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RESEARCH ARTICLE

ROSE VIRUSES IN THE BLACK SEA REGION OF TURKEY

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ABSTRACT

Rose is an economically important plant for Turkey and the world floral industry. A survey was conducted during 2010-2011 to identify viruses infecting rose plants in the Middle Black Sea Region of Turkey. Samples (210) from seven rose-growing regions (Terme, Çarşamba, Ilkadım, Atakum, Canik, Ondokuz Mayıs and Bafra) were collected. All samples were tested for *Apple mosaic virus* (ApMV), *Prunus necrotic ringspot virus* (PNRSV) and *Arabis mosaic virus* (ArMV) using double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA). The incidence of ApMV, ArMV and PNRSV among the samples tested was 13.3, 2.0 and 0.5%, respectively.

INTRODUCTION

Rose has an important position in the world floral industry. This cultivated rose plant is susceptible to many viruses, such as *Apple mosaic virus* (ApMV), *Prunus necrotic ringspot virus* (PNRSV), *Arabis mosaic virus* (ArMV), *Strawberry latent ringspot virus* (SLRVS) and *Tobacco streak virus* (TSV) (Thomas, 1980). The most important and widespread viruses in rose plants are PNRSV, ApMV and ArMV (Horst and Cloyd, 2007). Viruses that infect rose belong mainly to the genera *Ilarvirus* and *Nepovirus*. Among ilarviruses, ApMV (genus *Ilarvirus*, family *Bromoviridae*) is one of the important pathogens of rose. ApMV can infect, either experimentally or naturally, over 65 species in 19 families (Fulton, 1972). Natural hosts of ApMV include apple, rose, hazelnut, horse chestnut, raspberry, birch, hop (Rycbicki, 1995). PNRSV, a member of the genus *Ilarvirus* in the family *Bromoviridae*, occurs worldwide and is a serious pathogen of many plant species, including rose (Thomas, 1980). PNRSV has been isolated in many rose-growing regions worldwide (Fulton, 1972). Among nepoviruses, ArMV and SLRSV, alone or in complexes with ilarviruses, infect garden and greenhouse rose (Rakhshandehroo *et al.,* 2006). The diseases associated with the viruses ApMV, ArMV and PNRSV show symptoms that range from mild disorders, such as mosaic, chlorosis, yellow bands and spots, oak leaf pattern, leaf distortion or vein clearing, to more severe disorders, such as deformed flowers and colour break (Yardımcı and Culal, 2009). Rose is one of the most important commercial flower crop used in the floriculture and cut flower industry of Turkey (Yilmaz and Ekinci, 2011). The objective of this work was to test for the viruses ApMV, ArMV and PNRSV in rose plants in the Middle Black Sea Region of Turkey. Field inspections were conducted in rose-growing regions and leaf samples with suspicious virus symptoms were collected and tested for the above three viruses by ELISA and bioassay tests.

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MATERIALS AND METHODS

Survey and sample collection

Rose plantations visits and collection of samples were conducted from May 2010 to February 2011. A total of 210 leaf samples were collected from seven rose-growing regions (Terme, Çarşamba, İlkadım, Atakum, Canik, Ondokuz Mayıs, Bafra), as shown on Table 1. The locations surveyed are shown in Fig 1. Samples were collected in a proportional and representative way in accordance with quantities cultivated in each producing area of the region. Each sample was taken from a different plant that was selected different sites of the regions.

Serological tests

Rose leaf samples were tested for ApMV, ArMV and PNRSV. All viruses were tested in duplicate using DAS-ELISA, with antibodies from Bioreba (Bioreba, Basel, Switzerland) according to the Manufacturer's instructions. Absorbance values were read at 405 nm using a microplate reader (Tecan Spectra II, Salzburg, Austria). Samples were considered

