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Turnip mosaic virus infecting kale plants in Ordu, Turkey

El virus del mosaico del nabo infecta plantas de *Brassica oleracea* var. *acephala* en Ordu, Turquía

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Abstract. *Brassica oleracea* var. *acephala* L. (kale) is widely grown in the Black Sea Region of Turkey. Kale growing has not been common in the other regions of Turkey. A number of diseases can seriously affect *Brassica* crop production. Field surveys were done to determine the occurrence of viruses in kale-growing areas in Ordu in 2013-2014. Leaf samples were collected from kale plants and tested for the presence of *Turnip mosaic virus* (TuMV), *Cauliflower mosaic virus* (CaMV), *Turnip yellow mosaic virus* (TYMV), and *Cucumber mosaic virus* (CMV) by DAS-ELISA and bioassays. Result of serological and biological tests showed that 7.7% of these samples were infected with TuMV. However, CaMV, CMV, and TYMV were not detected in any of the tested kale plants. The occurrence of TuMV suggested that the virus might be an important threat for the kale crops in the province. This is the first report of occurrence of the virus on kale in Ordu, Turkey.

Keywords: *Brassica*; Kale; Survey; Disease; Virus.

Resumen. *Brassica oleracea* var. *acephala* L. (kale) crece ampliamente en la región del Mar Negro de Turquía. El crecimiento de kale no ha sido común en las otras regiones de Turquía. Varias enfermedades pueden afectar seriamente la producción de cosecha de *Brassica*. Se hicieron estudios de campo para determinar la ocurrencia de virus en áreas de crecimiento de kale en Ordu en 2013-2014. Se recolectaron muestras foliares de plantas de kale y se estudió en las mismas la presencia del virus del mosaico del nabo (TuMV), el virus del mosaico del coliflor (CaMV), el virus del mosaico amarillo del nabo (TYMV), y el virus del mosaico del pepino (CMV) por DAS-ELISA y bioensayos. Los resultados de pruebas serológicas y biológicas mostraron que el 7,7% de estas muestras estaban infectadas con TuMV. Sin embargo, CaMV, CMV, y TYMV no fueron detectados en ninguna de las plantas muestreadas de kale. La ocurrencia de TuMV sugirió que el virus podría ser una importante amenaza para las cosechas de kale en la provincia. Este es el primer informe sobre la ocurrencia del virus en kale en Ordu, Turquía.

Palabras clave: *Brassica*; Kale; Estudio; Enfermedad; Virus.

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INTRODUCTION

Kale (*Brassica oleracea* var. *acephala* L.) is a leafy herbaceous, biennial or perennial, plant in the family Brassicaeae. It is widely grown as a leafy green vegetable in the Black Sea Region of Turkey (Balkaya & Karaagac, 2005; Okumus & Balkaya, 2007). Annual kale production in Turkey is 85000 t according to 2013 recofards (TurkStat, 2013). Commercial growing for fresh production is mainly concentrated in the Black Sea region of Turkey (Balkaya & Yanmaz, 2005).

Cabbage vegetables, like the *Brassica* group, are perceived as very valuable food products. They have a very good nutritive value, high antioxidant activity and pro-healthy potential. Especially, kale is characterized by good nutritional and pro-healthy properties (Sikora & Bodziarczyk, 2012). Diseases are important factors limiting the production of leafy greens. Severe disease development can reduce quality to the point where the crop is unmarketable. Pathogens that cause the most common diseases of leafy greens are fungi, bacteria and viruses. A number of viral pathogens such as the *Turnip mosaic virus* (TuMV), *Cauliflower mosaic virus* (CaMV), *Turnip yellow mosaic virus* (TYMV) and *Cucumber mosaic virus* (CMV) can seriously affect *Brassica* crop production (Raybould et al., 1999; Moreno et al., 2004; Spence et al., 2007).

To date, there is no data on the incidence and distribution of these viruses in kale plants in the Ordu province, which has one of the largest kale production capacities in Turkey (TurkStat, 2013). This paper reports the results of a survey of kale plantings in Ordu, Turkey, during late summer 2013 and early spring 2014 to determine the incidence of common Brassica viruses (TuMV, CaMV, TYMV and CMV).

