Original Article

OCCURRENCE AND DISTRIBUTION OF *Apple mosaic virus* ON HAZELNUT CULTIVARS IN GIRESUN, TURKEY

OCORRÊNCIA E DISTRIBUIÇÃO DO VÍRUS MOSAICO DA MACIEIRA EM CULTIVARES DE AVELÃ EM GIRESUN, TURQUIA

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ABSTRACT: *Apple mosaic virus* (ApMV) is the most important viral pathogen of hazelnut. The Black Sea region is the main hazelnut producing area in Turkey. Hazelnut growing areas from Giresun which are located in the Black Sea region were surveyed for occurrence and distribution of ApMV in 2011-2012. Hazelnut leaf samples were collected randomly from non-symptomatic and symptomatic plants displaying yellow rings and lines, yellow flecking, oak leaf pattern and broad vein banding. Leaves from 229 samples collected in 35 fields were tested for the presence of ApMV using enzyme-linked immunosorbent assay (ELISA) with a commercial antiserum. Based on the ELISA results, 18 out of 229 samples (7.86%) were infected with ApMV.

KEYWORDS: *Apple mosaic virus.* Hazelnut. ELISA. Survey.

INTRODUCTION

Hazelnut (*Corylus avellana* L.) belongs to the *Betulaceae* family and is a popular nut tree worldwide. With respect to production and export of hazelnut, Turkey is by far the leading producer with 75% of world production (BABADOGAN, 2010). In Turkey, hazelnuts are cultivated in an area of around 350 000 ha. The production area is spread densely all along the Black Sea coast. Hazelnut orchards extend up to 30 km in land and up to 750-800 meters altitude due to being a shore plant. Giresun province, located in the Black Sea coast, is the second largest hazelnut-producing region in Turkey after Ordu province. Giresun had a hazelnut production of 139.764 tons in 2008. 'Tombul' and 'Sivri' are the main cultivars (KAFKAS et al., 2009) and are grown as bush-like trees called 'ocak' in the region. Hazelnuts make a significant contribution to Turkey's exports, as well as being a source of income for a large number of family farms in the Black Sea region (OZKUTLU at al., 2011).

Insects (SARUHAN; SEN, 2012), mites (OZMAN-SULLIVAN, 2006) and diseases (SNARE 2006; GENTIT et al., 2009) are major constraints to the production of hazelnuts throughout the world. *Apple mosaic virus* (ApMV; genus, *Ilarvirus*, family *Bromoviridae*) is one of the most important pathogens of hazelnut (EPPO, 2004). ApMV can infect, either experimentally or naturally, over 65 plant species in 19 families. Natural hosts of ApMV include apple, birch, hazelnut, hop, horse chestnut, raspberry and rose (RYBICKI, 1995). The pathogen is easily distributed with vegetative plant materials and is gradually spreading in hazelnut and oil rose plantations in Turkey (ERTUNC et al., 2011).

The present study was carried out to access the occurrence of ApMV on hazelnut in Giresun, Turkey.

MATERIAL AND METHODS

Survey for *Apple mosaic virus* was conducted during the 2011-2012 planting season in 35 locations in Giresun (Figure 1). Hazelnut leaf samples were collected randomly from non-symptomatic and symptomatic plants displaying yellow rings and lines, yellow flecking, oak leaf pattern and broad vein banding.

Leaf samples were taken from 229 shrub-like (ocak) hazelnut plants from two cultivars (Tombul and Sivri) collected at five locations each of the Bulancak, Central, Espiye, Kesap, and Tirebolu districts, six locations of Eynesil district and four locations of Gorele district (a minimum of three samples per location). Plant samples were placed in polyethylene bags, labeled, brought to the laboratory and kept at -20 ºC until using.

