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Detection of Some Virus Diseases of Edible Seed Squash (*Cucurbita pepo* L.) in Nevşehir Province, Turkey

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ABSTRACT

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DAS-ELISA, Edible seed squash, Nevşehir, WMV, Virus Squash is a member of Cucurbitaceae family. It is grown for fresh consuming and its seeds are used as a snack in Turkey like some Mediterranean countries and Germany, Hungary, Austria and China. Cucurbita pepo L. is mostly used for cultivating squash seeds in Turkey. Also, a small amount of seeds obtain from Cucurbita moschata Duch (Butternut squash or pumpkin). Virus diseases are one of the most destructive diseases on squash which is grown for seeds in Nevşehir province. In this study, it was aimed to determine the virus infections in major squash growing areas in Nevşehir province. Totally 134 plant samples with common virus symptoms like mosaic, curling, blistering, mottling, distortion, shoestring, stunting and vine decline were collected from squash plants during 2018. In this study. Double-Antibody Sandwich Enzyme-Linked Immunosorbent Assay (DAS-ELISA) method is used for identifying the virus infections on the plant samples. According to the results of the DAS-ELISA, 97.76 % of plant samples were infected with Zucchini yellow mosaic potyvirus (ZYMV), Watermelon mosaic potyvirus-2 (WMV), Cucurbit aphid borne yellow virus (CABYV), Cucumber mosaic cucumovirus (CMV), Papaya ringspot potyvirus-watermelon strain (PRSV-W) and Squash mosaic comovirus (SqMV). WMV was predominant in the research area with the ratio of 89.55 %. ZYMV was the second important virus disease in the surveyed area and it was detected on the samples at the ratio of 57.46 %. Also, mixed infections of those virus infections were detected commonly in squash. Especially, ZYMV+WMV (40.29 %) and WMV+ZYMV+PRSV-W (8.20 %) mixed infections were common.

1. Introduction

Squash seeds are one of the most nutritionally rich vegetable by-products out there, having a high content of unsaturated fat, protein, beta carotene, vitamin C, vitamin B1, fiber, iron, calcium, and potassium. These seeds were originally a main food for countries like China, United States, India, and Mexico and recently whole world has realized to the health benefits of these seeds. Although edible seed squash has been grown in Turkey for many years, there has been a rapid increase in the production area and quantity since 2004. As a result, in the year of 2018, edible seed squash production has reached 55.043 tons in about 73.789 ha. production area (TÜİK 2019). In our country, the most important reason for the increase in the production of edible seed squash is that this plant can be grown in both arid and irrigated field conditions. This plant is seen as an economic alternative product that can grow especially in arid conditions. For squash growing, one

of the most important problems is virus diseases. It's hard to estimate or calculate the amount of yield losses in crops due to virus diseases. According to the different calculations, 3-5% of overall cultivated vegetable crops are lost because of virus diseases, but these losses can be sometimes very high, where pest control is inadequate, particularly in developing countries (Caciagli 2010). Viruses can cause important economic losses in the world for cucurbit growing. Indeed, on cucurbits, more than 35 different species have been determined as pathogen (Provvidenti 1996). These pathogens cause complicated and dynamically varying problems (Nameth et al 1986). Edible seed squash is one of the most common vegetable crops which is grown in Nevşehir province in Turkey. It occupied 21.165 ha in Nevşehir in 2018, with a predicted production of 16.403 tons (Anonymous 2019). Previous studies from different parts of Turkey have reported different viruses such as Zucchini yellow mosaic potyvirus (ZYMV), Squash mosaic comovirus (SqMV) and Cucumber mosaic cucumovirus (CMV, Cucurbit aphid borne yellows polerovirus (CABYV), Papaya ring spot potyvirus - watermelon strain (PRSV-W),

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Watermelon mosaic potyvirus - 2 (WMV), Cucumber vein yellowing ipomovirus (CVYV), Tomato ring spot nepovirus (TRSV), Melon mosaic virus (MMV), and Tomato black ring nepovirus (TBRV) in the plants of family Cucurbitaceae. (Kurcman 1977; Davis & Yılmaz 1984; Nogay & Yorgancı 1984; Erdiller & Ertunç 1988; Yılmaz et al 1991; Yılmaz et al 1992; Fidan 1995; Gümüş et al 2001; Çağlar et al 2004).