MATERIALS AND METHODS

Surveys and sample collection. Kale, grown on an area of approximately 5532 ha, is one of the most important crops in Ordu province. Five districts were selected from the major kale-producing districts, based on the intensity of kale production in Ordu province. Surveys were conducted in Camas, Catalpinar, Fatsa, Gurgentepe, and Unye districts of Ordu province. Forty-seven sub-locations were selected from these 5 districts based on the size of the kale production. Samples were collected in a proportional and representative way in accordance with quantities cultivated of the region. A total of 234 samples of kale plants were randomly collected from five kale cultivation locations in Ordu province from late November 2013 to early March 2014 (Fig. 1). Leaf samples collected randomly from non-symptomatic and symptomatic plants (Fig. 2) were placed in plastic bags and brought to the laboratory. All collected samples were tested by DAS-ELISA in duplicate and bioassays.

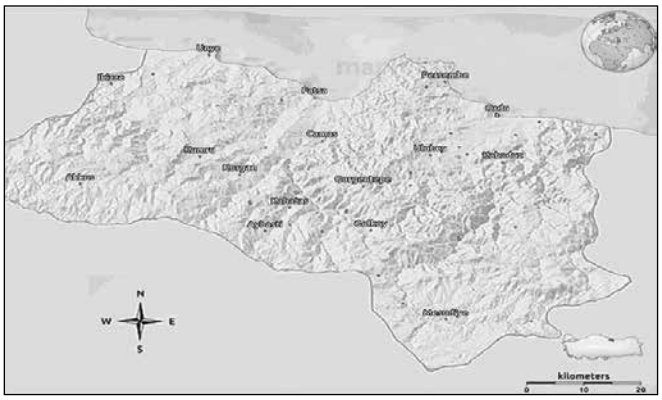

Fig. 1. Map of Ordu province of Turkey, showing surveyed locations.
Fig. 1. Mapa de la Provincia de Ordu en Turquía, mostrando los lugares muestreados.

Fig. 2. Symptoms associated with natural infection by TuMV on kale plant.
Fig. 2. Síntomas asociados con la infección natural por TuMV en plantas de *Brassica oleracea* var. *acephala*.

Serological analysis. Polystyrene 96-well plates (TPP) and polyclonal antiserum kits for TuMV, CaMV, TYMV, and *CMV* (Bioreba) were used in the study. Related chemicals of ELISA were provided from Laboratory of Virology, Department of Plant Protection, University of Ondokuz Mayis. Serological testing, the double antibody sandwich enzyme linked immuno-sorbent assay (DAS-ELISA), was used to detect viruses in kale samples. In the DAS-ELISA test, the leaves of kale plants were grounded (1 g leaf/5 mL buffer) in the extraction buffer (Phosphate Buffered Saline; pH: 7.4) containing 0.05% Tween-20, and 1% skimmed milk powder. ELISA Absorbance values were read at 405 nm using a microplate reader (Tecan Spectra II, Grodig/Salzburg, Austria). Tests were conducted using four negative controls on each plate for each virus. While negative controls were included healthy samples, a reaction was considered positive if ELISA readings were more than twice the average absorbance value of healthy controls (Spence et al., 2007).

Bioassays. Biological studies were done using TuMV isolates that gave the highest ELISA readings from different geographical locations (Camas, Catalpinar, and Unye). The saps obtained by grinding TuMV-infected kale leaves in 0.01 M phosphate buffer (pH: 7.0) (Korkmaz et al., 2008) were mechanically inoculated to indicator plants (i.e., *B. oleracea* var. *capitata, B. oleracea* var. *acephala, B. campestris* subsp. *rapa, Raphanus sativus* and *Chenopodium quinoa)* using carborundum powder as abrasive. The inoculated plants were maintained in a plant growth room at 24 ˚C. The occurrence and type of symptoms were investigated on those inoculated leaves. Inoculated plants were tested for the presence of viruses by DAS-ELISA.

