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Detection of *Cucumber mosaic virus* and Typing Using Serological and Molecular Methods in Razavi Khorasan Province

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Abstract: Investigation was conducted during 2006 and 2007 to detect and determine typing of *Cucumber mosaic virus* (CMV) in field-collected samples from Razavi Khorasan Province. Leaves showing chlorosis, mosaic, distortion and shoestring symptoms were collected from the Mashhad Regions (Astan-e-ghods and Khaje-rabie), Neishabour, Torbat-e-heidarye, Ghochan, Chenaran, Fariman, Shirvan, Kalat and Kashmar. Samples were transferred to laboratory in order to detect the virus in the collected samples, Samples were tested by DAS-ELISA. Typing was done for those serologically positive-reacted samples by RT-PCR-RFLP. Specific primers have amplified 650 bp fragments of RNA2, in RT-PCR assay. Digestion was performed by restriction enzyme MluI. Present research showed that all of collected samples were in subgroup IA.

Key words: CMV, ELISA, typing, RT-PCR-RFLP

INTRODUCTION

Cucumber mosaic virus (CMV) is one of the major factors causing reduction in different cucurbitaceous crops and is the most world widespread virus. It exists in both tropical and temperate weather regions. In Iran, 18.1% of cucurbit production is located in Khorasan Province. CMV has a very broad host range of wild and cultivated plants, with more than 1200 known hosts including some monocotyledonous and a great number of dicotyledonous plant hosts (Chen, 2003). The virus causes diseases and economic losses in cereals, fruits, vegetables, ornamentals and specially cucurbit crops. CMV produces different variety of symptoms depend on the host and isolate of the virus. The symptoms may include leaves and fruit distortion, blight, fanleaf, ringspot, fruit woodiness and necrosis followed by death of the host plant (Chen, 2003).

Cucumber mosaic virus (CMV) is the type species of the genus Cucumovirus belongs to family Bromoviridae. CMV is a multicomponent virus consisting of three genomic single-stranded RNA's each encapsidated individually in a 28 nm diameter icosahedral particle. CMV can be transmitted from plant to plant both mechanically by sap and by aphids in a stylet-borne nonpersistent manner (Gildow, 2008). It can also be transmitted in seeds and by the parasitic weeds, *Cuscuta* sp. (dodder). In plant tissue this virus makes characterisic viral inclusion bodies which can be diagnostic. They are hexagonal in shape. These inclusions are made up of virus particles.

Various strains of CMV that differ in their biological, serological and physico-chemical properties have been isolated from all over the world (Chen, 2003). Based on serological characters, nucleic acid hybridization, nucleic acid and/or protein sequence composition, RNAase protection assay and RT-PCR RFLP, CMV has been divided into two groups (I and II). Group I has been further divided into 2 subgroups IA and subgroup IB (Finetti-Sialar, 1999). This virus has been in Iran for many years. Rahimian (1978) based on symptomalogy, suggested 8 strains of CMV. Sobokkhz *et al*. (2003) reported occurrence of this virus in Khorasan. Bashir *et al*. (2006) reported occurrence of some strains of this virus. They preserved and propagated CMV-infected samples on *Nicotiana glutinosa* and varieties of *Nicotiana tabaccum* such as Turkish, Havana and Sumsun. To determine subgroups of the virus those already detected by DAS-ELISA, RT-PCR-RFLP has been used.

MATERIALS AND METHODS

Leaves of cucurbits showing chlorosis, mosaic, distortion and shoestring symptoms were collected from the Mashhad regions (Astan-e-ghods and Khaje- rabie), Neishabour, Torbat-e-heidarye, Ghochan, Chenaran, Fariman, Shirvan, Kalat and Kashmar areas.

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Samples were transferred to the laboratory in order to detect the virus. They were tested by DAS-ELISA according to the method by Clark and Adams (1977). Polyclonal antibodies of CMV were kindly supplied by DSMZ from Germany With dilution 1:1000. Table 1 describes the location, No. of samples collected and infected samples, crops and symptoms observed from the samples.

Total RNA was extracted by sedimentation with PEG6000, from infected samples, were tested by DAS-ELISA (Sabokkhiz *et al*., 2003).

Fermentas method (Sabokkhiz *et al*., 2003) used for synthesis of cDNA. Specific primers associated with RNA2.

