</b>



Very clear leaf deformation, mosaic and reduced growth were observed on watermelon plants grown in two fields in the Babaeski county of Kırklareli. Most of the leaves of watermelon plants showed disease symptoms. Deformation, mottling and yellow mosaic were observed on melon leaves. Mosaic-like symptoms were also observed in watermelon fields in the Center county of Kırklareli but, in comparison with the previous location, fewer plants with symptoms were found (location No. 9; Fig.1).

A total of 502 samples from 37 fields were collected and tested using DAS-ELISA with polyclonal antisera. ELISA results showed that 36 out of the 37 surveyed fields were infected at least by one of the viruses assessed in the present study. Out of the 19 watermelon fields, WMV-2 was detected in all 19, ZYMV in 18, and CMV in 9 fields (Table 1). Out of the 17 melon fields, ZYMV was detected in 14, WMV-2 in 13, and CMV in 9 fields (Table 2).

The highest infection rate was found in watermelon fields in Babaeski (90.2%), followed by fields in Tekirdag-Center (89.6%), Kesan (78.9%), Malkara (72.2%), Çorlu and Hayrabolu (70.0%), Meric (64.7%), Uzunköprü (56.0%), Havsa (51.7%), and Kırklareli-Center (37.5%) (Table 3) (results not shown). For melon, the highest infection rate was observed in Malkara county (85.7%), followed by Çorlu (77.3%), Uzunköprü (76.3%), Babaeski (74.2%), Hayrabolu (66.7%), Kesan (31.6%), Meric (30.0%), and Tekirdag-Center (27.3%) (Table 4).

The ELISA test results of 129 melon and 106 watermelon leaf samples indicated that 13.2% of melon and 31.1% of watermelon samples were infected with CMV, 0.8% of melon and 1.9% of watermelon samples were infected with SqMV, 34.1% of melon and 34.9% of watermelon samples were infected with WMV-2, 6.2% of melon and 6.6% of watermelon samples were infected with PRSV-W, 3.1% of melon and 0.9% of watermelon samples were infected with MNSV, while 52.7% of melon and 48.1% of watermelon samples were infected with ZYMV in the Tekirdag province (Tables 3 and 4). CGMMV was not detected using ELISA tests in this province. In the Edirne province, among the 61 melon and 126 watermelon leaf samples, 8.2% of melon and 8.7% of watermelon samples were infected with CMV, 3.3% of melon and 1.6% of watermelon samples were infected with SqMV, 24.6% of melon and 35.7% of watermelon samples were infected with WMV-2, 0.8% of watermelon samples were infected with PRSV-W, while 22.9% of melon and 34.9% of watermelon samples were infected with ZYMV (Tables 3 and 4). CGMMV and MNSV were not detected in samples from the Edirne province. Among the 31 melon and 49 watermelon sampled in the Kırklareli province, 20.4% of watermelon samples were infected with CMV, 54.8% of melon and 36.7% of watermelon samples were infected with WMV-2, and 61.3% of melon and 55.1% of watermelon samples were infected with ZYMV (Tables 3 and 4). SqMV, PRSV, CGMMV and MNSV were not detected in tested plants from Kırklareli.

**Table 3. Types of infection by cucurbit viruses in watermelon samples tested in 10 counties of the Thrace region in Turkey**

Infection types	Location of collected samples										Total
	Tekirdag-Center	Çorlu	Malkara	Hayrabolu	Kesan	Meric	Uzunköprü	Havsa	Kırklareli Center	Babaeski	
CMV	1	1	2	2	2	0	0	0	1	2	11
SqMV	0	0	0	0	0	0	0	2	0	0	2
WMV-2	10	3	1	2	10	8	7	5	1	6	44
CGMMV	0	0	0	0	0	0	0	0	0	0	0
PRSV-W	1	0	0	0	0	0	0	0	0	0	1
MNSV	0	0	0	0	0	0	0	0	0	0	0
ZYMV	10	1	4	2	6	7	2	7	0	18	57
CMV+ZYMV	5	4	2	1	7	0	0	0	0	1	20
CMV+WMV-2	0	1	0	1	2	0	0	0	1	2	7
WMV-2+ZYMV	6	2	2	3	2	7	5	0	0	5	32
CMV+PRSV-W	1	1	0	0	0	0	0	0	0	0	2
PRSV-W+ZYMV	1	0	0	0	0	0	0	0	0	0	1
SqMV+ZYMV	2	0	0	0	0	0	0	0	0	0	2
CMV+ PRSV-W+ZYMV	2	0	0	0	0	0	0	0	0	0	2
CMV+WMV-2+ZYMV	4	1	0	3	1	0	0	0	0	3	12
CMV+SqMV+ZYMV	0	0	1	0	0	0	0	0	0	0	1
CMV+MNSV+WMV-2+ZYMV	0	0	1	0	0	0	0	0	0	0	1
Positive	43	14	13	14	30	22	14	15	3	37	196
Negative	5	6	5	6	8	12	11	14	5	4	78
<b>Total</b>	<b>48</b>	<b>20</b>	<b>18</b>	<b>20</b>	<b>38</b>	<b>34</b>	<b>25</b>	<b>29</b>	<b>8</b>	<b>41</b>	<b>281</b>

