MOLECULAR AND SEROLOGICAL DETECTION OF *CUCUMBER MOSAIC VIRUS* IN PEPPER GROWING AREAS IN ISPARTA PROVINCE, TURKEY

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Abstract

The aim of the study was to investigate serological and molecula detection of *Cucumber mosaic virus* (CMV) in pepper growing areas in Isparta province, Turkey. During surveys conducted in 2013- 2014 growing season, field-grown pepper plants with symptoms of mosaic, chlorotic mottling, vein banding, blistering and leaf and fruit deformation were observed. A total of 239 leaf samples were collected from various locations in this region. The Leaf samples were tested by DAS-ELISA (Double antibody Sandwich Enzyme Linked Immunosorbent Assay) using specific kits to detect CMV. ELISA tests showed that among 239 samples, 10 were infected with CMV. To confirm the results of ELISA positive leaf samples were tested by RT-PCR method and the respected size of band with 678 bp was observed on agarose gel electrophoresis for CMV. As a result of the studies, ELISA test results and RT-PCR test results were confirmed each other.

Key words: pepper, *Cucumber mosaic virus*, ELISA, RT-PCR

Pepper (*Capsicum annum* L.) is a member of the *Solanaceae* family and is a hot and mild climate plant. It has been reported that the homeland of pepper is tropical South America, especially Brazil (Anonymous 2013).

Significant efficiency losses occur in pepper cultivation due to wrong or insufficient agricultural applications as well as living and non-living disease factors. There are many fungi, bacteria and virus based diseases in the vegetable cultivation areas which limit cultivation. Virus diseases are significant among these factors due to their chemical and physical structures, sizes, types of infection, symptom formation, transport and the lack of an effective struggle against them (Agrios G.N., 1997).

Symptomatologic studies based on the identification of the viruses should be supported by serological and molecular tests.

In the recent years, PCR techniques which allow precise and accurate diagnosis of pepper viruses from plant tissues have been developed (Jacobi V. *et al*, 1998).

Cucumber mosaic virus (CMV) was first reported in 1916 (Doolittle, S.P., 1916). The virus belongs to the Cucumovirus genus of the Bromoviridae family. The virus can be transported by aphids, seeds and mechanically by infected plants. More than 80 aphid species, including *Myzus persicae* (Sulz.) and *Aphis gossypii* (Glover.), are capable of transmitting the virus in a nonpersistent, stylet-borne manner (Hobbs H.A. *et al*, 2000).

In studies on viral diseases in peppers conducted in Turkey, CMV have been detected (Arlı Sökmen M. *et al*, 2005; Özaslan M. *et al*, 2006; Buzkan N., Yüzer D., 2009).

In this study, Reverse transcription polymerase chain reaction (RT-PCR) and DAS-ELISA (Double antibody sandwich enzyme linked immunosorbent assay) methods were used for the identification of CMV in pepper growing areas in Isparta province in Turkey.

MATERIAL AND METHOD

The main material of this study consisted of a total of 239 leaf samples from Isparta showing signs of the disease and thus suspected to be infected with the virus. The samples were taken from pepper production areas in June-September of 2013-2014. During the field surveys, virus-like symptoms including yellowing, mosaic pattern of light and dark green, yellow spotting, malformation symptoms on the leaves, ring-spots or line patterns on leaves or fruit were observed and symptoms are photographed. The collected samples were labeled in polyethylene bags, brought to the laboratory in ice boxes and kept in a freezer (-20ºC) until the necessary tests were made.

CMV-DAS-ELISA (BIOREBA AG, Switzerland) commercial kit was used in the study.

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The application was carried out in accordance with the procedure of the commercial company. Accordingly, 200 µl of IgG was added to each of the hole of ELISA plate, which was diluted in the coating buffer at a ratio of 1:1000 which was then kept at +4º C for overnight. Afterwards the ELISA plates were washed with the washing buffer. The washing was repeated 3 times. The plant extracts prepared by diluting at a ratio of 1/10 using extraction buffer was added to each hole as 200 µl and was kept overnight at +4º C. Washing was repeated the next day. After washing process, conjugated antibody was diluted at a ratio of 1:1000 in the conjugate buffer, 200 µl was added to each hole and was kept at 37º C for 4 hours. After the washing process, each substrate prepared as 1 mg/ml in the substrate solution was added to each hole as 200 µl and was left to wait at room temperature.

Values that give at least twice or higher readings in comparison with the negative control value according to the absorbance values read at 405 nm have been accepted as positive (Özaslan M. *et al,* 2006).

ELISA positive 10 leaf samples were used for total RNA extraction and were tested by RT-PCR for CMV. Total RNA was extracted from fresh leaves of pepper samples.

The isolation of total RNA was performed according to RNA extraction kit (Qiagen, RNeasy Mini Kit GmbH, Germany) protocol suggested by the manufacturer.

The reverse transcription and polymerase chain reaction amplification was performed using a One Step RT-PCR Kit (Bio Basic, Canada Inc). Reverse transcription was performed in a 50 µl reaction mixture containing, 21µl H2O, 25 µl 2x1 PrimeScript One Step RT-PCR buffer (containing dNTP mixture, One step Enhancer solution), 2 µl Prime Script 1 step enzyme mix, 1 µl 20 Mm primers.

RT-PCR of CMV coat protein gene portion of approximately 678 bp were amplified.

Primer I: F- 5-TTGAGTCGAGTCATGGACAAATC-3;
Primer II: R-5-AACACGGAATCAGACTGGGAG-3.

The primer set were synthesized by Bio Basic, Canada Inc. (Han-Xin L. *et al*, 2004).