RESULTS

Field survey. Eighteen out of 234 samples (7.7%) reacted positively in DAS-ELISA to TuMV from the viruses examined. The results of serological and biological tests showed that kale plants were only infected with TuMV in Ordu, but none of the samples were infected with CaMV, TYMV, and CMV in this region. Occurrence of the virus in kale plants was found in most *Brassica*-growing areas of Ordu province, including Camas, Catalpinar, and Unye districts. The highest infection rate was found in kale fields in Camas (24%), followed by fields in Unye (8.7%), and Catalpinar (3.3%) (Table 1).

Table 1. Occurrence of viruses in kale samples collected in Ordu in 2013-2014.
Tabla 1. Ocurrencia de virus en muestras de *Brassica oleracea* var. *Acephala* colectadas en Ordu en 2013-2014.

		Viruses*			
Regions	**Samples tested**	**TuMV**	**CaMV**	**TYMV**	**CMV**
Camas	50	12 (24.0%)	0	0	0
Catalpinar	60	2 (3.3%)	0	0	0
Fatsa	30	0 (0.0%)	0	0	0
Gurgentepe	48	0 (0.0%)	0	0	0
Unye	46	4 (8.7%)	0	0	0
Total	234	18 (7.7%)	0	0	0

*: TuMV (Turnip mosaic virus), CaMV (Cauliflower mosaic virus), TYMV (Turnip yellow mosaic virus), and CMV (Cucumber mosaic virus).

In the current study, TuMV was detected in kale plants showing symptoms of leaf mosaic, leaf distortion, mid rib narrowing, and reduction in overall plant growth. However, a few leaf samples that showed symptoms did not react with four antisera used in serological tests. The leaf samples were possibly infected with other disease-causing agents or the symptoms may have been caused by nutrient deficiencies.

Mechanical inoculation tests. Inoculated plants showed different symptoms (Table 2). Necrotic local lesions and mosaic in *B. oleracea* var. *capitata* subvar. *alba*, severe mosaic and leaf malformation in *B. oleracea* var. *acephala* and *B. campestris* subsp. *rapa,* mosaic in *R. sativus,* and necrotic local lesions in *C. quinoa* were observed.

Table 2. Symptoms caused by TuMV in test plants inoculated, and DAS-ELISA positive samples.
Tabla 2. Síntomas causados por TuMV en las plantas inoculadas, y muestras positivas a DAS-ELISA.

Plants	**Symptom*/ DAS-ELISA****
B. oleracea var. *capitata* var. *alba*	NLL, M / +
B. oleracea var. *acephala*	SM, LM / +
B. campestris subsp. *rapa*	SM, LM / +
R. sativus	M / +
C. quinoa	NLL / +

*: M: mosaic, SM: severe mosaic, LM: leaf malformation, NLL: necrotic local lesions
**: + = virus detected in DAS-ELISA.
*: M: mosaico, SM: mosaico severo, LM: hoja malformada, NLL: lesiones necróticas locales.
**: + = virus detectado en DAS-ELISA.

DISCUSSION

The kale is one of the most important vegetable crops in the Black Sea Region of Turkey (Balkaya & Yanmaz, 2005). A survey of kale plants for the presence of selected viruses in Ordu, Turkey was carried out in 2013-2014. All collected samples were tested by DAS-ELISA in duplicate and bioassays. Result of serological and biological tests showed that the kale plants were infected with TuMV in Ordu, located in the Middle Black Sea Region, Turkey. Occurrence of TuMV was recorded in Camas (24.0%), followed by kale fields in Unye (8.7%), and Catalpinar (3.3%). These results revealed that kale grown in commercial fields were infected quite commonly with TuMV. Likewise, a total of 142 samples collected from fields planted to Brassicaceae crops in the southwest Marmara region of Turkey during the 2004-06 growing seasons were tested by DAS-ELISA. Of those tested, 19 plants (13.4%) including white cabbage (7 plants), Brussels sprouts (1 plant), radish (7 plants), wild radish (3 plants) and wild mustard (1 plant) were infected with TuMV (Korkmaz et al., 2008). A total of 532 Brassicaceae reservoir weed samples were collected from plants with virus-like symptoms in Iran. The samples were tested for the presence of TuMV by DAS-ELISA using specific antibodies. Among those tested, 340 samples (64%) were in-

fected with TuMV (Farzadfar et al., 2009). Similarly, many reports have showed that one of the most widespread virus of *Brassica* crops was TuMV worldwide (Walsh & Jenner, 2002; Eiras et al., 20007; Gładysz & Hanus-Fajerska, 2009).

