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Seed Transmissibility of Viruses in Winter Squash Landraces Collected from the Black Sea Region of Turkey

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Abstract

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The presence of seed-borne viruses in winter squash landraces in the Black Sea region of Turkey were detected. The seed samples of landraces were tested by ELISA for *Cucumber mosaic virus* (CMV), *Zucchini yellow mosaic virus* (ZYMV), *Squash mosaic virus* (SqMV), *Tobacco ring spot virus* (TRSV), and *Cucumber green mottle mosaic virus* (CGMMV). Based on the ELISA results, 11.3% of the samples were infected with the viruses; ELISA, CMV was found at the rate of 5.1% in the winter squash samples, while incidence of ZYMV was determined as 6.2%. Moreover, none of the plants germinated from seeds were found to be infected with SqMV, TRSV, and CGMMV. This is for the first time that seed-borne viruses of winter squash landraces have been identified in the Black Sea region of Turkey.

Keywords: CMV; ZYMV; winter squash; serological test; seed transmission; Turkey

Winter squash is a member of the genus *Cucurbita* within the economically important *Cucurbitaceae* family. There are three economically important *Cucurbita* species, namely *C. pepo*, *C. maxima*, and *C. moschata*, which have different climatic adaptations and are widely distributed in agricultural regions worldwide (Paris & Brown 2005). *Cucurbita* spp. is collectively ranked among the 12 leading vegetable crops worldwide. Turkey is ranked the 12th in the world in terms of total squash and pumpkin production (FAOSTAT 2012). Winter squash is one of the most important cucurbit vegetable crops in Turkey. Annual winter squash production in Turkey is 95.076 t according to 2013 records. Ankara province has a big share (12.1%) of winter squash production with 11.498 t, followed by Sakarya (9.378 t), Duzce (8.081t), and Samsun (6.685 t) provinces (TurkStat 2013).

The Black Sea region in the north of Turkey is one of its richest regions as for the crop genetic resources diversity. Winter squash landraces are sometimes grown as unimproved populations in the Black Sea region. The exchange of seeds among farmers could support maintenance of the genetic diversity in these winter squash populations (Balkaya *et al.* 2009). These traditional landraces are an important genetic resource for plant breeders because of their considerable genotypic variation. There is a great diversity in morphological characteristics of winter squash populations found in the Black Sea region of Turkey, particularly in fruit length, fruit diameter, fruit shape, fruit brightness, flesh thickness, and fruit colour (Balkaya *et al.* 2010).

Viral diseases of cucurbit crops cause important economic losses throughout the world. More than 39 different viruses associated with cucurbit diseases have been reported (Ali *et al.* 2012). Seed transmission plays a pivotal role in the spread and survival of a number of important plant viral diseases. Infected seed is probably the most important source of viruses in commercial crop production (Sastry 2013). *Cucumber mosaic virus* (CMV), *Zucchini yellow mosaic virus* (ZYMV), *Squash mosaic virus* (SqMV), *Tobacco ring spot virus* (TRSV), and *Cucumber green mottle mosaic virus* (CGMMV) are the five most frequent

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and economically important seed-borne cucurbit viruses worldwide (Provvidenti 1996).

Cucumber mosaic virus has a broad host range, infecting more than 86 different families including *Cucurbitaceae*. The virus is distributed widely throughout most regions of the world. It can be transmitted from plant to plant both mechanically by sap and by aphids. It can also be transmitted in seeds (Lecoq *et al.* 1998). ZYMV was first detected in summer squash in northern Italy (Lisa *et al.* 1981). Subsequently, the virus spread worldwide, especially infecting squashes and pumpkins and causing significant yield reductions in these crops (Desbiez & Lecoq 1997; Svoboda & Polák 2002). The virus is easily non-persistently transmissible by aphids and at long distances via infected seeds (Fletcher *et al.* 2000). CGMMV, SqMV, and TRSV are readily seed-borne in cucurbit seeds and they are spread among plants by vectors (Ling *et al.* 2011).