The presence of ApMV was tested in duplicate subsamples using DAS-ELISA, with antibodies from Bioreba (Basel, Switzerland), according to the manufacturer's instructions. Samples were ground 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; 1 g sample per 10 ml buffer) containing 0.05% Tween-20, 1% polyvinyl pyrrolidone and 1% skimmed milk powder. Absorbance values were read at 405 nm using a microplate reader (Tecan Spectra II,

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Grödig/Salzburg, Austria). Samples were considered positive when the absorbance values at 405 nm (A405) exceeded the mean of the negative control (negative control from Bioreba) by a factor of at least three (ARAMBURU; ROVIRA, 1998).

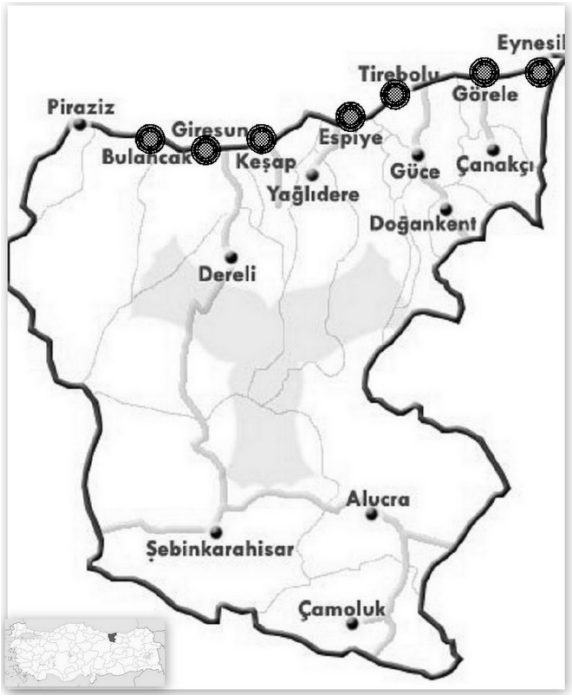

Figure 1. Map of Giresun, Turkey, showing the areas in which surveys were conducted in 2011 and 2012.

The results of ELISA were recorded and statistical differences among mean values from the two cultivars analyzed using the statistical package SPSS 12.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

A total of 35 fields were surveyed, and 229 leaf samples collected during the surveys were tested by DAS-ELISA (Table 1). Results as indicated in Table 1 revealed that 18 out of 229 samples (7.86%) contained ApMV. Twelve out of 124 (9.67%) 'Tombul' samples contained ApMV and six out of 105 (5.71%) 'Sivri' samples had the virus.

Bulancak district had the highest incidence of ApMV (four out of 36 samples, 11.11%), and Gorele district had the second highest (3 out of 28 samples, 10.71%). Incidence in the other districts ranged from 10.52 (Kesap) to 3.70% (Espiye) (Table 1).

Table 1. Occurrence of ApMV in hazelnut plants collected at seven districts of Giresun province, Turkey, in 2011-2012.

District	Number of locations	Hazelnut cultivars		ApMV incidence (%)
		Tombul	Sivri	
Bulancak	5	18/3*	18/1	11.11
Central	5	21/1	21/1	4.76
Kesap	5	17/2	21/2	10.52
Espiye	5	18/1	9/0	3.70
Tirebolu	5	19/2	10/0	6.89
Eynesil	6	19/1	10/1	6.89
Gorele	4	12/2	16/1	10.71
Total	**35**	**124/12**	**105/6**	**7.86**

*: Number of plants tested / infected.

There were statistically significant differences (P<0.05) between cultivars for the number of plants infected, with a higher incidence in 'Tombul' (12 out of 124 plants, 9.6%) than in 'Sivri' (six out of 105, 5.7%).

The presence of ApMV was confirmed in samples by transmission to indicator test plants (common bean, *Phaseolus vulgaris*). The symptoms observed in the indicator plants reflected the results of the DAS-ELISA.

DISCUSSION

Turkey is the most important country of origin for hazelnuts in the world. Very few countries in the world have a climate as favorable for hazelnut production as that of Turkey. Hazelnut plantations, which are a major source of income for the villagers in the eastern Black Sea region (DURKAYA; DURKAYA, 2009) are not able to provide sufficient income to the villagers due to pests (AK et al. 2005) and diseases (ALAY et al., 1973; AKBAS; DEGIRMENCI, 2009; SEZER; DOLAR, 2012).

