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Full Length Research Paper

Occurrence of pepper mild mottle virus in greenhouse-grown pepper (*Capsicum annuum* L.) in the West Mediterranean region of Turkey

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Severe systemic viral symptoms were observed on the leaves of infected pepper (*Capsicum annuum* L.) plants cultivated in Antalya located in the Mediterranean coast of Turkey, in 2008. The symptoms on the diseased pepper plants included, mosaic, mottle, chlorosis coupled with stunting, chlorotic spots, distortion of the leaves and fruits. Using *Pepper mild mottle tobamovirus* (PMMoV) specific polyclonal antisera, PMMoV was detected in symptomatic pepper plants by double antibody sandwich-enzyme linked immunosorbent assay (DAS-ELISA). Disease occurrence (the percentage of plants infected) of up to 30% was estimated in the region outbreaks. It was observed that, symptomatic pepper samples collected from Antalya province were infected with PMMoV (47.14%) according to the results of the study.

Key words: Virus, pepper, outbreak, double antibody sandwich-enzyme linked immunosorbent assay (DAS-ELISA), *Pepper mild mottle tobamovirus* (PMMoV), disease occurrence.

INTRODUCTION

Antalya is located in the Mediterranean coast of Turkey, with a wide range of vegetables and other crops being cultivated there. Pepper (*Capsicum annuum* L.) is one of the most commonly greenhouse-grown vegetable in the region (State Institute of Statistics, Turkey 2005). Pepper is among the world's most popular vegetable belonging to the family Solanaceae after potato and tomato and is mainly used as a spice (Berke, 2002). Currently, approximately 65 viruses have been reported to infect pepper throughout the world (Green and Kim, 1994). *Pepper mild mottle virus* (PMMoV) is one of the most important pathogens of pepper crops worldwide (Alonso et al., 1989; Oka et al., 2008). PMMoV was first reported in *C. annuum* L., from South Carolina, U.S.A. by McKinney (1952). The virus was first identified in Turkey in 1994, in fields of commercial pepper (Guldur et al., 1994; Palloix et al., 1994a). The virus is of economic importance for pepper crops grown under glasshouses and plastic tunnels (Wetter and Conti, 1988). It is easily transmitted by mechanical inoculation and by handling during cultivation. It is equally transmitted by grafting and contact between plants and contaminated seeds (Lewandowski, 1999; Genda et al., 2005; Svoboda et al., 2006). Mild leaf chlorosis and growth reduction occur in naturally infected commercial pepper cultivars. Fruits are small, malformed, mottled and sometimes develop necrotic depressed areas. Stunting is observed very severe in *Capsicum* spp. infected at early stages of growth. In field crops, infection may reach 100% which drastically reduces the yield of marketable fruits (Wetter and Conti, 1988; Green, 2003).

This study was conducted to monitor the prevalence and occurrence of PMMoV so that management strategies can be developed to minimize crop losses in Antalya, Turkey. Therefore, attempts were made in this study to analyze the etiology of the disease.

MATERIALS AND METHODS

Surveys and sample collection

In 2008, a survey of symptomatic greenhouse pepper plants was conducted in Kumluca district of Antalya located in the Mediterranean coast of Turkey (Figure 1). Leaf and fruit samples

Figure 1. Map of the West Mediterranean region of Turkey showing areas in which the surveys were conducted.

showing virus-like symptoms were taken from symptomatic pepper plants from each greenhouse in the same region. Each sample was placed in a plastic bag and symptom types were recorded and brought into the laboratory for virus analysis (Choi et al., 2005).

Biological testing

Inoculation for the biological tests was conducted in a climatic chamber at 23°C. The inoculum was extracted from naturally infected leaves of pepper in 0.01 M phosphate buffer (pH 7.0) and was mechanically inoculated onto carborundum-dusted leaves of the *C. annum* L plants. The plants were maintained for visual inspection of symptoms for at least 4 weeks and were confirmed by serology.

Serological testing

Double antibody sandwich-enzyme linked immunosorbent assay (DAS-ELISA) method was used to detect the virus in the pepper leaf and fruit samples and were applied according to the methods of Clark and Adams (1977) and the instructions of the antisera's manufacturer (Loewe Phytodiagnostica Biochemica, Sauerlach, Germany). In DAS-ELISA method, leaf and fruit samples with typical symptoms of virus infections were ground (1 g leaf or fruit /5 ml buffer) in extraction buffer (PBS: 0.13 M NaCl, 0.014 M KH_2PO_4, 0.08 M Na_2HPO_4, 0.002 M KCl, at pH 7.4) containing 0.05% Tween-20, were added to wells of microplate (Nunc Microwell, Roskilde, Denmark) after coating with PMMoV-specific polyclonal antisera diluted in carbonate buffer (pH 9.6) and were incubated at 4°C overnight. Plates were washed three times with PBS/Tween-20 buffer, were coated with alkaline phosphatase conjugated antibody diluted in the extraction buffer and were incubated for 2 h at 37°C. After washing, p-nitrophenyl phosphate (Sigma) in diethanolamine substrate buffer (0.5 mg/ml; pH: 9.8) was added to the wells and were incubated at room temperature for 30 to 180 min. Absorbance values were read at 405 nm using a microplate reader (Tecan). Virus-free pepper plants grown in a growth chamber and extraction buffer were used as negative controls. Samples were considered to be positive when the absorbance values at 405 nm (A_{405}) were at least two times higher than the negative controls (Hobbs et al., 2000) or the buffer controls (Cho et al., 1986; Montasser et al., 1998).