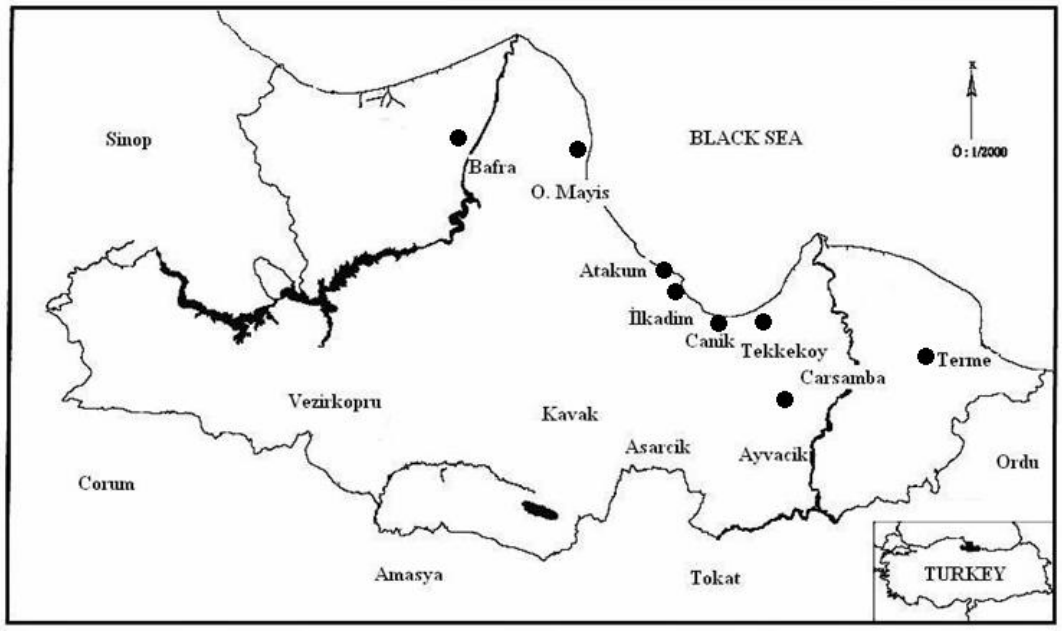

Fig 1. Map of the Middle Black Sea Region of Turkey showing areas in which surveys were conducted

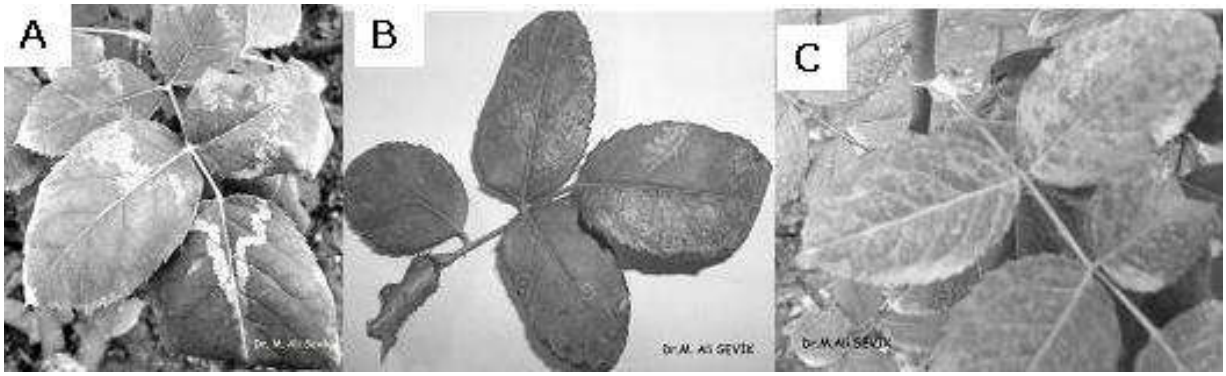

Fig 2. Natural symptoms of ApMV (A), PNRSV (B) and ArMV (C) on rose leaves under field conditions

Fig 3. Virus inoculated French bean (*P. vulgaris*) showing systemic chlorosis, necrotic lesions

Table 1. Occurrence of viruses on rose samples collected from different locations of the Black Sea Region of Turkey

Location	No. of samples tested	Virus		
		ApMV[a]	ArMV[b]	PNRSV[c]
Atakum	20	8	3	-
Bafra	10	-	-	-
Canik	5	1	-	-
Çarşamba	38	3	-	-
İlkadım	11	-	-	1
Ondokuz Mayıs	30	6	1	-
Terme	96	10	-	-
Total	210	28	4	1

[a] ApMV= *Apple mosaic viru;* [b] ArMV= *Arabis mosaic virus;* [c] PNRSV= *Prunus necrotic ringspot virus*

positive when the absorbance values of the two wells used for each tested sample at 405 nm exceeded the mean of the negative control by a factor of at least three (Kutluk-Yilmaz, 2010).

Biological tests

Three rose samples which gave high absorbance values in ELISA for ApMV were inoculated mechanically onto indicator plants of French bean (*P. vulgaris*) at the 2-4 leaf stage (three replicates) (Fulton, 1972; Arli-Sokmen *et al.,* 2005) and were maintained in a growth cabinet at 23ºC. Inoculated plants were tested for ApMV by ELISA to confirm the presence of this particular virus and the results of the serological tests performed on virus-symptomatic field plants.