3`Primer: 5`-GTTTATTTACAAGAGCGTACGG-3`(RW8)
5`Primer: 5-`GGTTCGAA(AG)(AG)(AT)ATAACCGGG-3`(RV11)

The first primer was the supplementary of 618th to 637th areas of the RNA2 of virus and second primer was the supplementary of the first to 22th part of the RNA2 of the virus. C-DNA was synthesised according to the method of Fermentas Company instruction. PCR reaction was done according to the method of Fermentas Company instructions. The reaction was conducted using the thermocycler at 94°C during 3 min (94°C for 30 sec, 55°C for 60 sec, 72°C for 120 sec) 35 cycle and 72°C for 10 min. The amplified products were electrophoresed on 1% agarose gel to determine the size of the amplicons. Gel electrophoresis of RT-PCR products revealed an expected size amplicon of approximately 650 bp. typing of CMV has been done using RFLP assay. RFLP assay was done according to Finetti Sialer *et al*. (1999) method.

RFLP assay was done according to Fermentas Co., method. In this assay λDNA was used as positive control. In order to prove accuracy of assays, have been used. RNA extraction has been done accordingly AccuPrep(R) viral RNA Extraction Kit. AccuPower(R) RT Pre Mix kit has been used for cDNA synthesis.

RESULTS AND DISCUSSION

Presence of the virus in infected leaves has been proved by ELISA (Table 1). CMV-Infected samples were mechanically transmitted, for virus propagation for further investigations i.e., serological and molecular assays. RT-PCR assay amplified a DNA fragment of approximately 650 bp from RNA2 for the three CMV subgroups. The band was between 600 bp and 700 bp of 100 bp DNA ladder as marker was used (Fig. 1).

Table 1: Description of the location, No. of samples collected and infected samples, crop and symptom observed of the samples

Location	No. of samples collected	No. of infected samples	Crops	Symptoms observed
Khaje-rabi and astan-e-ghods	150	61	Cucumber and squash	Mosaic, stunting
Fariman	45	12	Squash and melon	Sever mosaic
Neishabour	93	22	Cucumber and squash and melon	Sever mosaic
Chenaran	155	28	Cucumber and squash	Mosaic
Ghuchan	15	2	Squash	Mosaic
Shirvan	25	5	Squash	Mosaic
Kalat	59	22	Cucumber and squash and melon	Mosaic and shoe string
Kashmar	15	4	Cucumber	Mosaic

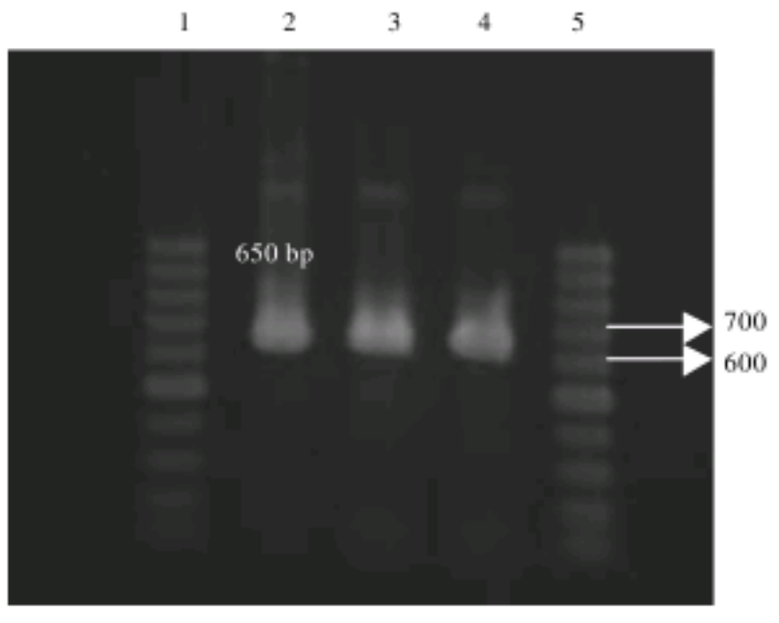

Fig. 1: Agarose electrophoresis of PCR products (650 bp) from suspended Infected leaves (Lanes 2 and 3), Lane 4 is positive control and Lane 1 and 5 is M100 bp molecular marker (1000, 900, 800, 700, 600, 500, 400, 300, 200 and 100 bp)