RESULTS AND DISCUSSION

In 2008, greenhouse-grown peppers in Antalya, Turkey were affected by a virus-like disease, where most fruits were severely mottled, reduced in size (Figure 2), deformed, and some showed off-colored sunken areas. The incidence of symptomatic plants was greater than 30% in two greenhouses. Symptomatic leaf and fruit samples were collected from pepper plants of green-houses from affected regions. Fruit and leaf samples from several plants were tested by DAS-ELISA with commercial antisera for PMMoV. The serological tests showed that, the surveyed greenhouses were infected with PMMoV. Tests were conducted using several negative controls for the virus. Therefore, the range of absorbance values of the negative controls varied from 0.078 to 0.092 and positive samples gave absorbance values of 0.820 to 2.235. 33 symptomatic leaf and fruit samples gave a positive result for PMMoV. It was shown that the symptomatic samples (47.14%) collected from Antalya province was infected according to study results. The results suggested that, the symptomatic pepper plants were infected with PMMoV. This record of PMMoV in the west Mediterranean region of Turkey appears to be particularly threatening because the outbreak was deve-

Figure 2. Small, malformed, and mottled pepper leaf and fruit caused by PMMoV.

loped in greenhouses.

Pepper is grown extensively in Turkey, however, infection with plant viruses such as *Tobacco mosaic virus* (TMV), *Cucumber mosaic virus* (CMV) (Heper, 1979; Yilmaz and Davis, 1985; Cicek and Yorganci, 1991), Potato virus *Y* (PVY) (Erkan, 1991), *Tobacco etch virus* (TEV) (Palloix et al., 1994b), *Tomato spotted wilt virus* (TSWV) (Yurtmen et al., 1999; Arli-Sokmen et al., 2005) and *Pepper mild mottle virus* (PMMoV) (Palloix et al., 1994a; Guldur et al., 1994; Guldur and Caglar, 2006) are considered the most limiting factors affecting their production. Recently, very severe symptoms were observed in greenhouse-grown peppers in the Kumluca district of Antalya, consisting of chlorosis and stunting, especially if the plants were infected when young; leaves and fruits were small, malformed and mottled (Figure 2). These symptoms were similar to those that were described previously for the infection of pepper by PMMoV (Wetter, 1987; Martínez-Ochoa et al., 2003; Guldur and Caglar, 2006). PMMoV of the genus *Tobamovirus* occurs worldwide and can drastically reduce fruit yield in peppers (Wetter, 1984; Alonso et al., 1989; Green, 2003). The data presented in this paper showed that, greenhouse-grown peppers were infected with PMMoV in Antalya province. In the study, viruses other than PMMoV likely infected the peppers in the region. 37 samples that showed virus-like symptoms did not react with the antiserum of PMMoV used in serological tests, indicating that they were possibly infected with other pepper viruses of which the antisera were not used in the study.

The results were similar to those reported in other studies in different regions. Disease incidence ranged from 60 to 95% and resulted in a 75 to 95% yield loss in Sanliurfa, Turkey (Guldur and Caglar, 2006). The fruit and leaf tissues from several plants were tested by ELISA using commercial kits for PMMoV. For the 42 symptomatic samples from five greenhouses, all gave a positive result for PMMoV. This is the first report of PMMoV in southeastern Anatolia and appears to be particularly threatening because of the environment (greenhouses) in which the outbreak developed and the high rates of seed transmission of the virus (Guldur and Caglar, 2006). PMMoV can be seed borne; consequently, the seedlings can be infected by mechanical contamination from their seed coats during transplanting or other cultural procedures (Martinez-Ochoa et al., 2003; Genda et al., 2005). Seed transmission may be a primary source of infection. Seed samples from twelve pepper plants also gave consistently positive ELISA result for PMMoV. Of the 120 seedlings tested for PMMoV 34 were positive, confirming transmissions through seed (Guldur and Caglar, 2006). These findings suggested that, seed companies may need to implement new screenings for PMMoV in pepper (Martinez-Ochoa et al., 2003).

In conclusion, the results of the study showed that PMMoV was one of the most frequent virus that infected greenhouse-grown peppers in Antalya, Turkey in 2008. The results of the tests and the surveys indicated that, the outbreak may have resulted from a seed introduction. PMMoV might cause severe damage in the future if pepper producers do not come up with preventive management practices.

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