**Table 4. Types of infection by cucurbit viruses in melon samples tested in 10 counties of the Thrace region in Turkey**

Infection types	Location of collected samples								Total
	Tekirdag-Center	Çorlu	Malkara	Hayrabolu	Kesan	Meric	Uzunköprü	Babaeski	
CMV	1	0	2	1	2	0	0	0	6
SqMV	0	0	0	0	0	0	0	0	0
WMV-2	0	6	4	3	1	0	5	4	23
CGMMV	0	0	0	0	0	0	0	0	0
PRSV-W	0	0	0	1	0	0	0	0	1
MNSV	0	0	1	0	0	0	0	0	1
ZYMV	8	8	11	5	2	3	0	6	43
CMV+ZYMV	1	1	1	0	0	0	0	0	3
CMV+WMV-2	0	0	1	0	0	0	2	0	3
WMV-2+ZYMV	1	2	10	2	6	0	3	13	37
CMV+PRSV-W	0	0	0	0	0	0	0	0	0
WMV-2+PRSV-W	0	0	1	1	0	0	0	0	2
PRSV-W+ZYMV	0	0	1	0	0	0	0	0	1
SqMV+ZYMV	0	0	0	0	1	0	0	0	1
MNSV+WMV-2	0	0	1	0	0	0	0	0	1
MNSV+ZYMV	0	0	1	0	0	0	0	0	1
CMV+ PRSV-W+ZYMV	0	0	0	1	0	0	0	0	1
CMV+WMV-2+ZYMV	1	0	1	0	0	0	0	0	2
CMV+ WMV-2+MNSV	0	0	1	0	0	0	0	0	1
Positive	12	17	36	14	12	3	10	23	127
Negative	32	5	6	7	26	7	3	8	94
<b>Total</b>	<b>44</b>	<b>22</b>	<b>42</b>	<b>21</b>	<b>38</b>	<b>10</b>	<b>13</b>	<b>31</b>	<b>221</b>

Out of the 281 watermelon samples tested, 196 were infected in the Thrace region (Table 3). Among these 281 samples, 115 were found to be infected only with one virus, 65 samples were infected with two viruses, 15 samples were infected with three viruses, and one sample was infected with four viruses. Seventy-eight watermelon samples tested negative.

Single viral infection was prevalent among the watermelon samples as 57 samples were infected with ZYMV, 44 samples with WMV-2, 11 samples with CMV, two samples with SqMV, and one sample with PRSV-W. The most prevalent double viral infection was WMV-2+ZYMV and it was found in 32 samples, while the pair CMV+ZYMV was detected in 20 samples. The triple infection types CMV+WMV-2+ZYMV and CMV+PRSV+ZYMV were detected in twelve and two samples, respectively (Table 3).

ZYMV was the most prevalent single infection type detected in 43 melon samples, followed by WMV-2 in 23 samples, and CMV in six samples. The WMV-2+ZYMV double infection was detected in 37 samples whereas the triple infection by CMV+WMV-2+ZYMV was detected in only two samples (Table 4).

## DISCUSSION

Viruses causing mosaic, leaf deformation and reduced growth were detected in melon and watermelon plants in the surveyed area. Reduced growth and yellowing symptoms caused by mineral deficiency were also observed in a few fields. Diseases symptoms were similar to the symptoms previously reported from virus-infected melon and watermelon fields worldwide (Alonso-Prados *et al.* 1997; Davis *et al.* 2002; Dodds *et al.* 1984; Lecoq *et al.* 1981; Luis-Arteaga *et al.* 1998; Makkouk and Lesemann 1980; Provvidenti 1996; Sammons *et al.* 1989). The presence of virus-transmitting vectors, availability of alternative virus-infected weeds and the growing of a wide range of crops species hosting cucurbit viruses in the region could increase the spread of viruses into melon and watermelon fields. SqMV and MNSV may be imported into an area via contaminated seeds and can then be spread mechanically (Alvarez and Campbell 1978; Faris-Mukhayyish and Makkouk 1983; Zitter *et al.* 1996).

