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Occurrence of *Squash mosaic virus* (SqMV) Infecting Pumpkin and Squash Growing in Samsun, Turkey

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ABSTRACT

Most of the cucurbits grown in Samsun showed symptoms of virus infection. The symptoms were characterized by severe or mild green mosaic, green vein-banding, deformation or reduction in leaf and fruit shape and size. The yield was also affected by the viruses. Surveys were conducted during 2009 to 2010 identify the SqMV infecting pumpkin and squash in Samsun, Turkey. Cucurbita species, namely *C. pepo* L., *C. moschata* Duch, and *C. maxima* Duch, samples were collected from 22 fields and tested by double antibody sandwich-enzyme-linked immunosorbent assay (DAS-ELISA). *Squash mosaic virus* (SqMV) was detected with 20.9% incidence in Cucurbita species after analysing the *symptomatic* samples by DAS-ELISA. This is the first report of SqMV in the Black Sea Region of Turkey.

Key words: SqMV, detection, epidemiology, seed transmission, virus

INTRODUCTION

Samsun is one of the main cucurbit-growing areas of Turkey. In Samsun province, approximately 90% of commercial pumpkin (*Cucurbita moschata* Duch. and *C. maxima* Duch.) and squash (*C. pepo* L.), are grown in the Carsamba, Bafra, Tekkekoy, and Terme districts (Provincial Directorate of The Ministry of Agriculture and Rural Affairs, *unpublished data*).

Virus diseases are a worldwide problem of cucurbits and a major limiting factor for cucurbit production. Almost 35 different viruses have been isolated from *Cucurbitaceae* (Provvidenti, 1996). Economically important viruses are *Zucchini yellow*

mosaic virus (ZYMV), *Watermelon mosaic virus* (WMV), *Papaya ringspot virus* (PRSV-W), *Cucumber mosaic virus* (CMV), and *Squash mosaic virus* (SqMV) (Lovisolo, 1980). Although CMV (Kurçman,1977; Nogay and Yorganci, 1984; Yılmaz and Davis, 1984; Yılmaz *et al.* 1994, 1995; Kaya and Erkan, 2007), PRSV-W (Erdiller and Ertunç, 1987 and 1988; Kaya and Erkan, 2007), WMV (Nogay and Yorgancı, 1984; Erdiller and Ertunç, 1988; Yılmaz *et al.* 1994; Kaya and Erkan, 2007), ZYMV (Yılmaz and Davis, 1984; Yılmaz *et al.* 1994, 1995; Kaya and Erkan, 2007), *Melon mosaic virus* (MMV) (Yılmaz *et al.* 1995), *Cucumber vein yellowing virus* (CVYV) (Yılmaz *et al.* 1991), *Tomato ringspot virus* (TRSV) and *Tomato black ring virus* (TBRV) (Fidan, 1995) have been reported in squash, pumpkin, and other cucurbit crops from many regions in Turkey, SqMV is reported in melon plants from Adana, Mersin, Urfa provinces and Thrace region (Caglar *et al.* 2004; Köklü and Yılmaz, 2006). In previous studies, SqMV was determined in squash in Turkey (Gümüş *et al.* 2001; Gümüş *et al.* 2004). But, information of SqMV on the Black Sea Region of Turkey is lacking.

SqMV is a member of the genus *Comovirus*, family *Comoviridae*, with isometric virus particles about 30 nm in diameter (Bruening, 1978). SqMV infects almost all species in the *Cucurbitaceae* family and is transmitted by beetles (Langham *et al.* 1997) and through seeds (Campbell, 1971; Franken *et al.* 1990). SqMV symptoms on cucurbit vary from mild mosaic or vein-banding to severe systemic mosaic and malformation of leaves, color change and deformation of fruit, and plant stunting (Davis and Muzuki, 1987).

In earlier studies, ZYMV, WMV and CMV were found to be the predominant viruses in Samsun, Turkey (Sevik and Sokmen, 2003). Some samples that showed virus-like symptoms did not react with three antisera used in serological tests. But, antisera specific to the other cucurbit viruses were not available in the study. A mosaic disease of pumpkin and squash was spread widely in Samsun, Turkey. Since the virus had not yet been identified, a study was conducted to identify the disease by symptomatological and serological tests in 2009 -2010.

MATERIALS and METHODS

Disease survey and collection of samples

Surveys were conducted in 2009 and 2010 to determine the virus associated with pumpkin and squash crops in Samsun, Turkey. Samples were collected from 22 fields in the five major cucurbit-growing areas including Carsamba, Bafra, Terme, Tekkekoy, Central districts (Figure 1). In each area, pumpkin and squash plants were randomly evaluated for virus-like symptoms such as vein clearing, vein-banding, mosaic, mottling, puckering, leaf and fruit malformation.