Raybould et al. (1999) reported four viruses (CaMV, TuMV, BWYV, and TYMV) in five natural populations of *B. oleracea* in Dorset (UK). All four viruses were common; 60% of plants were infected with CaMV, 43% with TuMV and BWYV, and 18% with TYMV. Alan (2012) tested winter vegetable species in the Eastern Mediterranean region of Turkey for the presence of a number of viruses, including TuMV and CaMV. Ten cabbage and one cauliflower plants were found to be infected with TuMV and CaMV, respectively. Contrarily to the reports of Raybould et al. (1999), and Alan (2012), CaMV, TYMV, and CMV were not detected in kale samples collected from Ordu, Turkey in the current study.

Positive findings for TuMV were observed in the biological tests to confirm the findings in DAS-ELISA. TuMV isolates were inoculated to test plants. Symptoms of isolates showed that TuMV caused necrotic local lesions and mosaic in cabbage, severe mosaic and leaf malformation in kale and turnip, mosaic in radish, and necrotic local lesions in quinoa. These symptoms were similar to those previously described for TuMV (Farzadfar et al., 2009).

Turnip mosaic virus belongs to the genus *Potyvirus* within the family Potyviridae (Fauquet et al., 2005), and is probably the most common and important virus in Brassicaceae crops throughout the world (Farzadfar et al., 2009). Aphids and weed hosts play a major role in the epidemiology of the virus. TuMV is transmitted in a non-persistent manner by at least 89 aphid species (Walsh & Jenner, 2002). Non-persistent transmission by aphids is the most difficult to avoid because once the aphids carrying virus have attacked the host plant, infection occurs immediately. However, the number of plants that can be infected is very low. Winter crops such as kale provide growers with an early season product because they grow well in cool weather. Harvesting goes from the end of November to the end of February each year in the surveyed regions (Balkaya & Yanmaz, 2005). Fortunately, the cold and wet conditions during winter have kept aphid populations low in the Black Sea Region of Turkey. In the present study, TuMV was detected in limited areas of the Ordu province and showed low incidence, most probably due to the reduced vector populations and activity (Sastry & Zitter, 2014).

TuMV is not seed-borne. It survives in weeds or volunteer host plants outside the growing season. Weed and wild plants also play a major role in the high incidence of the virus. Occurrence of the virus in weeds was found in most crop and ornamental *Brassica*-growing areas of Iran by Farzadfar et al. (2009). Samples collected from Brassicaceae weeds during 2003-2008 were tested by DAS-ELISA. The highest infection rate of TuMV was found for *Rapistrum rugosum* (82/82, 100%), followed by *Sisymbrium loeselii* (73%), *S. irio* (28.9%) and *Hirschfeldia incana* (20%) (Farzadfar et al., 2009). However, the role of weeds and wild plants in the epidemiology of TuMV was not studied in the present work.

In conclusion, virus diseases continue to be a problem in the production of *Brassica* crops in Turkey. This is particularly true of the diseases caused by aphid-borne viruses like TuMV, CaMV and CMV. However, TuMV causes the most serious damage to cabbage crops (Spence et al., 2007). This is the first report which used serological and biological methods to identify the viruses of kale crops in Ordu. We showed that TuMV was the major virus in kale crops at the Black Sea Region of Turkey during 2013 to 2014. Vectors and weeds may have a significant effect on virus epidemiology, and knowledge of weed reservoirs and vectors of viruses would be essential to understand the epidemiology. Therefore, further studies are needed to determine the role of weed hosts and the major vector species of TuMV in the region. To our knowledge, this is the first report of natural occurrence of TuMV on kale plants in Ordu, Turkey.

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