ZYMV, WMV, and PRSV-W are typical members of Potyvirus genus (Potyviridae), so they have flexuous filamentous particles, and single stranded positive sense RNA genome. Potyviruses can be transmitted efficiently by mechanical inoculation and vector aphid species. ZYMV can be transmitted with seed to a low level, while PRSV-W and WMV are not transmitted with seeds (Lisa & Lecoq 1984; Purcifull et al 1984a; Purcifull et al. 1984b). CMV is a polyhedral shaped virus that is member of the genus Cucumovirus in the Bromoviridae family and has a three-part genome consisting of ssRNA with positive polarity. This virus can be transmitted by seeds of some hosts, aphids and mechanically (Francki et al 1979). SqMV, which has a positive sense ssRNA genome, belongs to the genus Comovirus (Secoviridae). The particle of the virus is hexagonal formed of isometric subunits and can be transmitted by insect vectors, seed and mechanically (Campbell 1971). CABYV belongs to the genus Polerovirus in the family Luteoviridae (King et al 2012) and was first reported in France in 1992 (Lecoq et al 1992). The virus causes yellowing and thickening of the older leaves in cucurbit plants and is often mistakenly attributed as a nutrient deficiency. Although the major veins of younger leaves would remain green after the infection, plant yield may be reduced (Lecoq et al 1992). The virus is transmitted primarily by Aphis gossypii Glover and Myzus persicae Sulzer, and the transmission could be circulative, persistent, and nonpropagative (Dogimont et al 1996; Gray et al 2014). CABYV has been reported from cucurbit crops across different climatic regions of the world such as temperate, Mediterranean, and subtropical (Lecoq 1999), and no mechanical transmission has been reported (D'Arcy & Domier 2005). The main constraint for the management of diseases caused by members of Luteoviridae is that no effective strategy exists to cure plants after virus infection (Michelle & Veronique 2018).

In this study, one year of surveys were carried out for determining the incidence and distribution of viruses (CABYV, CMV, PRSV-W, WMV, SqMV, ZYMV) infecting edible seed squash crops grown in Nevşehir province.

2. Materials and Methods

2.1. Collecting of virus infected squash leaves

Surveys were conducted by collecting symptomatic squash leaf samples from main squash growing fields in 6 different districts (Center, Acıgöl, Avanos, Der-

inkuyu, Gülşehir and Ürgüp) of Nevşehir province during July through September in 2018 (Table 1). In order to samples to represent Nevşehir province, more than % 1 of total edible seed squash growing areas (2 130 da) of the province were surveyed. For this purpose, 24 edible seed squash fields were visited in the province. In these studies, the number of collected samples from each field was determined according to the amount of surface area of the field. So that, at least 5 samples were collected from the fields which have up to 50 da and 8 samples were collected from the fields which have more than 50 da growing areas. The samples were picked from plants which showed virus diseases symptoms like blistering-distortion, mottling, vein clearing, mosaic, yellowing, shoe-string, or stunting and fruit discoloration and deformation. In each field, it was tried to take samples from plants with different symptoms. Five different leaves from each plant showing symptoms of virus diseases were taken as a sample. The samples were tested to determine for the infections of CMV, ZYMV, WMV, SqMV, CABYV and PRSV-W. They were put in plastic bags, and kept in a deep-freezer (-20°C) until diagnostic tests.