The utilisation of plant genetic resources is one key to improving agricultural productivity and sustainability. Plant genetic resources for food and agriculture are part of the biological wealth indispensable for securing world food supplies, alleviating poverty and sustaining rural development (Morico *et al.* 1998). Intensive genetic improvement along with the development of agricultural inputs have led to the replacement of many local varieties by a few uniform modern cultivars in developing countries. For this reason, preservation of populations and landraces is very important (Balkaya & Yanmaz 2005).

There has been no comprehensive study on seed-borne viral diseases of cucurbit crops in the Black Sea region of Turkey. Until now, no data on the incidence and distribution of these viruses in winter squash seeds have been available in this region. The present study was carried out to detect the presence of seed-borne CMV, ZYMV, SqMV, TRSV, and CGMMV in winter squash landraces in the region. This is for the first time that seed-borne viruses of winter squash landraces in Amasya, Bolu, Samsun, and Sinop provinces have been investigated.

MATERIAL AND METHODS

Material. A comprehensive collecting programme for the winter squash populations of Turkey was initiated in 2005 (Balkaya *et al.* 2008). Ninety-six local populations of winter squash were collected from Amasya, Bolu, Samsun, and Sinop provinces of the Black Sea region (Balkaya *et al.* 2009). These genetic resources were preserved at the seed gene bank of the Agriculture Faculty, Ondokuz Mayis University, Samsun, Turkey. In this study, we used the seeds of winter squash populations from these gene pools.

Grow-out tests. The grow-out tests were carried out in the controlled plant growth cabin of the Department of Plant Protection during 2012–2013. Winter squash seeds were germinated in plastic pots. A total of 1920 (96 × 20 seeds) winter squash seedlings were tested in the pots kept in the growth room at 24°C under a 12 h photoperiod for one month and observed daily for symptoms development. At 3–4 leaf stages, all the plants germinated from seeds were tested by ELISA for seed-borne virus presence.

Serological tests. Polystyrene 96-well plates and polyclonal antiserum kits for CMV, ZYMV, SqMV, TRSV, and CGMMV (Bioreba, Reinach, Switzerland) were used in the study. All chemicals required for the ELISA testing were provided by the Laboratory of Virology. DAS-ELISA tests were applied to identify the seed-borne cucurbit viruses. In DAS-ELISA, the leaf samples obtained from plants germinated from seeds grown in the controlled growth cabin were ground (1 g leaf per 1 ml buffer) in extraction buffer (PBST, pH 7.4) containing 0.05% Tween-20, 0.1% skimmed milk powder, and 2% polyvinylpyrrolidone. Absorbance values were read at 405 nm using a microplate reader (Tecan Spectra, Grödig/Salzburg, Austria) and also confirmed visually after incubation at 24°C for 2 hours. Samples were 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 (Bananej & Vahdat 2008).

Bioassays. Mechanical inoculation tests were carried out by rubbing sap from virus source seedlings that gave high absorbance values in ELISA tests on the true leaves of test plants dusted with aluminum oxide. The sap was prepared by grinding CMV- and ZYMV-infected seedlings in a mortar and pestle with 0.1M phosphate buffer (pH = 7.2) (Usher *et al.* 2012). The isolates were mechanically inoculated onto test plants of the following species: winter squash (*C. maxima*), pumpkin (*C. moschata*), cucumber (*Cucumis sativus*), and watermelon (*Citrillus vulgaris*), maintained in the controlled growth cabin at 24°C and monitored daily for symptom expression (Svoboda *et al.* 2006). The winter squash seedlings for pathogenicity testing were obtained from seeds and were mechanically inoculated as mentioned previously for all tested plants. The inoculated plants were tested by ELISA method for the presence of virus.