RESULTS

During the surveys, most rose plants contained with viral symptoms in 2010-2011. Leaves of rose plants showed mosaic, chlorosis, yellow bands and spots, oak leaf pattern, leaf distortion (Fig 2). Therefore, our study was conducted to identify the major virus diseases and their incidence in rose plants using ELISA and bioassay. Among the samples tested, ApMV, ArMV and PNRSV were found in 13.3, 2.0 and 0.5% of the collection, respectively. The ApMV incidence was higher (>10%) in rose whereas very low incidence levels of ArMV (2.0%) and PNRSV (0.5) were found in only three locations. Symptoms occurred in 100% of positive plants and were characterized by mosaic, chlorosis, yellow bands and spots, oak leaf pattern. In a preliminary study, *Cucumis sativus* L., *Vigna unguiculata* (L.), *Phaseolus vulgaris* L., *Petunia hybrida pendula* Vilm., *Amaranthus retroflexus* L., *Gomphrena globosa* L., *Nicotiana tabacum* L. ''Xanthi nc'' or *N. clevalandii* L. were inoculated with ApMV. Only *P. vulgaris* (PI 212110, Washington, the USA) produced symptoms. Therefore, this species was used for virus isolation and maintenance. The presence of ApMV was verified in samples by transmission to indicator test plants, French bean (*P. vulgaris*). Bean plants mechanically inoculated with extracts of ELISA-positive plants showed systemic chlorosis and necrotic lines on leaves two weeks after inoculation (Fig 3). These symptoms were similar to those that were described previously for the virus. The symptoms observed on indicator plants for ApMV corresponded to the results of DAS-ELISA. In the study, ELISA tests were conducted using several negative controls for each virus. Therefore, the absorbance values varied from 0.3 to 0.6 for positive leaf samples and absorbance values equal to 0.06 was obtained for negative control. The highest infection rate was found in rose plantings in Atakum (55%), followed by plantings in Ondokuzmayis (23.3%), Canik (20%), Terme (10.4%), İlkadim (9.1%) and Carsamba (7.9%). ApMV, PRSV and ArMV were not detected in tested rose plants from Bafra (Table 1). In the present study, ArMV detected in only two regions, the percentages of rose plants infected with ArMV in Atakum and Ondokuzmayis were 15 and 3.3%, respectively. The virus was not detected in rose plants from other regions. In this study, PNRSV was detected only İlkadim (9.1%). The virus was not detected in any plant samples tested form other six regions. Mixed infections in combination with ApMV, PNRSV and/or ArMV were not detected by serological methods in this study. Interestingly, all virus-positive samples were single infected.

DISCUSSION

Viruses causing mosaic, chlorosis, yellow bands and spots and oak leaf pattern were detected in rose plants in the surveyed area in the Middle Black Sea Region. Diseases symptoms were similar to the symptoms previously reported from virus-infected rose plants worldwide (Rakhshandehroo *et al.,* 2006; Sertkaya, 2009). In the 210 rose leaf samples collected during surveys and tested by DAS-ELISA, three viruses (ApMV, PNRSV and ArMV) were detected. Of the plants tested, 15.71% were infected with at least one virus. The incidence of some virus infections varied between regions (Salem *et al.,* 2003). In this research, differences in infection rates and occurrence of viruses were also determined between viruses and locations. The presence of ApMV was verified in samples by transmission to indicator test plants (Arli-Sokmen *et al.,* 2005). The presence of ApMV, PNRSV and ArMV has also been reported from different countries on rose (Rakhshandehroo *et al.,* 2006). Our results indicate that ApMV, ArMV, and PNRSV are the three most prevalent viruses in the region, although PNRSV had the lowest incidence of these three viruses. Similarly, Yardımcı and Culal (2009) reported that ArMV, PNRSV, and ApMV were the most prevalent viruses on rose plants, with an incidence of respectively 51.8%, 35.7%, and 17.8% in the Lakes Region in 2006-2007.

ApMV was the most frequent virus found in rose plants in the regions. It is known that ApMV can infect over 65 species in 19 families (Fulton, 1972). The presence of other hosts in the locations surveyed, may explain the extensive occurrence of ApMV in rose samples collected in this survey. Previously, similar observations have been reported by Akpınar and Cıtır (2009). Although, PNRSV incidence was high in rose plants

elsewhere (Erdiller *et al.,* 1995; Sipahioglu *et al.,* 2001; Güran, 2007), our studies showed that PNRSV incidence was low relatively in rose-growing regions in the Middle Black Sea Region of Turkey. In Turkey, ApMV, ArMV and PNRSV have already been reported in rose plantations but the natural occurrence of ApMV and ArMV in rose is being reported for the first time in the Middle Black Sea Region of Turkey.

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