

VIRUS DISEASES OF EDIBLE SEED SQUASH (*CUCURBITA PEPO* L.) IN KONYA PROVINCE

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Abstract

Squash seeds have been used as a snack in Turkey like in some Mediterranean countries and Germany, Hungary, Austria and China. Viral diseases are very destructive especially on squash (*Cucurbita pepo* L.) which is grown for seeds in Konya province. This paper aims at determining the virus infections in major squash growing areas in Konya province (Turkey). Totally 334 plant samples with common virus symptoms like mosaic, curling, blistering, mottling, distortion, shoestring, stunting and vine decline were collected from squash plants during 2013. The viruses were identified by DAS-ELISA. The results showed that 80.53 % of plant samples were infected with *Zucchini yellow mosaic Potyvirus* (ZYMV), *Watermelon mosaic Potyvirus-2* (WMV-2), *Cucumber mosaic Cucumovirus* (CMV), *Papaya ringspot Potyvirus-watermelon strain* (PRSV-W) and *Squash mosaic Comovirus* (SqMV). ZYMV was predominant in the research area with the ratio of 60.18 %. WMV-2 was detected in squash at the ratio of 52.99 % and it was second important virus disease in the survey area. Also mixed infections of those virus infections were detected commonly in squash. *Cucumber green mottle mosaic Tobamovirus* (CGMMV) was not present in the research area.

Keywords: DAS-ELISA, edible squash seed, Konya, WMV-2, ZYMV.

Introduction

Cucurbits (the Cucurbitaceae family) include 119 genera and 825 species distributed primarily in tropical and subtropical regions of the world (Andres, 2004; Jeffrey, 1990) The major cultivated cucurbit species such as melon (*Cucumis melo* L.), cucumber (*Cucumis sativus* L.), squash (*Cucurbita pepo* L.), and watermelon (*Citrullus lanatus* (Thunb) Matsum.&Nakai) are important vegetable crops worldwide. According to the different estimates, 3-5% of overall vegetable production is lost due to virus infections, but losses can be occasionally very high, where pest control is insufficient, especially in developing countries (Caciagli, 2010). Virus diseases are a worldwide problem for cucurbit production and cause serious economic losses. Indeed, more than 35 different viruses have been isolated from cucurbits (Provvidenti, 1996). These viruses constitute complex and dynamically changing problems as described by Nameth et al. (1986). Edible seed squash is among the major vegetables grown in Konya province of Turkey. It planted on 1839 ha in the province during 2013, with an estimated production of 2093 tons (Anonymous, 2012). From different parts of Turkey, several virus diseases inducing mosaic symptoms were previously reported including *Cucumber mosaic Cucumovirus* (CMV) (Kurcman, 1977), *Watermelon mosaic Potyvirus-2* (WMV-2) (Nogay & Yorgancı, 1984), *Zucchini yellow mosaic Potyvirus* (ZYMV) (Davis & Yılmaz, 1984), *Papaya ringspot Potyvirus-watermelon strain* (PRSV-W) (Erdiller & Ertunç, 1988), *Cucumber vein yellowing Ipomovirus* (CVYV) (Yılmaz et al., 1991), *Cucurbit aphidborne yellows Polerovirus* (Yılmaz et al., 1992), *Melon mosaic virus* (MMV) (Yılmaz et al., 1995), *Tomato ringspot Nepovirus* (TRSV) and *Tomato black ring*

Nepovirus (TBRV) only in cucumber (Fidan, 1995), *Squash mosaic Comovirus* (SqMV) (Ça lar et al., 2004 Gümü et al., 2001; Gümü et al., 2004).

In the present research, one year of surveys were undertaken in order to evaluate the incidence and distribution of viruses (WMV-2, ZYMV, CMV, SqMV, PRSV-W, and CGMMV) infecting squash crops grown in the Konya province.