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Table 1. Incidence of viruses in the plants germinated from seeds collected from winter squash landraces in the Black Sea region of Turkey

Provinces	No. of populations	No. of seed samples tested	Winter squash plants germinated from infected seeds (%)		
			ZYMV	CMV	SqMV, TRSV, and CGMMV
Amasya	23	460	8.7	6.5	0.0
Bolu	24	480	4.1	4.1	0.0
Samsun	24	480	8.3	8.3	0.0
Sinop	25	500	4.0	2.0	0.0
Total	96	1920	6.2	5.1	0.0

RESULTS

The presence of seed-borne viral agents in winter squash landraces was determined by the aid of biological (grow-out and bioassay) and serological (ELISA) methods. A total of 1920 winter squash plants germinated from seeds were evaluated. ELISA test for detecting CMV, ZYMV, SqMV, TRSV, and CGMMV infection was performed for all the emerged seedlings. The plants germinated from seeds developed together with mosaic symptoms between two and three weeks of growth were positive by ELISA for ZYMV and CMV. The results of ELISA tests showed that the seedlings grown from winter squash seeds in the Black Sea region of Turkey were infected with CMV and ZYMV. The virus infection was detected in 11.3% of the populations sampled, while none of these agents were detected in 88.7% of the total samples. The ELISA absorbance values of negative samples ranged 0.091–0.301 at 405 nm, depending on the antiserum used. Positive samples gave absorbance values of 0.706–2.124.

According to the results of ELISA, 11.3% of the samples belonging to winter squash landraces were infected with ZYMV and CMV. Moreover, none of the plants germinated from seeds was found to be infected with SqMV, TRSV, and CGMMV. In the consequence of the tests conducted, CMV was found at the rate of 5.1% in the winter squash plants germinated from seeds, while incidence of ZYMV in winter squash plants germinated from seeds samples was at 6.2% (Table 1).

The seed samples were obtained from winter squash growing areas in Amasya, Bolu, Samsun, and Sinop provinces. They were found only positive for ZYMV and CMV by DAS-ELISA among five seed-borne cucurbit viruses tested. However, these viruses were detected at different percentages in the plants germinated from seeds from all the provinces. The greatest and the least incidence of seed-borne cucurbit viruses was recorded in Samsun province (16.7%) and Sinop province (6.0%). The infection rates of viruses in winter squash plants germinated from seeds were 15.2% in Amasya province and 8.2% in Bolu province.

ZYMV was the most prevalent virus in all winter squash plants germinated from seeds. The transmission rates of ZYMV in winter squash plants germinated from seeds were 8.7% in Amasya, 8.3% in Samsun, 4.1% in Bolu, and 4.0% in Sinop provinces. CMV was also detected in all four provinces and the infection rate of CMV in plants germinated from seeds was 2.0% in Sinop, 4.1% in Bolu, 6.5% in Amasya, and 8.3% in Samsun provinces (Figure 1). None of the plants germinated from seeds was positive for SqMV, TRSV, and CGMMV in this study.

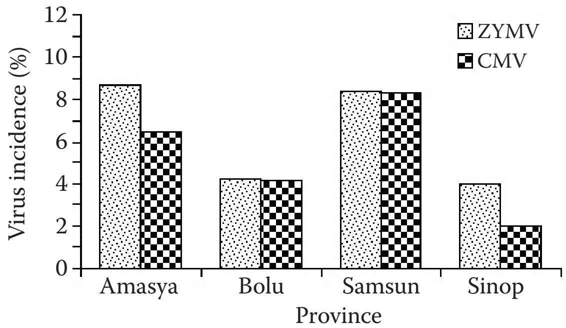

Figure 1. Incidence of seed-borne viruses in the plants germinated from seeds obtained from winter squash growing areas of Amasya, Bolu, Samsun, and Sinop provinces of Turkey

Table 2. Symptoms caused by ZYMV and CMV on test plants and ELISA positive samples

Species	Symptom/Back indexing ELISA	
	CMV	ZYMV
Winter squash	Mo/+	Mo, LM/+
Pumpkin	Mo, LM/+	Mo/+
Watermelon	NLL/+	NLL/+
Cucumber	Mo/+	Mo/+