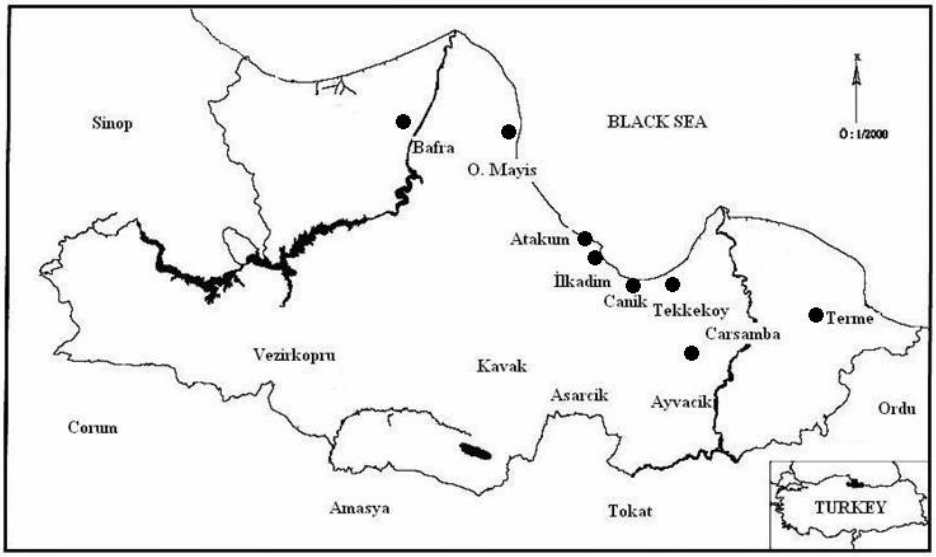

Figure 1. Map of the Black Sea Region of Turkey showing areas in which surveys were conducted (●: indicates the location of districts in which samples were collected).

Serological testing

The double-antibody sandwich ELISA procedure Clark and Adams, (1977) was used to test for leaf, fruit and seed infection with SqMV. DAS-ELISA was conducted by extracting plant sap by grinding the samples (leaf, fruit and fresh seed) and diluting the sap in extraction buffer (PBS: 0.13 M NaCl, 0.014 M KH_2PO_4, 0.08 M $Na_2HPO_4.12H_2O$, 0.002 M KCl, pH: 7.4) at a ratio of 1:5 (vol/vol, sap: buffer). This sap was loaded into the polyclonal antibody-coated wells of microplate (TPP, Switzerland). The wells were incubated at 4°C for 12 h, washed using phosphate-buffered saline Tween (PBST), and dried on paper towels. The washed and dried wells were loaded with alkaline phosphatase conjugated anti-virus antibody and incubated at 37°C for 4 h. The wells were washed and loaded with *p*-nitrophenol phosphate and incubated at room temperature (23°C) for 30-180 min. Absorbance values were read at 405 nm using a microplate reader (Tecan Spectra II, Grodig/Salzburg, Austria) and also confirmed visually. All samples tested in two replicate wells and the absorbance value greater than three times that of a negative control and with a visually detectable yellow colour was rated as positive (Avgelis and Katis, 1989; Dahal *et al.* 1997; Jossey and Babadoost, 2008).

RESULTS

During the surveys, most cucurbit fields contained plants with viral symptoms in 2009-2010. Leaves of infected pumpkin plants showed green systemic mosaic, green

vein-banding and malformation (Figure 2). Average incidence of plants with symptoms ranged from 30% to 70% on pumpkin and on squash. The leaf and fruit samples from symptomatic plants collected from pumpkin and squash were analyzed using DAS-ELISA to determine the presence of SqMV in 22 areas of Samsun province of Turkey. In the study, ELISA tests were conducted using several negative controls for SqMV. Therefore, the range of absorbance values of negative controls varied from 0.091- 0.112 at 405 nm. Positive samples gave absorbance values of 0.682- to 2.353 after 2 h substrate incubation. The results of serological tests showed that cucurbit plants were infected with SqMV. The symptomatic leaf and fruit samples (20.9%) reacted positively in DAS-ELISA with SqMV (Table 1). Overall, 21.11% of pumpkin and winter squash and 20% of zucchini squash were tested positive for the virus infection during the survey. While Çarşamba district was the first in distribution of SqMV with infecting of 8 out of 30 samples, Tekkeköy district was the second with the infected 7 out of 30 samples. It was estimated as 20, 15 and 10% of SqMV contamination ratio for Bafra, Central and Terme districts, respectively (Figure 3). These results revealed that cucurbits grown in commercial fields were infected quite commonly with the virus, but a significant percentage of samples (79.1%) from symptomatic cucurbit plants did not react with the antiserum against the virus, what may be due to the presence of the other cucurbit viruses. In a previous study in the region, ZYMV, WMV, CMV were found to be the predominant viruses in cucurbits (Sevik and Sokmen, 2003). On the other hand, the fresh seeds from infected pumpkin were tested for SqMV, but the virus was not detected in seed samples.