Thermocycling was carried out as follows: 50ºC for 30 min., 94ºC for 2 min., then 30 cycles of 94ºC for 30 second, 55ºC for 30 second and 72ºC for 1 min., followed by 72 ºC for 3 min. PCR products were separated in 1% agarose gel by electrophoresis, stained with 0.5 µg/ml ethidium bromide solution.

An image was captured after exposing the ethidium-bromide-stained gel on a transilluminator with a digital camera (UVP-Doc-It). DNA markers (100 bp DNA ladder, Fermentas) were used in each electrophoretic run.

RESULTS AND DISCUSSIONS

In survey studies conducted between 2013 and 2014, leaf samples were collected from Isparta province.

Leaf deformation, curliness, vein banding, discoloration of the leaves, necrosis, mosaic and plant stunting, ring-spot symptoms were common in surveys. Photographs of these plants are presented in *figure 1*.

Observation of the presence of these symptoms indicated that pepper plants in the visited fields were possibly infected with CMV or some other viruses. Similar observations were reported in different studies (Avgelis, A.D. 1987; Palukaitis P. *et al,* 1992; Svoboda J., Svobodová-Leišová L., 2012).

All pepper leaf samples were tested for CMV using commercially available DAS-ELISA kits with negative and positive controls for virus. The results showed that 10 of 239 samples evaluated by DAS-ELISA were infected with CMV.

Several researchers have used ELISA tests to reveal the plant viruses (Cherian S., Muniyappa N.,1998; Sanchez-Navarro J.A. *et al,* 2006; Şevik M.A., 2011; ELISA is a routine and reliable test to diagnose plant viruses (Clark M.F., Adams A.N., 1977).

To confirm the results of ELISA positive leaf samples (10 samples) were tested by RT-PCR method. In RT-PCR, a single fragment of about 678 bp was amplified from total RNA extracts of pepper plants infected with CMV (*figure 2 a, b*). These 10 samples giving positive results in RT-PCR studies, gave positive results in the previous DAS-ELISA tests.

The results obtained in this study were compatible with the studies by conducted (Han-Xin L. *et al,* 2004; Eyvazi A. *et al*, 2015) designed for CMV using the primer pairs. The same researchers obtained 678 bp bands in their molecular studies.

In the light of these findings, it was shown that the RT-PCR method can be more successfully used.

The presence of CMV was confirmed using DAS-ELISA and RT-PCR method.

Figure 1 **Leaf deformation and mosaic symptoms observed in pepper**

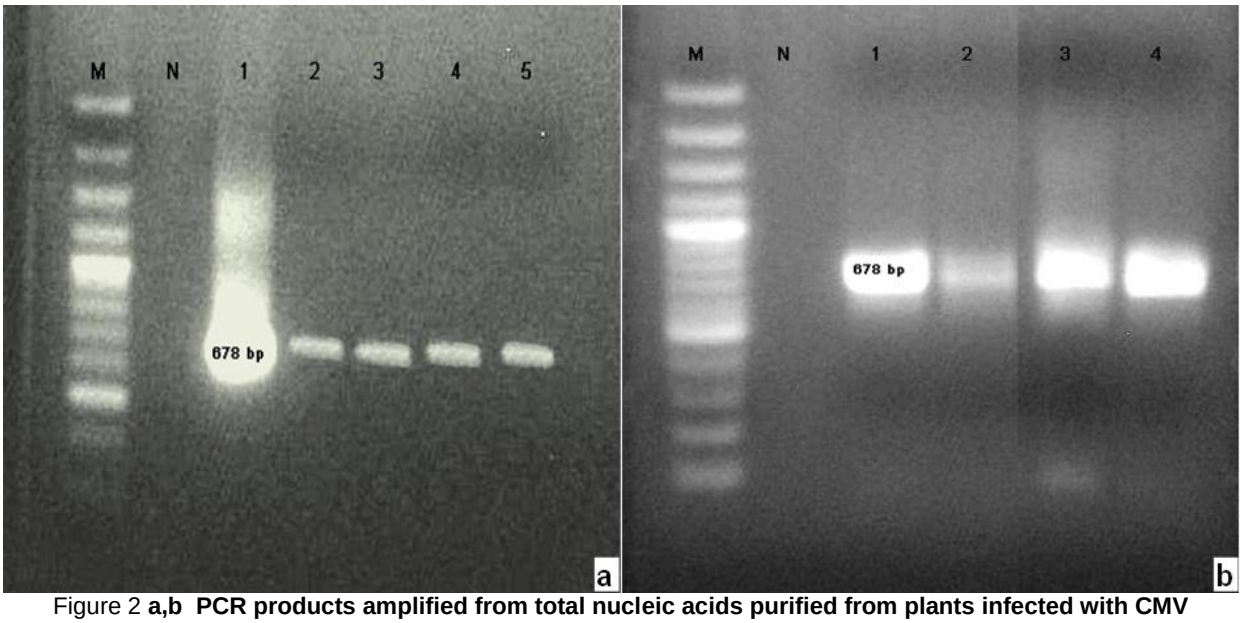

Figure 2 **a,b PCR products amplified from total nucleic acids purified from plants infected with CMV**

CONCLUSIONS

In this study, DAS-ELISA and molecular assays revealed that *Cucumber mosaic virus* was present in the region. Serological and molecular assays have generally been used for identification of vegetable viruses. Although ELISA is the preferred assay for routine virus detection, RT-PCR has increasingly been used for detection and identification of viruses due to higher level of sensitivity (Sanchez-Navaro J.A. *et al*, 2006).

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