Mo – mosaic; LM – leaf malformation; NLL – necrotic local lesion; + virus detected in DAS-ELISA

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Pathogenicity assays were also performed by inoculating the seedling extract onto seedlings of indicator plants, i.e. winter squash, pumpkin, cucumber, and watermelon. CMV and ZYMV produced mosaic, leaf malformation, and necrotic local lesion symptoms on cucurbit plants 1–2 weeks post inoculation and they were also detected positive by DAS-ELISA (Table 2).

DISCUSSION

Seed-borne viral pathogens of cucurbit cause high damages to yield and seed quality on cucurbit crops (Lecoq *et al.* 1998). In the present study, the seed-borne viral pathogens were determined in the plants germinated from seeds collected from winter squash populations in the Black Sea region of Turkey. At the end of this study, two different seed-borne viruses were only determined (ZYMV and CMV) in the populations, but none of the samples was positive to SqMV, TRSV, and CGMMV. ZYMV was detected with the highest ratio of 4.0–8.7% in winter squash plants germinated from seeds. Similarly, a total of 62 seed lots originating from pumpkin fruit exhibiting typical symptoms of virus infection were tested by Tobias *et al.* (2008). Of those, 25 seed lots showed seed-borne virus transmission of ZYMV and CMV that ranged from 0.3% to 15.3% of the plants germinated from seeds. The occurrence of ZYMV in winter squash plants germinated from seeds was determined as 6.2% in the present study. Similarly, Fletcher *et al.* (2000) observed seed transmission rates of 3.5% for ZYMV in winter squash using DAS-ELISA.

Seed transmissions of ZYMV in various *Cucurbita* spp. have been recorded by many researchers (Simmons *et al.* 2011). Davis and Mizuki (1986) found ZYMV seed transmission in seedlings of squash at a maximum rate of 80.7% when based on individual infected fruits. Seed transmission of ZYMV was ascertained by ELISA in 7 out of 12 summer squash seed lots. Transmission rates ranged between 1 and 5% (Riedle-Bauer *et al.* 2002). ZYMV can be transmitted by seed in holl-less seeded oil pumpkin, although at a very low rate (Tobias & Palkovics 2003).

In the current study, CMV was found at the rate of 5.1% in the winter squash samples. Similarly, a study was conducted to identify virus transmission in winter squash plants germinated from seeds by Al-Tamimi *et al.* (2009). The seeds were collected from symptomatic and symptomless fruits in Syria. CMV was the most commonly encountered virus in all seedlings (0.5%) and in symptomatic fruits (2.4%).

Cucurbits can easily become infected with more than one virus. Mixed infections of cucurbits by CMV and potyviruses exhibit a synergistic interaction (Wang *et al.* 2002). However, we did not detect CMV+ZYMV mixed infection in any winter squash plants germinated from seeds while the single-virus infections were detected in the present study.

A majority of the viruses that are seed-borne in cucurbits are also insect-transmitted. Accordingly, infected grown-out plants can serve as initial infection foci for secondary virus spread among field grown plants (Sastry 2013). Examples of such viruses include the aphid-transmitted ZYMV and CMV, which infected 6.2 and 5.1% of the winter squash plants germinated from seeds, respectively (Table 1). Previously, colonising aphids were observed in the regions where surveyed (Arli-Sokmen *et al.* 2005). Transmission by insect vectors remains the most likely potential source of CMV and ZYMV.

In conclusion, the results clearly showed that CMV and ZYMV can be transmitted from winter squash seeds contaminated with virus to plants germinated from seeds, highlighting the risk of using seeds from virus-infected plants and the potential for seed transmission to contribute to the further spread of virus. This is the first time that seed-borne viruses were determined on winter squash plants germinated from seeds in the Black Sea region, the most important winter squash growing region in Turkey.

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