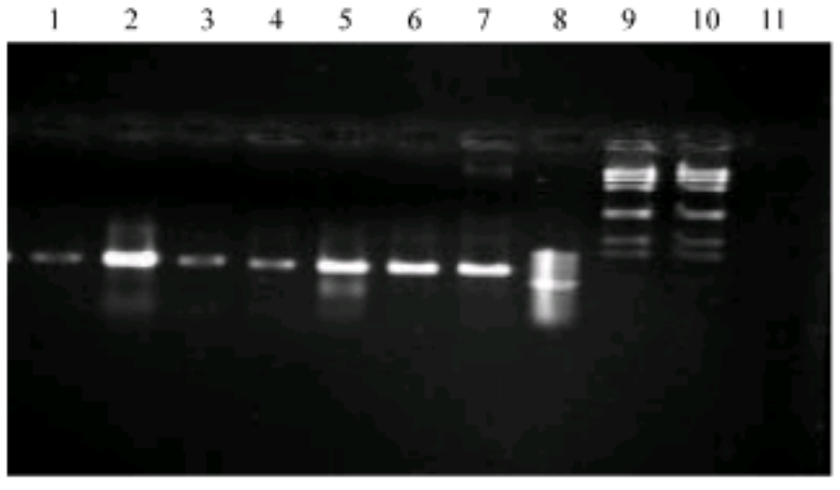

Fig. 2: RT-PCR-RFLP results on 1.2% agarose gels. Infected samples (Lanes 1-7), Lanes 9 and 10 is λ DNA (control), Lane 11 is negative control (Healthy plant) and Lane 8 is M100 bp molecular marker (1000, 900, 800, 700, 600, 500, 400, 300, 200 and 100 bp)

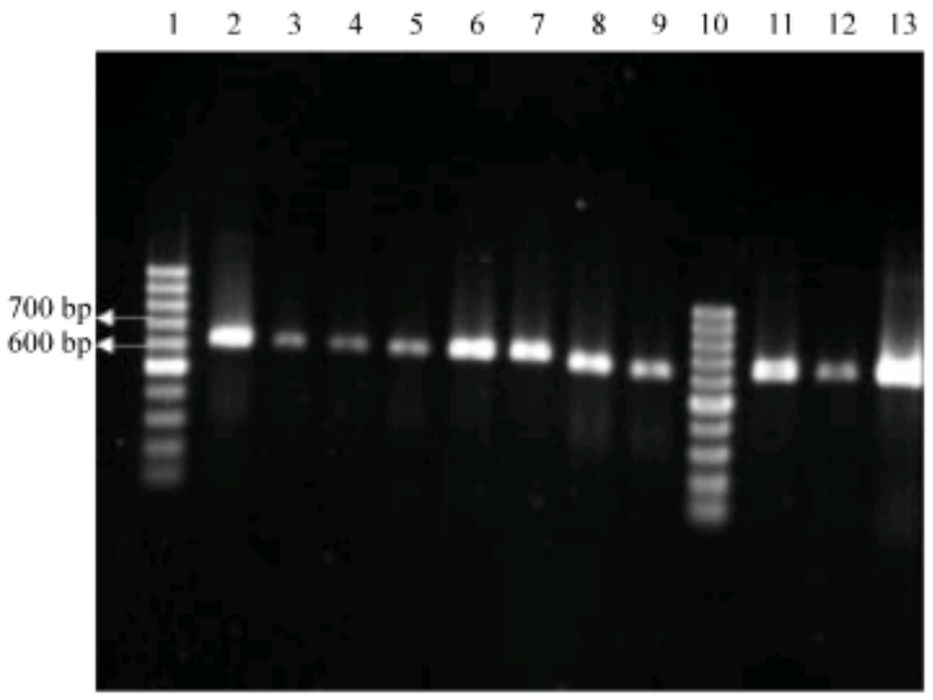

Fig. 3: RT-PCR-RFLP result on 1.2% agarose gels. Infected samples (Lanes 2-9 and 11-13), Lane 1 and 10 is M100 bp molecular marker (1000, 900, 800, 700, 600, 500, 400, 300, 200 and 100 bp)

RFLP results have been observed on 1.2% agarose and all of the bands were approximately 650 bp, without any cutting on its sequence (Fig. 2, 3). Enzyme restriction results showed one band approximately 650 bp of the subgroup IA, two fragments of approximately 470 and 160 bp of subgroup IB and three fragments of approximately 320, 170 and 150 bp of group II. λDNA was used as positive control. In RFLP assay with enzyme digestion, it showed that all of the collected samples were in subgroup IA.

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