2.2. Testing by DAS-ELISA

For determining the virus infections (CABYV, PRSV-W, CMV, WMV, SqMV, and ZYMV) on the squash leaf samples, Double-Antibody Sandwich Enzyme-Linked Immunosorbent Assay (DAS-ELISA) test method was used (Clark and Adams 1977). The antibodies were provided from commercial companies and utilized with respect to the instructions of them. Before the homogenization of the leaf samples, microplates were coated with virus IgG that were diluted in carbonate buffer (pH 9.6), and incubated for 4 h at 37°C. The squash leaf samples were grinded in a mortar with the addition of the sample extraction phosphate buffer solution at a ratio of 1:10 (PBS, pH 7.4). After washing the microplates with washing buffer (PBST) thrice, the extracted samples were added to wells and incubated overnight in a refrigerator (+4°C). Alkaline phosphatase (APP) conjugated antibody diluted in conjugate buffer (pH 7.4) was added after washing the plates, and incubated for 4 h at 37°C. Substrate buffer (pH 9.8) with Para-nitrophenylphosphate (P-NPP) was added to each well and then, incubated for 30 to 90 min. at dark and room temperature (Fig. 1). Absorbance values were determined at 405 nm by Anthos 2010 Microplate Reader (Biochrom Ltd., Cambridge, UK). Test was assessed as positive when the average absorbance value of tested sample was greater than two times of healthy (uninfected) control (Abou-Jawdah et al 2000; Yeşil & Ertunç 2012).

2.3. Determining infection rates of the viruses

Numbers of infected plant samples for each virus were determined by DAS-ELISA tests. Infection rates of each virus were calculated by simple proportion. Therefore, for each virus, numbers of sum of single, double and multiple virus infected plant samples were divided to numbers of total tested samples then the Table 1

Surveyed districts and number of collected plant samples

results were multiplied with 100. In this way, infection rates of each virus were calculated as percentage.

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Districts	Number of collected samples	Number of surveyed fields	Total areas of the fields (da				
Nevşehir (Center district)	15	3	130				
Acıgöl	20	4	400				
Avanos	53	7	855				
Derinkuyu	11	3	195				
Gülşehir	19	4	250				
Ürgüp	16	3	300				
Total	134	24	2 130				

Figure 1

Steps of DAS-ELISA tests. A: Coating wells with virus IgG. B: Extracted samples were added to wells and incubated overnight in a refrigerator (+4°C). C: Substrate (*P*-NPP) added microplates, incubated for 30 to 90 min. at dark and room temperature. D: End of the incubation, the positive wells got yellow and absorbance values were determined by ELISA reader.

3. Results and Discussion

In this study, totally 134 edible seed squash leaf samples were tested by DAS-ELISA. The incidences of the different viruses which infect squash are given in Tables 2 and 3. They obviously show that WMV and ZYMV are the most common squash viruses in the survey area. According to the results of the DAS-ELISA 97.76 % of the samples were infected with CABYV, PRSV-W, ZYMV, CMV, WMV, and SqMV. WMV was the most common virus in the survey area with the ratio of 89.55 %. The second important virus disease in the research area was ZYMV; it was detect-

ed on the samples at the ratio of 57.46 %. They are followed by PRSV-W, SqMV, CABYV and CMV 14.18, 2.99, 2.23 and 0.75% in all tested samples, respectively (Table 2). On 82 of all the samples were determined mixed virus infections. Mixed infections of WMV + ZYMV were the most frequently detected ones in the samples with the ratio 40.29 % (Table 3). Double infections with WMV + PRSV-W, WMV + SqMV and ZYMV + PRSV-W were detected as 5.22, 2.98 and 1.49 %, respectively. Triple infections of WMV + ZYMV + PRSV-W (8.20%) and WMV + ZYMV + CABYV (1.49%) were detected in 11 and 2 samples, respectively. According to the DAS-ELISA test results, all of the samples which were collected from Nevşehir Center, Acıgöl, Derinkuyu and Ürgüp were determined as virus infected. WMV was the most commonly detected in samples from Ürgüp (100%), Acıgöl (95%), Gülşehir (94.74%) and Derinkuyu (90.91%). As for ZYMV, PRSV-W, SqMV, and CABYV were frequently detected in samples from Nevşehir Center (80%), Gülşehir (47.37%), Ürgüp (25%), and Avanos (5.66%), respectively. CMV infection was only determined on one sample from Acıgöl (5%).

