Characterization of Cucumber mosaic virus from mint in Tehran province

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ABSTRACT
Cucumber mosaic virus (CMV) is one of the causal agents of viral diseases that infected mint (Mentha spp.). CMV is a member of the cucumovirus genus in Bromoviridae family. The virus is naturally transmitted by aphids in a non-persistent manner. During growing season 2007-2008, leaf samples from plants of mint fields with symptoms of viral disease were collected in mint fields in Tehran province. The polyclonal antibodies were used to the samples by DAS-ELISA. Some of the tested samples, showed positive reaction against cucumber mosaic virus (CMV) specific antibody. Some of the positive samples which are mechanically inoculated on test plants. Some leaf samples from mint plants with symptoms of yellows and mosaic were showed positive reaction against cucumber mosaic virus specific antibody. To confirm the identity of the virus detected using a specific primer pair of CMV which amplify part of coat protein of CMV. A fragment about 700bp was amplified in Immunocapture reverse transcriptase polymerase chain reaction (IC-RT-PCR).

Key words: Cucumber Mosaic Virus ;Mint;DAS-ELISA; IC-RT-PCR.

Introduction
Cucumber mosaic virus (CMV) is one of the causal agents of viral diseases that infected mint (Mentha spicata). CMV is a member of the cucumovirus genus in Bromoviridae family. The virus is naturally transmitted in a non-persistent manner by several species within Aphididae. CMV is a typic genus of Bromoviridae. (Francki et al.,1991). About 1000 species from 85 families Such as vegetables, ornamentals and medicinal plants are hostes of CMV. Symptoms of CMV is vary. In some plants show systemic symptoms and in other show local lesion. Experimentally infected hosts mainly show symptoms of mosaic, cholorisis and local lesion. CMV is an RNA plant virus with a tripartite genome and extremely broad host range. (Roosmnick,2001). Virions are polyhedral, not enveloped. 26 nm in diameter. Genome is ssRNA, linear, tripartite and at the same size. (Qwen et al.,1988). The molecular weight of coat protein of CMV is 24500D. Many strains of CMV is reported. (Pedea & Symons,1973) CMV has a tripartite genome of single – stranded RNAs of plus sense that are packaged in small spherical virions. (Palukaitis and Gracia- Arenal,2003;Palukaitis et al.,1992) Density is 1.367 g/cm-3 in cscl. The Sedimenting coefficient is 99(S20w). (Francki et al.,1968).
Properties of particles in sap are such as; TIP: 55-70°C, LIV: 1-10 days, DEP: log 10 minus 3-6. (Lot et al., 1976) This virus has a satellite RNA. CMV transmitted by a vector; an insect; more than 85 spp. Including Myzus persicae Sulzer, Aphis gossypii Glover; Aphididae. (Palukaitis et al., 1992) Transmitted in a non-persistent manner. Transmitted by mechanical inoculation. Transmitted by seeds (20 species, but in variable extents) and transmitted by some weeds. (Francki et al., 1979). About 10 species of cucurbit fruit virus can replicate and transmit CMV. (Bennet, 1944). Mint (Mentha spicata) is from the family of Labiatea and including important species (about 30 species) of medicinal and aromatic plants. It has been used for centuries for both medicinal and culinary purposes. (Dormat et al., 2003). There is a little knowledge about the viruses that infect the crop, other than reports identifying mint as a host. (DeAngelis et al., 1993). At the present, mint is attacked by several plant pathogens including the viruses. Studies were done on the field infection for Cucumber mosaic virus (CMV), Alfalfa mosaic virus (AMV), Arabis mosaic virus (ArMV), Strawberry latent ring spot virus (SLRV), Tobacco ring spot virus (TRSV), Mint virus 1 (MV1), Mint virus 2 (MV2), Mint virus x (MVX), Mint vein banding associated virus (MVBaV). (Tzanetakis, 2005). The first report of the occurrence of CMV in mint is from New Zealand by Fletcher (2001). Cucumber mosaic virus (CMV) is the most important virus in this case that infected the mint in several fields.

**Materials and Methods**

**Sampling**

During 2007-2008, leaf samples from mint plants with symptoms of viral diseases such as (yellows, chlorosis mosaic) were collected from botanical nationpark of Tehran province in Iran. The virus were transmitted to the Laboratory of Faculty of Agriculture, section of plant pathology and were tested by some measurement.

**Serological methods**

The polyclonal antiserum from (DSMZ, Germany) such as PVX, CMV, TMV, ToMV, TSWV, TSV, AMV were used to the samples by DAS-ELISA at the base of the method of Adams & Clark (1977). Then some samples tested by ACP-ELISA for detection of Potyviruses. Some of the tested samples, that showed positive reaction against CMV were choosed and tested by monoclonal antibody of CMV in the method of TAS-ELISA (Jordan, 1990). The last stage of this project was the identification of molecular weight of coat protein of this virus.

**Protein Analysis**

The Molecular weight of the viral coat protein was estimated by SDS PAGE (Sodium dodecyl sulfate polyacrylamid gel electrophoresis) as previously described (6). Total soluble proteins of healthy and infected plants were extracted in a Tris-borate buffer, pH 8.0 containing 5% glycerol, 1.5% polyclar AT and 0.01% β-mercaptoethanol, and fractionated on discontinuous 12% SDS–polyacrylamide gels. The separated proteins were electro-transferred onto nitrocellulose membrane (15), and after blocking of the membranes with 2% skim milk powder, membranes were incubated with CeMV IgG (DSMZ, AS-0148) at 2μg/ml followed by immunodetection of the bound CeMV IgG with alkaline-phosphatase labeled goat anti-rabbit IgG (DAKO). The molecular weight of the viral cp was determined by comparing the migration distance of the standard marker proteins in a log curve. For there used from the method of SDS-PAGE (Sambrook et al., 1989). Then for research one protein through other proteins used the method of Wester blotting (Electroblot immunoassay). In this method were applied a specific antibody for detection the same protein.
Maintenance and Host range of the virus

Test plants were dusted with carborandum (600 mesh) prior to inoculation with tissue homogenized in 0.1 M phosphate buffered saline (PBS) pH: 7 containing PVP (poly vinyl pyrrolidone 2%) at a wt/rol ratio of 1:10.