Table 1. Occurrences of SqMV in pumpkin and squash plants collected from Samsun

DISTRICTS Villages	**No. of fields surveyed**	**No. of samples tested**	**SqMV**
CARSAMBA			
Karaca	2	10	2
Köklük	2	10	3
Kızılot	2	10	3
BAFRA			
Eminli	4	20	4
TEKKEKOY			
Aşağıçinik	4	20	6
Ovabaşı	2	10	1
TERME			
Gölyazı	2	10	1
CENTRAL			
Tepecik	2	10	2
Derebahçe	2	10	1
TOTAL	**22**	**110**	**23**
% Infection	**-**	**-**	**20.9**

Figure 2. Green vein-banding of pumpkin leaf caused by SqMV

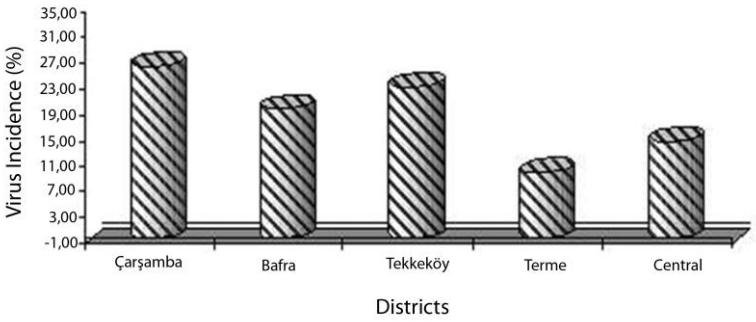

Figure 3. Rates of infection of SqMV in cucurbit-growing districts of Samsun in 2009- 2010.

DISCUSSION

The previous studies performed in Turkey have reported the existence of ZYMV (Davis, 1986; Yılmaz *et al.* 1995; Çıtır *et al.* 1998; Bostan *et al.* 2002; Sertkaya *et al.* 2004), WMV (Çıtır *et al.* 1998; Sevik and Arli-Sokmen, 2003) and CMV (Ertunç, 1992; Vargün and Ertunç, 1994; Yılmaz *et al.* 1995; Çıtır *et al.* 1998; Uçar and Ertunç, 1998) in squash plants. There has been one report on the detection of SqMV from melon in the southern part of Turkey (Caglar *et al.* 2004). Widespread symptoms of a possible virus disease were repeatedly observed in melon crops grown in the provinces of Mersin, Adana, and Urfa (south-west Turkey). ELISA tests of leaf extracts from naturally infected melon plants gave positive reactions with antiserum to SqMV (Çağlar *et al.*

2004). Prior to the present study, no data was available on the occurrence, incidence and distribution of SqMV infecting cucurbit plants in the Black Sea Region of Turkey. Therefore, we conducted a survey to determine the virus involved in mosaic disease and to estimate its relative occurrence. Leaf and fruit extracts of some pumpkin and squash samples from Samsun, Turkey, 20.9% reacted with SqMV antiserum. But, SqMV was not determined in the fresh seeds from infected pumpkin fruit. Plant virus infection rates and symptoms vary from year to year and region to region (German *et al.* 1992). Incidence of SqMV in the symptomatic cucurbit plants varied in the different regions and among fields of the same regions in Samsun, as previously reported (Farzadfar *et al.* 2007). In our study, the percentages of cucurbit plants infected with SqMV in Carsamba, Tekkekoy, Bafra, Central and Terme districts were 26.66, 23.33, 20, 15, and 10%, respectively. The incidence of the disease was maximum at Carsamba region (26.66%), the minimum disease incidence was recorded at Terme region (10%).

Although, SqMV incidence was low in cucurbits elsewhere, our studies showed that SqMV occurrence was relatively high in cucurbits grown fields in Samsun. Infected seed is considered to be the main source of SqMV, therefore seed transmission may be important for introducing the virus to new areas. A survey of cucurbitaceous vegetables from 1992 to 1994 in Nepal, indicated that these crops were heavily affected with various virus-like symptoms, but none of the samples reacted with SqMV (Dahal *et al.* 1997). Based on ELISA tests, SqMV was not detected in any cucurbit samples tested in Cyprus (Papayiannis *et al.* 2005). Although, SqMV previously had been recovered from cucurbits in Iran (Izadpanah, 1983), cucurbit crops in several Iranian regions were surveyed for the incidence of SqMV and the other viruses in 2002–2004. SqMV was not detected in any samples (Massumi *et al.* 2007). Nevertheless, SqMV was detected in Guilan province of Iran in 2006-2007 (Gholamalizadeh *et al.* 2008). Moreover, In Greece, high incidences of cucurbit-infecting viruses have also been reported, including WMV (67%), ZYMV (27%), CMV (20%), PRSV (3%) and SqMV (2%) (Papvassiliou *et al.* 2002).