Table 2

According to DAS-ELISA tests results, the number of single virus infections

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District	No. Tested	Healthy	CMV	SqMV	WMV	PRSV-W	ZYMV	CABYV
Nevşehir (Center)	15	0	0	0	3	0	2	0
Acıgöl	20	0	0	0	5	0	0	0
Avanos	53	2	0	0	15	0	6	0
Derinkuyu	11	0	0	0	6	0	0	0
Gülşehir	19	1	0	0	7	0	0	0
Ürgüp	16	0	0	0	5	0	0	0
Total	134	3	0	0	41	0	8	0

Table 3

According to DAS-ELISA tests results, the number of multiple virus infections

		Double virus infections					Triple virus infections	
District	WMV +ZYMV	WMV+ PRSV-W	WMV +SqMV	ZYMV+ PRSV- W	ZYMV+ CMV	WMV+ CABYV	ZYMV+ WMV + PRSV- W	ZYMV+ WMV+ CABYV
Nevşehir (Cen- ter)	9	0	0	0	0	0	1	0
Acıgöl	11	0	0	0	1	0	3	0
Avanos	23	2	0	1	0	1	1	2
Derinkuyu	2	2	0	1	0	0	0	0
Gülşehir	2	3	0	0	0	0	6	0
Ürgüp	7	0	4	0	0	0	0	0
Total	54	7	4	2	1	1	11	2

Edible seed squash is economically important in Nevşehir province, but have a high incidence of viruslike symptoms. During the surveys, different symptoms were observed such as leaf deformations (crinkle, blistering, shoe-string, etc.), different chlorotic patterns on leaves (mosaic, ring spot, oak leaf, etc.), fruit deformations and growth reductions on squash plants (Fig.2). Also, symptoms of mineral deficiencies like growth reductions, wilting and yellowing were observed in some fields. The observed diseases symptoms in this study were similar to the symptoms previously reported from virus-infected cucurbits fields worldwide (Makkouk & Lesemann 1980; Lecoq et al 1981; Sammons et al 1989; Provvidenti 1996; Luis-Arteaga et al 1998; Yuki et al 2000; Davis et al 2002; Massumi et al 2007; Malandraki et al 2014). The occurrence and incidence of viruses on cucurbit plants have been determined in different parts of Turkey. The infection of CMV, CABYV, ZYMV, PRSV-W, WMV, SqMV, and ToMV has been reported in previous studies (Çağlar et al 2004; Davis & Yılmaz 1984; Erdiller and Ertunç 1988; Fidan 1995; Korkmaz et al 2018; Köklü & Yılmaz 2006; Nogay & Yorgancı 1984; Şevik & Balkaya 2015; Topkaya & Ertunç 2012; Yeşil & Ertunç 2012; Yeşil 2014; Yılmaz et al. 1991). But there are a few reports on virus diseases of edible seed squash plants (Yeşil and Ertunç, 2012; Yeşil 2014; 2019a; b). With this study, WMV and ZYMV were determined as the most prevalent viruses in research area. They are effectively transmitted by either infected sap or vector

aphids. These two viruses have been accepted as the most common viruses of cucurbits in the world (Al-Ali et al 2013). Similarly, in a study which was carried out in different provinces of Turkey by Yılmaz et al (1992) WMV and ZYMV were the most widespread viruses among the tested viruses (ZYMV, WMV, CMV, CABYV and PRSV-W). The similar results were reported by Kızmaz et al (2016). These researchers were conducted a survey in cucurbit fields of Mardin and Divarbakır provinces and they reported that the incidences of WMV (60.00%), CMV (43.13%), ZYMV (39.38%), PRSV-W (21.25%) and CABYV (16.25%). Also, a survey was carried out in Konya province, 334 edible seed squash leaf samples were tested by DAS-ELISA, and ZYMV, WMV and CMV were determined on the samples with the ratios of 60.18%, 52.99% and 13.77%, respectively (Yeşil 2014). The similar results were reported by the Özaslan et al (2006); they carried out a survey study to determine infections of cucurbit viruses in Gaziantep province of Turkey. They reported that ZYMV was the most common virus species on cucurbit plants and, also, the infections of CMV and Potato potyvirus Y (PVY) on cucurbits are common. To reveal viruses of cucurbits in Tokat province, a survey performed by Korkmaz et al (2018). Totally 146 squash plant samples were tested by DAS-ELISA and they found WMV (38.35%), ZYMV (26.71%), ToMV (Tomato mosaic tobamovirus) (22.53%), CMV (13.01) and PRSV-W (5.47%) infections on the samples. Also,

they didn't determine any infection of TMV (*Tobacco mosaic tobamovirus*), SqMV and PVY.