Some differences on the incidence of the most prevalent viruses in different countries have been reported (Davis *et al.* 2002; Dodds *et al.* 1984; Luis-Arteaga *et al.* 1998; Yoshida *et al.* 1980; Yuki *et al.* 2000). The occurrence and incidence of cucurbit viruses on melon and watermelon have been studied

worldwide, and the presence of CMV, PRSV-W, WMV-2 and ZYMV has been reported from Mediterranean countries (Alonso-Prados *et al.* 1997; Luis-Arteaga *et al.* 1998; Makkouk and Lesemann 1980).

Our results indicate that ZYMV, WMV-2, CMV and PRSV-W are the four most prevalent viruses in the region, in decreasing order. The survey of field-grown watermelon and melon in the Thrace region of Turkey indicated that mosaic and leaf deformation-causing viruses should be of special concern in all surveyed areas even if viral incidences are low in a few locations. It is important to use certified seeds to prevent transmission, particularly because many farmers produce their own seeds in the region studied. Additionally, weed control in seedling-growing greenhouses may help prevent early infections. The high infection rates detected in watermelon in the Babaeski, Tekirdag-Center, Kesan, Malkara, Çorlu, Hayrabolu and Meric counties may indicate that infection occurred at the seedling stage or through infected seeds in addition to the expected vector transmission. Dispersal of plants with viral symptoms in many surveyed fields was irregular, and unique symptomatic plants were observed without symptoms on adjacent plants. Spraying in order to control vector populations, mainly for aphid-transmitted viruses, seems important to prevent virus transmission to both cucurbits and weed natural hosts. The fact that some symptomatic plants did not react to ELISA antisera could be explained by the presence of other mosaic viruses and/or by the alteration of the suspected viruses.

Luis-Arteaga *et al.* (1998) reported that CMV and WMV-2 were the most prevalent viruses on melon, with an incidence of respectively 78% and 65%, in 1995, and of respectively 58% and 55% in 1996. ZYMV was found in 13% of surveyed fields, infection by a single virus was reported for 87% of infected plants, while CMV and WMV-2 were detected in 94% of the mixed infections. PRSV-W and ZYMV were the most frequently found viruses, their incidence accounting for 49.1% and 24.8%, respectively (Luis-Arteaga *et al.* 1998). ZLCV, CMV and WMV-2 were detected in 7.8%, 6.0% and 4.5% of the samples tested in Brasil, respectively (Yuki *et al.* 2000). Sevik and Arlı-Sökmen (2003) reported that WMV-2, ZYMV and CMV infection occurred on cucurbits for 53.9%, 38.8%, and 20.6% of the 165 samples tested in Samsun province of Turkey. Nogay and Yorgancı (1984) reported that CMV, WMV-2 and CMV+WMV-2 infections occurred respectively in 52.8%, 43.9% and 0.03% of 269 symptomatic cucurbit samples collected in the Marmara region in 1979 and 1980. Sammons *et al.* (1989) conducted a research to determine the incidence of CMV, PRSV-W, WMV-2, SqMV and TRSV and found that the infection rate of WMV-2 can be up to 100% and, in some cases, that this rate can change from region to region in South Carolina. Purcifull *et al.* (1984) reported that among 39 samples with mosaic symptoms, 17.9% were infected by PRSV-W (WMV-1) and 53.8% were infected by WMV-2.

In this research, differences in infection rates and occurrence of viruses were determined between fields and locations in the Thrace region of Turkey. The global infection rate was up to 89% in some fields, the most prevalent viruses in melon and watermelon fields

being ZYMV, WMV-2 and CMV. With melon samples, the infection rate differed among locations, the highest being from samples collected in Malkara (85.7%), followed by Çorlu (77.3%), Uzunköprü (76.3%) and Babaeski (74.2%). A few melon fields showed severe yellowing symptoms on older leaves in the Kesan and Malkara counties. Infection by a single virus was found in 69.75% of watermelon and 52.1% of melon samples, while double infections and triple infections were also found. Considering these results, it seems that the spread by vectors was the most important transmission mechanism. SqMV and MNSV were identified in only a few fields, indicating a putative low rate of seed transmission. The occurrence and very low incidence of SqMV and MNSV indicate transmission by seeds in the region (Hibi 1996; Tomassoli and Barba 2000). The absence of reactions to CGMMV strongly suggests that this virus is absent in the region or is present in very low numbers.

ZYMV and WMV-2 are the most destructive viruses infecting cucurbits. These viruses are easily transmitted and widely disseminated in a given region. Control of the vectors may restrict virus transmission at early growing stages for field-grown melons and watermelons. The presence of MNSV in Malkara and SqMV in Malkara and Tekirdag-Center is suspected and has to be further investigated.

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