Material and methods

Collection of infected plant material

Symptomatic leaf samples were collected during the period July-October 2013 in the main edible seed squash-growing areas of Konya province. Each sample consisted of the youngest fully developed leaf from plants exhibiting symptoms such as mosaic, mottling, vein clearing, blistering distortion, shoestring, stunting or yellowing and fruit discoloration and deformation. All samples were tested for the presence of ZYMV, WMV-2, CMV, PRSV-W, CGMMV and SqMV. All collected samples were placed in plastic bags, and stored in a freezer (-20°C) until use.

DAS-ELISA

All collected samples were subjected to DAS-ELISA and were tested for the presence of ZYMV, WMV-2, PRSV-W, CMV, SqMV and CGMMV using the double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) tests as described by Clark & Adams (1977). The antisera and conjugates were purchased from BIOREBA AG (Reinach, Switzerland) (ZYMV, WMV-2, SqMV, CMV, and PRSV-W), and ADGEN Phytodiagnosics (Neogen Europe Ltd., Scotland, UK) (CGMMV) and used according to the instructions of the companies. The leaf 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 KH₂PO₄, 2.9 g Na₂HPO₄.12H₂O, 0.2 g KCl, 0.2 g NaN₃, 20 g polyvinylpyrrolidone-25 per L, pH 7.4) at a ratio of 1:8. Plates (Nunc Microwell, Roskilde, Denmark) were precoated with virus IgG that were diluted in carbonate buffer (1.59 g Na₂CO₃, 2.93 g NaHCO₃, 0.2 g NaN₃ per L, pH 9.6), and incubated for 4 h at 37°C. After washing the plates with PBST buffer (8.0 g NaCl, 0.2 g KH₂PO₄, 2.9 g Na₂HPO₄.12H₂O, 0.2 g KCl, 0.2 g NaN₃, 0.5 mL Tween-20 per L) three times, samples were added to wells and incubated overnight at 4°C. Alkaline phosphatase conjugated antibody diluted in conjugate buffer (PBST+2% polyvinylpyrrolidone-40 + 0.2% egg albumin (Sigma A-5503) pH 7.4) was added after washing the plates, and incubated for 4 h at 37°C. *P*-nitrophenylphosphate in substrate buffer (97 mL diethanolamine, 0.2 g NaN₃ L⁻¹, pH 9.8) was added to each well and incubated for 30 to 90 min. at dark and room temperature. Absorbance values were measured at 405 nm using an Anthos 2010 Microplate Reader (Biochrom Ltd., Cambridge, UK) at the laboratory. Tests were considered positive when the mean absorbance value of tested samples were greater than twice the healthy control (Abou-Jawdah et al., 2000; Cradock et al., 2001; Ertunç, 1992; Paylan & Erkan, 2011; evik & Arlı-Sökmen, 2003).

Results and discussion

In this study, 334 plant samples were tested by DAS-ELISA. The relative frequencies of the different viruses infecting squash are reported in Tables 1 and 2. The data clearly showed that ZYMV and WMV-2 are the most widespread cucurbit viruses in the research area. Their incidence reached 60.18 and 52.99% in squash, respectively. They are followed by CMV, SqMV, and PRSV-W 13.77, 1.8, and 1.2% in all tested samples, respectively. CGMMV were not detected in any of the tested squash samples. Double virus infection was detected in 153 of the samples. Among double infected plants, 69.28 % were infected with ZYMV+WMV-2, the most frequently detected viruses in the samples. Double infections with CMV+ZYMV (13.07%), CMV+WMV-2 (11.76%), PRSV-W+ZYMV (2.61%),

SqMV+ZYMV (1.96%), and SqMV+WMV-2 (1.31%) were detected. Triple infections were not detected in tested samples. Among the surveyed districts, the highest incidences of the viruses were detected in Altinekin and Yunak with the ratio of 86.66%. ZYMV was the most commonly detected in samples from Yunak (75%), Altinekin (61.66%) and Ak ehir (56.25%). As for, WMV2-2, CMV, and SqMV were frequently detected in samples from Konya-Merkez (60%), Yunak (20%), and Altinekin (6.66%), respectively.