Also, mixed virus infections were determined on 82 of the plant samples with this study (Table 3). In previous studies, mixed virus infections on cucurbits were reported (Kaya & Erkan 2011; Topkaya et al 2019; Yeşil & Ertunç 2012; Yeşil 2014; 2019a;b; Yuki et al 2000).

Some of the cucurbit viruses can be transmitted by seeds such as CMV, ZYMV, SqMV, TRSV, and CGMMV. A research was performed to detect the presence of seed borne viruses in pumpkin seed lots collected from Samsun, Sinop, and Bolu provinces during 2013-2014. According to the results of this research, the seed samples were only infected with ZYMV (12.5%) and CMV (4.1%). Moreover, any infection wasn't determined on the seeds of SqMV, TRSV, and CGMMV. With another study about determining seed infections of some viruses in major cucurbit growing areas in Konya, Karaman and Aksaray provinces of Turkey during 2009 and 2010. The results of this study showed that 8,7% of seed samples were infected with ZYMV (4,3%), WMV (3,3%) and CMV (1,1%). PRSV-W, SqMV and CGMMV were not determined in any of the tested samples and were not present in the tested cucurbit seeds lots (Yeşil & Ertunç 2016). As can be seen in the above mentioned studies, the reason of occurring frequently infections on cucurbits by the viruses such as ZYMV, WMV and CMV may be infected seeds.

In the present study, WMV and ZYMV were detected as most common viruses. In previous studies, similar viruses were detected in different incidences (Erdiller & Ertunc 1988; Fidan 1995; Korkmaz et al 2018; Köklü & Yılmaz 2006; Nogay & Yorgancı 1984; Şevik & Balkaya, 2015; Yeşil & Ertunç, 2012; Yeşil 2014; 2019a; b; Yılmaz et al 1991). It may be two main reasons for this. Firstly, although other cucurbit crops are grown only in irrigated fields, edible seed squash plant can be grown in either irrigated or semi-arid conditions. The second reason is regional differences. It's normal that, different viruses infect to the same plant species in different environmental conditions. Because differentiations in environmental conditions determine significantly some factors which effect virus epidemiology. These factors are populations and varieties of weed and vector species in or near fields, plant species which are grown in adjacent fields and plant vitality.



Figure 2

Virus diseases symptoms on edible seed squash plants. A: Mosaic symptoms on the leaf caused by WMV. B: Severely leaf deformations and blisters on the leaf because of ZYMV+WMV double infection. C: Shoestring symptoms on

squash leaves because of ZYMV+CMV double infections and (D) blisters on fruit because of ZYMV+WMV double infections.

4. Conclusions

The presences of CABYV, CMV, PRSV-W, WMV, ZYMV, and SqMV on edible seed squash were firstly detected in Nevşehir with the study. The results showed that one of the most important problems in squash growing in the province is virus infections. Because, during the survey studies, symptoms of virus diseases were observed almost each edible seed squash fields in the province. According to the results of this study, for reducing or eradication of virus diseases in squash production areas in Nevşehir province and can be produced more yielded and more quality edible squash seeds the following suggestions must be regarded.

First of all, healthy, non-infected, pathogen-free and certified seed should be used.

For controlling virus diseases efficiently, it is very important to know about transmission ways and infection sources of the viruses. It's known that, except of SqMV, all viruses detected in our study were spread by mechanical inoculation and aphids (Kaper & Waterworth 1981). Unfortunately, squash growers in the province neither know symptoms of virus diseases nor know transmission ways of the viruses from plant to plant. Therefore, they are not able to efficiently control virus diseases as they can't prevent the spread of viruses via vector aphid species.

Also, some of the common weed species in squash growing areas have a great importance in the epidemiology of virus diseases because they role as reservoir plants for virus diseases (Zitter 2002; Yeşil & Ertunç 2015). For preventing virus infection of cucurbits, weeds must be controlled.

As well in other plant crops production, in squash production cultural practices are very important. If all conditions which are necessary for growing healthy plant can be obtained, possibility of chance of phytopathological problems occurrence will be minimum. Therefore, cultural practices such as tilling, planting, fertilizing and irrigation should be done properly.

The plants which show virus diseases symptoms should be eradicated as soon as seen. Since, they act as infection sources for later infections.

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