In the present study, 79.1% of the symptomatic pumpkin and squash plant samples tested negative for SqMV. Jossey and Babadoost (2008) found that CMV, PRSV, SqMV, TRSV, WMV, and ZYMV produced similar symptoms in single and mixed infections in all cucurbit host plants. So, the symptoms may be due to the presence of the other cucurbit viruses.

The planting of infected seed is considered the most important primary source of inoculum (Anonymous, 1999). SqMV is seedborne in cucurbit plants and the virus may spread during transplanting and at harvest after primary infections via infected seeds. Some growers in Samsun province produce and use their own pumpkin seed and this may contribute to the spread of the virus in cucurbit crops. In our study, the fresh seeds from infected pumpkin were tested for SqMV, but the ELISA absorbance values were low relatively. Because of A405 nm values were low than three times that of negative

control, the pumpkin seed samples were considered virus-negative. Seed transmission has been studied by several authors (Nelson and Knuhtsen, 1973; Franken *et al.* 1990**).** Long-distance spread of the virus, of course, is possible with seeds. A research was conducted in the Çukurova Region of Turkey in 1999-2002. SqMV was identified by serological and biological methods in the imported melon seeds (Çağlar and Yılmaz, 2002). SqMV was consistently detected by ELISA in germinated seeds from imported melon seed batches (Çağlar *et al.* 2004). SqMV was isolated from melon plants raised from imported seed in Greece. Seed testing by ELISA detected SqMV in melon imported from the USA (Avgelis and Katis, 1989). The rates of infection in squash seed samples were 6.66% (Gümüş *et al.* 2001), and 18.5% (Gümüş *et al.* 2004) for SqMV in squash in Turkey. Therefore, seed transmission may be important for introducing the virus to the Black Sea Region.

It has been reported that SqMV is distributed by seeds, insect vectors and many weeds is known to be hosts for SqMV. It survives in infected cucurbit weed hosts, in infected seed, and in overwintering beetles (Langham *et al.* 1997; Jossey and Babadoost, 2008). Although no work to demonstrate the presence of the beetle vector was carried out in this study, the identification of SqMV in the region emphasizes the importance of efficient phytosanitary controls. Effective control of virus diseases in cucurbits can only be achieved by adapting an integrated approach (Ali *et al.* 2004). Control of SqMV is normally through the use of virus-free seeds (Anonymous, 2010) and insecticides for the control of beetles. An important alternative for the disease control would be the use of cultivars that carry a gene for a resistance to the infecting viruses (Gholamalizadeh *et al.* 2008; Pang *et al.* 2000).

The present study provides information on the occurence of SqMV in cucurbit fields in Samsun, Turkey. The results obtained in this investigation clearly demonstrate that SqMV is widely distributed in different areas of the Black Sea Region of Turkey and provide evidence that this virus causes serious damages in cucurbit crops. Further studies are needed to characterise SqMV, its epidemiology, yield loss, and management in Turkey. Although SqMV has been reported in surveys of cucurbit crops in the other region of Turkey, This is the first report of SqMV from commercial squash and pumpkin fields in the Black Sea Region of Turkey.

ÖZET

SAMSUN İLI KABAK ÜRETIM ALANLARINDA *Squash mosaic virus* (SqMV)' nün BULUNUS ORANLARININ BELIRLENMESI

Samsun ili kabak üretim alanlarının büyük bir kısmında, yapraklarda hafif veya şiddetli mozayik, koyu yeşil damar bantlaşması, yaprak ve meyvelerde şekil bozukluğu veya yaprak ayasının daralması şeklinde virus simptomları gözlenmiştir. Kabakgil üretimi, virüsler tarafından önemli oranda etkilenmektedir. Samsun ilinde kabakgil

bitkilerinde SqMV'yi saptamak amacıyla 2009 ve 2010 yılında kışlık ve yazlık kabak üretim alanlarında sürveyler yapılmıştır. Üretimin yoğun olarak yapıldığı 22 alandan virus belirtisi gösteren sakız (*C. pepo* L.), bal (*C. moschata* Duch) ve kestane (*C. maxima* Duch) kabağı örnekleri toplanarak DAS-ELISA yöntemi ile test edilmiştir. DAS-ELISA yöntemine göre simptomatik bitkilerin %20,9'nun *Squash mosaic virus* (SqMV) ile enfekteli olduğu belirlenmiştir. Bu çalışma ile SqMV, Karadeniz Bölgesi'nde yetiştirilen kabaklarda ilk kez tespit edilmiştir.

Anahtar kelimler: SqMV, tespit, epidemiyoloji, ELISA

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