Table 1. Number of plants infected by the following viruses: ZYMV, WMV-2, CMV, PRSV-W, SqMV or CGMMV as determined by DAS-ELISA

Location	No. Tested	Healthy	CMV	SqMV	WMV-2	ZYMV	CGMMV	PRSV-W
Akşehir	32	9	1	0	2	2	0	0
Altinekin	60	8	3	1	9	16	0	0
Çeltik	50	12	0	0	6	13	0	0
Çumra	72	13	3	0	14	10	0	0
Güneysınır	40	10	1	0	7	9	0	0
Konya Merkez	20	5	0	0	4	3	0	0
Yunak	60	8	0	0	3	9	0	0
Total	334	65	8	1	45	62	0	0

Table 2. Number of samples double infected by the following viruses: ZYMV, WMV-2, CMV, PRSV-W, or SqMV as determined by DAS-ELISA

Location	Double virus infection					
	WMV-2+ ZYMV	CMV+ WMV-2	CMV+ ZYMV	PRSV-W+ ZYMV	SqMV+ WMV-2	SqMV+ ZYMV
Ak ehir	13	2	2	1	0	0
Altinekin	14	1	3	2	1	2
Çeltik	13	2	3	0	0	1
Çumra	21	5	4	1	1	0
Güneysınır	11	2	0	0	0	0
Konya Merkez	6	2	0	0	0	0
Yunak	28	4	8	0	0	0
Total	106	18	20	4	2	3

In Konya province, edible seed squash is economically important but has a high incidence of virus-like symptoms. Viruses causing mosaic, leaf deformation, fruit deformation and reduced growth were observed in squash plants in the province. 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 cucurbits fields worldwide (Abou-Jawdah et al., 2000; Alonso-Prados et al., 1997; Davis et al., 2002; Dodds et al., 1984; Lecoq et al., 1981; Luis-Arteaga et al. 1998; Makkouk & Lesemann 1980; Massumi et al., 2007; Provvidenti, 1996; Sammons et al. 1989; Yuki et al., 2000). The occurrence and incidence of viruses on cucurbit plants have been studied in different parts of Turkey. The presence of ZYMV, WMV-2, CMV, PRSV-W and SqMV has been reported by different researchers (Ça lar et al., 2004; Davis & Yılmaz, 1984; Erdiller & Ertunç, 1988; Fidan, 1995; Köklü & Yılmaz, 2006; Kurcman, 1977; Nogay & Yorgancı, 1984; Özasan et al., 2006; Yılmaz & Davis, 1985; Yılmaz et al., 1991; Yılmaz et al., 1995). ZYMV and WMV-2 were the most widespread viruses in our study. Similarly, Yılmaz et al., (1992) found that they were the most common viruses among five viruses (CMV, WMV-2,

ZYMV, PRSV-W and CABYV) in different provinces of Turkey. Also, a survey was performed by Evik & Arlı-Sökmen (2003) on 165 cucurbits in Samsun province, 53.9% WMV-2, 38.8% ZYMV and 20.6 CMV of 165 samples were determined. In the survey conducted in 33 fields in the Gaziantep province of Turkey for viruses infecting cucurbits, ZYMV was found in higher incidence than two other viruses, CMV and *Potato Potyvirus Y* (PVY) (Özaslan et al., 2006).

Conclusion

With this study, the presences of ZYMV, WMV-2, CMV, PRSV-W and SqMV on squash were determined in the province. The results revealed that edible seed squash grown in commercial fields commonly were infected with viruses in Konya. According to the results obtained, the following recommendations should be considered for Konya Province

1. Certified and virus-free seed must be used.
2. Except for SqMV, all viruses detected were spread efficiently by aphids and mechanical inoculation (Hollings et al., 1981; Kaper and Waterworth, 1981). Unfortunately, growers in the province are not aware of spreading the viruses from plant to plant and don't know about control measures for virus dissemination.
3. Weeds play an important role on virus epidemiology and are a common problem in vegetable-growing areas in the province. In order to control the virus infection, weeds should be controlled.
4. Cultural practices are very important in cucurbits. For this purpose, cultural practices such as sowing, fertilizing and irrigation should be performed properly.
5. Virus infected plants should be destroyed promptly to prevent them from serving as sources of further infections.

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