In the present study, the occurrence and distribution of viruses in hazelnuts in the Giresun province (Black Sea region) of Turkey was investigated. Symptoms of yellow flecking, oak leaf pattern yellow rings and lines were observed in hazelnut plants in the surveyed area in 2011 and 2012. These symptoms were similar to those previously reported from virus-infected hazelnut plants in other countries (KOBYLKO et al., 2005; SNARE, 2006).

ApMV is one of the most important pathogens of hazelnut. It causes mosaic on the leaves and may have an effect on yield and vigour (EPPO, 2004). The effect of infection on hazelnut trees was examined in three different provinces collecting data on plant yield from selected trees (AKBAS; DEGIRMENCI, 2009). Crop losses ranged from 19.7 to 35.6% (28.2% in average) and infected trees showed a slight growth reduction. In another study, the susceptibility of seven hazelnut cultivars to ApMV was compared for four years in an experimental orchard (GENTIT et al., 2009). Observations were carried out on different criteria regarding symptom severity on leaves as well as yield and growth. All cultivars were sensitive to ApMV with different level of symptoms. The yield and the growth were affected by ApMV infection (GENTIT et al., 2009).

In previous studies, ApMV incidence in hazelnut varied considerably among the provinces in different parts of Turkey (ERTUNC et al., 2011). A survey of 213 orchards was carried out in 2005-2008 in the west Black Sea region (AKBAS; DEGIRMENCI, 2009). The average infection rate was 13.6%. The highest infection rate was found in Duzce (14.83%), followed by Bartın (13.4%), Zonguldak (12.74%), Sakarya (12.66%), and Kocaeli (12.5%). In a study of 62 orchards in Samsun province in April-May 2002-2003, it was shown that the percentage of infected hazelnut samples determined by ELISA varied between 10 and 18.3% (ARLI-SOKMEN et al., 2004). Similarly, in the present study, average infection rate was 7.86% in Giresun province.

Weeds are abundant in hazelnut orchards in the Black Sea region due to poor weed control by growers. Our study did not include experiments to elucidate the mechanism of ApMV transmission to weeds in nature. However, it was reported by Hunter et al. (1958) that root grafts may cause the spread of ApMV. In a previous study, weed samples belonging to *Scandix* sp., *Artemisia sp.*., *Campanula* sp., *Galeopsis* sp., *Salvia sp.*, *Prunella* sp., *Clematis* sp., and *Rubus* sp. were found to be infected by ApMV (ARLI-SOKMEN et al., 2005). Weeds ought to be controlled not only because of economic losses produced by weed-crop competition, but also because they are alternative hosts of viruses. From an epidemiological perspective, management of weed control ought to include not only *weeds* within the crop, but also weeds surrounding the upwind edges of the field (ORMENO et al., 2006).

CONCLUSION

The presence of ApMV in hazelnut plants of the studied area was confirmed by serological methods. These results demonstrate that ApMV is widely distributed in different hazelnut growing areas of Giresun.

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RESUMO: O virus do mosaico da macieira (APMV) é o patógeno viral mais importante da avelã. A região do Mar Negro é a principal área produtora de avelã na Turquia. Áreas de cultivo de avelã de Giresun, que estão localizadas na região do Mar Negro foram levantadas para a ocorrência e distribuição de APMV em 2011-2012. Amostras de folhas de avelã foram colhidas aleatoriamente a partir de plantas com e sem sintomas, onde se visualizava anéis amarelos alinhados na superfície foliar, amarelecimento em pontos localizados redução do limbo foliar e amarelecimento das nervuras. . Folhas coletadas para 229 amostras em 35 áreas (locais) foram analisadas para a presença do vírus do mosaico da macieira (VMM) usando ELISA com um antissoro comercial. Baseando na análise de ELISA 18 amostras foram positivas para o vírus (7,86 %) num total de 229.

PALAVRAS-CHAVES: Vírus do mosaico da macieira. Avelã. ELISA. Pesquisa.

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