The plant species inoculated at experimental green house were such as: Chenopodium quinoa, Chenopodium amaranticolor, Chenopodium album, Cucumis sativus cv. Dominus, Vigna unguiculata, Vicia faba, Phaseolus vulgaris cv. Bountiful, Coasisum frutescence, Nicotiana tabacum cv. White Burley, Nicotiana glutinosa, Nicotiana rustica, Nicotiana tabacum cv. Samsun, Lycopersicum esculentum, Petunia x hybrida. Five plants per treatment of each indicator plant were inoculated with plant tissue. Vigna unguiculata, at experimental green house. The condition of this green house such as temperature, pH, sterilization and control of insects and pathogens.

Immunocapture reverse transcriptase polymerase chain reaction (IC-RT-PCR)

To confirm the identity of the virus detected using a specific primer pair of CMV which amplify part of coat protein gene. A fragment about 700bp was amplified in Immunocapture reverse transcriptase polymerase chain reaction (IC-RT-PCR). The nucleotide sequence of forward primer and reverse primer were (5' - TTG AGT CGA GTC ATG GAC AAA TC - 3') and (5' - AAC ACG GAA TC A GAC TGG GAG - 3') respectively with 700 bp expected size (Han-Xin, 2004).

RESULTS

Some leaf samples from mint plants with symptoms of yellows and mosaic were showed positive reaction against cucumber mosaic virus specific antibody which tested by polyclonal antibody in DAS-ELISA method (DSMZ, Germany (AS-0475)). The result of TAS-ELISA by used of a specific antibody was positive that showed, Mint infected samples were infected by CMV.

Five of the herbaceous hosts (indicators) used for mechanical transmission of CMV developed visible symptoms. positive samples which are mechanically inoculated on Nicotiana tabacum cv. Burley, Nicotiana rustica, Capsicum frutescens and Petunia x hybrid caused systemic symptoms after 15 days and on vigna unguiculata caused local lesions after 7 days (Fig.1:1a,1b,1c,1d,1e,1f).

The results of SDS-PAGE showed that the molecular weight of coat protein of this virus is about 26.91 kD (Fig.2) and western blotting supported this protein. (Fig.3)

A fragment about 700 bp was amplified in Immuno Capture reverse transcription Polymerase Chain Reaction (IC-RT-PCR). (Fig.4)

CMV is the most important pathogens that has very wide host range. In this project we emphasis on studing the CMV on Mint and symptoms. Mentha spp. is an important Medicinal plant, that have very application. This is the first report of accurence of CMV in Mint in Iran.

and we were detected the region of infection and symptoms of CMV on Mint and we were tired ti indentification symptoms and some biological and molecular characterization of cucumber Mosaic virus on Mint in Tehran province in Iran.

In this pages, we identified that CMV in mint, causes some symptoms such as yellows and mosaic.
Symptoms on tested plants are vary and in some of them causes systemic and in some of them causes local lision. Five of the thirty plants that were inoculated with tissue plants became infected by CMV, and this result by attention of the wide host ranges of CMV is unfamiliarity.

To attention, that CMV has a wide host range. The mint field from other plants such as ornamental and vegetable plants that they are hostes of this virus and then the best we should protect the mint field from Insects specially Aphids. by used of insecticides some samples that collected from fields that they used for daily uses didn't show symptoms of infection and in experimental tests hadn't any viruses. thus we resulted that this field hadn't enough time for infection and they harvested very soon and didn't go to flowering. This subject is the first report of occurrence of CMV in Mint in Iran.

The presence of infected plants with CMV as a sources of CMV inoculums increases the risk of disease problems in this important medicinal crop.

**Conclusion**

Some leaf samples of mint with symptoms of yellows and mosaic were showed positived reaction against cucumber mosaic virus specific antibody and also confirm by other tests. This virus is very important because it naturally transmitted by Aphids. Studies on diseases of medical plants is important. This is the first study on this virus in Iran on mint (Tehran Province).

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Figure 1 systemic mosaic infected by CMV on (from left to right) 1a. *Mentha* spp. Leaves, 1b. *Nicotiana tabacum* cv. White Barley, 1c. *capsicum frutescence*, 1d. *Nicotiana rustica*, 1e. *Petunia× hybrid* and 1f. local lesion on *Vigna unguiculata*. 
Fig. 2 SDS-PAGE: Lane M: protein marker, Lane 1: infected mint by CMV, Lane 2: infected pepper by CMV, Lane 3: minipurified sample, Lane 4: healthy pepper plant.
Fig. 3 Western Blotting; Lane M: proteinal marker, Lane 1: infected mint by CMV, Lane 2: infected piper by CMV, Lane 3: minipurificated sample, Lane 4: healthy piper plant
Fig. 4 Agarose gel of electrophoresis of IC-RT-PCR product representing the CMV coat protein gene: Lane M: 100 bp DNA ladder, Lane 1: empty, Lane 2 and 3: CMV-infected mint leaves Lane 4: healthy sample from mint

References


